12 December 2024
EMA/CHMP/14178/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Paxneury

International non-proprietary name: guanfacine

Procedure No. EMEA/H/C/006312/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

ADHD	Attention deficit/ hyperactivity disorder
API	Active Pharmaceutical Ingredient
ASMF	Active Substance Master File
AUC	Area Under the Concentration-time Curve
BCS	Biopharmaceutics classification system
CHMP	Committee for Medicinal Products for Human use
Cmax	Maximum value of the concentration time curve
CNS	Central nervous system
CYP450	Cytochrome P450
DDD	Defined daily dose
DSC	Differential scanning calorimetry
EC	European Commission
EU	European Union
FDA	Food and Drug Administration
GC	Gas chromatography
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
HRMS	High resolution mass spectrometry
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-OES	Inductively coupled plasma optical emission spectroscopy
IR	Infra-red
IPC	In-process control
LDPE	Low density polyethylene
MAA	Marketing authorization application
NADPH	Nicotinamide adenine dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance
NMT	Not more than
MS	Mass spectrometry
PDE	Permitted daily exposure

PE	Polyethylene
Ph.Eur.	European Pharmacopoeia
PVC	Polyvinyl chloride
PVDC	Polyvinylidene chloride
RH	Relative Humidity
QC	Quality control
QP	Qualified person
Q(SAR)	Qualitative structure activity relationship
QTc	Corrected QT Interval
SmPC	Summary of Product Characteristics
t1/2	Half-life
TGA	Thermogravimetric analysis
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopoeia
UV	Ultraviolet

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Neuraxpharm Pharmaceuticals S.L. submitted on 6 October 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Paxneury, through the centralised procedure under Article 3 (3) of Regulation (EC) No. 726/2004 – 'Generic of a Centrally authorised product'. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 30 March 2023.

The application concerns a hybrid medicinal product as defined in Article 10(3) of Directive 2001/83/EC and refers to a reference product, as defined in Article 10 (2)(a) of Directive 2001/83/EC, for which a marketing authorisation is or has been granted in the Union on the basis of a complete dossier in accordance with Article 8(3) of Directive 2001/83/EC.

The applicant applied for the following indication:

Paxneury is indicated for the treatment of attention deficit hyperactivity disorder (ADHD) in children and adolescents 6-17 years old for whom stimulants are not suitable, not tolerated or have been shown to be ineffective.

Paxneury must be used as a part of a comprehensive ADHD treatment programme, typically including psychological, educational and social measures.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Hybrid application (Article 10(3) of Directive No 2001/83/EC).

The application submitted is composed of administrative information, complete quality data and a bioequivalence study with the reference medicinal product Intuniv instead of non-clinical and clinical unless justified otherwise.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

Product name, strength, pharmaceutical form:

Intuniv 1 mg prolonged-release tablets, Intuniv 2 mg prolonged-release tablets, Intuniv 3 mg prolonged-release tablets, Intuniv 4 mg prolonged-release tablets

- Marketing authorisation holder: Takeda Pharmaceuticals International AG Ireland Branch
- Date of authorisation: 17-09-2015
- Marketing authorisation granted by:
  - Union
- Union Marketing authorisation number: EU/1/15/1040/001-009

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form:

Intuniv 1 mg prolonged-release tablets, Intuniv 2 mg prolonged-release tablets, Intuniv 3 mg prolonged-release tablets, Intuniv 4 mg prolonged-release tablets

- Marketing authorisation holder: Takeda Pharmaceuticals International AG Ireland Branch
- Date of authorisation: 17-09-2015
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/15/1040/001-009

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form:
- Intuniv 1 mg prolonged-release tablets, Intuniv 2 mg prolonged-release tablets, Intuniv 3 mg prolonged-release tablets, Intuniv 4 mg prolonged-release tablets
- Marketing authorisation holder: Takeda Pharmaceuticals International AG Ireland Branch
- Date of authorisation: 17-09-2015
- Marketing authorisation granted by:
  - Union
  - Marketing authorisation number(s): EU/1/15/1040/001-009
- Bioavailability study number(s): GUA-1122-133, GUA-1022-120, GUA-0123-6, GUA-1122-134, GUA-T1221/118, GUA-0722-87, GUA-1122-135, GFC-BEMD-01-NXP/23, GUA-T1221/117

1.3. Information on paediatric requirements

Not applicable

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Scientific advice

The applicant did not seek Scientific advice from the CHMP.

1.6. Steps taken for the assessment of the product

The Rapporteur appointed by the CHMP were:

Rapporteur: Christian Gartner Co-Rapporteur: N/A

The application was received by the EMA on	6 October 2023

The procedure started on	26 October 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	15 January 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	26 January 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 February 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	08 August 2024
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on	24 September 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	03 October 2024
The CHMP agreed on a list of outstanding issues <in writing and/or in an oral explanation> to be sent to the applicant on	17 October 2024
The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on	11 November 2024
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	27 November 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Paxneury on	12 December 2024

2. Scientific discussion

2.1. Introduction

Disease and diagnosis

ADHD is one of the most common neurobehavioural disorders of childhood, with a worldwide pooled prevalence of around 5.3% in children and adolescents (and 2.8% in adults). It is a neurodevelopmental disorder that runs a chronic course and causes significant impairments across various domains of everyday functioning, such as peer and social functioning, academic functioning, and occupational functioning across the lifespan. A comprehensive assessment including detailed history, mental state, and physical examination is needed to reach the diagnosis and estimate the severity and impact of the disorder on the patient and caregivers. Comorbid developmental, psychiatric and medical disorders, as well as psychosocial or environmental factors such as family discord, parenting, and parental substance abuse that are directly relevant to the management should be assessed as far as possible. The diagnosis should preferably be based on the diagnostic criteria as per prevailing nosological systems for the symptoms of inattention, hyperactivity, and impulsivity.

Current therapies

According to the clinical practice guidelines, the management of ADHD consists of non-pharmacological interventions, including behavioural therapy and pharmacological options, including administration of stimulants (long- and short-acting stimulants, e.g., methylphenidate or derivatives and amphetamines) and non-stimulants (e.g., atomoxetine, clonidine or guanfacine). As per the updated recommendations on the pharmacological approach, for children with ADHD aged 4-6 years, the first-line treatment should include parent training in behaviour management and/or behavioural classroom interventions (if available). Guanfacine is recommended for oral use, as a second-line therapy, in children aged 5 years and over and young people if they cannot tolerate methylphenidate or lisdexamfetamine or their symptoms have not responded to separate 6-week trials of psychostimulants, having considered alternative preparations and adequate doses. Extended release guanfacine should be used along with parental training in behaviour management, behavioural classroom interventions or preferably both educational interventions. In case, patients have sustained orthostatic hypotension or fainting episodes, the dose should be reduced or switching to another ADHD medication may be recommended.

About the product

Guanfacine hydrochloride is a highly selective α2A-AR agonist, with very little affinity for other noradrenergic receptors, including α2B- and α2C-ARs. Guanfacine preferentially binds to postsynaptic α2A-ARs in the prefrontal cortex, while other mediators of noradrenergic transmission used in ADHD, such as stimulants or α2A-ARs rather than atomoxetine, act on presynaptic postsynaptic. The development and clinical application of guanfacine has been strongly driven by its modulation of postsynaptic α2A-ARs of the dorsolateral prefrontal cortex (dlPFC), with subsequent control over working memory and other executive functions (e.g., organisation, planning, response inhibition, etc.) primarily affected in neurodevelopmental disorders like ADHD. The catecholaminergic arousal system is involved in the coordination between arousal and cognitive functions, modulating, among other aspects, the relationship between exposure to stressors and executive functioning performance. Guanfacine modulates dlPFC connectivity via feedforward calcium (Ca2+)-cyclic adenosine monophosphate (cAMP) signalling opening potassium (K+) channels. Activating α2A-ARs in the dlPFC, guanfacine closes these receptor-coupled ion channels, lowering their intracellular signalling. This specific effect strengthens the connectivity of the recurrent dlPFC excitatory circuits involved in the shaping of the contents of working memory and is considered to form the basis of the therapeutic effect of guanfacine over executive functions. Guanfacine is not a central nervous system (CNS) stimulant.

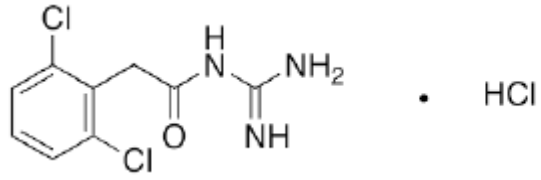


Figure 1: Chemical structure of guanfacine hydrochloride

Guanfacine hydrochloride was first described in the literature in 1974. It was initially approved by the United States Food and Drug Administration (US-FDA) under the brand name Tenex® in 1986, as an immediate-release (IR) tablet formulation, for the treatment of hypertension in patients older than 12 years. Like clonidine, it has been used clinically (and "off-label") for the treatment of ADHD and pervasive developmental disorders, which are often complicated by hyperactivity and impulsivity. Guanfacine hydrochloride, in the form of extended-release tablet, was later approved in the US on 2 September 2009 and in the European Union (EU) through a centralised procedure on 23 July 2015, for the treatment of ADHD in children and adolescents 6-17 years old for whom stimulants are not suitable, not tolerated or have been shown to be ineffective, under the brand name Intuniv®

prolonged-release tablets for once-daily use (MAH: Shire Pharmaceuticals Ireland Ltd.) It must be used as a part of a comprehensive ADHD treatment programme, typically including psychological, educational and social measures.

Posology

Careful dose titration and monitoring are necessary at the start of treatment since clinical improvement and risks for several clinically significant adverse reactions (syncope, hypotension, bradycardia, somnolence and sedation) are dose- and exposure-related. Patients should be advised that somnolence and sedation can occur, particularly early in treatment or with dose increases; if they are judged to be clinically concerning or persistent, a dose decrease or discontinuation should be considered.

For all patients, the recommended starting dose is 1 mg of guanfacine, taken orally once a day. The dose may be adjusted in increments of not more than 1 mg per week. Dose should be individualised according to the patient's response and tolerability. Depending on the patient's response and tolerability for guanfacine the recommended maintenance dose range is 0.05-0.12 mg/kg/day. The recommended dose titration for children and adolescents is provided below:

Table 1: Dose titration schedule for children aged 6-12 years

Dose titration schedule for children aged 6-12 years				
Weight Group	Week 1	Week 2	Week 3	Week 4
25 kg and up Max Dose= 4 mg	1 mg	2 mg	3 mg	4 mg

Table 2: Dose titration schedule for adolescents aged 13-17 years

Dose titration schedule for adolescents (aged 13-17 Years)							
Weight Group[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
34-41.4 kg Max Dose= 4 mg	1 mg	2 mg	3 mg	4 mg			
41.5-49.4 kg Max Dose= 5 mg	1 mg	2 mg	3 mg	4 mg	5 mg		
49.5-58.4 kg Max Dose= 6 mg	1 mg	2 mg	3 mg	4 mg	5 mg	6 mg	
58.5 kg and above Max Dose= 7 mg	1 mg	2 mg	3 mg	4 mg	5 mg	6 mg	7 mg[b]

[a] Adolescent subjects must weigh at least 34 kg.
[b] Adolescents weighing 58.5 kg and above may be titrated to a 7 mg/day dose after the subject has completed a minimum of 1 week of therapy on a 6 mg/day dose and the physician has performed a thorough review of the subject's tolerability and efficacy.

The development programme/compliance with CHMP guidance/scientific advice

The applicant did not receive CHMP scientific advice pertinent to the clinical investigation.

However, the applicant received Scientific Advice from the Spanish Medicines Agency. This advice concerned the requirements for the bioequivalence study program. The applicant sought advice regarding the proposal of performing the single dose studies with the highest demonstrated safe strength in healthy volunteers (4 mg), and the multiple dose study with the highest strength (7 mg) following an up- and down-titration design for safety assurance in healthy volunteers, the proposed

bioequivalence study package considering a bracketing approach, and the proposed standard meal for the conduct of the single dose studies under fed conditions in alignment with the SmPC of the reference product Intuniv.

The applicant did follow the advice with regard to the studies for the 1 mg and 2 mg strengths (submitted under the generic pathway (Article 10.1 of Directive 2001/83/EC)) for which it was stated that 4 studies are needed (1 mg fasted single dose, 2 mg fasted single dose, fed single dose and fasted multiple dose), and meal conditions. However, concerning the 3 mg and 4 mg strengths, for which a generic authorisation (Article 10.1 of Directive 2001/83/EC) is sought, and the 5 mg, 6 mg, and 7 mg strengths, for which a hybrid authorisation under Article 10.3 of Directive 2001/83/EC is sought owing to the fact that only the 1-4 mg strengths are approved for the reference medicinal product Intuniv, initially, the applicant did not follow the advice. Paxneury's 1 mg and 2 mg strengths have a different formulation (i.e., differing active pharmaceutical ingredient to excipients proportion, which means that the composition of the strengths are not quantitatively proportional) compared to the strengths 3 mg, 4 mg, 5 mg, 6 mg, and 7 mg (which is due to the overall weight of the tablets as with a unique composition; tablets of 5, 6 and 7 mg strength would be extremely big and not suitable for administration to children, according to the applicant); hence, a different set of bioequivalence studies would be required according to the BE guideline (CPMP/EWP/QWP/1401/98 Rev.1). In addition, the applicant had noted cardiovascular safety risks with the maximal dose of 7 mg or any dose beyond 4 mg, but proposed to perform a multiple dose study with the 7 mg strength in healthy volunteers following an up- and down-titration design for safety assurance. Therefore, it was advised to conduct 4 studies for the 3-7 mg strengths: 3 mg fasted single dose, 4 mg fasted and fed single dose and 7 mg fasted multiple dose. However, in contrast, this 7 mg study had not been initially presented, which left the hybrid-strengths (5-7 mg) without any robust clinical data and the generic strengths 3 and 4 mg without a bioequivalence demonstration in a multiple dose setting. The latter aspect changed as the applicant provided data of a 7 mg multiple-dose BE study under fasting conditions following an up- and down-titration approach.

Clinical development program

To support the application, the applicant submitted 9 bioequivalence studies. As outlined above not only the formulations of Intuniv tablets and Paxneury tablets differ but also the formulations within some of both Intuniv's and Paxneury's strengths differ (i.e., the compositions of the strengths are not quantitatively proportional). Indeed, the applicant developed a differing formulation for the strengths 1 mg and 2 mg (termed lower strengths) and the strengths 3 mg, 4 mg, 5 mg, 6 mg, and 7 mg (termed higher strengths) as with a unique formulation for all strengths, tablets of 5, 6 and 7 mg strength would be extremely big and not suitable for administration to children. Hence, the clinical study requirement, as outlined in the Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CHMP/EWP/280/96 Rev1), of single-dose under fasted and fed conditions as well as multiple-dose under fasted conditions apply for the lower (1 mg and 2 mg) and higher strengths (3 mg to 7 mg) separately. Therefore, dedicated bioequivalence trials evaluating the above-mentioned points are expected for each respective strength range (low and high) to support the current MAA.

To fulfil this requirement, the applicant submitted 4 studies for the lower strengths: study GUA-T1221/117 examining 1 mg fasted single dose, study GUA-1122-133 (NXPGUAN/22/BQ-12) examining 2 mg fasted single dose, study GUA-1022-120 (NXPGUAN/22/BQ-9) examining 2 mg fed single dose and study GUA-0123-6 (NXPGUAN/23/BQ-1) examining 2 mg fasted multiple dose).

Concerning the higher strengths, the applicant had initially submitted 4 studies: study GUA-1122-134 (NXPGUAN/22/BQ-11) examining 3 mg fasted single dose, study GUA-T1221/118 examining 4 mg fasted single dose, study GUA-0722-87 examining 4 mg fasted single dose, and study GUA-1122-135

examining 4 mg fed single dose. The 4 mg fasted single dose was investigated in two studies; study GUA-0722-87 was conducted to repeat bioequivalence evaluation for 4 mg single dose under fasted conditions after the preceding Study GUA-T1221/118 failed to demonstrate bioequivalence. With the responses to the Day 120 LoQ, the applicant submitted an additional 5th (in total 9th) study GFC-BEMD-01-NXP/23 (NXPGUAN/23/BQ-11), examining 7 mg fasted multiple dose.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as prolonged release tablets containing guanfacine hydrochloride equivalent to 1, 2, 3, 4, 5, 6, or 7 mg of guanfacine free base.

Other ingredients are hypromellose (2208), microcrystalline cellulose, colloidal anhydrous silica, lactose monohydrate, povidone K30, crospovidone (Type A), methacrylic acid-ethyl acrylate copolymer (Type A), sodium laurilsulfate, polysorbate 80, fumaric acid, and glycerol dibehenate.

The product is available in PVC/PE/PVDC/aluminium blisters as described in section 6.5 of the SmPC.

2.2.2. Active substance

General information

The chemical name of guanfacine hydrochloride is *N*-amidino-2-(2,6-dichlorophenyl)-acetamide monohydrochloride corresponding to the molecular formula $C_9H_9Cl_2N_3O.HCl$. It has a relative molecular mass of 282.55 g/mol and the following structure:

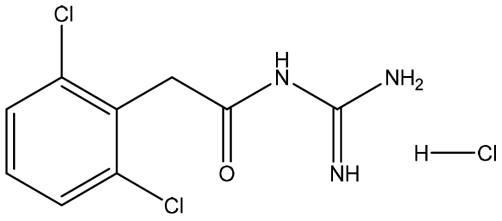


Figure 2: Active substance structure

The chemical structure of guanfacine hydrochloride was elucidated by a combination of IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, UV spectroscopy, and elemental analysis. The solid-state properties of the active substance were measured by x-ray diffraction, DSC and TGA.

The active substance is a white to off-white crystalline solid, not appreciably hygroscopic and sparingly soluble in aqueous media across the pH range. It is achiral. It has been demonstrated that the same polymorph is isolated irrespective of the solvent used to crystallise it, including the isopropanol/water mixture used in the proposed commercial manufacturing process.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

The active substance is manufactured by one manufacturer. A letter of access to the ASMF has been submitted, along with a QP declaration by the applicant stating GMP compliance based on a recent audit.

345Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised. All residual solvents used during synthesis of the active substance are adequately controlled. A risk assessment for nitrosamines and declaration for the absence of nitrosamines in the drug substance is given. Evaluation of the mutagenic potential of potential- and actual impurities in line with ICH M7 was performed. A risk assessment on elemental impurities is provided.

The active substance is packaged in double LDPE bags, sealed inside a polyethylene drum. The primary contact material complies with Commission Regulation (EU) 10/2011, as amended.

Specification

The active substance specification applied by the finished product manufacturer3 includes tests for identity loss on drying, sulphated ash assay, impurities, residual solvents and particle size distribution.

3Overall the specification for the active substance is acceptable.

Impurities limits have been set according to ICH Q3A. In the initial submission, the mutagenic risk assessment was considered deficient as the potential impurities had only been assessed by one Q(SAR) methodology resulting in a major objection. In response, the applicant updated the assessment to provide input from a second complementary Q(SAR) methodology and classified all relevant impurities according to ICH M7. Potential mutagenic impurities are now controlled according to ICH M7. Residual solvents are controlled in accordance with ICH Q3C.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. The stability indicating nature of related substances assay methods has been demonstrated.

Batch analysis data 3 production scale of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from multiple batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 60 months under long term conditions (25 ºC / 60% RH, 7 batches) and for up to 6 months under accelerated conditions (40 ºC / 75% RH, 4 batches) according to the ICH guidelines were provided. The following parameters were tested: appearance, related substances, assay, and loss on drying. All tested parameters complied with the specifications.

Photostability testing according to the ICH guideline Q1B was performed and results under stressed conditions were also provided. No significant degradation was observed on exposure to acidic conditions, oxidative conditions, temperature, humidity and light, but the active substance degrades to generate 2,6-dichlorophenylacetic acid in alkaline conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 60 months in the proposed container.

2.2.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is presented as prolonged release tablets containing 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg or 7 mg of guanfacine. The different strengths are differentiated by size, shape and inscription as per below table.

Table 3: Finished product description

Guanfacine 1 mg prolonged-release tablets are white, 8 mm round, biconvex tablets with the inscription "I" on one side.
Guanfacine 2 mg prolonged-release tablets are white, 14 x 6 mm oblong, biconvex tablets with the inscription "II" on one side.
Guanfacine 3 mg prolonged-release tablets are white, 6 mm round, biconvex tablets with the inscription "3" on one side.
Guanfacine 4 mg prolonged-release tablets are white, 7 mm round, biconvex tablets with the inscription "IV" on one side.
Guanfacine 5 mg prolonged-release tablets are white, 8 mm round, biconvex tablets with the inscription "V" on one side.
Guanfacine 6 mg prolonged-release tablets are white, 9 mm round, biconvex tablets with the inscription "VI" on one side.
Guanfacine 7 mg prolonged-release tablets are white, 12.5 x 6.5 mm oblong, biconvex tablets with the inscription "7" on one side.

The 7 strengths are made from 2 common blends. The quantitative and qualitative composition of the tablets was provided**Error! Reference source not found.**.

The finished product is a hybrid of reference product Intuniv which is available in 1-4 mg strengths. The current application adds 3 additional strengths based on the dosing regimen of the product. The tablets were designed to be essentially equivalent to the reference product. Important physico-chemical properties of the active substance such as particle size, solid state properties, specific surface area, hygroscopicity and pH dependent solubility of guanfacine were studied. Guanfacine is a BCS class II compound, exhibiting low solubility – as such, the active substance is micronized so improve solubility and content uniformity.

The hybrid tablets contain the same qualitative composition as the reference product but a different quantitative composition. Hypromellose, methacrylic acid–ethyl acrylate copolymer and glycerol dibehenate are the excipients principally responsible for active substance release rate.

A quality target product profile was applied to identify the active substance attributes that might be critical and impact the product quality. The excipients' impact on critical quality attributes of the finished product was evaluated and control strategies were proposed, as necessary. Several modifications of batch composition were studied, including the use of different polymer materials, the addition of surfactants, the use of different concentrations of methacrylic acid–ethyl acrylate copolymer and the use of different active substance to excipient ratios in the formulation. Each modification step was evaluated by a comparative dissolution study between the test and reference product.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.2.1 of this report.

Excipient compatibility studies show that the active substance is susceptible to degradation under humid conditions. Therefore, a process based on dry blending and compression was selected.

Bioequivalence of the test and reference products was demonstrated in clinical studies between 1, 2, 3 and 4 mg tablets (5 mg, 6 mg and 7 mg tablets were not used in the clinical study). A comparative dissolution rate study was conducted for the 1 mg, 2 mg, 3 mg and 4 mg tablets vs the same strengths of the reference product. In addition, dissolution rate of the 7 mg tablet was compared to dissolution rate of the 3 + 4 mg reference product tablets. All batches (other than the 7 mg tablet) were those used in the clinical bioequivalence trial. Furthermore, the dissolution rates between tablets manufactured from the same blends were compared (1 vs 2 mg tablets and between 3-7 mg tablets) in 4 different dissolution media: pH 1.2, 4.5 and 6.8 and the quality control (QC) dissolution medium (pH 2.2). Dissolution profiles were found to be similar. For further information and assessment concerning the strength waiver and bioequivalence studies please refer to the non-clinical and clinical sections of this report.

A dissolution method was developed with pharmacopoeial equipment and parameters and release was assessed at regular timepoints up to 20 hours. In the initial submission, the information provided regarding the dissolution method was considred deficient: the dissolution medium and proposed specifications (different for each strength) were not considered justified and the discriminatory power had not been adequately investigated resulting in a major objection.

In response, the applicant explained that media of different pH had been tested and the most appropriate selected based on achieving sink conditions and low variability. The discriminatory power of the method was investigated by comparing batches with different quantities of matrix components responsible for active substance release to biobatches of 1 mg, 2 mg, 3 mg and 7 mg. These strengths were selected due to representing extremes of tablets manufactured using the 2 common blends. The method was able to distinguish between tablets of different compositions and is thus considered to be suitably discriminatory. However, no distinction could be made between batches manufactured with different active substance particle size distributions or with different tablet hardnesses. This is considered reasonable since the release is mainly governed by the excipients responsible for release rate. The specification limits were amended in line with CHMP comments. As a result, the major objection is considered resolved.

The primary packaging is PVC/PE/PVDC/aluminium blisters. The materials comply with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The finished product is manufactured at one site. Satisfactory GMP documentation has been provided. The manufacturing process consists of three main steps: blending, sieving and mixing of guanfacine with defined amounts of pre-blended excipients, tableting and packaging. Due to the low concentration of active substance in 1 mg and 2 mg tablets and the prolonged release dosage form, the manufacturing process is a non-standard process.

Major steps of the manufacturing process have been validated by a number of studies with a focus on blending and compression steps. Three batches of the low strength final blend and 5 batches of the high strength final blend were manufactured on production scale and then compressed into the

respective tablets. Three batches each of the 1 and 2 mg tablets were manufactured. The 5 batches of high strength blend were used to make 3 batches of the 3 and 7 mg tablets and 1 batch each of the 4, 5 and 6 mg tablets. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Bulk holding times have been defined as 40 days for the final blends and up to 9 months for the bulk tablets. The hold times have been justified with appropriate stability data.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form including appearance and colour, water content, dissolution, uniformity of dosage units, identification, assay, degradation products and microbial contamination. 4Limits for related substances (specified, unspecified and total impurities) and dissolution are justified.

The potential presence of elemental impurities in the finished product was assessed following a risk-based approach as per ICH Q3D. Batch analysis data on 3 batches of 1 mg tablets and 3 batches of 7 mg tablets using a validated ICP-OES method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. It is not necessary to include any elemental impurity controls in the finished product specification.

In the initial submission, the applicant submitted a risk assessment concerning the potential presence of nitrosamine impurities. Despite the indication that secondary amines could be present as impurities in the active substance, and that excipients known to contain trace nitrite are used in the finished product, it was concluded that there was negligible risk of nitrosamine presence. This was not accepted by CHMP resulting in a major objection. In response, the applicant updated the risk assessment. It was clarified that the information regarding amines in the active substance had been incorrect. No amines susceptible to nitrosation are used in the active substance manufacturing process and are thus not present in the active substance. Based on the updated information, it is concluded that there is no risk of nitrosamines being present in the finished product and thus, no need to include any specific controls.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 3 production scale batches of 1, 2, 3 and 7 mg tablets and 1 each of the 4, 5 and 6 mg batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data from multiple production scale batches of finished product stored for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The stability batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. A bracketing approach was as follows and is considered acceptable:

Tablet strength/mg	Number of batches
1	4
2	3
3	3
4	2
5	1
6	1
7	3

Samples were tested for appearance, colour, identity, water content, dissolution and related substances. The analytical procedures used are stability indicating. Under long term conditions, no changes were observed to any of the measured parameters other than a small increase in water content. Under accelerated conditions, water content also increased slightly and there was a small increase in impurities in the lower strengths, though the amount remained acceptable. The wider shelf-life limits for impurities and water content are considered justified.

In addition, 4 batches of high and low strength tablets of each blend were exposed, unprotected, to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Paxneury is not photosensitive.

Stress testing was carried out by exposure of samples to acid, alkali, oxidant, and heat. Degradation occurring under all conditions, more so in the presence of oxidant or alkali. The results indicate that the impurities and assay analytical methods are stability indicating.

Based on available stability data, the proposed shelf-life of 30 months without specific storage conditions as stated in the SmPC (section 6.3 and 6.4) is acceptable.

Adventitious agents

It is confirmed that the lactose is produced from milk from healthy animals in the same condition as those used to collect milk for human consumption and that the lactose has been prepared without the use of ruminant material other than calf rennet according to the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. Three major objections were raised during the procedure, which were satisfactorily resolved. The first major objection on the mutagenic impurity risk assessment was resolved by provision of additional Q(SAR) predictive data. The second major objection relating to potential nitrosamine impurities was also resolved via an updated risk assessment. The third major objection relating to the development, discriminatory power, and specification limit of the dissolution method was resolved by provision of additional development data and by tightening specification limits.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. The impurity profile has been discussed and was considered acceptable.

Pharmacodynamic, pharmacokinetic and toxicological properties of guanfacine are well known. As Guanfacine is a widely used, well-known active substance, the applicant has not provided additional studies, and further studies are not required. Overview based on literature review is, thus, appropriate.

Therefore, the CHMP agreed that no further non-clinical studies are required.

2.3.2. Pharmacology

No pharmacology studies have been conducted.

2.3.3. Pharmacokinetics

No studies pharmacokinetics studies have been conducted.

2.3.4. Toxicology

No toxicology studies have been conducted.

2.3.5. Ecotoxicity/environmental risk assessment

No Environmental Risk Assessment studies were submitted. Based on the revised EMA Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Rev. 1) the applicant provided a justification that the environmental exposure to guanfacine hydrochloride will not increase upon the authorization of Paxneury. This is substantiated by the fact that maximum daily dose, therapeutic indication and treatment duration of the hybrid medicinal product will be the same as of medicinal products already marketed in the Member States. For this medicinal product, based on the current scientific knowledge it could be agreed that it is not expected to pose increased

risk to the environment. However the applicant is reminded of their responsibility to complement ERA in accordance with the above mentioned recently revised guideline.

2.3.6. Discussion on non-clinical aspects

The applicant provided a comprehensive literature-based review on the pharmacodynamics, secondary pharmacodynamics and safety pharmacology of guanfacine which is considered adequate for a hybrid application according to Article 10(3).

The general toxicology of guanfacine hydrochloride was extensively evaluated in a series of studies in various animal models as well as in a battery of specific in vitro assays during the early development programme of the innovator product. An overview of the publicly available data was presented by the applicant as part of this MAA.

2.3.7. Conclusion on the non-clinical aspects

There are no objections to approval of guanfacine prolonged-release tablets from a non-clinical point of view. The SmPC of guanfacine prolonged-release tablets is identical to the reference product.

2.4. Clinical aspects

2.4.1. Introduction

This is an application for Paxneury prolonged-release tablet containing guanfacine. To support the application, the applicant submitted 9 bioequivalence studies. No new clinical efficacy or safety studies were submitted by the applicant and none of those are required for this application. It is noteworthy that doses up to 7 mg daily of guanfacine hydrochloride are approved (reference medicinal product Intuniv).

The finished product under submission are prolonged-release tablets of guanfacine hydrochloride equivalent to 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg and 7 mg guanfacine designed for once-a-day oral administration. Importantly, two different formulations have been developed for the current finished product: one for the lower strengths (1 mg and 2 mg) and a second one for the higher strengths (3 mg to 7 mg). Hence, in line with the Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CHMP/EWP/280/96 Rev1) the requirement of single-dose under fasted and fed conditions as well as multiple-dose under fasted conditions apply for the lower (1 mg and 2 mg) and higher strengths (3 mg to 7 mg) separately. Therefore, dedicated bioequivalence trials evaluating the above-mentioned points are expected for each respective strength range (low and high) to support the current MAA. Notably, the 4 mg study under fasted conditions was conducted twice, as the first study (GUA-T1221/118) failed to demonstrate bioequivalence and it was consequently repeated (GUA-0722-87).

For the clinical assessment the Guideline on the Investigation of Bioequivalence CPMP/EWP/QWP/1401/98) and the Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CHMP/EWP/280/96 Rev1) in their current versions, are of particular relevance.

The applicant provided a clinical overview outlining the pharmacokinetics and pharmacodynamics as well as efficacy and safety of guanfacine based on published literature. The SmPC is in line with the SmPC of the reference product.

No CHMP scientific advice pertinent to the clinical development was given for this medicinal product.

GCP aspect

The applicant has provided statements confirming that the clinical trials GUA-T1221/117, GUA-1122-133 (NXPGUAN/22/BQ-12), GUA-1022-120 (NXPGUAN/22/BQ-9), GUA-0123-6 (NXPGUAN/23/BQ-1), GUA-1122-134 (NXPGUAN/22/BQ-11), GUA-T002 (GUA-T1221/118), GUA-0722-87 (NXPGUAN/22/BQ-8), and GUA-1122-135 (NXPGUAN/22/BQ-10) were conducted outside the European Union (Jordan) whereas GFC-BEMD-01-NXP/23 (NXPGUAN/23/BQ-11) was conducted in Romania (EU) under ethical requirements that are equivalent to those of Directive 2001/20/EC and were performed as per ICH-GCP standards.

In addition, the applicant has provided a statement confirming that the clinical trial GUA-0123-6 was conducted outside the European Union (Jordan) under ethical conditions of Regulation 536/2014 and was performed as per ICH-GCP standards.

In this regard, it is worth noting that the Clinical Trials Regulation repealed the Clinical Trials Directive on January 31st 2022.

No concerns have been raised during the assessment about compliance with GCP.

2.4.2. Exemption

The applicant requested a biowaiver for the strengths 5 mg and 6 mg. These strengths are not approved for the reference medicinal product Intuniv and are thus applied for via the hybrid approach under Article 10.3 of Directive 2001/83/EC.

Applicant's rationale for omitting bioequivalence studies with the 5 mg and 6 mg strengths

The applicant justifies this biowaiver by arguing that the results of the studies conducted with 4 (single-dose) and 7 mg (multiple-dose) strengths can be extrapolated to the additional higher strengths 5 and 6 mg.

Comparative dissolution studies for strength biowaiver

The tests were carried out in the following conditions:

Table 4: In vitro dissolution data for biowaiver request

Dissolution Conditions	Apparatus	Paddle Apparatus (Ph. Eur)
	RPM	50
	Medium	Hydrochloric acid 0.1 N pH 1.2 Hydrochloric acid buffer pH 2.2 Acetate buffer pH 4.5 Phosphate buffer pH 6.8
	Volume	900 ml
	Temperature	37 °C ± 0.5 °C
	Surfactant	--

Sampling times: 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours.

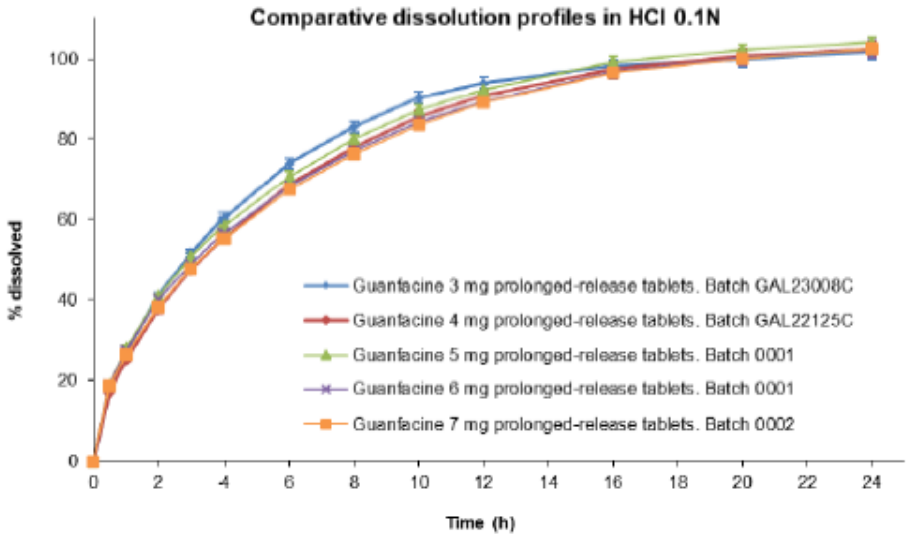


Figure 3: *Comparative dissolution profiles in hydrochloric acid (HCl) buffer pH 1.2*

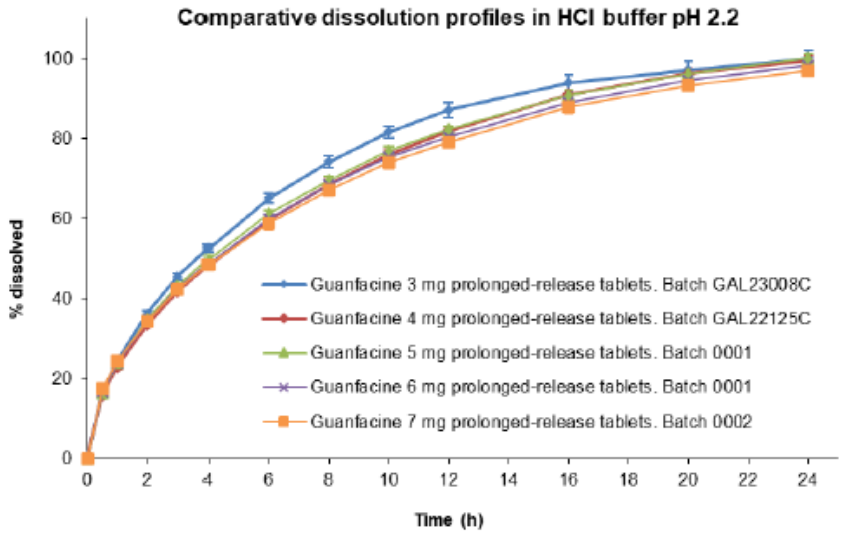


Figure 4: Comparative dissolution profiles in hydrochloric acid (HCl) buffer pH 2.2

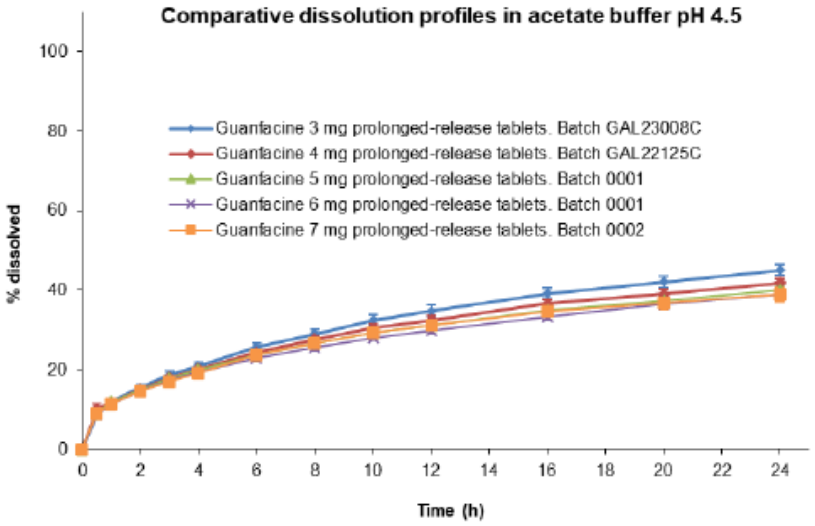


Figure 5: Comparative dissolution profiles in acetate buffer pH 4.5

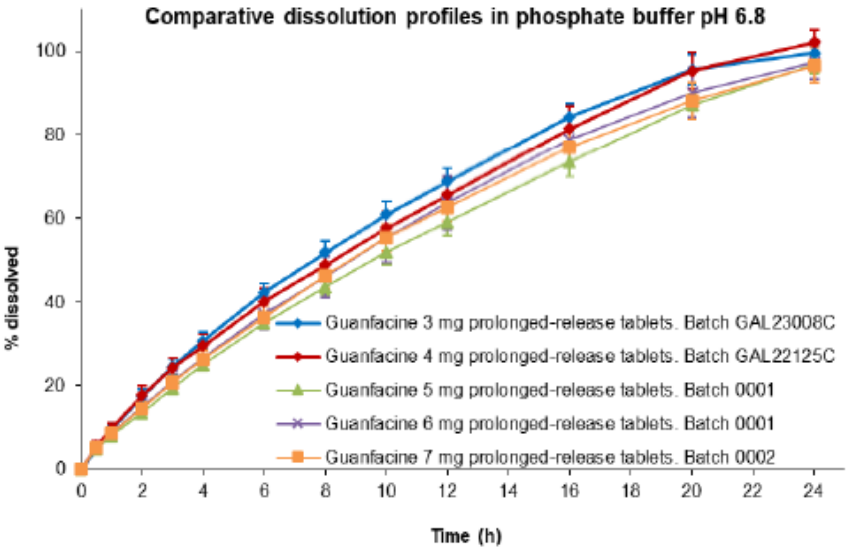


Figure 6: Comparative dissolution profiles in phosphate buffer pH 6.8

Conclusion:

Rationale for omitting bioequivalence studies with the 5 mg and 6 mg strengths

It is noteworthy that not only the formulations of Intuniv tablets and Paxneury tablets differ but also the formulations within some of both Intuniv's and Paxneury's strengths differ (i.e., the composition of the strengths are not quantitatively proportional). Indeed, the applicant developed a differing formulation (i.e. differing active pharmaceutical ingredient to excipients proportion) for the strengths 1 mg and 2 mg (termed lower strengths) compared to the strengths 3 mg, 4 mg, 5 mg, 6 mg, and 7 mg (termed higher strengths), as with a unique formulation for all strengths, the tablets of 5, 6 and 7 mg strength would be extremely big and not suitable for administration to children. However, this approach entails the requirement for a different set of bioequivalence studies for the lower strengths and higher strengths according to the BE-guideline (CPMP/EWP/QWP/1401/98 Rev.1).

Initially, the applicant requested a strength biowaiver for the additional higher strength 5 mg, 6 mg and 7 mg. The applicant's justification was based on safety concerns (in particular cardiovascular) for healthy volunteers, which was deemed unacceptable and raised a major objection. It was not understood why the applicant did not examine the 7 mg strength in a fasted, multiple-dose setting in healthy volunteers following an up- and down-titration design (as also initially proposed and deemed acceptable in the national SA issued). Without such a study, no clinical bioequivalence demonstration for the strengths 5, 6, and 7 mg would have been available. Waiving essential comparative clinical data in adult healthy volunteers due to safety concerns appeared inadequate considering that the medicine's intended population includes children and adolescents (who are particularly vulnerable) and that without such comparative clinical data the outcome is that the bioequivalence conclusion (and thus conclusion on equivalent efficacy and safety) of the new strengths would have relied solely on dissolution data, leaving this vulnerable patient group at risk of in the worst case a drug with even higher safety risks than already known. Consequently, clinical evaluation of the new higher strengths, for which the hybrid application pathway is sought, was requested and raised as major objection. Upon this request, the applicant submitted data from a new bioequivalence multiple-dose study (study GFC-BEMD-01-NXP/23) with the highest strength of 7 mg under fasted conditions. Notably, that study's protocol was already finalized prior the start of the MAA procedure indicating that the applicant foresaw the potential need to clinically investigate the highest dose strength.

Comparative dissolution studies for strength biowaiver

The applicant performed comparative *in vitro* dissolution studies between the lower strength 1 mg and 2 mg as well as between the higher strengths 3 mg, 4 mg, 5 mg, 6 mg and 7 mg to support the strength biowaiver request. In addition, comparative *in vitro* dissolution studies between Test and Reference product Intuniv were performed for the strength 1 mg, 2 mg, 3 mg, 4 mg and 7 mg, whereby 7 mg Guanfacine Test product was compared to 3 mg + 4 mg Reference product Intuniv.

Dissolution profiles were initially evaluated in hydrochloric acid buffer pH 2.2, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The dissolution of guanfacine prolonged release tablets was pH-dependent: complete dissolution at pH 2.2 and pH 6.8, incomplete dissolution at pH 4.5 (40-45 %) after 24 hours. According to the Bioequivalence guideline (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**), comparative in vitro dissolution experiments should follow "current compendial standards", including 50 rpm in paddle apparatus, 900 mL, n=12 and within the range of pH 1–6.8, normally pH 1.2, 4.5, and 6.8, unless otherwise justified. Upon request, the applicant also provided comparative dissolution data at pH 1.2.

The dissolution variability (relative standard deviation – RSD) of the strengths 4 mg, 6 mg and 7 mg exceeded 10 % at certain time points, other than the first time point, in phosphate buffer pH 6.8. Thus, the requirements of the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1 – January 2010) regarding the calculation of the f2 similarity factor have not been fulfilled. Therefore, the applicant applied bootstrap analysis as an alternative method to evaluate dissolution similarity between the higher strengths 3 mg, 4 mg, 5 mg, 6 mg and 7 mg. Notably, Q&As 3.11 and 3.13 from the EMA Q&A on Clinical pharmacology and pharmacokinetics (https://www.ema.europa.eu/en/human-regulatory-overview/research-and-development/scientific-guidelines/clinical-pharmacology-and-pharmacokinetics/clinical-pharmacology-and-pharmacokinetics-questions-and-answers) outline expectations pertaining to conducting and reporting bootstrap methodology for the f2 value estimation. However, no information regarding the applied bootstrap method had been initially provided. Only f2 similarity factor values and, presumably, a confidence interval derived from bootstrap analysis were presented; but also, the level of uncertainty (e.g. 90% CI) was not further specified in the presentation of the results. In addition, it was unclear whether certain time points were excluded from the analysis. This further raised doubts to what extent the conducted bootstrap analysis was pre-specified. This issue was initially raised as major objection, as

the assessment of comparative dissolution data between the higher strengths 3 mg, 4 mg, 5 mg, 6 mg and 7 mg was not feasible. The applicant addressed all uncertainties and provided the outstanding documentation. In all cases where applicable, calculation of similarity estimates was repeated using the requested methodology (see above). It is further considered that the software (Pheq_bootstrap) used to perform the bootstrap analysis is sufficiently validated for its intendent purpose. The lower bound of the 90% bootstrap CI for the expected f2 similarity factor was above 50 in all instances showing dissolution similarity. It is thus agreed that similarity in dissolution profiles has been sufficiently demonstrated between the higher strengths at all conditions.

Upon assessment of the applicant D120 responses, it was agreed that similarity in dissolution profiles has been sufficiently demonstrated, also in cases that required bootstrap methodology and the strength biowaiver criteria are now considered to be formally fulfilled. Hence, the strength biowaiver for the hybrid strengths of 5 mg and 6 mg is in principle acceptable. Notably, the 7 mg strength was only investigated as multiple dose study under fasted conditions. However, as testing 7 mg in healthy volunteers requires an up-titration period due to safety reasons, difficulties in performing a single dose study are acknowledged. Therefore, demonstrating bioequivalence between the 7 mg guanfacine test formulation and 3 mg + 4 mg Intuniv after multiple dosing is considered sufficient to address outlined concerns.

Tabular overview of clinical studies

To support the application, the applicant submitted 9 bioequivalence studies. No new clinical efficacy or safety studies were submitted by the applicant and none of those are required for this application. It is noteworthy that doses up to 7 mg daily of guanfacine hydrochloride are approved (reference medicinal product Intuniv).

Table 5: Tabular overview of clinical studies

Study Code (Protocol No.)	Study Title	No. of Subjects
Study Code no.: GUA-T1221/117 Protocol Code no.: GUA-T001	Comparative Randomized, Single Dose, Two-Way Crossover Open Label Study To Determine The Bioequivalence Of Guanfacine **1 mg** Prolonged-Release Tablet After An Oral Administration To Healthy Male Adults **Under Fasting Conditions**	To be enrolled 18 subjects plus 1-2 alternates; enrolled
Study Code No. GUA-1122-133 Protocol Code No. GUA-T007	COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAY CROSSOVER OPEN LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF GUANFACINE **2 MG** PROLONGED-RELEASE TABLET AFTER AN ORAL ADMINISTRATION TO HEALTHY MALE ADULTS **UNDER FASTING CONDITIONS**	To be enrolled 34 male subjects plus 1-2
Study Code no.: GUA-1022-120 Protocol Code no.: GUA-T004	Comparative Randomized, Single Dose, Two-Way Crossover Open Label Study To Determine The Bioequivalence Of Guanfacine **2 mg** Prolonged-Release Tablet After An	To be enrolled 34 male subjects plus 1-2 alternates

	Oral Administration To Healthy Male Adults **Under Fed Conditions**	
Study Code no.: GUA-0123-6 Protocol Code no.: GUA-T008	Comparative Randomized, Multiple-Dose, Two-Way Crossover Open Label Study To Determine The **Steady State** Bioequivalence Of Guanfacine **2 mg** Prolonged-Release Tablet After An Oral Administration To Healthy Male Adults **Under Fasting Conditions**	To be enrolled 28 subjects plus 1-2 alternates
Study Code No. GUA-1122-134 Protocol Code No. GUA-T006	COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAY CROSSOVER OPEN LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF GUANFACINE **3 MG** PROLONGED-RELEASE TABLET AFTER AN ORAL ADMINISTRATION TO HEALTHY MALE ADULTS **UNDER FASTING CONDITIONS**	To be enrolled 34 male subjects plus 1-2
Study Code no.: GUA-T1221/118 Protocol Code no.: GUA-T002	Comparative Randomized, Single Dose, Two-Way Crossover Open Label Study To Determine The Bioequivalence Of Guanfacine **4 mg** Prolonged-Release Tablet After An Oral Administration To Healthy Male Adults **Under Fasting Conditions**	To be enrolled 18 subjects plus 1-2 alternates; enrolled
Study Code no.: GUA-0722-87 Protocol Code no.: GUA-T003	Comparative, Randomized, Single Dose, Four-Period, Crossover, Open-Label, Full-Replicate Study To Determine The Bioequivalence Of Guanfacine **4 mg** Prolonged-Release Tablet After An Oral Administration To Healthy Male Adults **Under Fasting Conditions**	To be enrolled 38 subjects plus 1-2 alternates
Study Code no.: GUA-1122-135 Protocol Code no.: GUA-T005	Comparative Randomized, Single Dose, Two-Way Crossover Open Label Study To Determine The Bioequivalence Of Guanfacine **4 mg** Prolonged-Release Tablet After An Oral Administration To Healthy Male Adults **Under Fed Conditions**	To be enrolled 34 male subjects plus 1-2 alternates
CRO's Protocol identification.: GFC-BEMD-01-NXP/23 Sponsor study code: NXPGUAN/23/BQ-11	AN OPEN LABEL, TWO PERIODS, TWO SEQUENCES, CROSSOVER, RANDOMIZED MULTIPLE DOSE BIOEQUIVALENCE STUDY OF Guanfacine **7 mg** prolonged-release	To be enrolled 42 male subjects

	tablets (TEST FORMULATION) vs. EQUAL DOSE OF intuniv® prolonged-release tablets (REFERENCE FORMULATION) IN HEALTHY MALE VOLUNTEERS **UNDER FASTING CONDITIONS**	

2.4.3. Clinical pharmacology

2.4.3.1. Pharmacokinetics

Study GUA-T1221/117: Comparative randomised, single dose, two-way crossover open label study to determine the bioequivalence of guanfacine 1 mg prolonged-release tablet after an oral administration to healthy male adults under fasting conditions

Objectives

Primary objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 1 mg prolonged-release tablets to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fasting conditions.

Study sites of GUA-T1221/117: Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T001
STUDY CODE:	GUA-T1221/117
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 25/05/22
STUDY COMPLETION:	Last Subject Last Visit: 12/06/22
STUDY PERIODS:	Screening commencement: 25/05/22
	Dosing Period I: 29/05/22
	Dosing Period II: 08/06/22
ANALYSIS DATES:	Analysis start date: 13/06/22
	Analysis end date: 25/06/22
DATE OF VERSION 01 REPORT:	20-11-22 (updated final report)

Table 6: Study report versions

Version	Date	Major Comments
Final Report	21/08/22	NA
Version 01 Report	20/11/22	• New Quality Statement was issued in section 16.1.8. • Sponsor approval was updated in the report. • Signature of IPRC Investigators were updated and signature of Principal Investigator was updated • The following subsections were added to section 16.2: 16.2.11. Medical History 16.2.12. Physical Examination 16.2.13. Alcohol Test 16.2.14. Drug of Abuse Test 16.2.15. Drug Administration Time for All Subjects and By Period

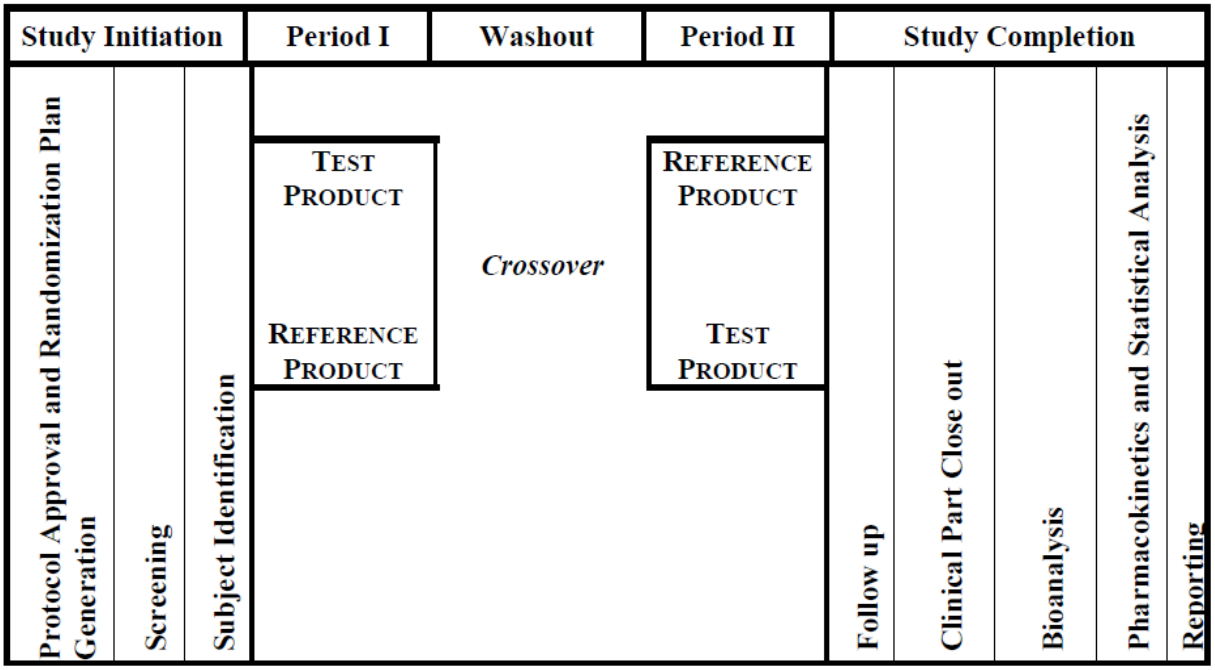


Figure 7: Study plan

The first screening examination was performed on 25/05/22. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan. The first administration of the study drug (Test or Reference drug product) and the first blood collection for drug analysis took place on 29/05/22 (first day of Period I). After a washout period of 10 days, on 08/06/22 subjects were given the second administration of the study drug (Test or Reference drug product). The last blood sample for drug analysis was collected on 12/06/22. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study.

Food and fluid-intake were identical in both study periods, starting from the dinner served at least 11 hours before study drug administration on study day -1 until the end of confinement. Meals were standardized in composition and amount in both periods. The subjects were not allowed to consume any additional beverages or foodstuffs other than those provided throughout the period of confinement. The subjects received their standardized meals at the following times:

Table 7: Standardized diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hours after study drug administration
1	Snack	8 hours after study drug administration
1	Dinner	12 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan. The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One tablet of guanfacine 1 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One tablet of Intuniv® 1 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 6 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 6 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 6 ml) after administration of study drugs, the total number of blood draws during the study was 42. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples. The total amount of blood draw during the whole study did not exceed 264.5 ml: [(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations. This volume did not include discarded blood before

sample withdrawal, samples for clinical laboratory repeats or samples for ensuring subjects safety based on the judgment of the principal investigator. The total volume did not exceed 420 ml through the whole study.

In the morning of study day 1 of each study period and before study drug administration, a cannula was inserted into the subject's forearm vein to collect samples up to 24 hours post-dose. Subsequent samples (+48, +72 and +96 hour post-dose) were drawn by venipuncture.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain plastic tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the -70°C freezer. All samples were collected into suitably labeled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 20/01/22 and approval for version 01 was given on 06/02/22.

Table 8: Protocol version history and summary of changes:

Version	Description of change	Change carried out on pages	Reason of Change
ORG	NA	NA	NA
01	Orthostatic hypotension test will be performed at screening and the following will be added to the exclusion criteria: Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test) Vital signs will be measured for all subjects every hour in the first five hours in addition to the original times already present in the study protocol, so vital signs will be measured before dosing and approximately at the following times after dosing: 1st, 2nd, 3rd, 4th, 5th, 8th, 11th and 24th hour Subjects will be monitored closely for any side effects, especially sedation and syncope	19, 23, 26 and 27	Clinical Trial Committee Recommendations

A few minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

There were collection time anomalies reported. The maximmun delays reported on collection times were 2 hours and 32 minutes. However PK calculation were performed using actual times. Two subjects did not show up at clinical site in Period I and one in Period II.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

- **Test and reference products**

Table 9: Test and reference product information of GUA-T1221/117

Product Characteristics	Test product	Reference product
Name	*Guanfacine 1 mg prolonged-release tablets*	*intuniv® 1 mg*
Strength	Guanfacine hydrochloride equivalent to 1 mg of guanfacine	Guanfacine hydrochloride equivalent to 1 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	04/22	NA
Batch number	GAL22001C	AM7640AL10
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	100%	100.4%
Commercial Batch Size	110000 tablets	
Expiry date (Retest date)	07/22	03/24
Member State where the reference product is purchased from:		Germany
This product was used in the following trials:	GUA-T1221/117	GUA-T1221/117

- **Population(s) studied**

Description of number of subjects included in the study, number of subjects included in PK- and statistical analysis, drop-outs (reason why in detail), ethnicity, gender, age, BMI, health status, etcetera Study subjects were healthy male middle eastern volunteers, between 18-45 years of age. To be considered for participation in the study, subjects had to meet all the inclusion criteria and none of the exclusion criteria. Subjects were informed, by an CRO representative, about the aim of the study and any associated potential risks. Subjects signed a written Informed Consent Form before any screening procedure was carried out. Screening procedures to determine subjects' eligibility for participation in the study were to be performed within the 14 days prior to the first dosing, and they included medical history, demographic data, complete physical examination, orthostatic hypotension test and vital signs measurements, ECG, haematology, biochemistry, serology and urinalysis. Enrolled subjects were free to withdraw at any time during the course of the study.

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HBsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Subject disposition

18 subjects plus 1-2 alternates were planned to be admitted; 28 subjects were screened. 04 subjects dropped out from the study and 05 subjects were not included due to screening failure. A total of 18 subjects plus 1 alternate were enrolled before study drug administration in Period I. One subject was excluded by CRO staff due to protocol requirement after study drug administration in Period I. 18 subjects were dosed in period I, 1 subject withdrew after study drug administration in period I and before study drug administration in period II for personal reason. 17 subjects were dosed in period II and completed the study.

Data sets analyzed

Data from the 17 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 27 days, from 29.05.2022 till 12.06.2022 (clinical part) and from 14.06.2022 till 25.06.2022 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	18
Total number of samples collected	697
Total number of samples analyzed	697
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, Guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Observations and comments
No Sample reassays for guanfacine were done.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 70 samples for each subject and study period (~ 10% of total samples analysed); 70 out of 70 ISR samples (100%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

The pharmacokinetic parameters were calculated for guanfacine.

Primary pharmacokinetic parameters:

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.
- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

As per the study protocol (version 01):

- No value of AUC0-∞ was to be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.
- The actual times of blood sampling were to be used for these.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured datapoints from the time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}]$, where C_{last} is the last quantifiable concentration.

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

As per the study protocol (version 01):

Samples from all subjects who complete the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence was to be used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics.

Exclusion of data from statistical analysis included:

1. A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).
2. Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}.

- Fixed effects model:

$$Y = \mu + Sequence + Formulation + Period$$

- Random effects model is the nested term:

Subject (Sequence)

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

Determination of sample size

Sample size calculation is based on the power of Schuirmann's two one-sided tests procedure for interval hypotheses using the ± 20 rule for the assessment of average bioequivalence.

Changes in the conduct of the study or planned analysis

No changes were made during the conduct of the study or on the planned analysis.

Handling of dropouts or missing data

Concentration data from the withdrawn one subject were presented in the individual listings and did not include in the descriptive statistics.

Missing drug concentration data (value below LLOQ) were treated as follows:

- Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.
- Any missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

- ***Results***

Data from the 17 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

Table 10: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	**Test**		**Reference**	
	arithmetic mean	**SD**	**arithmetic mean**	**SD**
$AUC_{(0-t)}$ (pg.h/ml)	15068.3	4998.91	13602.8	3705.82
$AUC_{(0-\infty)}$ (pg.h/ml)	16301.4	4975.69	14687.7	3818.65
C_{max} (pg/ml)	506.865	121.55	505.372	123.35
T_{max}* (h)	4.50	4.50- 8.00	4.50	4.00- 7.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 11: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	**Geometric Mean Ratio Test/Reference %**	**Confidence Intervals 90%**	**CV%***
$AUC_{(0-t)}$	108.23	97.33-120.35	17.76
$AUC_{(0-\infty)}$	108.88	97.08-122.10	19.21
C_{max}	100.31	94.12-106.91	10.60
* estimated from the Residual Mean Squares			

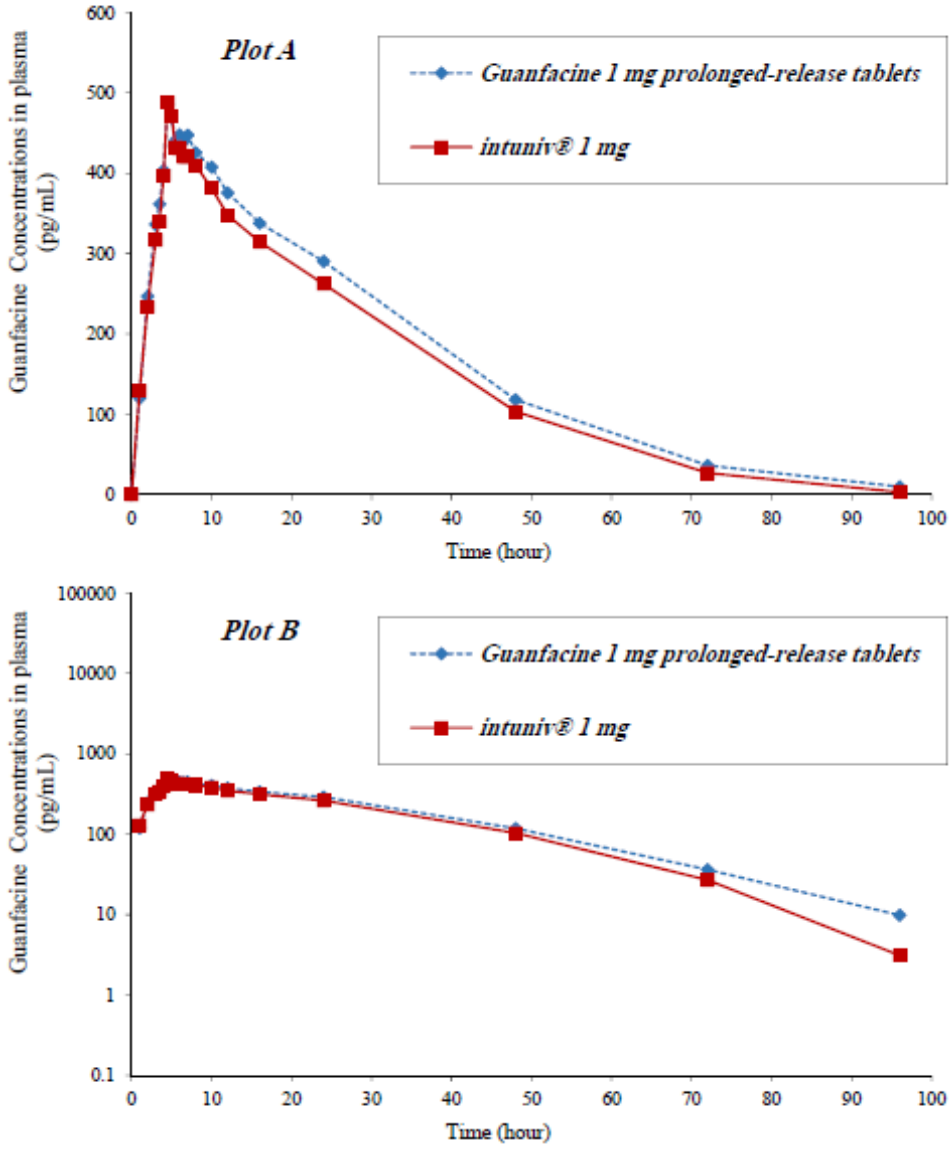


Figure 8: Linear presentation (Plot A) and semi-logarithmic presentation (Plot B) for guanfacine means after a single oral dose administration of one tablet from both treatments Guanfacine 1 mg prolonged-release tablets under fasted conditions.

- **Safety data**

Adverse events

There were no AEs during the study.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical

assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period II.

Clinical assessment for all subjects was carried out to evaluate their tolerability to the study medication. No serious adverse events were reported during the conduct of this study. Study subjects demonstrated good tolerance to the study drugs.

Study GUA-1122-133 (NXPGUAN/22/BQ-12): Comparative randomised, single dose, two-way crossover open label study to determine the bioequivalence of guanfacine 2 mg prolonged-release tablet after an oral administration to healthy male adults under fasting conditions

Objectives

Primary objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 2 mg prolonged-release tablets to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fasting conditions.

Study sites of GUA-1122-133 (NXPGUAN/22/BQ-12): Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T007
STUDY CODE:	GUA-1122-133
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 16/01/23
STUDY COMPLETION:	Last Subject Last Visit: 09/02/23
STUDY PERIODS:	Screening commencement: 16/01/23
	Dosing Period I: 26/01/23
	Dosing Period II: 05/02/23
ANALYSIS DATES:	Analysis start date: 15/02/23
	Analysis end date: 26/02/23
DATE OF FINAL REPORT:	26/03/23

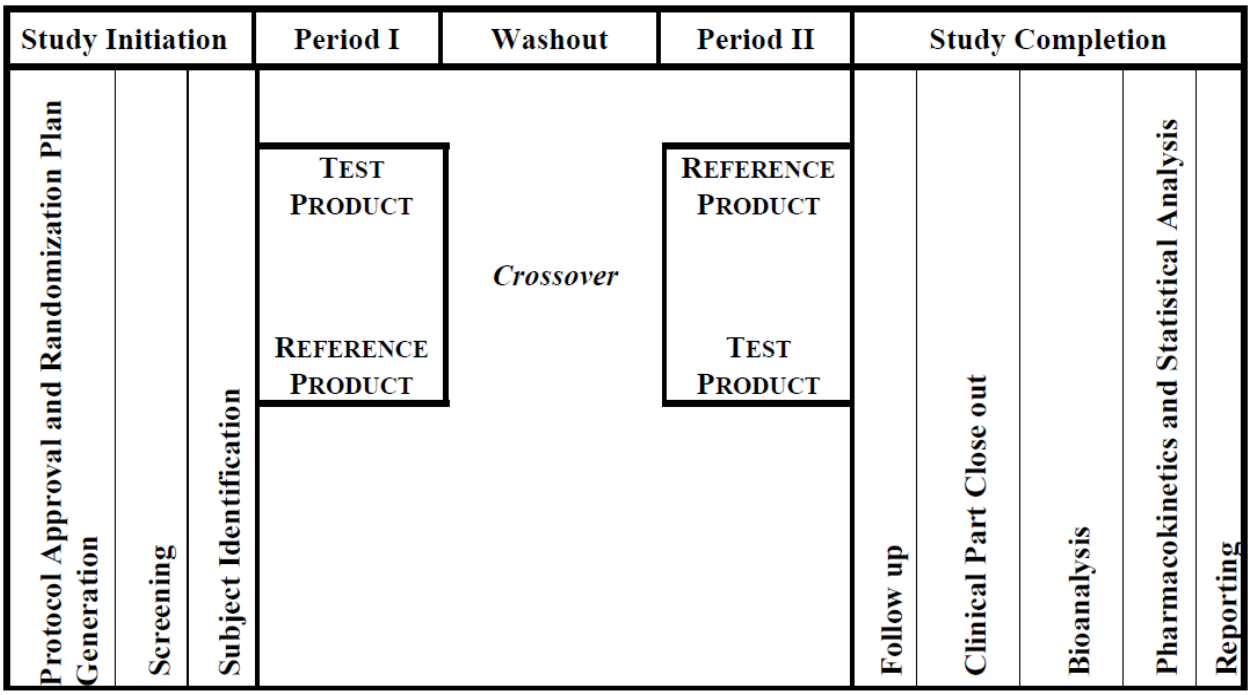


Figure 9: Study plan

The first screening examination was performed on 16/01/23. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan. The first administration of the study drug (Test or Reference drug product) and the first blood collection for drug analysis took place on 26/01/23 (first day of Period I). After a washout period of 10 days, on 05/02/23 subjects were given the second administration of the study drug (Test or Reference drug product). The last blood sample for drug analysis was collected on 09/02/23. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study.

Food and fluid-intake were identical in both study periods, starting from the dinner served at least 11 hours before study drug administration on study day -1 until the end of confinement. Meals were standardized in composition and amount in both periods. The subjects were not allowed to consume any additional beverages or foodstuffs other than those provided throughout the period of confinement. The subjects received their standardized meals at the following times:

Table 12: Standardized diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hours after study drug administration
1	Snack	8 hours after study drug administration
1	Dinner	12 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan. The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One prolonged-release tablet of guanfacine 2 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One prolonged-release tablet of Intuniv® 2 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 6 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 6 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 6 ml) after administration of study drugs, the total number of blood draws during the study was 42. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples. The total amount of blood draw during the whole study did not exceed 264.5 ml: [(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations. This volume did not include discarded blood before sample withdrawal, samples for clinical laboratory repeats or samples for ensuring subjects safety based on the judgment of the principal investigator. The total volume did not exceed 420 ml through the whole study.

In the morning of study day 1 of each study period and before study drug administration, a cannula was inserted into the subject's forearm vein to collect samples up to 24 hours post-dose. Subsequent samples (+48, +72 and +96 hour post-dose) were drawn by venipuncture.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain polypropylene tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the around -70°C freezer. All samples were collected into suitably labelled tubes (subject no., part one from study code, the year the

study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 22/12/22.

A few minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

Some samples after confinement period were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

The maximmun delays reported on collection times were 2 hours and 46 minutes. Two subjects did not show up at clinical site in Period I and five in Period II.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

- **Test and reference products**

Table 13: Test and reference product information of GUA-1122-133 (NXPGUAN/22/BQ-12)

Product Characteristics	Test product	Reference product
Name	*Guanfacine 2 mg prolonged-release tablets*	*intuniv® 2 mg*
Strength	Guanfacine hydrochloride equivalent to 2 mg of guanfacine	Guanfacine hydrochloride equivalent to 2 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	10/22	NA
Batch number	0001	AP1291AL6
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	97.7%	99.2%
Commercial Batch Size	110000 tablets	
Expiry date (Retest date)	04/23	12/24
Member State where the reference product is purchased from:		Spain
This product was used in the following trials:	GUA-1122-133	GUA-1122-133

- **Population(s) studied**

Study subjects were healthy male middle eastern volunteers, between 18-45 years of age. To be considered for participation in the study, subjects had to meet all the inclusion criteria and none of the exclusion criteria. Subjects were informed, by an CRO representative, about the aim of the study and any associated potential risks. Subjects signed a written Informed Consent Form before any screening procedure was carried out. Screening procedures to determine subjects' eligibility for participation in the study were to be performed within the 14 days prior to the first dosing, and they included medical history, demographic data, complete physical examination, orthostatic hypotension test and vital signs measurements, ECG, haematology, biochemistry, serology and urinalysis. Enrolled subjects were free to withdraw at any time during the course of the study.

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range5 is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HBsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Subject disposition

34 subjects plus 1-2 alternates were planned to be admitted; 59 subjects were screened. 06 subjects dropped out from the study and 17 subjects were not included due to screening failure. A total of 34 subjects plus 2 alternates were enrolled before study drug administration in Period I. One subject was excluded due to personal reason and 1 subject was excluded by CRO staff due to protocol requirement after study drug administration in Period I. 34 subjects were dosed in period I, period II and completed the study.

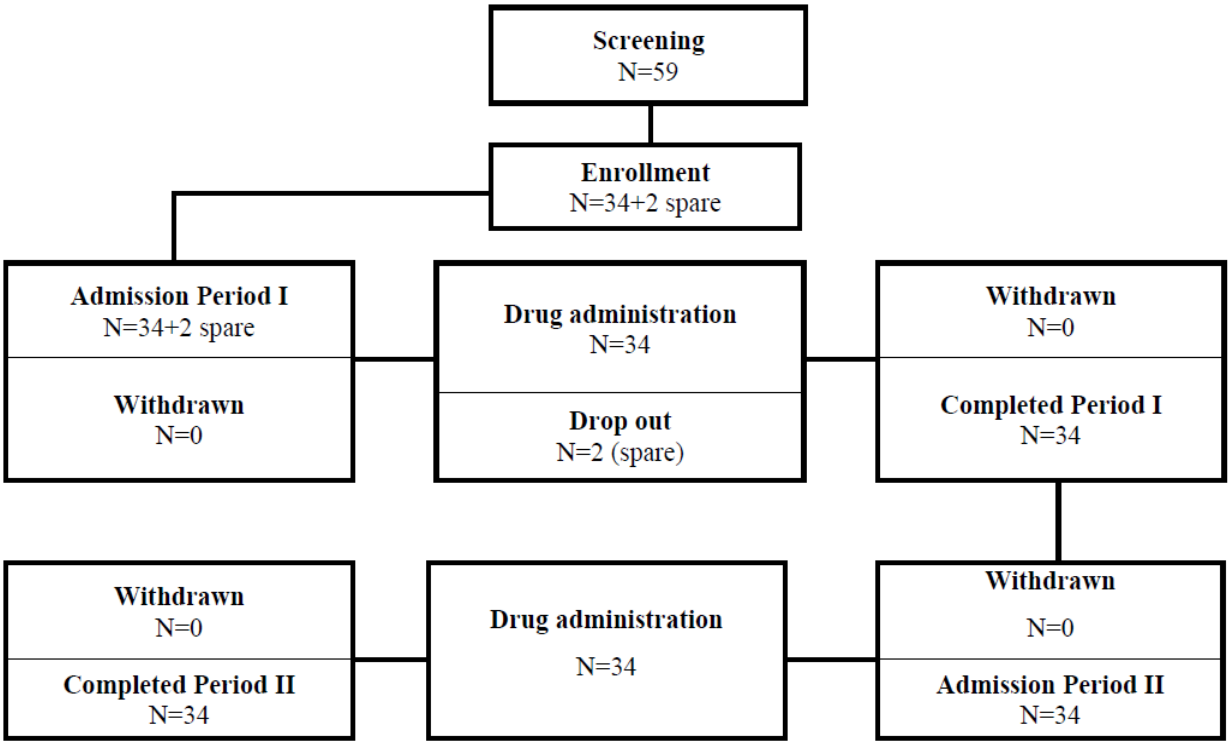


Figure 10: Disposition of subjects

Data sets analyzed

Data from the 34 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence

- **Analytical methods**

The study lasted 31 days, from 26.01.2023 till 09.02.2023 (clinical part) and from 16.02.2023 till 26.02.2023 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 Periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	34
Total number of samples collected	1353
Total number of samples analyzed	1353
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-13C15N3; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, Guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

Sample reassays for guanfacine were done on 7 samples (0.5%). All reassays are in accordance with the presented SOP and the relevant guideline.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 136 samples for each subject and study period (10% of total samples analysed); 136 out of 136 ISR samples (100%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

The pharmacokinetic parameters were calculated for guanfacine.

Primary pharmacokinetic parameters:

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.

- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

As per the study protocol:

- No value of AUC0-∞ was to be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.
- The actual times of blood sampling were to be used for these.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured datapoints from the time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}]$, where C_{last} is the last quantifiable concentration.

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

As per the study protocol:

Samples from all subjects who complete the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence was to be used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics.

Exclusion of data from statistical analysis included:

1. A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).
2. Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}. Fixed effects were used for all terms.

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

Determination of sample size

The following estimates were considered for the computation of sample size:

- T/R ratio: 108 %
- Intra-Subject C.V (%) ~ 17.76%
- Significance Level = 5%
- Power =90%

Based on the above estimate, a sample size of 28 subjects would be sufficient to establish bioequivalence between formulations with adequate power. However, considering the drop-out or withdrawal, a sample size of 34 subjects was considered for two-way crossover study.

Changes in the conduct of the study or planned analysis

No changes were made during the conduct of the study or on the planned analysis.

Handling of dropouts or missing data

There were no withdrawals in this study.

Missing drug concentration data (value below LLOQ, missing value) were treated as follows:

- Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.
- Any missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

- ***Results***

Table 14: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	arithmetic mean	SD	arithmetic mean	SD
$AUC_{(0-t)}$ (pg.h/ml)	39203.2	9979.19	38885.8	9184.23
$AUC_{(0-\infty)}$ (pg.h/ml)	40699.7	10772.43	40690.3	10205.18
C_{max} (pg/ml)	1372.389	355.07	1269.571	328.22
T_{max}* (h)	4.50	4.50-6.50	4.50	3.50-24.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 15: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference %	Confidence Intervals 90%	CV%*
$AUC_{(0-t)}$	100.73	94.09-107.83	16.70
$AUC_{(0-\infty)}$	100.04	93.30-107.26	17.09
C_{max}	108.29	101.51-115.52	15.83
* estimated from the Residual Mean Squares			

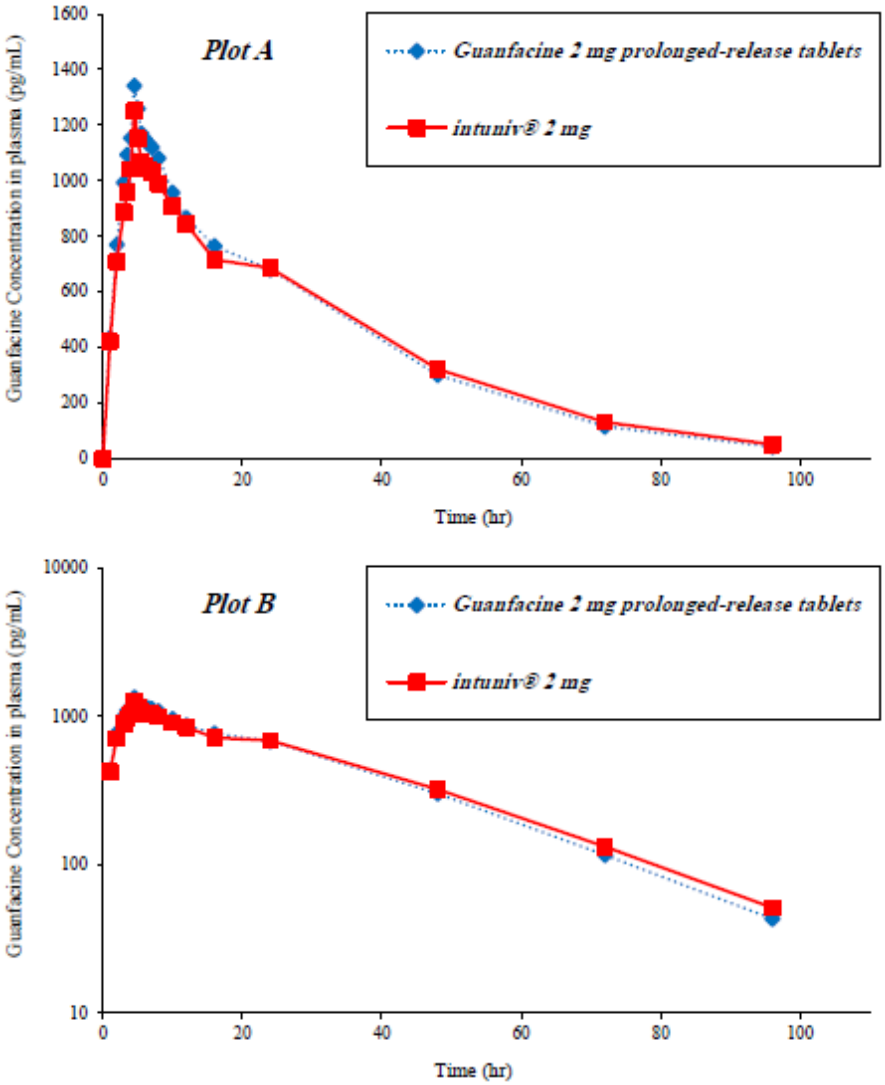


Figure 11: Linear presentation (Plot A) and semi-logarithmic presentation (Plot B) for guanfacine means after a single oral dose administration of one tablet from both treatments Guanfacine 2 mg prolonged-release tablets under fasted conditions.

Table 16: P-values obtained from guanfacine ANOVA results after single dose administration of Test Product and Reference Product

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{max} (N=34)	2.91×10^{-2}	1.00×10^{-4}	4.49×10^{-2}	7.95×10^{-2}
$AUC_{0\rightarrow t}$ (N=34)	5.90×10^{-1}	4.00×10^{-4}	8.58×10^{-1}	4.82×10^{-2}
$AUC_{0\rightarrow \infty}$ (N=34)	4.51×10^{-1}	3.00×10^{-4}	9.93×10^{-1}	2.75×10^{-2}

There was statistically significant subject (sequence) effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ for guanfacine, in addition to period and drug effect observed from the ANOVA for the logarithmically transformed primary PK parameter C_{max} and sequence effect observed from the ANOVA for the logarithmically transformed primary PK parameters $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.9 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate sequence, subject (sequence), drug and period effects in this study were performed, and therefore, we consider that though there is a sequence, subject (sequence), drug and period effects, this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during two periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (10 hrs before dosing and 24 hrs after dosing) and were maintained seated upright for the first two hours after dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the two periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the sequence, subject (sequence), drug and period effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for tmax using Wilcoxon signed-rank tests, there were no differences observed between T and R after the statistical analysis was applied.

- ***Safety data***

Adverse events

No adverse events occurred during the study.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period II.

Clinical assessment for all subjects was carried out to evaluate their tolerability to the study medication. No serious adverse events were reported during the conduct of this study. Study subjects demonstrated good tolerance to the study drugs.

Study GUA-1022-120 (NXPGUAN/22/BQ-9): Comparative randomised, single dose, two-way crossover open label study to determine the bioequivalence of guanfacine 2 mg prolonged-release tablet after an oral administration to healthy male adults under fed conditions

Objectives

Primary Objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 2 mg prolonged-release tablets to healthy male adults under fed conditions.

Secondary Objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fed conditions.

Study sites of GUA-1022-120 (NXPGUAN/22/BQ-9): Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T004
STUDY CODE:	GUA-1022-120
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 30/11/22
STUDY COMPLETION:	Last Subject Last Visit: 21/12/22
STUDY PERIODS:	Screening commencement: 30/11/22
	Dosing Period I: 07/12/22
	Dosing Period II: 17/12/22
ANALYSIS DATES:	Analysis start date: 22/12/22
	Analysis end date: 28/12/22
DATE OF FINAL REPORT:	05-02-23

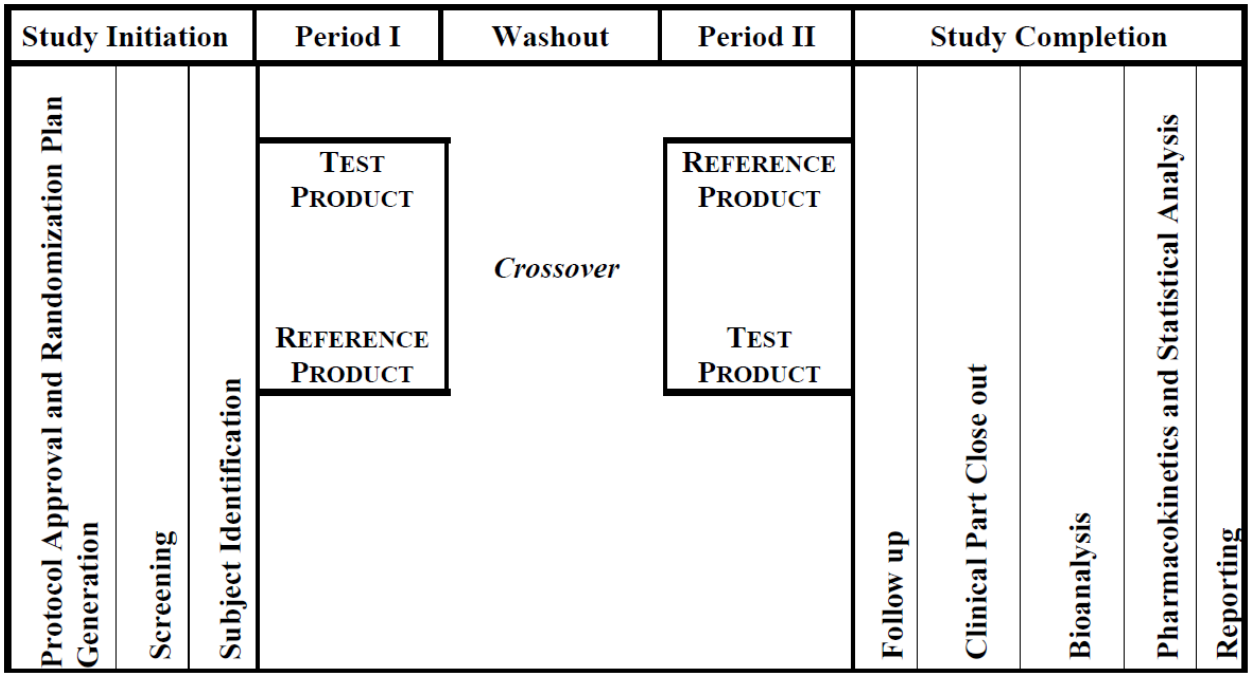


Figure 12: Study plan

The first screening examination was performed on 30/11/22. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan. The first administration of the study drug (Test or Reference drug product) and the first blood collection for drug analysis took place on 07/12/22 (first day of Period I). After a washout period of 10 days, on 17/12/22 subjects were given the second administration of the study drug (Test or Reference drug product). The last blood sample for drug analysis was collected on 21/12/22. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study.

Food and fluid-intake were identical in both study periods, starting from the dinner served at least 11 hours before study drug administration on study day -1 until the end of confinement. Meals were standardized in composition and amount in both periods. The subjects were not allowed to consume any additional beverages or foodstuffs other than those provided throughout the period of confinement. The subjects received their standardized meals at the following times:

Table 17: Standardized diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Breakfast	0.5 an hour before study drug administration
1	Lunch	5 hours after study drug administration
1	Snack	9 hours after study drug administration
1	Dinner	13 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan. The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One prolonged-release tablet of guanfacine 2 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One prolonged-release tablet of Intuniv® 2 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 6 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 6 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 6 ml) after administration of study drugs, the total number of blood draws during the study was 42. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples. The total amount of blood draw during the whole study did not exceed 264.5 ml: [(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations. This volume did not include discarded blood before sample withdrawal, samples for clinical laboratory repeats or samples for ensuring subjects safety based on the judgment of the principal investigator. The total volume did not exceed 420 ml through the whole study.

In the morning of study day 1 of each study period and before study drug administration, a cannula was inserted into the subject's forearm vein to collect samples up to 24 hours post-dose. Subsequent samples (+48, +72 and +96 hour post-dose) were drawn by venipuncture.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain plastic tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the -70°C freezer. All samples were

collected into suitably labelled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 13/11/22.

A few minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

Some samples after confinement period were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

The maximmun delays reported on collection times were 3 hours. Five subjects did not show up at clinical site in Period I and three in Period II.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

- **Test and reference products**

Table 18: Test and reference product information of GUA-1022-120 (NXPGUAN/22/BQ-9)

Product Characteristics	Test product	Reference product
Name	*Guanfacine 2 mg prolonged-release tablets*	*intuniv® 2 mg*
Strength	Guanfacine hydrochloride equivalent to 2 mg of guanfacine	Guanfacine hydrochloride equivalent to 2 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	10/22	NA
Batch number	0001	AP1291AL6
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	97.7%	99.2%
Commercial Batch Size	110000 tablets	
Expiry date (Retest date)	04/23	12/24
Member State where the reference product is purchased from:		Spain
This product was used in the following trials:	GUA-1022-120	GUA-1022-120

- **Population(s) studied**

Study subjects were healthy male middle eastern volunteers, between 18-45 years of age. To be considered for participation in the study, subjects had to meet all the inclusion criteria and none of the exclusion criteria. Subjects were informed, by an CRO representative, about the aim of the study and any associated potential risks. Subjects signed a written Informed Consent Form before any screening procedure was carried out. Screening procedures to determine subjects' eligibility for participation in the study were to be performed within the 14 days prior to the first dosing, and they included medical history, demographic data, complete physical examination, orthostatic hypotension test and vital signs measurements, ECG, haematology, biochemistry, serology and urinalysis. Enrolled subjects were free to withdraw at any time during the course of the study.

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HbsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Subject disposition

34 subjects plus 1-2 alternates were planned to be enrolled, 57 subjects were screened. 10 subjects dropped out from the study and 12 subjects were not included due to screening failure. A total of 34 subjects plus 1 alternate were enrolled before study drug administration in Period I. One subject was excluded by CRO staff due to protocol requirement after study drug administration in Period I. 34 subjects were dosed in period I, one subject withdrew after study drug administration in period I and before study drug administration in period II for personal reason. 33 subjects were dosed in period II and completed the study.

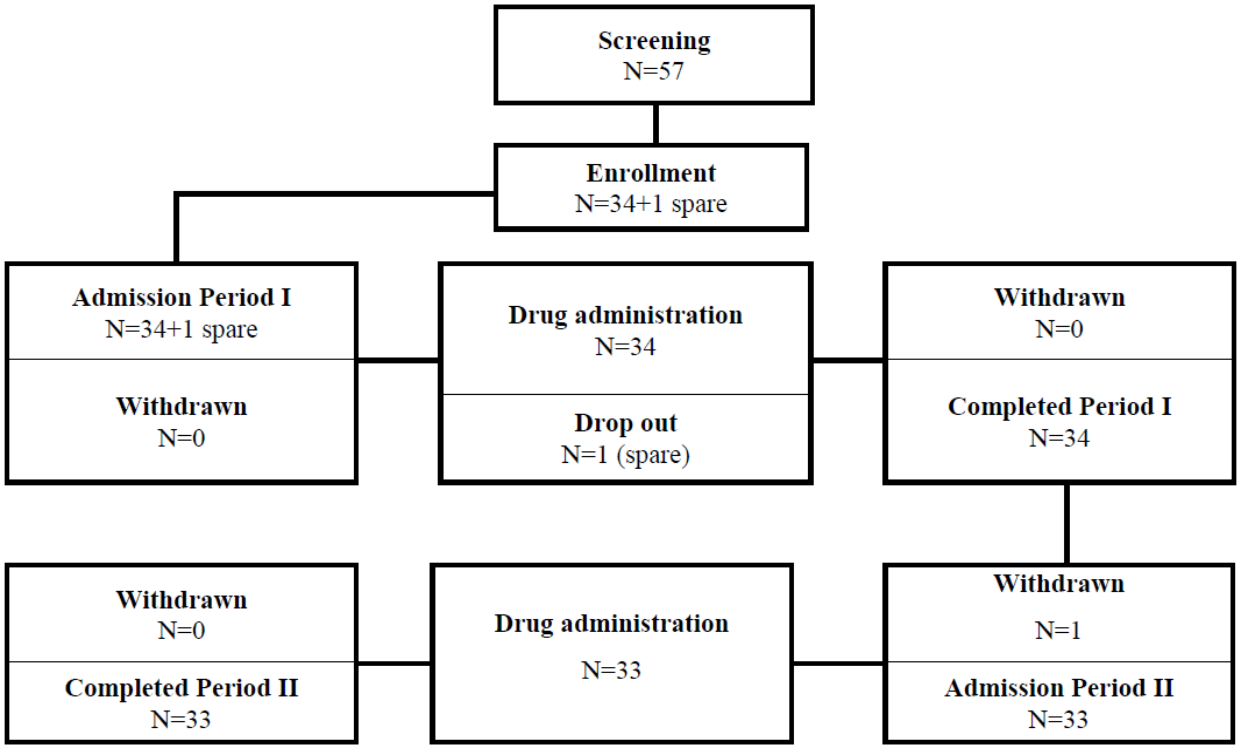


Figure 13: Disposition of subjects:

Data sets analyzed

Data from the 33 subjects who completed the crossover study, excluding one withdrawn subject, were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 21 days, from 07.12.2022 till 21.12.2022 (clinical part) and from 23.12.2022 till 28.12.2022 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	34
Total number of samples collected	1332
Total number of samples analyzed	1332
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

No Sample reassays for guanfacine were done.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 122 samples for each subject and study period (~ 10% of total samples analysed); 122 out of 122 ISR samples (100%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

The pharmacokinetic parameters were calculated for guanfacine.

Primary pharmacokinetic parameters:

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.

- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

As per the study protocol:

- No value of AUC0-∞ was to be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.
- The actual times of blood sampling were to be used for these.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured datapoints from the time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}]$, where C_{last} is the last quantifiable concentration.

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

As per the study protocol:

Samples from all subjects who complete the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence was to be used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics.

Exclusion of data from statistical analysis included:

1. A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).
2. Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

Tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}. Effects were used for all terms.

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

Determination of sample size

The following estimates were considered for the computation of sample size:

- T/R ratio: 108 %
- Intra-Subject C.V (%) ~ 17.76%
- Significance Level = 5%
- Power =90%

Based on the above estimate, a sample size of 28 subjects would be sufficient to establish bioequivalence between formulations with adequate power. However, considering the drop-out or withdrawal, a sample size of 34 subjects was considered for two-way crossover study.

Changes in the conduct of the study or planned analysis

No changes were made during the conduct of the study or on the planned analysis.

Handling of dropouts or missing data

Concentration data from the withdrawn one subject were presented in the individual listings and did not include in the descriptive statistics.

Missing drug concentration data (value below LLOQ) were treated as follows:

- Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.
- Any missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

- ***Results***

Table 19: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	arithmetic mean	SD	arithmetic mean	SD
$AUC_{(0-t)}$ (pg.h/ml)	37709.8	11289.97	35907.6	10696.88
$AUC_{(0-\infty)}$ (pg.h/ml)	38860.8	11647.01	37318.7	11419.31
C_{max} (pg/ml)	1435.322	317.91	1473.026	298.51
T_{max}* (h)	5.50	3.50- 24.00	5.50	3.50- 8.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 20: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference %	Confidence Intervals 90%	CV%*
$AUC_{(0-t)}$	105.07	97.02-113.79	19.26
$AUC_{(0-\infty)}$	104.39	96.11-113.38	19.98
C_{max}	97.25	91.32-103.57	15.16
* estimated from the Residual Mean Squares			

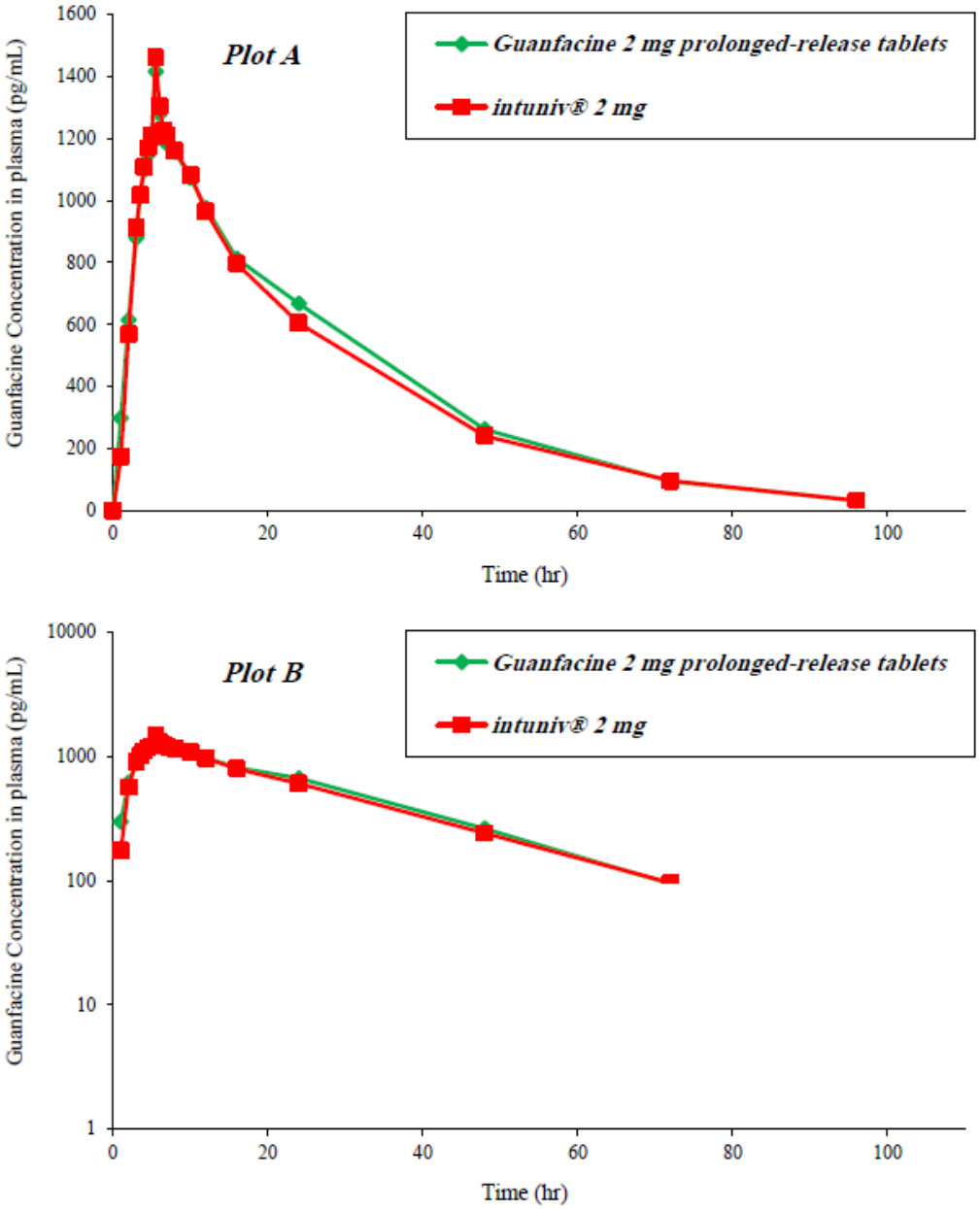


Figure 14: Linear presentation (Plot A) and semi-logarithmic presentation (Plot B) for guanfacine means after a single oral dose administration of one tablet from both treatments Guanfacine 2 mg prolonged-release tablets under fed conditions.

Table 21: P-values obtained from guanfacine ANOVA results after single dose administration of Test Product and Reference Product

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{max} (N=33)	2.53×10^{-2}	1.6×10^{-3}	4.59×10^{-1}	3.44×10^{-1}
$AUC_{0\to t}$ (N=33)	5.11×10^{-1}	5.00×10^{-4}	3.01×10^{-1}	1.43×10^{-1}
$AUC_{0\to\infty}$ (N=33)	5.21×10^{-1}	9.00×10^{-4}	3.85×10^{-1}	1.27×10^{-1}

There was statistically significant subject (sequence) effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for guanfacine. In addition to period effect observed from the ANOVA for the logarithmically transformed primary PK parameter C_{max} for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.0 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate subject (sequence) and period effects in this study were performed, and therefore, we consider that though there is a subject (sequence) and period effects, this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during two periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (10 hrs before dosing and 24 hrs after dosing) and were maintained seated upright for the first two hours after dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the two periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the subject (sequence) and period effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for tmax using Wilcoxon signed-rank tests, there were no differences observed between T and R after the statistical analysis.

- ***Safety data***

Adverse events

No adverse events occurred during the study.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period II.

Clinical assessment for all subjects was carried out to evaluate their tolerability to the study medication. No serious adverse events were reported during the conduct of this study. Study subjects demonstrated good tolerance to the study drugs.

Study GUA-0123-6 (NXPGUAN/23/BQ-1): Comparative randomized, multiple-dose, two-way crossover open label study to determine the steady state bioequivalence of guanfacine 2 mg prolonged-release tablet after an oral administration to healthy male adults under fasting conditions

Objectives

Primary objective: To investigate the steady state bioequivalence of Test Product relative to Reference Product after a multiple oral dose administration of 2 mg guanfacine prolonged-release tablet to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations

Methods

- **Study design**

This study was a single centre, open-label, randomized, multiple-dose study with two-way crossover design to investigate the steady state bioequivalence of guanfacine of the test product and the reference product in healthy adult male subjects under fasting conditions.

Study sites of GUA-0123-6 (NXPGUAN/23/BQ-1): Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T008
STUDY CODE:	GUA-0123-6
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 07/05/23
STUDY COMPLETION:	Last Subject Last Visit: 07/06/23
STUDY PERIODS:	Screening commencement: 07/05/23
	Dosing Period I: 14/05/23 - 20/05/23
	Dosing Period II: 31/05/23 - 06/06/23

ANALYSIS DATES: Analysis start date: 08/06/23

Analysis end date: 16/06/23

DATE OF FINAL REPORT: 24-07-23 /Final Report

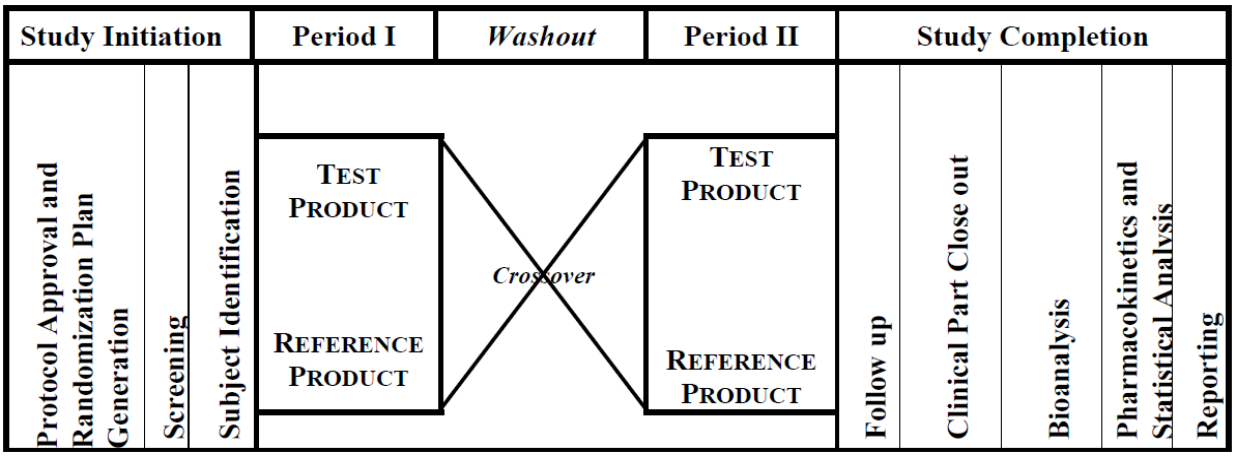


Figure 15: Study plan

The first screening examination was performed on 07/05/23. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan.

The dose administrations and blood collection for period I was done between 14/05/23 and 21/05/23, after a washout interval of 11 days between last dose of period I and first dose of period II, dose administrations and blood collection for period II was done between 31/05/23 and 07/06/23. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, the subjects were admitted to the study site before study drug administration on study day 1 or and day 18 of study periods I and II respectively and confined until the 24-hours sample after dose 7 and dose 14 was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects at least 48 hours prior to PK sampling days of either study periods until the end of each PK blood sampling day (up to 24 hours after that dosing). Xanthine-containing beverages and food were allowed during the other dosing days (except for 48h prior to the PK sampling days (Day 7 with Dose 7 and Day 24 with Dose 14) and until 24h after that dosing (Dose 7 and Dose 14)) not exceeding 300 ml of caffeinated products (coffee, tea, cola, energy drinks etc.) per day. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for two weeks before first dosing and until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks before first dosing and until donating the last sample of the study.

A standardized dinner was served at least 11 hours before study drug administration in the morning of study day 1 and day 18 of period I and period II respectively.

The subjects maintained fasting state of at least 10 hours prior to each dosing during the study.

The meals during the study were provided as follows:

- At the days of PK blood sampling (days 7, 24), standardized meals were provided approximately at 4, 8 and 12 hours after each dose.
- The meals for rest of the study days for both periods were provided approximately at 2, 6, 9 and 12 hours after each dose.
- Food type on day 1 of period I was identical to the food type on day 18 of period II, day 2 of period I was identical to day 19 of period II, and so on.

No water or fluids were permitted from 1 hour before each study drug administration until 1 hour after each dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

Following the overnight fast of at least 10 hours before each dosing, the study drugs were administered according to the randomization plan. The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One prolonged-release tablet of guanfacine 2 mg prolonged-release tablets, TEST PRODUCT, was administered every 24 hours on seven consecutive days with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One prolonged-release tablet of Intuniv® 2 mg, REFERENCE PRODUCT, was administered every 24 hours on seven consecutive days with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

The drug administrations in each period referred to the zero time of the first drug administration of that period.

Sampling schedule and sample handling

Period I (days 1-7, doses no. 1-7):

Twenty blood samples (each sample 8 ml) were collected in K3EDTA tubes at the following timepoints: Before dose no.1, 5, 6 and 7 (four pre-dose blood samples at 0.00 hour (within the last 5 minutes before dosing)) and at the following times after dose no.7: 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00 and 24.00 hours.

Period II (days 18-24, doses no. 8-14):

Twenty blood samples (each sample 8 ml) were collected in K3EDTA tubes at the following timepoints: Before dose no.8, 12, 13 and 14 (four pre-dose blood samples at 0.00 hour (within the last 5 minutes before dosing)) and at the following times after dose no.14: 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00 and 24.00 hours

Before dose no. 1, 5, 6, 8, 12, 13, blood samples were collected through direct veinpuncture.

Before and after dose no. 7 and 14, blood samples were collected using a cannula inserted into the subject's forearm vein up to the 24-hour blood sample. If cannula is blocked and sample cannot be drawn through cannula, direct veinpuncture was used to withdraw this sample. Before every blood sample collection from the cannula, the content of the cannula, approximately 0.2 mL (around 4 drops) of blood was discarded to remove the heparin For each subject, the total number of blood draws during the study were 43, and the total volume of blood drawn [(4 x 8 ml for pre-dose samples) + (16 x 8 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening, a maximum of 11 ml at end of confinement of period I and a maximum of 11 ml for follow up examinations (at end of confinement of

period II), did not exceed 355.5 ml. This volume doesn't include discarded blood before sample withdrawal, samples of repeated tests or samples for ensuring subjects safety based on the judgement of the Principal Investigator or the Study Physician. The total volume of blood drawn did not exceed 420 ml through the whole study.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain polypropylene tubes. These samples were immediately stored at the clinical site at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the around -70°C freezer. All samples were collected into suitably labelled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, multiple dose, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until donating the last sample of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until donating the last sample of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study), but one subject was given one capsule of ibuprofen (400 mg) during period I, after hours from study drug administration on that day and one capsule of ibuprofen (400 mg) during period I, after hours from study drug administration on that day. These concurrent medications were judged to have no effect on the subject health as per principal investigator decision.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 12/03/23.

A few minor variations from the study protocol concerning sampling time and concomitant medications were observed during study conduct.

Collection anomalies:

On the PK Day of period I, one sample after dosing were not withdrawn on scheduled time (for reason of cannula blockage). Nevertheless, the effect of this time deviation (2 minutes) in collection time on results is minimal because it was taken into consideration in statistical analysis.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations on day 1 or on day 18 of period I and period II, respectively.

- **Test and reference products**

Product Characteristics	Test product	Reference product
Name	*Guanfacine 2 mg prolonged-release tablets*	*intuniv® 2 mg*
Strength	Guanfacine hydrochloride equivalent to 2 mg of guanfacine	Guanfacine hydrochloride equivalent to 2 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	Laboratorios Lesvi, S.L., Spain	Shire Pharmaceuticals Ireland Limited, Ireland
Batch number	0001	AP1291AL6
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	97.7%	99.2%
Commercial Batch Size	110000 tablets	
Expiry date (Retest date)	04/24	12/24
Member State where the reference product is purchased from:		Spain
This product was used in the following trials:	GUA-0123-6	GUA-0123-6

- **Population(s) studied**

Study subjects were healthy male middle eastern volunteers, between 18-45 years of age. To be considered for participation in the study, subjects had to meet all the inclusion criteria and none of the exclusion criteria. Subjects were informed, by an CRO representative, about the aim of the study and any associated potential risks. Subjects signed a written Informed Consent Form before any screening procedure was carried out. Screening procedures to determine subjects' eligibility for participation in the study were to be performed within the 14 days prior to the first dosing, and they included medical history, demographic data, complete physical examination, orthostatic hypotension test and vital signs measurements, ECG, haematology, biochemistry, serology and urinalysis. Enrolled subjects were free to withdraw at any time during the course of the study.

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range 5 is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 48 hours prior to PK sampling days of either study periods until the end of each PK blood sampling day (up to 24 hours after that dosing). Xanthine-containing beverages and food will be allowed during the other dosing days (except for 48h prior to the PK sampling days (Day 7 with Dose 7 and Day 24 with Dose 14) and until 24h after that dosing (Dose 7 and Dose 14)) not exceeding 300 ml of caffeinated products (coffee, tea, cola, energy drinks etc.) per day.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration of each period until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beats per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breaths per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HBsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Subject disposition

28 male subjects plus 1-2 alternates were planned to be enrolled, 54 subjects were screened. 09 subjects dropped out from the study and 15 subjects were not included due to screening failure. A total of 28 subjects plus 2 alternates were enrolled before first study drug administration in Period I. 2 alternate subjects were excluded by CRO staff due to protocol requirement after first study drug administration in Period I. 28 subjects were dosed in the first day of period I, one subject withdrew after the first drug administration of period I (dose no. 1) for personal reason. 27 subjects completely dosed in period I. One subject withdrew after study drug administration in period I and before study drug administration in period II for personal reason. 26 subjects completely dosed in period II and completed the study.

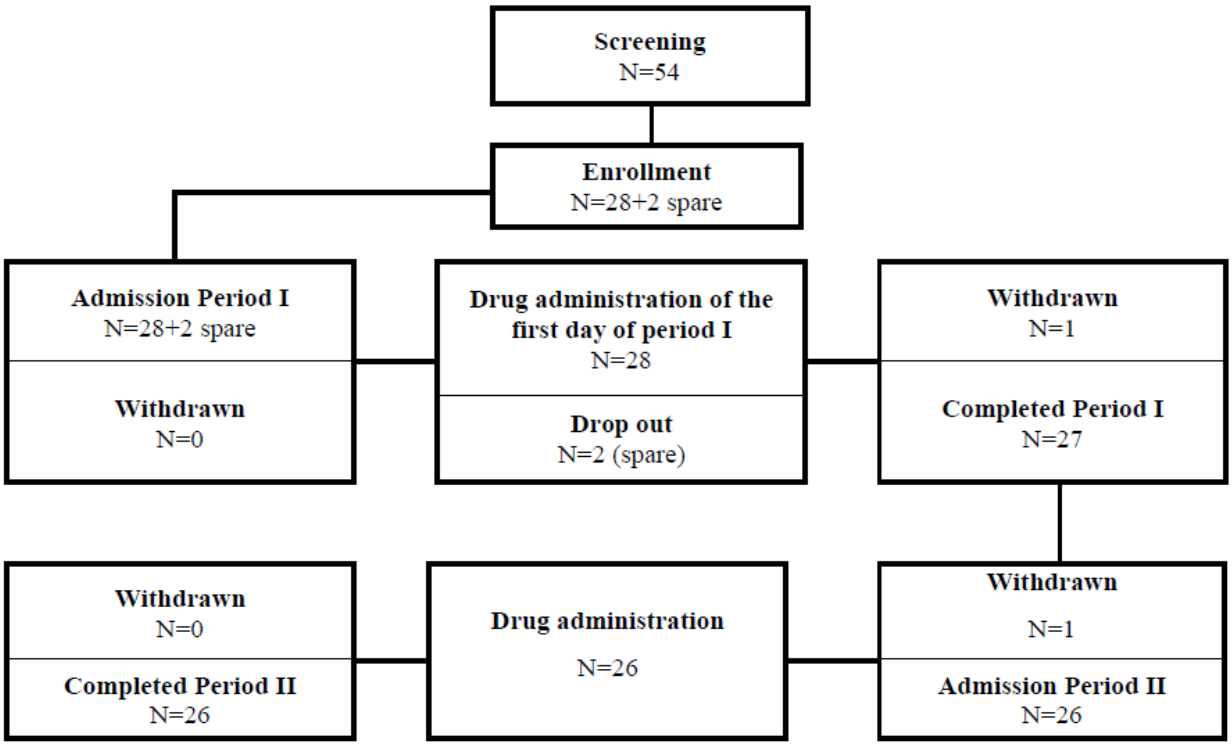


Figure 16: Disposition of subjects

Data sets analyzed

Data from the 26 subjects who completed the crossover study, excluding two withdrawn subjects, were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 33 days, from 14.05.2023 till 07.06.2023 (clinical part) and from 09.06.2023 till 16.06.2023 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	27
Total number of samples collected	1061
Total number of samples analyzed	1060
Maximum no. of injections in the analyzed runs	111
Validated batch size	161

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

Sample reassays for guanfacine were done on 2 samples (0.2%). All reassays are in accordance with the presented SOP and the relevant guideline.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 106 samples for each subject and study period (~ 10% of total samples analysed); 105 out of 106 ISR samples (~99%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

The pharmacokinetic parameters were calculated for guanfacine.

Primary pharmacokinetic parameters:

- $C_{max,ss}$: Maximum plasma concentration at steady state.
- AUC0-τ: AUC during a dosage interval at steady state, as calculated by the linear trapezoidal method.
- $C_{\tau,ss}$: Concentration at the end of the dosing interval at steady state.

Secondary pharmacokinetic parameter:

- Cmin,ss: Minimum plasma concentration at steady state.
- Cavg: Average concentration during a dosing interval (AUC0-τ / τ).
- % fluctuation: [(C_{max} -C_{min})/Cavg] %.
- Tmax,ss: Time until $C_{max,ss}$ is reached.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration at steady state ($C_{max,ss}$) and the time to peak plasma concentration at steady state ($t_{max,ss}$) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve during a dosage interval at steady state. (AUC0-τ) was calculated from measured data points by the linear trapezoidal rule.

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

As per study protocol:

Samples from all subjects who completed the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analyzed if the profile of at least one period could be determined. If necessary, an unequal number of subjects per sequence were to be used.

The pharmacokinetic results from withdrawals who did not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics.

Exclusion of data from statistical analysis included:

1. A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).
2. Subjects with non-zero (pre-dose) baseline concentrations on day 1 > 5% of $C_{max,ss}$ value of that subject (after dose no. 7 on day 7) or subjects with non-zero (pre-dose) baseline concentrations on day 18 > 5% of $C_{max,ss}$ value of that subject (after dose no. 14 on day 24)

Statistical analysis

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed $C_{max,ss}$, $C_{\tau,ss}$ and AUC0-τ parameters.

Non-parametric methods for $T_{max,ss}$. Descriptive statistic by treatment was provided for $T_{max,ss}$.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln $C_{max,ss}$, Ln $C_{\tau,ss}$ and Ln AUC0-τ. Fixed effects were used for all terms.

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for $C_{max,ss}$, $C_{\tau,ss}$ and AUC0-τ to conclude bioequivalence between formulations.

Determination of sample size

The following estimates were considered for the computation of sample size:

- T/R ratio: 92 %
- Intra-Subject C.V (%) ~ 17%
- Significance Level = 5%
- Power =80%

Based on the above estimate, a sample size of 20 subjects would be sufficient to establish bioequivalence between formulations with adequate power. However, considering the drop-out or withdrawal, a sample size of 28 subjects is considered for two-way crossover steady state study.

Changes in the conduct of the study or planned analysis

No changes were made during the conduct of the study or on the planned analysis.

Handling of dropouts or missing data

Concentration data from the withdrawn subject who completed at least one period (one subject) were presented in the individual listings and did not include in the descriptive statistics.

Missing drug concentration data (value below LLOQ) were treated as follows:

- Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.

- ***Results***

Table 22: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	arithmetic mean	SD	arithmetic mean	SD
$C_{max,ss}$ (pg/ml)	2237.916	553.49	2072.734	453.64
AUC0-τ (pg.h/ml)	37463.7	10638.05	35548.6	9185.95
Cτ,ss (pg/ml)	1212.726	471.21	1100.626	370.45
Tmax,ss * (h)	4.50	3.00-8.00	4.50	4.50-8.00
Cavg (pg/ml)	1560.986	443.25	1481.191	382.75
Cmin,ss (pg/ml)	906.959	366.28	839.517	296.28
% fluctuation	88.573	19.15	85.589	17.74

$C_{max,ss}$ Maximum plasma concentration at steady state.

AUC0-τ AUC during a dosage interval at steady state, as calculated by the linear trapezoidal method.

Cτ,ss Concentration at the end of the dosing interval at steady state.

Tmax,ss Time until $C_{max,ss}$ is reached.

Cavg Average concentration during a dosing interval (AUC0-τ / τ).

Cmin,ss : Minimum plasma concentration at steady state.

% fluctuation $[(C_{max} - C_{min})/Cavg]$ %.

Table 23: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference %	Confidence Intervals 90%	CV%*
$C_{max,ss}$	107.98	100.35-116.18	15.53
$C_{τ,ss}$	109.47	96.52-124.16	27.01
AUC0-τ	105.58	97.37-114.48	17.19
* estimated from the Residual Mean Squares			

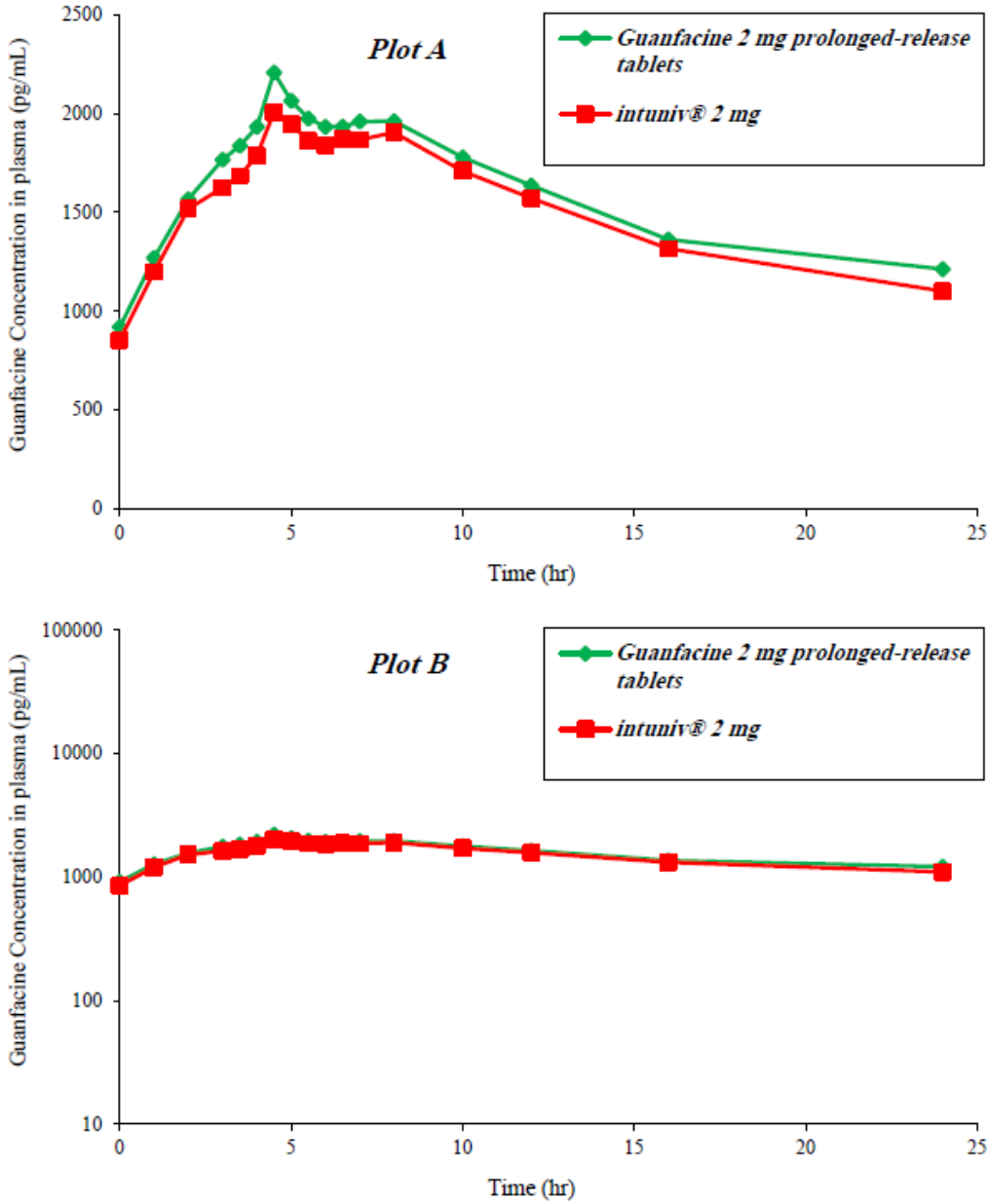


Figure 17: Linear presentation (Plot A) and semi-logarithmic presentation (Plot B) for guanfacine means after a multiple oral dose administration of one tablet from both treatments Guanfacine 2 mg prolonged-release tablets under fasted conditions.

Table 24: P-values obtained from guanfacine ANOVA results after multiple dose administration of Test Product and Reference Product

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{maxss} (N=26)	2.86×10^{-1}	1.20×10^{-3}	8.56×10^{-2}	9.53×10^{-1}
$C_{\tau,ss}$ (N=26)	5.70×10^{-1}	2.50×10^{-3}	2.31×10^{-1}	9.31×10^{-1}
$AUC_{0\rightarrow\tau}$ (N=26)	6.57×10^{-1}	3.00×10^{-4}	2.63×10^{-1}	7.65×10^{-1}

There was statistically significant subject (sequence) effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max} ss, $C_{\tau,ss}$ and AUC0-τ for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.0 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate subject (sequence) effects in this study were performed, and therefore, we consider that though there is a subject (sequence), this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during two periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (12 hrs before each dosing and 24 hrs after dose no. 7 and dose no. 14) and were maintained seated upright for the first two hours after each dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the two periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the subject (sequence) effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for t_{maxss} using Wilcoxon signed ranks test, there were no differences observed between T and R after the statistical analysis was applied.

- ***Safety data***

Adverse events

During the study, there was eight subjects who were reported to manifest adverse events.

Five subjects experienced an increase in SGPT (ALT) and one subject experienced toothache twice during period I. Two subjects and experienced an increase in SGPT (ALT) and SGOT (AST) and 3 subjects experienced an increase in SGPT (ALT) during period II. These adverse events were judged to have no effect on the subject health as per principal investigator decision where complete recovery were observed for those subjects.

There were no deaths or other significant adverse events during the study period.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) were performed at the end of confinement of period I (approximately 24 hours after last dosing on previous day). Laboratory tests of (haematology and biochemistry) were performed for follow up examination (At end of confinement of Period II, approximately 24 hours after last dosing on previous day).

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical assessment as well as vital signs evaluation and ECG at end of confinement of period I and at the follow up examination (At end of confinement of Period II, approximately 24 hours after last dosing on previous day).

Subjects were monitored closely for any side effects, especially sedation and syncope.

Clinical assessment for all subjects was carried out to evaluate their tolerability to the study medication. No serious adverse events were reported during the conduct of this study. Study subjects demonstrated good tolerance to the study drugs.

Study GUA-1122-134 (NXPGUAN/22/BQ-11): Comparative randomised, single dose, two-way crossover open label study to determine the bioequivalence of guanfacine 3 mg prolonged-release tablet after an oral administration to healthy male adults under fasting conditions.

Objective

Primary objective: To compare the absorption and disposition kinetics of Test Product relative to Reference Product after a single oral dose administration of guanfacine 3 mg prolonged-release tablets to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fasting conditions.

Study site(s) of GUA-1122-134: Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T006
STUDY CODE:	GUA-1122-134
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 13/04/23
STUDY COMPLETION:	Last Subject Last Visit: 12/05/23
STUDY PERIODS:	Screening commencement: 13/04/23
	Dosing Period I: 27/04/23
	Dosing Period II: 08/05/23
ANALYSIS DATES:	Analysis start date: 15/05/23

Analysis end date: 28/05/23

FINAL REPORT: Final report 26/06/23

The plan of study is depicted in the Figure below:

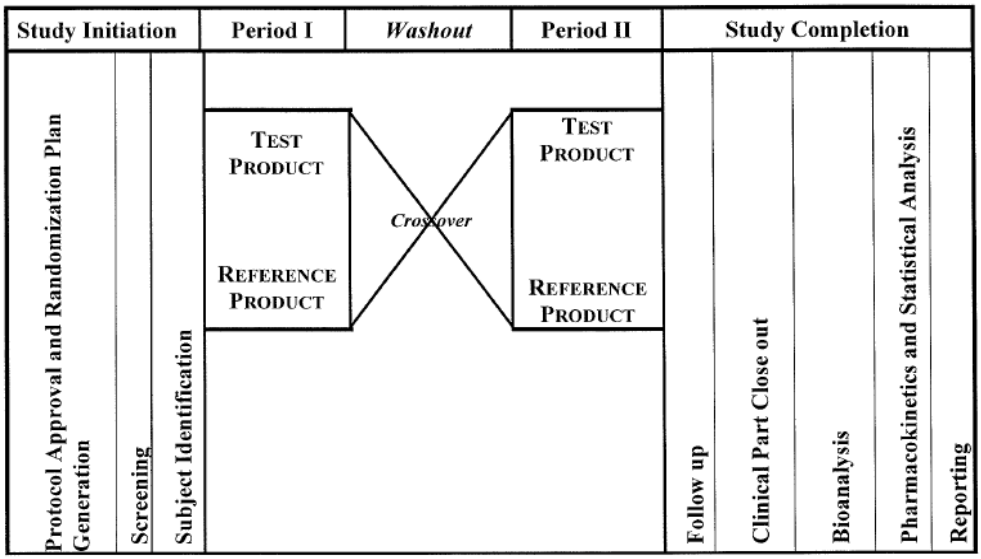


Figure 18: Study plan

The first screening examination was performed on 13/04/23. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan. The first administration of the study drug (Test or Reference drug product) and the first blood collection for drug analysis took place on 27/04/23 (first day of Period I). After a washout period of 11 days, on 08/05/23 subjects were given the second administration of the study drug (Test or Reference drug product). The last blood sample for drug analysis was collected on 12/05/23. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. The subjects received their standardized meals at the following times:

Table 25: Standardised diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hours after study drug administration
1	Snack	8 hours after study drug administration
1	Dinner	12 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

At least 10-days was be allowed between the two doses, as a washout period.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan. The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One prolonged-release tablet of guanfacine 3 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One prolonged-release tablet of Intuniv® 3 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

Blood samples were to be collected in K:EDTA tubes before dosing (one pre-dose at 0.00 hour) and at the following times after the dose: 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours.

For each subject, the total number of blood draws during the study was to be 42, and the total volume of blood drawn [(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations, was not to exceed 264.5 ml. This volume does not include discarded blood before sample withdrawal, samples of repeated tests or samples for ensuring subjects safety based on the judgement of the Principal Investigator. The total volume of blood drawn was not to exceed 420 ml through the whole study.

Blood samples were collected at the times specified under the sampling schedule, and centrifuged (using refrigerated centrifuge) as soon as possible after collection. Following centrifugation, the resulting plasma were transferred directly into plain polypropylene tubes. These samples were immediately be stored at clinical site at around -70°C using dry ice till transferred to the freezers area to be stored in the around -70°C freezers. All plasma samples will be divided into two (2) aliquots. All samples will be collected into suitably labelled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.).

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at

(www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II) but at period II one subject was given one tablet of paracetamol (500 mg) after hours from study drug administration. This concurrent medication was judged to have no effect on the subject health as per principal investigator decision. Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol (No. GUA-T006) and approval was given on 18/12/22 and approval for version 01 was given on 12/03/23.

Minor variations from the study protocol concerning sampling time and concurrent medications were observed during study conduct.

Collection anomalies:

Some samples were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

- **Test and reference products**

Table 26: Test and reference product information of GUA-1122-134

Product Characteristics	Test product	Reference product
Name	*Guanfacine 3 mg prolonged-release tablets*	*intuniv® 3 mg*
Strength	Guanfacine hydrochloride equivalent to 3 mg of guanfacine	Guanfacine hydrochloride equivalent to 3 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	02/23	NA
Batch number	GAL23008C	AS0250AL1
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	101.6%	98.9%
Commercial Batch Size	160000 tablets	
Expiry date (Retest date)	08/24	07/26

Member State where the reference product is purchased from:		Germany
This product was used in the following trials:	GUA-1122-134	GUA-1122-134

- **Population(s) studied**

34 male subjects plus 1-2 alternates were planned to be admitted in the study. An alternate subject was to be dosed by the same sequence as the withdrawn subject only if any subject of the first 34 subjects withdraws before the first study drug administration. Withdrawals after study drug administration were not to be replaced. The subjects were volunteers selected from the Jordan population, 18-45 years of age, weighing at least 50 kg, who are within the acceptable range of Body Mass Index (BMI).

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range5 is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HBsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Sample size

The following estimates were considered for the computation of sample size:

- T/R ratio: 108 %
- Intra-Subject C.V (%) ~ 17.76%
- Significance Level = 5%

- Power =90%

Based on the above estimate, a sample size of 28 subjects would be sufficient to establish bioequivalence between formulations with adequate power. However, considering the drop-out or withdrawal, a sample size of 34 subjects was considered for two-way crossover study.

Subject disposition

56 healthy male subjects were screened to evaluate fulfilment of selection criteria described in the study protocol. 34 subjects plus 2 alternates were admitted to the study. Two subjects were excluded by CRO staff due to protocol requirement after study drug administration in Period I. 34 subjects were dosed in period I, period II and completed the study. Data from the 34 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

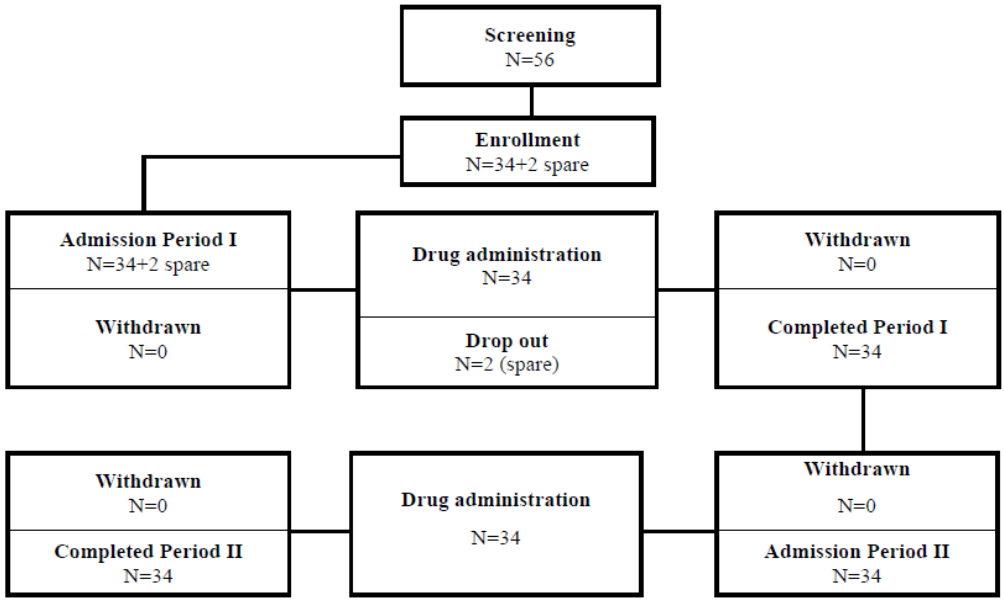


Figure 19: Disposition of subjects

Data sets analyzed

Data from the 34 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 31 days, from 27.04.2023 till 12.05.2023 (clinical part) and from 16.05.2023 till 28.05.2023 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	34
Total number of samples collected	1352
Total number of samples analyzed	1352
Maximum no. of injections in the analyzed runs	111
Validated batch size	161

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, Guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

No Sample reassays for guanfacine were done.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 122 samples for each subject and study period (~ 10% of total samples analysed); 120 out of 122 ISR samples (~98%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

Primary pharmacokinetic parameters

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.
- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter

- tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

No value of $AUC_{0-\infty}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

The actual times of blood sampling were to be used for these.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured datapoints from the time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}].$$

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

Samples from all subjects who complete the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence was to be used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics. Exclusion of data from statistical analysis included:

1) A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).

2) Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}. Fixed effects were used for all terms.

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

No changes were made during the conduct of the study or on the planned analysis.

Handling of dropouts or missing data

There were no withdrawals in this study.

Missing drug concentration data (value below LLOQ, missing value) were treated as follows:

- Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.
- Any missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

- ***Results***

Data from the 34 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

Table 27: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	Arithmetic mean	**SD**	**Arithmetic mean**	**SD**
$AUC_{(0-t)}$ (pg.h/ml)	65128.7	14909.99	58928.5	17710.95
$AUC_{(0-\infty)}$ (pg.h/ml)	68022.3	15897.00	61475.0	19322.37
C_{max} (pg/ml)	2236.856	537.75	1994.321	605.30
T_{max}* (h)	4.50	3.00-5.50	4.50	4.00-8.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 28: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference %	Confidence Intervals 90%	CV%*
$AUC_{(0-t)}$	112.48	103.15-122.66	21.32
$AUC_{(0-\infty)}$	112.83	103.25-123.30	21.85
C_{max}	114.00	105.34-123.37	19.40
* estimated from the Residual Mean Squares			

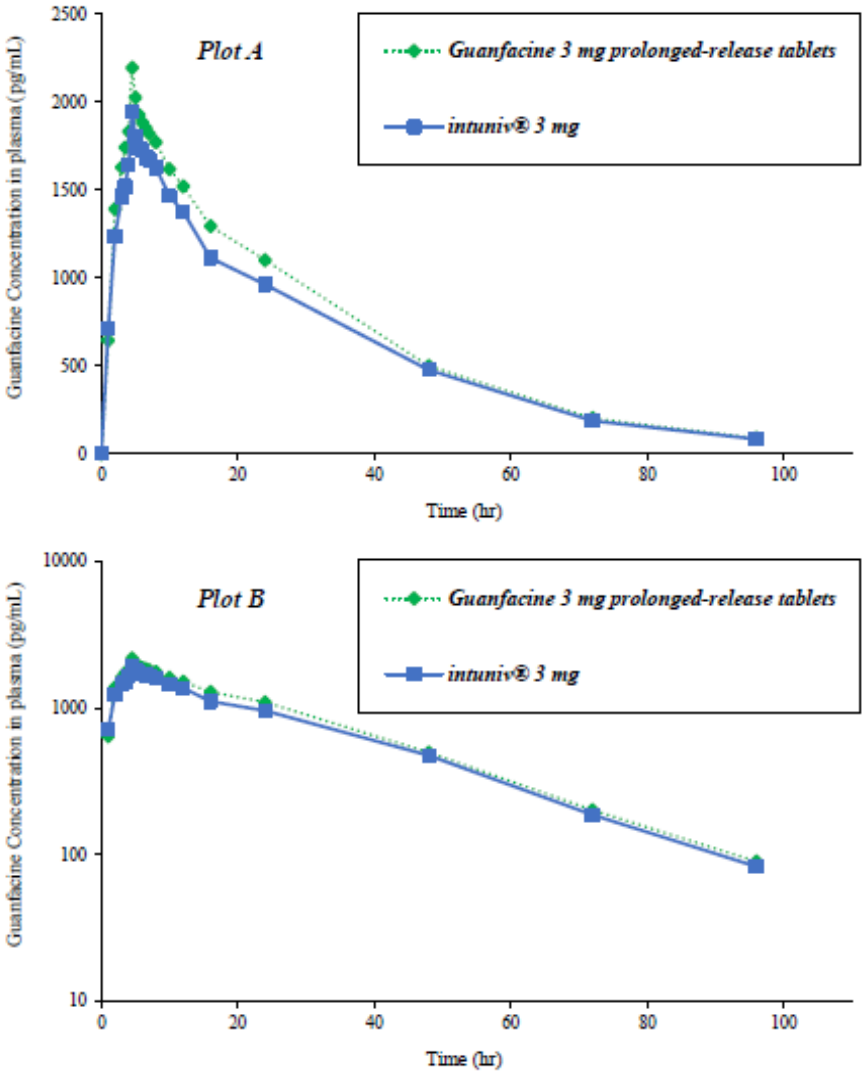


Figure 20: Linear (Plot A) and semi-logarithmic (Plot B) presentation for guanfacine means after single oral dose

Table 29: P-values obtained from ANOVA results

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{max} (N=34)	3.07×10^{-1}	2.90×10^{-3}	8.40×10^{-3}	1.97×10^{-1}
$AUC_{0 \to t}$ (N=34)	2.86×10^{-1}	4.88×10^{-2}	2.81×10^{-2}	7.25×10^{-1}
$AUC_{0 \to \infty}$ (N=34)	3.07×10^{-1}	3.66×10^{-2}	2.78×10^{-2}	6.80×10^{-1}

There was statistically significant subject (sequence) and drug effect observed from the ANOVA for the logarithmically transformed primary PK parameter C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.9 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination

and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate subject (sequence) and drug effect in this study were performed, and therefore, we consider that though there is subject (sequence) and drug effect, this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during two periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (10 hrs before dosing and 24 hrs after dosing) and were maintained seated upright for the first two hours after dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the two periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the subject (sequence) and drug effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for t_{max} using Wilcoxon signed-rank tests, there were no differences observed between T and R after the statistical analysis was applied.

- ***Safety data***

Adverse events

During the study, there was one subject who manifested an adverse event.

This subject reported headache during the study. This adverse event was judged to have no effect on the subject health as per principal investigator decision (AE was assessed as mild in intensity and related to study drug) and complete recovery was observed for that subject.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period II.

No clinically significant changes in vital signs, physical examination, electrocardiogram and laboratory measurements related to the study treatments were observed for all subjects.

Study GUA-T1221/118: COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAY CROSSOVER OPEN LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF GUANFACINE 4 MG PROLONGED-RELEASE TABLET AFTER AN ORAL ADMINISTRATION TO HEALTHY MALE ADULTS UNDER FASTING CONDITIONS

Objective

Primary objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 4 mg prolonged-release tablets to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fasting conditions.

Study site(s) of GUA-T1221/118: Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T002
STUDY CODE:	GUA-T1221/118
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 26/05/22
STUDY COMPLETION:	Last Subject Last Visit: 12/06/22
STUDY PERIODS:	Screening commencement: 26/05/22
Dosing Period I:	29/05/22
Dosing Period II:	08/06/22
ANALYSIS DATES:	Analysis start date: 13/06/22
	Analysis end date: 21/06/22
DATE OF VERSION 01 REPORT:	20-11-22

The clinical part of the study was conducted at the CRO clinical site. The first screening examination was performed on 26/05/22. After the screening examination and assessment for eligibility, subjects were given a subject enrolment number. The subjects were assigned to one of the two treatment sequences AB or BA according to a previously generated randomization plan. The first administration of the study drug (Test or Reference drug product) and the first blood collection for drug analysis took place on 29/05/22 (first day of Period I). After a washout period of 10 days, on 08/06/22 subjects were given the second administration of the study drug (Test or Reference drug product). The last

blood sample for drug analysis was collected on 12/06/22. Blood sampling in each study period was carried out as per the blood sampling schedule detailed in the study protocol.

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. The subjects received their standardized meals at the following times:

Table 30: Standardised diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hours after study drug administration
1	Snack	8 hours after study drug administration
1	Dinner	12 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan.

The administration of the study drugs was documented in the drug administration forms. Study drugs were administered by the clinical staff of CRO as follows:

Treatment A: One tablet of guanfacine 4 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One tablet of Intuniv® 4 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 6 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 6 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 6 ml) after administration of study drugs, the total number of blood draws during the study was 42. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples.

The total amount of blood draw during the whole study did not exceed 264.5 ml:

[(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations. This volume did not include discarded blood before sample withdrawal, samples for clinical laboratory repeats or samples for ensuring subjects safety based on the judgment of the principal investigator. The total volume did not exceed 420 ml through the whole study.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain plastic tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the -70°C freezer. All samples were collected into suitably labeled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 30/01/22 and approval for version 01 was given on 13/02/22:

Table 31: Protocol version history and summary of changes

Version	Description of change	Change carried out on pages	Reason of Change
ORG	NA	NA	NA
01	Orthostatic hypotension test will be performed at screening and the following will be added to the exclusion criteria: Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test) Vital signs will be measured for all subjects every hour in the first five hours in addition to the original times already present in the study protocol, so vital signs will be measured before dosing and approximately at the following times after dosing: 1st, 2nd, 3rd, 4th, 5th, 8th, 11th and 24th hour Subjects will be monitored closely for any side effects, especially sedation and syncope	19, 23, 26 and 27	Clinical Trial Committee Recommendations

Minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

There were collection time anomalies reported.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

Table 32: Study report versions

Version	Date	Major Comments
Final Report	21/08/22	NA
Version 01 Report	20/11/22	• New Quality Statement was issued in section 16.1.8. • Sponsor approval was updated in the report. • Signature of IPRC Investigators were updated and signature of Principal Investigator was updated • The following subsections were added to section 16.2: 16.2.11. Medical History 16.2.12. Physical Examination 16.2.13. Alcohol Test 16.2.14. Drug of Abuse Test 16.2.15. Drug Administration Time for All Subjects and By Period

- **Test and reference products**

Table 33: Test and reference product information of GUA-T1221/118

Product Characteristics	Test product	Reference product
Name	*Guanfacine 4 mg prolonged-release tablets*	*intuniv® 4 mg*
Strength	Guanfacine hydrochloride equivalent to 4 mg of guanfacine	Guanfacine hydrochloride equivalent to 4 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	03/22	NA
Batch number	GAL22002C	AM9790AL2
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	99.1%	101.3%
Commercial Batch Size	138750 tablets	
Expiry date (Retest date)	09/22	06/24
Member State where the reference product is purchased from:		Germany
This product was used in the following trials:	GUA-T1221/118	GUA-T1221/118

- **Population(s) studied**

24 healthy male subjects were screened to evaluate fulfilment of selection criteria:

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HBsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Sample size

Sample size calculation is based on the power of Schuirmann's two one-sided test procedure for interval hypotheses using the ± 20 rule for the assessment of average bioequivalence.

Subject disposition

During this study, 24 subjects were screened. 3 subjects dropped out from the study and 1 subject was not included due to screening failure. A total of 18 subjects plus 2 alternates were enrolled before study drug administration in Period I. Two subjects were excluded by CRO staff due to protocol requirement after study drug administration in Period I. 18 subjects were dosed in period I, II and completed the study.

Data sets analyzed

If a subject was withdrawn before the first study drug administration, he was to be replaced by the next qualifying alternate. The replacing alternate subject received the study products in the same sequence and under the same conditions as the dropped subject, and he underwent the entire protocol procedure. Withdrawals after study drug administration were not replaced.

When a subject withdrew from the study, the reasons were to be stated on the Case Report Form and a final evaluation of the subject was performed.

According to the study protocol, samples from the subjects who completed the study were analyzed for the plasma concentrations. Data from the 18 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 23 days, from 29.05.2022 till 12.06.2022 (clinical part) and from 13.06.2022 till 21.06.2022 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	18
Total number of samples collected	720
Total number of samples analyzed	720
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from a aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods
Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, Guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments
Sample reassays for guanfacine were done on 9 samples (~1.3%). All reassays are in accordance with the presented SOP and the relevant guideline.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 72 samples for each subject and study period (10% of total samples analysed); 70 out of 72 ISR samples (~97%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

Primary pharmacokinetic parameters

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.
- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

No value of $AUC_{0-\infty}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

The actual times of blood sampling were to be used for these.

Pharmacokinetic parameters of guanfacine were estimated using standard non-compartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) of guanfacine were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured datapoints from the time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}].$$

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

Samples from all subjects who complete the study were to be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were to be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence was to be used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not to be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were to be presented in the individual listings but were not to be included in the summary statistics. Exclusion of data from statistical analysis included:

1) A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).

2) Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}.

- Fixed effects model:

Y = μ + Sequence + Formulation + Period

- Random effects model is the nested term:

Subject (Sequence)

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted. For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

Handling drop-out or missing data

Missing drug concentration data (value below LLOQ) were treated as follows:

Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.

- ***Results***

Data from the 18 subjects who completed the crossover study were used for descriptive statistics and in the statistical evaluation of bioequivalence.

Table 34: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	**Test**		**Reference**	
	arithmetic mean	**SD**	**arithmetic mean**	**SD**
$AUC_{(0-t)}$ (pg.h/ml)	57186.0	16683.76	52574.2	27464.65
$AUC_{(0-\infty)}$ (pg.h/ml)	59050.0	17255.48	54196.5	28385.76
C_{max} (pg/ml)	2043.263	646.90	1839.875	817.00
T_{max}* (h)	4.50	4.50- 5.00	4.75	4.50- 12.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours>			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 35: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	**Geometric Mean Ratio Test/Reference**	**Confidence Intervals 90%**	**CV%***
$AUC_{(0-t)}$	115.90	97.52-137.75	30.34
$AUC_{(0-\infty)}$	116.03	97.73-137.77	30.15
C_{max}	115.16	97.65-135.81	28.92
* estimated from the Residual Mean Squares			

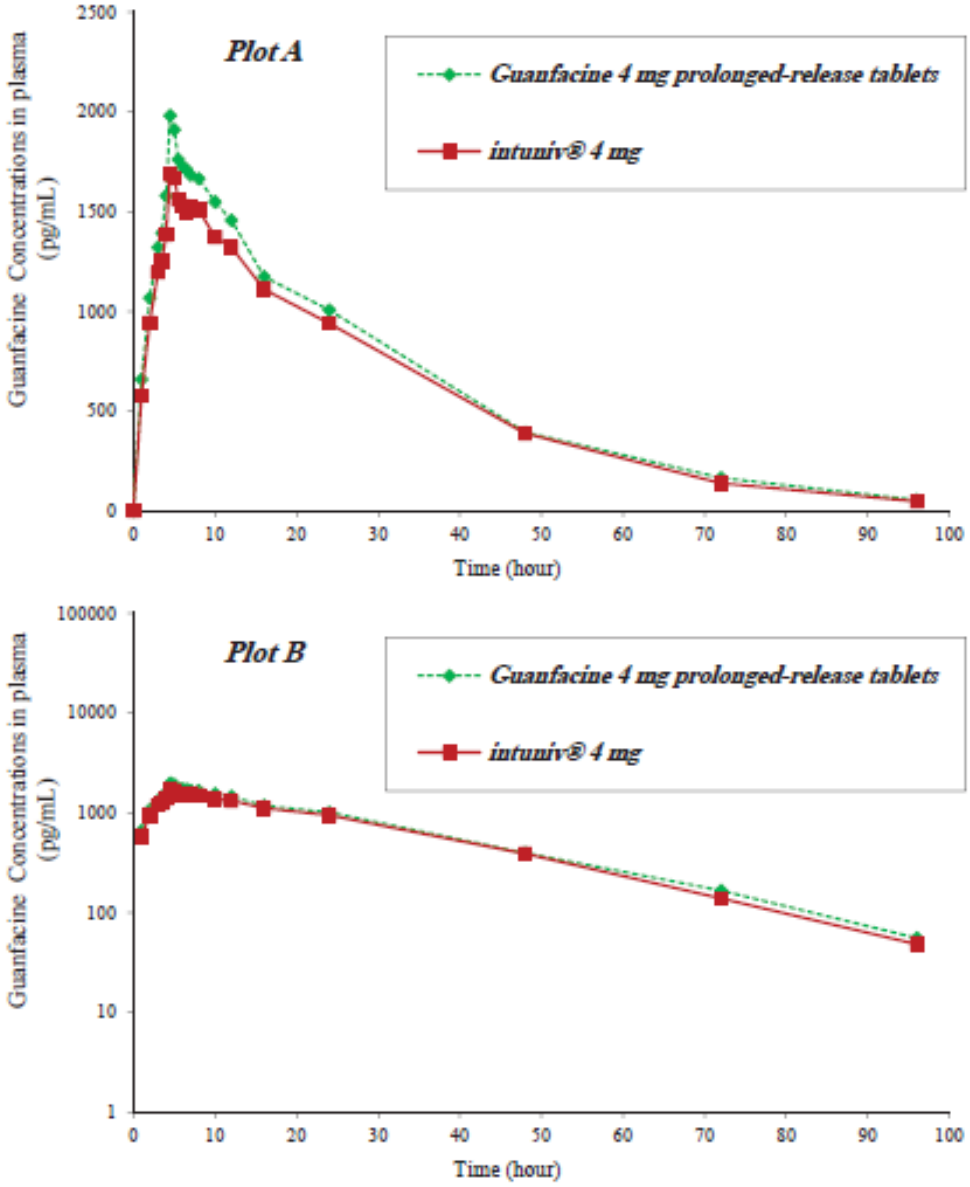


Figure 21: Linear (Plot A) and semi-logarithmic (Plot B) presentation for guanfacine means after single oral dose.

- ***Safety data***

Adverse events

During the study, there was one subject who manifested an adverse event. This subject reported headache during the study. This adverse event was classified as mild in intensity and judged to have no effect on the subject health as per principal investigator decision. Complete recovery was observed for this subject.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which was performed within 24 hours of collecting the last sample in period II.

Study GUA-0722-87: COMPARATIVE, RANDOMIZED, SINGLE DOSE, FOUR-PERIOD, CROSSOVER, OPEN-LABEL, FULLREPLICATE STUDY TO DETERMINE THE BIOEQUIVALENCE OF GUANFACINE 4 MG PROLONGEDRELEASE TABLET AFTER AN ORAL ADMINISTRATION TO HEALTHY MALE ADULTS UNDER FASTING CONDITIONS

Objective

Primary objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 4 mg prolonged-release tablets to healthy male adults under fasting conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with four-way full replicate design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fasting conditions. The washout interval of 10 days between period I and II, between period II and III and between period III and period IV was deemed appropriate based on the elimination half-life of approximately 18 hours.

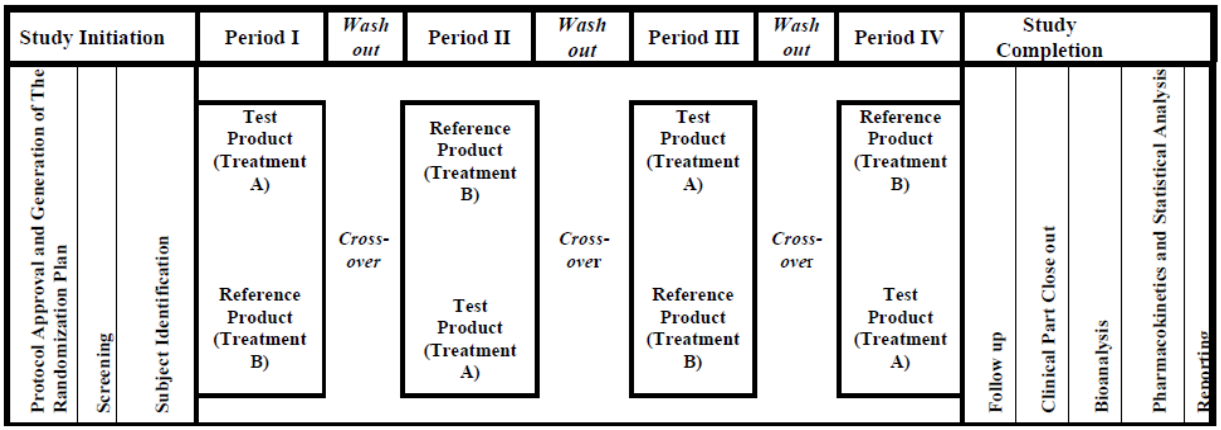


Figure 22: Study design and plan

The dose of 4 mg guanfacine as guanfacine hydrochloride was considered safe when given as four single doses to healthy subjects at 10 days between period I and II, between period II and III and between period III and period IV.

Study site(s) of GUA-0722-87: Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T003
STUDY CODE:	GUA-0722-87
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 19/10/22
STUDY COMPLETION:	Last Subject Last Visit: 29/11/22
STUDY PERIODS:	Screening commencement: 19/10/22
Dosing Period I:	26/10/22
Dosing Period II:	05/11/22
Dosing Period III:	15/11/22
Dosing Period IV:	25/11/22
ANALYSIS DATES:	Analysis start date: 30/11/22
	Analysis end date: 18/12/22
DATE OF REPORT/VERSION	12-02-23 / Final report

According to the study protocol, in each study period, the subjects were admitted to the study site before study drug administration on study day 1 and confined until the 24-hour blood sample was collected.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of each study period until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. The subjects received their standardized meals at the following times:

Table 36: Standardised diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Lunch	4 hours after study drug administration
1	Snack	8 hours after study drug administration
1	Dinner	12 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan.

Test Product (first and second administration of Treatment A):

One prolonged-release tablet of guanfacine 4 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Reference Product (first and second administration of Treatment B):

One prolonged-release tablet of Intuniv® 4 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 5.5 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 5.5 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 5.5 ml) after administration of study drugs, the total number of blood draws during the study was 83. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples. The total amount of blood draw during the whole study did not exceed 475.5 ml.

This volume did not include discarded blood before sample withdrawal, samples of repeated tests or samples for ensuring subjects safety based on the judgement of the Principal Investigator. The total volume of blood drawn was not to exceed 500 ml through the whole study.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain plastic tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the -70°C freezer. All samples were collected into suitably labelled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analysed the samples blindly.

Randomization and blinding

The study was randomized as a four-way, two-sequence crossover design. Administration was done according to a plan of randomization generated using software (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference/ Test/Reference (ABAB) and Reference/Test/ Reference/Test (BABA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period IV). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample of Period IV). The consumption of any medication or food which, may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

There were no concurrent medications taken during the study.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 11/09/22.

Minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

> Some samples after confinement period were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

Non-zero pre-dose anomalies:

> There were no non-zero pre-dose concentrations.

- **Test and reference products**

Table 37: Test and reference product information of GUA-0722-87

Product Characteristics	Test product	Reference product
Name	*Guanfacine 4 mg prolonged-release tablets*	*intuniv® 4 mg*
Strength	Guanfacine hydrochloride equivalent to 4 mg of guanfacine	Guanfacine hydrochloride equivalent to 4 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	Manufacturer: Laboratorios Lesvi, S.L., Spain	Shire Pharmaceuticals Ireland Limited, Ireland
Batch number	GAL22125C	AP1289AL4
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	99.4%	100.2%
Commercial Batch Size	138750 tablets	
Expiry date (Retest date)	07/23	01/25.
Member State where the reference product is purchased from:		Spain
This product was used in the following trials:	GUA-0722-87	GUA-0722-87

- **Population(s) studied**

67 healthy male subjects were screened to evaluate fulfilment of selection criteria:

Inclusion criteria

1. Healthy male subjects, age 18 to 45 years, inclusive.

2. Body Mass Index (BMI) range5 is within 20.0 – 29.9 Kg/m2.

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine

are accepted if below the reference range after being evaluated by the physician as clinically not

significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least 24 hours prior to the study drug administration of each study period until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study.

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120 days, 1500 ml in 180 days, 2000 ml in 270 days, 2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HbsAg), or Hepatitis C.

22. Subject has a difficulty fasting or consuming standard meals.

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test)

Sample size and subject disposition

The following estimates were considered for the computation of sample size:

• T/R ratio: 90 %

• Intra-Subject C.V (%) ~ 30%

• Significance Level = 5%

• Power =80%

Based on the above estimate, a sample size of 34 subjects were sufficient to establish bioequivalence between formulations with adequate power. However considering the dropout or withdrawn, a sample size of 38 subjects was considered full replicate study.

During this study, 67 subjects were screened. 38 subjects plus 2 alternates were enrolled before study drug administration in Period I. 2 subjects were excluded by CRO staff due to protocol requirement after study drug administration in Period I (alternates). 38 subjects were dosed in period I and period II. One subject withdrew after study drug administration in period II and before study drug administration in period III for personal reason. A total of 37 subjects were dosed in period III and IV and completed the study.

Data sets analyzed

Data from the 37 subjects who completed the full replicate crossover study and one withdrawn subject who completed the first two periods were used in the statistical evaluation of bioequivalence. In total, data from 38 subjects was included in the statistical analysis.

Protocol amendments and deviations

Minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

Some samples after confinement period were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

Non-zero pre-dose anomalies:

There were no non-zero pre-dose concentrations.

- **Analytical methods**

The study lasted 53 days, from 26.10.2022 till 29.11.2022 (clinical part) and from 01.12.2022 till 18.12.2022 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	4 periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	38
Total number of samples collected	2999
Total number of samples analyzed	2999
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Analytical Methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

Sample reassays for guanfacine were done on 2 samples (0.1%). All reassays are in accordance with the presented SOP and the relevant guideline.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 200 samples for each subject and study period (~ 6.7% of total samples analysed); 200 out of 200 ISR samples (100%) were within 20% from the mean value.

- **Pharmacokinetic variables**

Primary pharmacokinetic parameters

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.
- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.
- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

No value of $AUC_{0-\infty}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

The actual times of blood sampling will be used for these calculations as per internal SOPs.

The pharmacokinetic parameters of guanfacine were estimated using standard noncompartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured data points from the time of administration to time of last quantifiable concentration (Clast) by the linear trapezoidal rule.

It was checked that no time point ≤ tmax was used in the calculation.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was

calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}].$$

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

Samples from all subjects who complete the study will be analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, will be analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence will be used. The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products will not be included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects will be presented in the individual listings but will not be included in the summary statistics.

Exclusion of data from statistical analysis included:

1) A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low

plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).

2) Subjects with non-zero (pre-dose) baseline concentrations > 5% of C_{max}.

Statistical analysis was performed using WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ parameters.

Tmax was compared between formulations using Friedman test.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}.

Confidence intervals

The point estimates (Test/Reference) and the 90% Confidence Intervals for bioequivalence are set to be within the accepted limits of 80.00-125.00% for logarithmically transformed data for AUC_{0-t} and $AUC_{0-\infty}$. The confidence interval applied to parameter C_{max} of guanfacine will depend on the observed intrasubject variability for C_{max} of the Reference product. The intrasubject variability for Reference product will be calculated by excluding the Test formulation from the model specifications. Criteria applied will be the following:

In case the within-subject variability for C_{max} of the Reference product in the study (considering data from all study periods) is ≤30%, the confidence interval of logarithmically transformed TEST/REFERENCE ratio for C_{max} of guanfacine will be set to be within 80.00-125.00%.

In case the within-subject variability for C_{max} of the Reference product in the study (considering data from all study periods) is >30%, the confidence interval of logarithmically transformed TEST/REFERENCE ratio for C_{max} of guanfacine will be widened. The extent of the widening will be defined based upon the within-subject variability seen in the bioequivalence study using scaled-average-bioequivalence according to [U, L] =exp [±k·swR], where U is the upper limit of the acceptance range, Lis the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and swR is the within-subject standard deviation of the log-transformed values of C_{max} of the Reference product. The table below gives examples of different acceptance limits obtained based on different levels of variability using the above-mentioned methodology:

Within-subject CV (%)* (of guanfacine in the Reference product used in the study)	**Lower limit**	**Upper limit**
30	80.00	125.00
35	77.23	129.48
40	74.62	134.02
45	72.15	138.59
≥50	69.84	143.19

The geometric mean Test/Reference ratio should lie within the conventional acceptance range 80.00 - 125.00%.

The acceptance criteria for the 90% confidence interval of the geometric mean Test/Reference ratio for C_{max} will be widened according to the calculation above to a maximum of 69.84-143.19%.

Handling drop-out or missing data

Concentration data from the withdrawn one subject who completed two periods (first administration of treatment A and treatment B) was presented in the individual listings and included in the descriptive statistics and statistical evaluation.

Missing drug concentration data (value below LLOQ) were treated as follows:

Missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.

- ***Results***

Data from the 37 subjects who completed the full replicate crossover study and one withdrawn subject who completed the first two periods were used in the statistical evaluation of bioequivalence. In total, data from 38 subjects was included in the statistical analysis.

Bioequivalence was demonstrated for guanfacine, within the prescribed 90% confidence interval for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ with respect to the parametric method on log-transformed data of guanfacine.

Table 38: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	Arithmetic mean	SD	Arithmetic mean	SD
$AUC_{(0-t)}$ (pg.h/ml)	66982.9	21645.16	66768.1	22133.77
$AUC_{(0-\infty)}$ (pg.h/ml)	70190.7	23558.45	70151.8	24411.43
C_{max} (pg/ml)	2228.189	640.35	2308.608	826.60
T_{max}* (h)	4.50	3.00-12.00	4.50	2.00-24.00
AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours			
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity			
C_{max}	maximum plasma concentration			
T_{max}	time for maximum concentration (* median, range)			

Table 39: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference %	Confidence Intervals 90%	CV%* for reference product (Logarithmic)
$AUC_{(0-t)}$	100.54	94.35-107.15	22.96
$AUC_{(0-\infty)}$	100.38	94.02-107.17	24.37
C_{max}	98.57	92.03-105.58	25.22

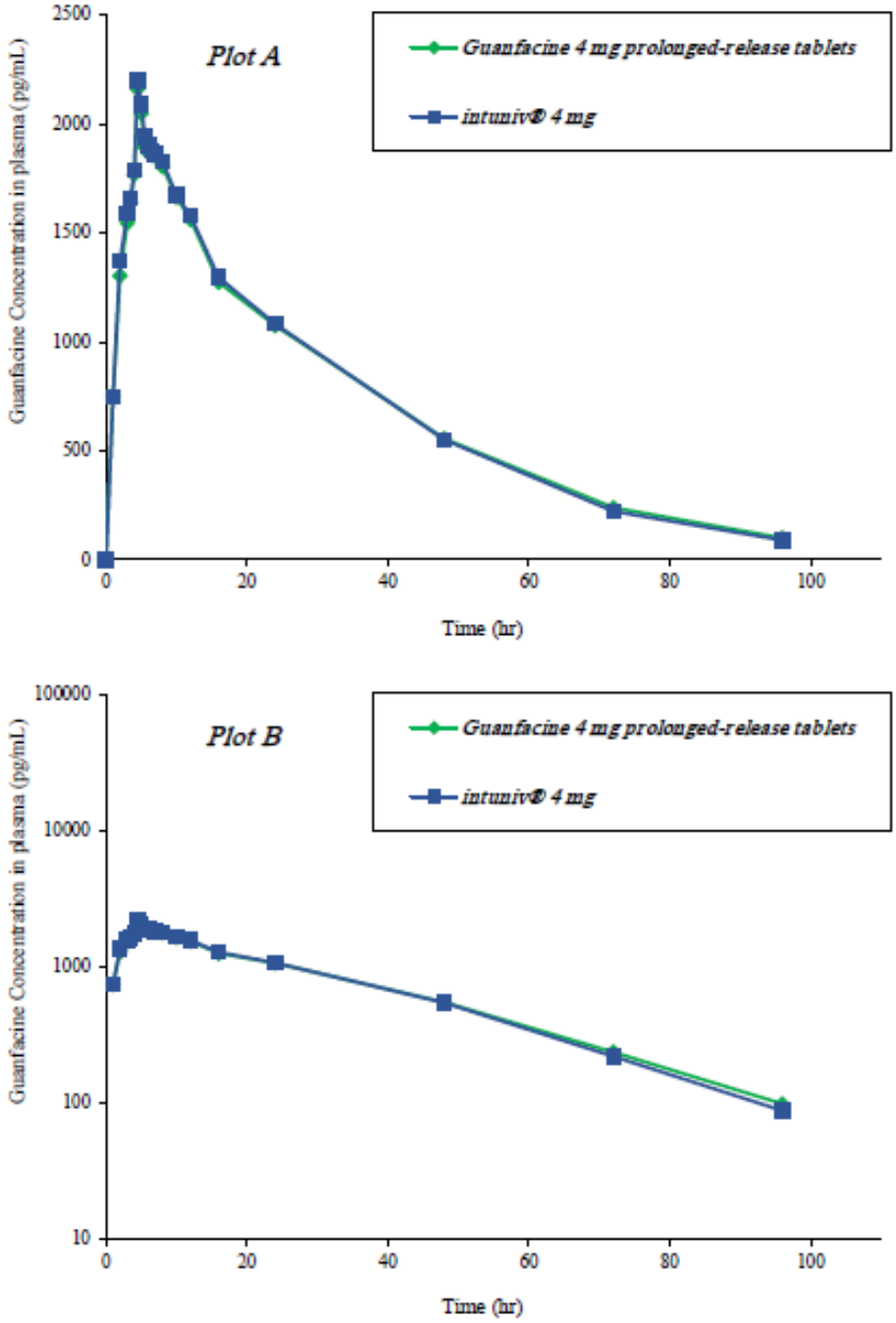


Figure 23: Linear (Plot A) and semi-logarithmic (Plot B) presentation for guanfacine means after a single oral administraion

Table 40: P-values obtained from guanfacine ANOVA results

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{max} ♣	$2.41x\ 10^{-1}$	0.000	$7.29x\ 10^{-1}$	$1.30x\ 10^{-3}$
$AUC_{0\rightarrow t}$ ♣	$9.40x\ 10^{-1}$	0.000	$8.88x\ 10^{-1}$	0.000
$AUC_{0\rightarrow\infty}$ ♣	$9.57x\ 10^{-1}$	0.000	$9.24x\ 10^{-1}$	0.000

♣number of observations for the test and reference=150

There was statistically significant subject (sequence) and sequence effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.0 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate subject (sequence) and sequence effect in this study were performed, and therefore, we consider that though there is a sequence, subject (sequence) effects, this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during four periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (10 hrs before dosing and 24 hrs after dosing) and were maintained seated upright for the first two hours after dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the four periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the sequence, subject (sequence) effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for t_{max} using Friedman test, there were no differences observed between Test and Reference product after the statistical analysis.

- ***Safety data***

Adverse events (AEs)

There were no AEs during the study.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) were performed for each subject before admission to Period III. Laboratory tests of

(haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period IV.

Each subject received a thorough physical assessment, orthostatic hypotension test and vital signs evaluation, and ECG. ECG was performed for each subject before admission to Period III. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period IV.

Study GUA-1122-135: COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAY CROSSOVER OPEN LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF GUANFACINE 4 MG PROLONGED-RELEASE TABLET AFTER AN ORAL ADMINISTRATION TO HEALTHY MALE ADULTS UNDER FED CONDITIONS

Objective

Primary objective: To investigate the bioequivalence of Test Product relative to Reference Product after a single oral dose administration of guanfacine 4 mg prolonged-release tablets to healthy male adults under fed conditions.

Secondary objective: To investigate the safety and tolerability of the formulations.

Methods

- **Study design**

This study was a single centre, open-label, randomized, single-dose study with two-way crossover design to compare the bioavailability of guanfacine from the test product and the reference product in healthy adult male subjects under fed conditions. The washout interval of 10 days was deemed appropriate based on the elimination half-life of approximately 18 hours.

The dose of 4 mg guanfacine as guanfacine hydrochloride was considered safe when given as two single doses to healthy subjects at 10 days from the first study drug administration.

Study site(s) of GUA-1122-135: Clinical, bioanalytical, PK and statistical parts of the study were performed in a CRO in Jordan inspected by several EU and other competent authorities.

PROTOCOL CODE NO.:	GUA-T005
STUDY CODE:	GUA-1122-135
DEVELOPMENT PHASE OF STUDY:	Phase I –Bioequivalence Study
STUDY INITIATION:	First Signed Informed Consent Form: 17/01/23
STUDY COMPLETION:	Last Subject Last Visit: 11/02/23
STUDY PERIODS:	Screening commencement: 17/01/23
	Dosing Period I: 28/01/23
	Dosing Period II: 07/02/23
ANALYSIS DATES:	Analysis start date: 15/02/23

Analysis end date: 26/02/23

DATE OF FINAL REPORT: 28-03-23

The study was conducted in fed conditions according to the EMA Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CHMP/EWP/280/96 Rev1). According to this Guideline – Section 6, a single-dose fed study using a high fat meal comparing test and reference drug is required. According to section 5.1.4.1, the meal should be a high-fat (approximately 50 percent of total caloric content of the meal) and high calorie (approximately 800 to 1000 kcal) meal. This test meal should derive approximately 150,250, and 500-600 kcal from protein, carbohydrate and fat, respectively.

Recommendations of Intuniv@ regarding the method of administration is that guanfacine can be administered with or without food but should not be administered with high fat meals, due to increased exposure as it has been shown that high fat meals have a significant effect on the absorption of guanfacine. In adults, the mean exposure of guanfacine increased (C_{max} -75% and AUC ~40%) when guanfacine was taken together with a high fat meal, compared to intake in the fasted state. This significant food effect could be clinically significant, because many of the important adverse effects of Intuniv@ occurred in a dose-related manner. Considering the possible increase of adverse effects when administering a high fat meal, the single dose study in fed conditions was performed but under the conditions concerning concomitant food intake recommended in the SmPC for the originator product. In this case, a standard meal (normal meal without high-calorie high-fat content) was given to the subjects 30 minutes prior to administration of the drug product.

No consumption of beverages or foods containing methylxanthines, e.g. caffeine (coffee, tea, cola, energy drinks, chocolate, etc.) was permitted for the subjects for at least 24 hours prior to the study drug administration of either study periods until the end of confinement. In addition, the consumption of any beverages or foods containing grapefruit was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study. Consumption of alcohol containing beverages and foods was prohibited for at least two weeks prior to first study drug administration until donating the last sample of the study.

Food and fluid-intake were identical in both study periods, starting from the dinner served at least 11 hours before study drug administration on study day -1 until the end of confinement. The subjects received their standardized meals at the following times:

Table 41: Standardised diets served during the study

Study Day	Standardized Diet	Time Received
-1	Dinner	Finished by a minimum of 10 hours before the scheduled time of study drug administration in the morning of study day 1
1	Breakfast	0.5 an hour before study drug administration
1	Lunch	5 hours after study drug administration
1	Snack	9 hours after study drug administration
1	Dinner	13 hours after study drug administration

No water or fluids were permitted from 1 hour before study drug administration until 1 hour after the dose, no fluid intake was allowed apart from the 240 ml of water used for the administration of the study drug. Following 1 hour, the subjects were allowed to drink water as desired.

Treatments

On study day 1 of each study period, following the overnight fast of at least 10 hours, the study drugs were administered according to the randomization plan.

Treatment A: One prolonged-release tablet of guanfacine 4 mg prolonged-release tablets, TEST PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Treatment B: One prolonged-release tablet of Intuniv® 4 mg, REFERENCE PRODUCT, was given with 240 ml of water. Water was at ambient temperature and was measured with a graduated cylinder.

Sampling schedule and sample handling

The volume of blood taken was 6 ml per sample. Blood samples for the determination of drugs concentration were collected immediately in K3EDTA tubes before study drug administration (1 × 6 ml) at 0.00 hr (pre- dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00 and 96.00 hours (19 × 6 ml) after administration of study drugs, the total number of blood draws during the study was 42. After each blood sampling the cannula was injected with 0.1 ml of heparin solution it is concentration 50 IU/ml to prevent blood coagulation. The number of blood collections in each study period for drug analysis was 20 samples. The total amount of blood draw during the whole study did not exceed 264.5 ml:

[(1 x 6 ml for pre-dose sample) + (19 x 6 ml for post dose samples)] x 2 plus a maximum of 13.5 ml for screening and a maximum of 11 ml for follow up examinations. This volume did not include discarded blood before sample withdrawal, samples for clinical laboratory repeats or samples for ensuring subjects safety based on the judgment of the principal investigator. The total volume did not exceed 420 ml through the whole study.

Blood samples were collected at the times specified under study design, centrifuged (using refrigerated centrifuge) as soon as possible after collection at approximately 3500 r.p.m for around 10 minutes. Following centrifugation, the resulting plasma was transferred directly into two plain polypropylene tubes. These samples were immediately stored at the clinical site in a freezer at a temperature around -70°C using dry ice till transferred to the freezers area to be stored in the around -70°C freezer. All samples were collected into suitably labeled tubes (subject no., part one from study code, the year the study was assigned and the number of the study, study period, sample no. and aliquot no.). This assured that the analysts at CRO analyzed the samples blindly.

Randomization and blinding

The study was randomized as a two-way, two-sequence, crossover design. Administration was done according to a plan of randomization generated using the randomization plan generators available at (www.randomization.com). Subjects were assigned to one of the two treatment sequences Test/Reference (AB) and Reference/Test (BA) according to the plan of randomization.

The study was an open-label study in terms of the drug and the dose. The randomization plan and dispense records were freely available to CRO clinical staff. None of the laboratory staff had access to the randomization since the bioassay was performed blinded with regard to the sequence of product administrations.

Prior and concomitant therapy

According to the study protocol, no medication including over-the-counter products was to be taken starting at least 2 weeks before the first study drug administration until the end of the study (collection of the last sample of Period II). Vitamins taken as nutritional supplements were discontinued at least two days before first study drug administration until the end of the study (collection of the last sample

of Period II). The consumption of any medication or food which may affect CYP3A4/5 enzymes was prohibited at least two weeks prior to first study drug administration until donating the last sample of the study.

Any deviation from the above would have been recorded on the CRF.

Protocol amendments and deviations

The Institutional Review Board of study site reviewed the study protocol and approval was given on 04/12/22.

Minor variations from the study protocol concerning sampling time were observed during study conduct.

Collection anomalies:

Some samples after confinement period were not withdrawn on scheduled time. Nevertheless, the effect of this time deviation in collection time on results is minimal because it was taken into consideration in statistical analysis.

Non-zero predose anomalies:

There were no non-zero pre-dose concentrations.

- **Test and reference products**

Product Characteristics	Test product	Reference product
Name	*Guanfacine 4 mg prolonged-release tablets*	*intuniv® 4 mg*
Strength	Guanfacine hydrochloride equivalent to 4 mg of guanfacine	Guanfacine hydrochloride equivalent to 4 mg of guanfacine
Dosage form	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	07/22	NA
Batch number	GAL22125C	AP1289AL4
Batch size (Biobatch)	110000 tablets	
Measured content(s) (% of label claim)	99.4%	100.2%
Commercial Batch Size	138750 tablets	
Expiry date (Retest date)	07/23	01/25
Member State where the reference product is purchased from:		Spain
This product was used in the following trials:	GUA-1122-135	GUA-1122-135

- **Population(s) studied**

60 healthy male subjects were screened to evaluate 115ulfilment of selection criteria:

Inclusion criteria:

1. Healthy male subjects, age 18 to 45 years, inclusive

2. Body Mass Index (BMI) range is within 20.0 -29.9 Kg/m2

3. Subject does not have a known allergy to the drug under investigation or any of its ingredients or any other related drugs.

4. Standard ECG assessment is normal (No QTc Prolongation).

5. Medical history and physical examination within medically acceptable criteria.

6. Results of laboratory investigations within laboratory reference ranges (ALP and creatinine are accepted if below the reference range after being evaluated by the physician as clinically not significant). Haematology tests within 5% of reference limits.

7. Subject is capable of consent.

Exclusion criteria

1. Medical demographics performed not longer than two weeks before the initiation of the clinical study with significant deviations from the normal ranges.

2. Presence of any clinically significant results from laboratory tests, however, ALP and creatinine will be accepted if below the reference range after being evaluated by the physician as clinically not significant. Haematology tests with deviation of more than 5% of the reference limits. Laboratory tests are performed not longer than two weeks before the initiation of the clinical study.

3. History of drug or alcohol abuse.

4. Subject is a light or heavy smoker (more than 5 cigarettes per day).

5. Subject does not agree to not taking any prescription or non-prescription drugs within at least two weeks before first study drug administration and until donating the last sample of the study.

6. Subject does not agree to not taking any vitamins taken for nutritional purposes within at least two days before first study drug administration and until donating the last sample of the study.

7. Subject is on a special diet (for example subject is vegetarian).

8. Subject consumes large quantities of alcohol or beverages containing methyl-xanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.).

9. Subject does not agree to not consuming any beverages or food containing alcohol at least two weeks prior to first study drug administration until donating the last sample of the study.

10. Subject does not agree to not consuming any beverages or food containing methylxanthines e.g. caffeine (coffee, tea, cola, energy drinks, chocolate etc.) at least24 hours prior to the study drug administration of either study periods until the end of confinement.

11. Subject does not agree to not consuming any beverages or food containing grapefruit at least two weeks prior to first study drug administration until donating the last sample of the study.

12. Subject has a history of severe diseases which have direct impact on the study

13. Participation in a bioequivalence study or in a clinical study within the last 80 days before first study drug administration.

14. Subject intends to be hospitalized within 3 months after first study drug administration.

15. Subjects who donated blood or its derivatives in the past 3 months or who through completion of this study, would have donated more than 1250 ml in 120days, 1500 ml in 180 days,2000 ml rn270 days,2500 ml of blood in 1 year.

16. Subject has a history of significant asthma, peptic or gastric ulcer, sinusitis, pharyngitis, renal disorder (impaired renal function), hepatic disorder (impaired hepatic function), cardiovascular disorder, neurological disease such as epilepsy, haematological disorders or diabetes, psychiatric, dermatologic or immunological disorders.

17. Subject does not agree to not be engaged in strenuous exercise at least one day prior to study drug administration until donating the last sample in each respective period.

18. Subject having at screening examination a pulse outside the normal range of (60-100 beat per minute) or a body temperature outside the normal range of (35.0-37.2 °C) or a respiratory rate outside the normal range of (14-20 breath per minute) or a sitting blood pressure less than 100/60 mm Hg or more than or equal to 140/90 mm Hg.

19. Subject has history of difficulties in swallowing or any gastrointestinal disease which could affect the drug absorption.

20. The subject is a female.

21. Positive blood screen for HIV, Hepatitis B surface antigen (HbsAg), or Hepatitis C

22. Subject has a difficulty fasting or consuming standard meals

23. Subject does not agree to not consuming any medication or food which may affect CYP3A4/5 enzymes at least two weeks prior to first study drug administration until donating the last sample of the study.

24. Subject with orthostatic hypotension (blood pressure falls by more than 20 mmHg and/or the pulse rises by more than 20 beats per minute and/or subject growing dizzy or losing consciousness during orthostatic test).

Sample size

The following estimates were considered for the computation of sample size

T/R ratio = 108 %

Intra-Subject C.V (%) ~ 17.76%

Significance Level = 5 %

Power = 90 %

Based on the above estimate, a sample size of 28 subjects would be sufficient to establish bioequivalence between formulations with adequate power. However, considering the dropout or withdrawal, a sample size of 34 subjects is considered for two-way crossover study.

Subject disposition

During this study, 60 subjects were screened. 12 subjects dropped out from the study and 12 subjects were not included due to screening failure. A total of 34 subjects plus 2 alternates were enrolled before study drug administration in Period I. Two subjects were excluded by CRO staff due to protocol requirement after study drug administration in Period I. 34 subjects were dosed in period I, one subject withdrew after study drug administration in period I and before study drug administration in period II for personal reason. 33 subjects were dosed in period II and completed the study.

Data sets analyzed

According to the study protocol, samples from the subjects who completed the study and samples from withdrawal were analyzed for the plasma concentrations.

Data from the 33 subjects who completed the crossover study, excluding one withdrawn subject, were used for descriptive statistics and in the statistical evaluation of bioequivalence.

- **Analytical methods**

The study lasted 29 days, from 28.01.2023 till 11.02.2023 (clinical part) and from 16.02.2023 till 26.02.2023 (analytical part); study samples were obtained stored at a nominal temperature of -70ºC.

Description	Numbers
Periods	2 Periods
Theoretical number of samples for each subject per study period to be analyzed	20
Total number of subjects to be analyzed	34
Total number of samples collected	1328
Total number of samples analyzed	1328
Maximum no. of injections in the analyzed runs	111
Validated batch size	160

Analytical methods

The analyte was guanfacine.

Internal standard was guanfacine-$^{13}C^{15}N_3$; samples were extracted from an aliquot of K_3EDTA human plasma by liquid extraction. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 20.00 pg/mL to 5000.00 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Data on long term stability are provided.

QC samples at different concentrations (Low and High QC levels) were stored at -70ºC for 54 days. Stability calculated by comparing stored samples with the nominal concentrations for each Low and High levels and calculated using a freshly prepared standard calibration curve.

With regard to the data, Guanfacine is stable in human plasma up to 54 days after stored at -70ºC.

Observations and comments

Sample reassays for guanfacine were done on 8 samples (0.5%). All reassays are in accordance with the presented SOP and the relevant guideline.

Incurred sample reanalysis (ISR) of guanfacine has been performed on 72 samples for each subject and study period (~ 5,4% of total samples analysed); 70 out of 72 ISR samples (~97%) were within 20% from the mean value.

All chromatograms were provided.

- **Pharmacokinetic variables**

Primary pharmacokinetic parameters

- C_{max}: Maximum measured plasma concentration over the time span specified. Determined directly from the plasma concentration-time curve.

- AUC_{0-t}: The area under the plasma concentration versus time curve, from time (0) to the last measurable concentration (t), as calculated by the linear trapezoidal method.
- $AUC_{0-\infty}$: The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

Secondary pharmacokinetic parameter:

tmax: Time of the maximum measured plasma concentration. Determined directly from the plasma concentration-time curve. If the maximum value occurs at more than one time point, tmax is defined as the first time point with this value.

No value of $AUC_{0-\infty}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

The actual times of blood sampling will be used for these calculations as per internal SOPs.

The pharmacokinetic parameters of guanfacine were estimated using standard noncompartmental methods. The maximum plasma concentration (C_{max}) and the time to peak plasma concentration (tmax) were taken directly from the measured data.

The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from measured data points from the time of administration to time of last quantifiable concentration (Clast) by the linear trapezoidal rule.

The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_{last} / [Ln\ (2) / t_{½el}],$$

The pharmacokinetic calculations were performed by WinNonlin Statistical Software, version 8.3.4.

- **Statistical methods**

Samples from all subjects who complete the study were analyzed for the plasma concentrations and considered for statistical analysis. Samples from withdrawals, if any, were analysed if the profile of at least one period can be determined. If necessary, an unequal number of subjects per sequence were used.

The pharmacokinetic results from withdrawals who do not provide evaluable data for both the test and reference products were not included in statistical evaluation. Concentration data and pharmacokinetic parameters from such subjects were presented in the individual listings but were not included in the summarv statistics.

Exclusion of data from statistical analysis included:

1) A subject with lack of any measurable concentrations or only very low plasma concentrations for reference medicinal product. A subject is considered to have very low plasma concentrations if its AUC is less than 5%o of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).

2) Subjects with non-zero (pre-dose) baseline concentrations > 5 % C_{max}.

Statistical analysis was performed using the WinNonlin Statistical Software, version 8.3.4.

The statistical evaluation of bioequivalence included analysis of variance (ANOVA) of the primary parameters, calculation of formulation ratios (point estimates) and parametric 90% confidence intervals for ln-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} parameters.

Tmax was compared between formulations using Wilcoxon signed rank tests.

Analysis of variance (ANOVA)

An analysis of variance (ANOVA) tested for sequence, period, subject (sequence) and treatment effect was used. ANOVA was performed on Ln AUC_{0-t}, Ln $AUC_{0-\infty}$ and Ln C_{max}. Fixed effects were used for all terms.

Confidence intervals

A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach recommended by Steinijans and Diletti based on the inclusion of the shortest 90% confidence interval in the bioequivalence range was adopted.

For the parametric analysis of bioequivalence for Ln-transformed data, the 90% confidence interval for the ratio of (Test /Reference) was to be contained within the acceptance boundaries of 80.00-125.00% for AUC_{0-t} and $AUC_{0-\infty}$ (that defines the extent of absorption) and for C_{max} (parameter that reflects rate of absorption) to conclude bioequivalence between formulations.

Handling drop-out or missing data

Concentration data from the withdrawn one subject were presented in the individual listings and did not include in the descriptive statistics.

Missing drug concentration data (value below LLOQ) were treated as follows:

> Values below LLOQ were treated as zero for all pharmacokinetic and statistical analyses.
>
> Any missing concentration values were treated as missing and not included in the pharmacokinetic calculations.

- ***Results***

Data from the 33 subjects who completed the crossover study, excluding one withdrawn subject, were used for descriptive statistics and in the statistical evaluation of bioequivalence.

Table 42: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	arithmetic mean	SD	Arithmetic mean	SD
$AUC_{(0-t)}$ (pg.h/ml)	82480.7	30001.44	89582.8	22728.83
$AUC_{(0-\infty)}$ (pg.h/ml)	85661.9	32918.94	91963.3	24011.76
C_{max} (pg/ml)	3306.283	732.15	3439.825	833.97
T_{max}* (h)	5.50	2.00- 8.00	5.50	3.00- 8.00

AUC_{0-t}	area under the plasma concentration-time curve from time zero to t hours
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity
C_{max}	maximum plasma concentration
T_{max}	time for maximum concentration (* median, range)

Table 43: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference	Confidence Intervals 90%	CV%*
$AUC_{(0-t)}$	89.85	80.09-100.78	28.04
$AUC_{(0-\infty)}$	90.51	80.52-101.73	28.55
C_{max}	97.22	89.13-106.05	21.03
* estimated from the Residual Mean Squares			

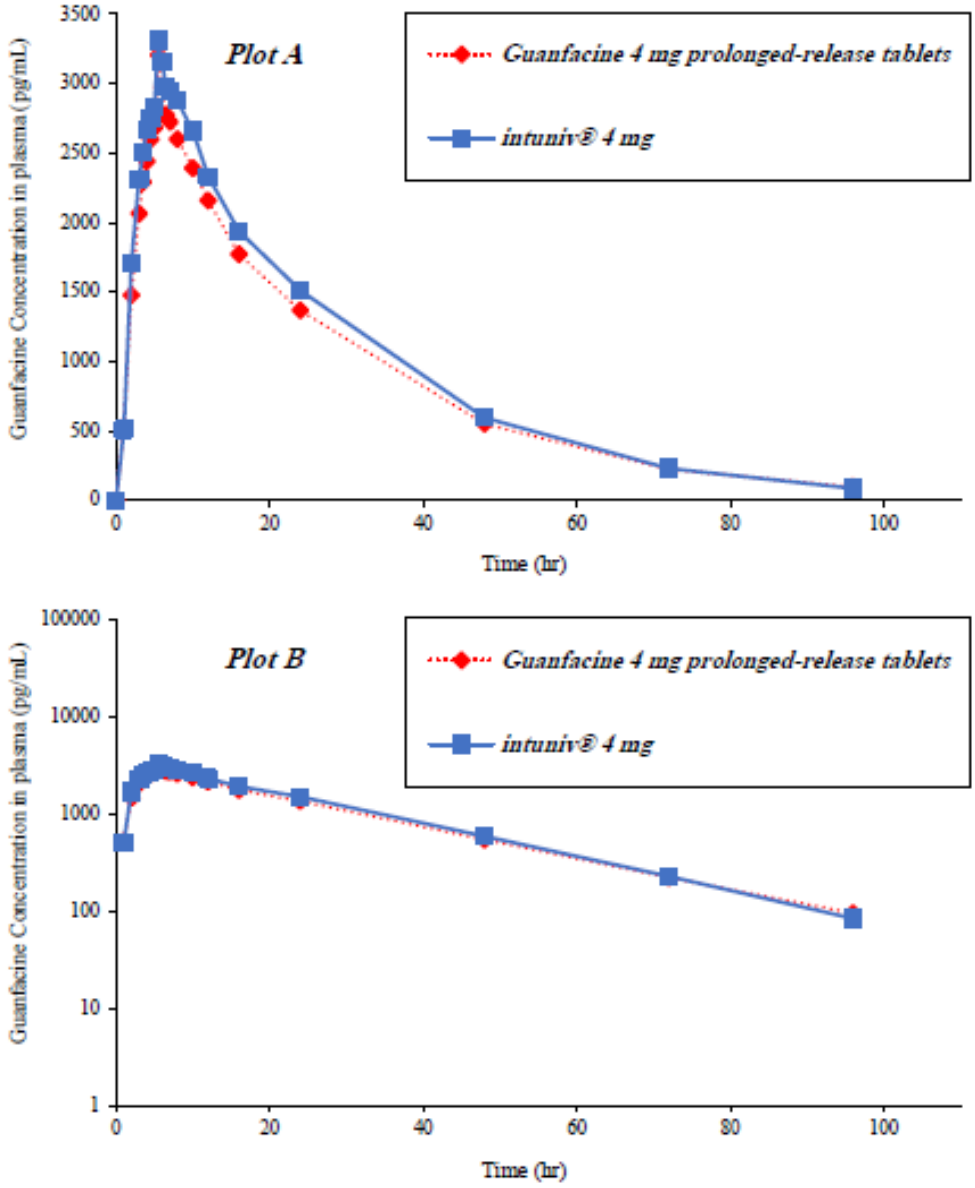


Figure 24: Linear (Plot A) and semi-logarithmic (Plot B) presentation for guanfacine means after a single oral administraion

Table 44: P-values obtained from guanfacine ANOVA results after single dose administration of Test Product and Reference Product

Pharmacokinetic Parameter	Period	Subject (sequence)	Drug	Sequence
Transformed to natural logarithm (neperian)				
C_{max} (N=33)	7.541×10^{-1}	1.99×10^{-2}	5.865×10^{-1}	5.00×10^{-4}
$AUC_{0\rightarrow t}$ (N=33)	7.703×10^{-1}	6.66×10^{-2}	1.242×10^{-1}	9.2×10^{-3}
$AUC_{0\rightarrow\infty}$ (N=33)	8.065×10^{-1}	5.29×10^{-2}	1.580×10^{-1}	1.24×10^{-2}

There was statistically significant sequence effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for guanfacine, in addition to subject (sequence) effect observed from the ANOVA for the logarithmically transformed primary PK parameters C_{max} for guanfacine.

In order to reduce the impact of these factors on the study, the protocol established as inclusion criteria that all participants must be healthy within 18-45 years old and 20.0 to 29.0 kg/m2 body mass index. During the screening procedures, all selected volunteers had normal ECG, physical examination and all their lab results were within normal range. The exclusion criteria were drugs and alcohol abuse, smoking, as well as other limiting conditions such as hypersensitivity, other medications, health problems, special diet regimes, previous participation in other studies in the last 80 days, etc. During the conduction of the study, and immediately before dosing, vital signs determination was performed to all participants. All measurements intended to eliminate subject (sequence) and sequence effects in this study were performed, and therefore, we consider that though there is a subject (sequence) and sequence effect, this does not impact the outcome of the study.

On the other hand, the study's protocol established details on how to control the variables like: positioning, timing, degree of physical activity, composition of food, beverages, temperature of water administered during dosing, psychological status of subjects during two periods in turn effecting the bowel transit and drug absorption. The conduction of the study as observed in the study's report and study's master file, proved that the subjects tested were healthy and their vital signs were recorded in each period. The volunteers were confined at site for an adequate time period (10 hrs before dosing and 24 hrs after dosing) and were maintained seated upright for the first two hours after dosing. No exercises were allowed during the confinement periods. Dosing and sample withdrawals were performed the same way for the two periods as scheduled in the study's protocol and actual times for all events were documented accordingly. Food and beverages consumption was the same as observed in meals' records. Therefore, we are confident the subject (sequence) and sequence effects appear to be a random occurrence and had no effect on the study outcome.

Based on non-parametric methods for t_{max} using Wilcoxon signed-rank tests, there were no differences observed between T and R after the statistical analysis was applied.

- ***Safety data***

Adverse events

There were no adverse events that occurred in the study.

Clinical laboratory evaluation

Medical history and the clinical laboratory tests (haematology, biochemistry, serology and urinalysis) were all performed for each subject on screening examination. Laboratory tests of (haematology and biochemistry) for follow up examination were performed within 24 hours of collecting the last sample in period II.

Each subject received a thorough physical assessment, orthostatic hypotension test, vital signs evaluation and ECG on screening examination. Drugs of abuse test and alcohol screening test were performed for each subject on admission to the study. The subjects received the same physical assessment as well as vital signs evaluation and ECG at the follow up examination, which were performed within 24 hours of collecting the last sample in period II.

Study GFC-BEMD-01-NXP/23 (NXPGUAN/23/BQ-11): MD fasting BE study of guanfacine 7 mg prolonged-release tablets vs equal dose of Intuniv® in healthy males

Objectives

Primary objective: The aim of the study was to demonstrate the steady state bioequivalence between the guanfacine TEST prolonged release tablets (7 mg strength) and a corresponding dose of REFERENCE prolonged-release tablets (one 3mg tablet and one 4 mg tablet administered simultaneously in order to match the strength of the TEST formulation). During the cross-over investigational period, the TEST and REFERENCE products were administered under fasting conditions for 7 consecutive days each. The bioequivalence assessment was based on plasma drug levels of guanfacine.

Secondary objective: the safety and tolerability of the formulations were assessed.

GCP aspects

The study was conducted in agreement with the Helsinki Declaration (1964 and following amendments), ICH-GCP (1996), EEC rules concerning human experimentation (No. 91/507/EEC) and Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 following the laws, regulation and administrative provisions of the Member States relating to the implement of good clinical practice in the conduct of clinical trials on medicinal products for human use.

Methods

- **Study design**

This was an open label, two periods, two sequences, crossover, randomized, multiple dose bioequivalence study on healthy male volunteers at steady state and under fasting conditions.

Study sites of GFC-BEMD-01-NXP/23: Clinical and bioanalytical parts of the study were performed in a CRO in Romania inspected by several EU and other competent authorities.

CRO's Protocol identification (code or number): GFC-BEMD-01-NXP/23

Sponsor study code: NXPGUAN/23/BQ-11

Development phase of study: Bioequivalence study

First volunteer Informed Consent Form signature: 01 February 2024

First volunteer enrolled: 05 February 2024

Last volunteer completed: 13 May 2024

The study began on 31 January 2024. The first subject was dosed on 06 February 2024 and the last subject provided the final PK blood sample on 08 April 2024.

This multiple dose study was a comparative bioavailability study performed with the purpose to demonstrate bioequivalence between the TEST formulation (Guanfacine 7 mg prolonged-release tablets) and a corresponding dose of REFERENCE medicinal product (Intuniv® 3 mg + Intuniv® 4 mg, prolonged-release tablets - one tablet of each, administered simultaneously), each IMP being administered under fasting conditions for 7 consecutive days.

In order to prevent the occurrence of an adverse event during the study as much as possible, the following measures were taken:

1. For safety reasons, a dose titration approach was used. The starting dose for up-titration was of 2 mg guanfacine daily as single dose for four (4) consecutive days, followed by 3 mg guanfacine daily as single dose for four (4) consecutive days, 4 mg guanfacine daily as single dose for four (4) consecutive days, 5 mg guanfacine daily as single dose for four (4) consecutive days and, respectively, 6 mg guanfacine daily as single dose for an additional four (4) consecutive days. Starting with study day 21, daily doses of 7 mg guanfacine were administered for 14 consecutive days (Steady State BE assessment performed at this dose level).

Also, after completion of the cross-over investigational period, tapering down was performed by administering authorized auxiliary medicinal products at the same five dose levels used during the up-titration but now in decreasing order (6 mg, 5 mg, 4 mg, 3 mg and 2 mg, for three (3) consecutive days each) as a measure to prevent the occurrence of withdrawal symptoms.

2. Drop-out/withdrawn subjects also completed the tapering-down process from the last dosage received down to 2 mg.

3. In the study only adult male healthy volunteers were enrolled having a minimal weight of 58.5 kg (to ensure that the maximum recommended maintenance dose of 0.12 mg/kg/day was not exceeded in any study participant) and no history of hypersensitivity reaction to guanfacine or any excipients contained in the formulations.

4. Inclusion/exclusion criteria for volunteers have been properly defined in order to make sure that participation in the study was only allowed for subjects that based on current health status and medical history did not pertain to a high-risk population subcategory relating to guanfacine use; discontinuation criteria have been properly defined in order to address specific issues that might arose with the use of guanfacine;

5. In the up-titration and tapering down days, the breakfast administered to the volunteers at 2 hours post dose was low-fat.

6. Body temperature and vital signs measurements, ECG determinations and well-being checks were followed periodically for all subjects and along the entire period of confinement.

7. The Investigator / Responsible Physician checked on each volunteer's health status prior to his discharge from the clinic (physical examination, ECG and body temperature and vital signs measurement). Additionally, during the period when subjects have self-administered the tapering-down medication in ambulatory conditions (Days 39 to 49), they were contacted by the investigational team in order to undergo telephonic visits for interview regarding their well-being state after each dose reduction (evenings of Days 41, 44, 47).

8. The Investigator / Responsible Physician checked on each subject's well-being prior to his discharge from the clinic. If necessary, some subjects could remain at the clinic under medical observation for longer time.

9. The clinical center was equipped with emergency equipment and medication.

Subjects were confined from the evening of Day -1 until Day 38 (after administration of the first 5 mg tapering down dose and conduct of the pre-discharge physical examination and safety checks (body temperature, vital signs measurement including orthostatic blood pressure and ECG) conducted at approximately 5 hours post-dose). Based on the results, it was decided for each subject if he can be discharged or should remain at the clinic, under medical supervision, for an additional period of time (at the discretion of the Investigator).

On Days 39 to 49, the subjects discharged by the Investigator were to self-administer the tapering-down medication in ambulatory conditions. Throughout this period, subjects were contacted by the investigational team (e.g., undergo telephonic visits) for interviews regarding their well-being state after each dose reduction (evenings of Days 41, 44, 47).

In the morning before dosing on each dosing day (Days 1 to 38), vital signs (SAP, DAP, HR) as well as body temperature were measured prior to morning dosing and the subjects were interviewed with respect to their well-being and eventual other medication taken.

A summary of all study procedures performed during screening, experimental period and follow-up visit is presented below:

Table 45: Study flow chart

PROCEDURE	SCREENING[1]	STUDY DAYS					FOLLOW UP
		DAYS -1 to 20 (Admission and titration)	DAYS 21-27 (Period I)	DAYS 28-34 (Period II)	DAYS 35-38 (Tapering-down at clinic)	DAYS 39-49 (Ambulatory tapering-down)	
Inclusion/exclusion criteria	X						
Discontinuation criteria		X[2]					
Demography	X						
Medical history	X						
Physical examination	X				X[3]		X
Vital signs	X	X[4]	X[4]	X[4]	X[3, 4]		X
ECG	X	X[4]	X[4]	X[4]	X[3, 4]		X
Clinical chemistry	X						X
Hematology	X						X
Urinalysis	X						X
Hepatitis B, C and HIV tests	X						
COVID19 test	X	X[2]					
Confinement at clinic		X	X	X	X		
Phone contact visits						X	
Drug abuse test	X	X[2]					
Alcohol test	X	X[2]					
Product administration T or R			X	X			
Auxiliary products administration (up-titration)		X					
Auxiliary products administration (tapering-down)					X	X	
Blood collection for drug concentrations measurement		X[5]	X	X	X[6]		
Concomitant treatments recording	X	X	X	X	X	X	X
Adverse Events recording	X	X	X	X	X	X	X

[1] Screening procedures may start only after volunteer's informed consent is obtained
[2] To be performed at check-in (Day -1)
[3] To be performed on Day 38, before discharge
[4] At scheduled intervals and whenever considered necessary for safety reasons
[5] One sample only, pre-dose on Day 1
[6] One sample only, pre-dose on Day 35

Standardized meals served during Days 1 to 20 and Days 35 to 38* (up-titration and tapering down days).

The food served for breakfast, lunch, snack and dinner during each day of Period I was exactly replicated during the corresponding day of Period II in order to ensure standardized study conditions with respect to meals during the build-up and PK profiling days.

Treatments

For safety reasons, a dose titration approach (with intuniv® authorized auxiliary medicinal products) was used before administering the 7 mg guanfacine IMPs. The total duration of the titration period was 20 days (intuniv® being administered in single daily doses of 2 mg, 3 mg, 4 mg, 5 mg and 6 mg for 4 consecutive days each, in ascending dose order).

The study medication, 7 consecutive once daily doses of TEST formulation, Guanfacine 7 mg prolonged-release tablets, taken in the morning (T) or 7 consecutive treatment days with REFERENCE formulation, intuniv® 3 mg prolonged-release tablets and intuniv 4 mg prolonged-release tablets, taken at once, in the morning (R) was administered in Days 21 to 27 for Period I and Days 28 to 34 for Period II, under fasting conditions.

After the last dosing day of Period II (last dose of 7 mg guanfacine IMP), as a measure to prevent/ attenuate withdrawal symptoms, tapering down was performed. The total duration of the tapering down period was 15 days (intuniv® being administered in single daily doses of mg, 4 mg, 3 mg and 2 mg for 3 consecutive days each, in descending dose order).

Treatments administered

Days 1-20

The authorized auxiliary medicinal product intuniv® 2 mg prolonged-release tablets were administered under fasting conditions, in four (4) mornings, between 6:00 to 10:00 a.m. (one prolonged release tablet daily) (Days 1 to 4), or intuniv® 3 mg prolonged-release tablets were Authorized auxiliary medicinal products for up-titration and tapering-down (A1, A2, A3, A4) prolonged release tablet daily) (Days 5 to 8), or intuniv® 4 mg prolonged-release tablets were administered under fasting conditions, in four (4) mornings, between 6:00 to 10:00 a.m. (one prolonged release tablet daily) (Days 9 to 12), or intuniv® 1 mg prolonged-release tablets (one tablet) and intuniv® 4 mg prolonged-release tablets (one tablet) were co-administered under fasting conditions, in four (4) mornings, between 6:00 to 10:00 a.m. (two prolonged release tablets daily) (Days 13 to 16), or two intuniv® 3 mg prolonged-release tablets were coadministered under fasting conditions, in four (4) mornings, between 6:00 to 10:00 a.m. (two prolonged release tablets daily) (Days 17 to 20), under the direct observation of the Clinical Investigator/ Responsible Physician. On each treatment day the subjects received the treatment at the same time (+10 min) as in the first steady-state build-up day (Day 21).

The product was administered orally with 240 ml of still bottled water. The volunteers kept a sitting position during the treatment intake in Days 1 to 8, then they kept a standing position in Days 9 to 20.

During Days 1 to 8, the subjects stayed in bed in half supine position in the 4 hours following each administration (in this interval they avoided lying completely down unless medically necessary). After that, they could resume normal non-strenuous activity such as standing or walking but when standing-up they had to do this very slowly after waiting for 30 to 60 seconds in a sitting position at the bed margin. All subjects received a low-fat breakfast, 2 hours after dosing (served at bedside in Days 1 to 8 only).

Days 21-27 (Period I) and days 28-34 (Period II):

Each subject received in a random order (according to the randomization table), the following treatment in Period I and Period II: either seven (7) consecutive treatment days with TEST formulation (Guanfacine 7 mg prolonged-release tablets, 1 tablet in the morning, in fasting conditions) OR seven (7) consecutive treatment days with REFERENCE formulation (one intuniv® 3 mg prolonged-release tablet + one intuniv® 4 mg prolonged-release tablet daily (swallowed at once, to reach the target daily dose of 7 mg guanfacine)). After the end of Period I, a direct switch was performed to the cross-over IMP on Day 28. IMPs were administered between 6:00 to 10:00 a.m., under the direct observation of the Clinical Investigator/ Responsible Physician. On each treatment day the subjects received the treatment at the same time (+10 min) as in the first steady-state build-up day (Day 21). The product was administered orally with 240 ml of still bottled water. The volunteers kept a standing position during the treatment intake and thereafter remain in a semi-reclined position for 4 hours post-dose. All subjects received a low-fat breakfast, 2 hours after dosing (on Days 21-26 and Days 28-34) or 4 hours after dosing (Day 27 and Day 34).

Days 35-37:

The authorized auxiliary medicinal products intuniv® 3 mg prolonged-release tablets (two tablets) were co-administered under fasting conditions (after at least 10 hours of fasting), in three (3) consecutive mornings, between 6:00 to 10:00 a.m. (two prolonged release tablets daily, swallowed at

once, to reach the target daily dose of 6 mg guanfacine), under the direct observation of the Clinical Investigator/ Responsible Physician. On each treatment day the subjects received the treatment at the same time (+10 min) as in the first steady-state build-up day (Day 21).

The products were administered orally with 240 ml of still bottled water. The volunteers kept a standing position during the treatment intake. All subjects received a low-fat breakfast, 2 hours after dosing.

Day 38:

The authorized auxiliary medicinal products intuniv® 1 mg prolonged-release tablets (one tablet) and intuniv® 4 mg prolonged-release tablets (one tablet) were co-administered under fasting conditions (after at least 10 hours of fasting), between 6:00 to 10:00 a.m. (two prolonged release tablets, swallowed at once, to reach the target daily dose of 5 mg guanfacine), under the direct observation of the Clinical Investigator/ Responsible Physician. The subjects received the treatment at the same time (+10 min) as in the first steady-state build-up day (Day 21). The products were administered orally with 240 ml of still bottled water. The volunteers kept a standing position during the treatment intake. All subjects received a low-fat breakfast, 2 hours after dosing.

On Day 38, at approximately 5 hours after administration of the first 5 mg tapering down dose, the pre-discharge physical examination and safety checks were conducted (body temperature, vital signs measurement including orthostatic blood pressure and ECG). Based on the results, it was decided for each subject if he could be discharged or should remain at the clinic, under medical supervision, for an additional period of time (at the discretion of the Investigator) (no such case occurred).

Days 39 to 49 (study days in which dosing was performed ambulatory):

During the two study periods (Days 21 to 34), all volunteers had free access to water until 1.0 hour before each study drug administrations and they were not allowed to drink water (or any other liquids) until 1.0 hour after each study drug administration (with the exception of 240 mL of still bottled water for study drug intake). After that, the subjects received water ad libitum.

Auxiliary medicinal products

A1.	
Name of the product:	**intuniv® 1 mg prolonged-release tablets**
Pharmaceutical form:	Prolonged-release tablets
Strength:	1 mg
Active ingredient:	Guanfacine hydrochloride equivalent to 1 mg of guanfacine
Mode of administration:	Oral, administered as follows: - 1 tablet administered concomitantly with one tablet of A4, daily, for 4 consecutive days (Days 13-16) for titration at 5 mg dose level; - 1 tablet administered concomitantly with one tablet of A4, daily, for 3 consecutive days (Days 38-40) for tapering down at 5 mg dose level;
Manufacturer:	Shire Pharmaceuticals Ireland Limited
Country of manufacturer:	Ireland, EU
Marketplace of purchase:	Spain, EU

A2.

Name of the product:	**intuniv® 2 mg prolonged-release tablets**
Pharmaceutical form:	Prolonged-release tablets
Strength:	2 mg
Active ingredient:	Guanfacine hydrochloride equivalent to 2 mg of guanfacine
Mode of administration:	Oral, administered as follows: - 1 tablet for 4 consecutive days (Days 1-4) for titration at 2 mg dose level; - 1 tablet for 3 consecutive days (Days 47-49) for tapering down at 2 mg dose level;
Manufacturer:	Takeda Pharmaceuticals International AG Ireland Branch
Country of manufacturer:	Ireland, EU
Marketplace of purchase:	Germany, EU

A3.

Name of the product:	**intuniv® 3 mg prolonged-release tablets**
Pharmaceutical form:	Prolonged-release tablets
Strength:	3 mg
Active ingredient:	Guanfacine hydrochloride equivalent to 3 mg of guanfacine
Mode of administration:	Oral, administered as follows: - 1 tablet for 4 consecutive days (Days 5-8) for titration at 3 mg dose level; - 2 tablets administered concomitantly, daily, for 4 consecutive days (Days 17-20) for titration at 6 mg dose level; - 2 tablets administered concomitantly, daily, for 3 consecutive days (Days 35-37) for tapering down at 6 mg dose level; - 1 tablet for 3 consecutive days (Days 44-46) for tapering down at 3 mg dose level;
Manufacturer:	Takeda Pharmaceuticals International AG Ireland Branch
Country of manufacturer:	Ireland, EU
Marketplace of purchase:	Austria and Germany, EU

A4.

Name of the product:	**intuniv® 4 mg prolonged-release tablets**
Pharmaceutical form:	Prolonged-release tablets
Strength:	4 mg
Active ingredient:	Guanfacine hydrochloride equivalent to 4 mg of guanfacine
Mode of administration:	Oral, administered as follows: - 1 tablet for 4 consecutive days (Days 9-12) for titration at 4 mg dose level; - 1 tablet administered concomitantly with one tablet of A1, daily, for 4 consecutive days (Days 13-16) for titration at 5 mg dose level; - 1 tablet administered concomitantly with one tablet of A1, daily, for 3 consecutive days (Days 38-40) for tapering down at 5 mg dose level; - 1 tablet for 3 consecutive days (Days 41-43) for tapering down at 4 mg dose level;
Manufacturer:	Shire Pharmaceuticals Ireland Limited
Country of manufacturer:	Ireland, EU
Marketplace of purchase:	Netherland, EU

Sampling schedule and sample handling

Blood samples for the quantification of guanfacine (5 mL per samples, 46 samples in total) were drawn at the following times:

• Pre-dose on Day 1 (collected within 2 hours before dosing);

• Pre-dose on Day 21 (collected within 5 minutes before dosing);

• Pre-dose on Days 25-27 (collected within 5 minutes before dosing);

• Post-dose on Day 27 at: 0.5 (30min.); 1.0; 2.0; 3.0; 3.5 (3h 30min.); 4.0; 4.5 (4h 30min.); 5.0; 5.5 (5h 30min.); 6.0; 6.5 (6h 30min.); 7.0; 8.0; 9.0; 10.0; 12.0; 16.0; 20.0 and 24.0* hours after administration of the Day 27 morning dose. *Collected within 5 minutes before dosing on Day 28 (detected value to be used also as CD28-T0);

• Pre-dose on Days 32-34 (collected within 5 minutes before dosing);

• Post-dose on Day 34 at: 0.5 (30min.); 1.0; 2.0; 3.0; 3.5 (3h 30min.); 4.0; 4.5 (4h 30min.); 5.0; 5.5 (5h 30min.); 6.0; 6.5 (6h 30min.); 7.0; 8.0; 9.0; 10.0; 12.0; 16.0; 20.0 and 24.0* hours after administration of the Day 34 morning dose. *Collected within 5 minutes before dosing on Day 35 (detected value to be used also as CD35-T0).

Total amount of blood withdrawn for each subject for PK determinations (46 samples of 5 mL), cannula washing during vein catheter usage (40 samples x 0.5 mL) was approximately 250 mL per study.

Unless otherwise specified in the detailed study-specific »Instructions for PK samples collection, processing and storage at the clinical centre« issued by the analytical laboratory before the start of the experimental period, the following standard procedures were to be used:

- the 5 mL blood samples will be collected in blood sampling tubes containing anticoagulant;
- each blood sample will be centrifuged for 10 minutes at 4°C nominal and 1500 (±5) g;
- after centrifugation, the plasma will be separated and put in labelled, duplicate test tubes (two aliquots of plasma obtained from each PK blood sample collected, approximately 1.0 mL plasma transferred as aliquot 1 and the remaining plasma transferred as aliquot 2);
- the plasma test tubes will be securely closed and racked;
- within 60 minutes from collection (within the tolerance windows specified in Appendix 4) the plasma aliquots will be frozen for storage at -20 °C nominal ([-10°C] to [-30°C]);
- the two aliquots of each sample will be stored in separate freezers;
- along the entire period of samples storage at the clinical centre, the temperature will be monitored and recorded with temperature loggers;
- the transport of the analytical samples (plasma samples) from the clinical centre to the analytical laboratory will be performed in thermo-isolated boxes containing dry ice to keep plasma samples frozen; the temperature during the transport will be monitored by a temperature logger (electronic device); at first only one set of plasma tubes will be sent to the analytical laboratory (the second set can be either sent with a new shipment to the analytical lab, or still be kept at the clinical centre until required).

The samples were stored at -20°C or cooler at the analytical laboratory until submitted to analysis. The two aliquots of each sample were stored in separate freezers. The temperature during the storage period in the analytical laboratory will be monitored by temperature logger.

Randomization and blinding

The dosage regimen was randomized; the randomization of the study sequences (TR and RT) was generated by SAS, by blocks of subjects.

This is an open-label study, but the randomization table was to be available only to the clinical staff. In contrast, the analytical laboratory personnel analyzed the PK samples blindly (the analyst was kept blind in respect of the treatment: TEST or REFERENCE).

The randomization list was transferred electronically to the Sponsor in view of arranging the correct labeling and packing of the study medication (IMPs and authorized auxiliary medicinal products), according to the randomization list.

Prior and concomitant therapy

During the experimental period, due to safety, analytical and pharmacokinetic considerations (analytical interference or drug-drug pharmacokinetic interactions), no concomitant medication is permitted.

However, it may happen that a subject self-medicate or that the Clinical Investigator decide to administer concomitant medication in case of an adverse event that requires treatment. In such cases the Investigator will decide if the subject is to be withdrawn or if he is allowed to continue the study; the latter case must be registered as a deviation from protocol. At discharge, each subject will be informed that he should contact the investigational team for medical advice in case concomitant treatment is needed while taking guanfacine.

All the concurrent medication administered during the study will be thoroughly registered in source documents (specific section of AE descriptive charts if occurring during the confinement period or Subject Diary if occurring during the ambulatory tapering down period) and reported in the CRF and Clinical Study Report.

Protocol amendments and deviations

No change in the conduct of the study or in the planned analysis was implemented during the present study.

The study was performed in compliance with the clinical protocol, GCPs and GLPs guidelines, the applicable regulations and internal SOPs and study-specific instructions for handling PK samples.

Some protocol deviations were registered in the present study:

Two subjects did not eat the entire meal (one for breackfast and one for snack) and one subject received his lunch and dinner modified due to an AE (diarrhoea). There was no impact on PK results.

Table 46: Deviations regarding concomitant medication

Concomitant medication	Reason
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Tooth pain
Smecta – 2 sachets of 3 g powder for oral solution diosmectite orally administered	Diarrhoea
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered and Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Tooth pain
Buscopan – 1 dragee of 10 mg butylscopolammonii bromidum orally administered	Abdominal pain
Buscopan – 1 dragee of 10 mg butylscopolammonii bromidum orally administered	Abdominal colic
Buscopan – 1 dragee of 10 mg butylscopolammonii bromidum orally administered	Abdominal pain
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache
Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Neuralgia (dental)
Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Tooth pain
4 LAX - 1 suppository containing 2100 mg glycerinum each, rectally administered	Constipation
4 LAX – 1 suppository containing 2100 mg glycerinum each, rectally administered	Constipation
Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Tooth pain
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache
Nurofen - 3 dragees of 200 mg ibuprofenum orally administered	Tooth pain
4 LAX - 1 suppository containing 2100 mg glycerinum rectally administered	Constipation
4 LAX - 1 suppository containing 2100 mg glycerinum rectally administered	Constipation
Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Tooth pain
Nurofen - 1 dragee of 200 mg ibuprofenum orally administered	Tooth pain
Paracetamol – 1 tablet of 500 mg paracetamolum orally administered	Headache

Deviations regarding study schedules:

One subject was lost to follow-up, due to personal reasons: has left the country for a job.

The Clinical Investigator considered that these deviations did not have any effect on the results of the study and therefore the subjects were allowed to continue the study.

- **Test and reference products**

Product Characteristics	TEST Product	REFERENCE Products	
Name	Guanfacine 7 mg prolonged-release tablets	**R1.** intuniv® 3 mg prolonged-release tablets	**R2.** intuniv® 4 mg prolonged-release tablets
Strength	7 mg	3 mg	4 mg
Dosage form	Prolonged-release tablets	Prolonged-release tablets	Prolonged-release tablets
Manufacturer	Neuraxpharm Pharmaceuticals, S.L.	Shire Pharmaceuticals Ireland Limited	Shire Pharmaceuticals Ireland Limited
Batch number	0002	AQ0992AL4	AP1289AL4
Batch size (Bio batch)	110 000 tablets	N.A.	N.A.
Measured content(s) (% of label claim)	98.9%	98.9%	100.2%
Commercial batch size	N.A.	N.A.	N.A.
Retest date (TEST)/ Expiry date (REFERENCE)	05/2024	06/2025	01/2025
Member State where the reference product is purchased from	N.A.	Spain, EU	Spain, EU
This product was used in the following trials	GFC-BEMD-01-NXP/23		

- **Population(s) studied**

For this study 42 subjects were to be enrolled and the sample size accounted for a potential dropout rate of up to 15%. No additional subjects were to be enrolled (withdrawals and drop-outs after the first study drug administration will not be replaced).

Inclusion criteria (applicable at screening)

1. Healthy Caucasian male volunteers of at least 18 years of age but not older than 55 years.

2. Subject with the Body Mass Index within 20.0 and 29.0 kg/m2 and body weight not lower than

58.5 kg.

3. Normal haematology, clinical chemistry and urinalysis (some deviations outside the normal limits may be acceptable if judged so by the Investigator).

4. Absence of renal and hepatic impairment.

5. Absence of cardiovascular diseases.

6. Non-smokers or ex-smokers that gave up smoking for at least two years prior to the study.

7. The subject agrees to abstain from alcohol and from beverages or food

containing methylxanthines (coffee, tea, cola, energy drinks, chocolate etc.) and chewing-gum for 48 hours prior to study Day 1 and until the end of the tapering down period (Day 49).

8. The subject has not consumed and agrees to continue to abstain from St John's Wort, vitamins and herbal remedies for two weeks prior to study Day 1 and until the end of the tapering down period (Day 49).

9. The subject has not consumed and agrees to continue to abstain from beverages or food containing orange, grapefruit or pomelo for two weeks prior to study Day 1 and until the end of the tapering down period (Day 49).

10. The subject agrees to abstain from driving or using machines from discharge (Day 38) and until 48 hours after the last guanfacine administration on Day 49.

11. The subject does not wish to procreate in the near future and agrees to use condoms during heterosexual intercourse from discharge (Day 38) and until 1 month after the last guanfacine administration on Day 49 (8 condoms will be provided at discharge and the stock can be replenished whenever necessary).

12. Ability to understand the full nature and purpose of the study, including possible risks and side effects; ability to cooperate with the Clinical Investigator and to comply with the requirements of the entire study.

13. Informed written consent given voluntarily before the initiation of the study screening.

14. No clinically significant abnormal findings at the physical examination.

15. Negative result to the COVID19 test.

16. Based on the opinion of the Clinical Investigator, subject is medically fit for participation in the study.

<u>Exclusion Criteria (applicable at screening)</u>

1. History of hypersensitivity to guanfacine or to the inactive ingredients.

2. Subjects with rare hereditary problems of galactose intolerance, total lactase deficiency, or glucose-galactose malabsorption.

3. Subject intends to be hospitalized within 3 months after last study drug administration.

4. Participation in a clinical study with an investigational product in the preceding three months or in a clinical study with a generic product in the preceding two months.

5. Hospitalization for any reason within eight weeks prior to the study initiation.

6. Donation of 450 ml or more of blood, within eight weeks prior to the study initiation.

7. Intake of any prescription or non-prescription drugs during the two weeks prior to the first dosing.

8. Depot injection or an implant of any drug within 3 months prior to the first dosing.

9. History or presence of any relevant medical condition including cancer, significant disease of the renal, hepatic, gastrointestinal, respiratory, cardiovascular, endocrine or locomotor systems, and any metabolic, hematological (see also exclusion criteria 10 to 11) or neurological disorder.

10. History or actual presence of coagulation disorders such as thromboembolic diseases (e.g. thrombophlebitis, pulmonary embolism or coagulation factors deficiency), cerebrovascular diseases, angina pectoris, myocardial infarction or coronary arterial disease, porphyria.

11. History of hemophilia (A or B or C).

12. ECG evidence of any clinically significant abnormality.

13. History of heart block, bradycardia, QT-prolongation, arrhythmia.

14. Family history of sudden cardiac death /unexplained death.

15. Subject with heart rate outside the normal range of 50-100 beats per minute or a body temperature outside the normal range of 35.5-37.4 °C or a respiratory rate outside the normal range of 14-20 breaths per minute or blood pressure less than 90/50 mmHg or more than 140/90 mmHg measured in supine position after 5 minutes of rest at the screening examination

16. Subject with history or current findings suggestive of postural hypotension (i.e., symptoms of dizziness, visual 'black out' or syncope and/or a drop in systolic blood pressure by ≥ 20 mm of Hg and/or-diastolic blood pressure by ≥ 10 mm of Hg on assuming the upright standing position from the supine lying down posture).

17. History of syncope or exercise-related cardiac events (including pre syncope or clinically significant bradycardia).

18. History or risk of dehydration (subject not accustomed to drink plenty of fluids, as necessary to ensure proper hydration).

19. Any recent history (within the last two years) of drug or alcohol abuse, psychiatric disorder or use of psychotropic medicines (within the last six months).

20. History of suicidal attempt, suicidal ideation or behavior.

21. History or current tendency towards aggressive behavior or hostility.

22. Family history of obesity or personal history of difficulty in maintaining weight below the overweight threshold (29.9 kg/m2).

23. History of gastrointestinal bleeding or perforation; history of partial or total gastrectomy.

24. Subjects with history of achlorhydria or who have had surgery that bypasses or excludes the duodenum.

25. History of recurrent gastritis, peptic or duodenal ulcer.

26. History of significant gallbladder/ biliary tract disease, liver tumors or any liver function disorders.

27. Chronic inflammatory bowel disease (Crohn's disease, ulcerative colitis).

28. Significant bilateral renal artery stenosis or renal artery stenosis in a single functioning kidney.

29. Renal failure with or without haemodialysis.

30. Active virus infection during previous 4 weeks.

31. Immunization during previous 2 weeks.

32. History or any current condition or other disease known to interfere with the absorption, distribution, metabolism or excretion of investigational medicines.

33. Presence of any acute or chronic infectious disease.

34. Positive results to the HIV, hepatitis C or hepatitis B tests.

35. Positive results to the breath alcohol test at screening.

36. Positive results to the drug abuse checks (urine test for: amphetamines, phencyclidine, cannabinoids, opiates, benzodiazepines, barbiturates and cocaine) at screening.

37. Subject is vegetarian or follows particular diets.

Removal of subjects from study or assessment

Subjects were informed that they were free to withdraw from the study at any time. The participation of a subject in the study might had been discontinued for any of the following reasons:

- subject's own wish; (as was the case of 4 subjects)

- significant non-compliance with study protocol and procedures; (no such cases)

- inter-current illness which interfered with the progress of the study; (no such cases)

- intolerable adverse events, including laboratory findings or results from vital signs measurements or other safety determinations considered abnormal and clinically significant (where in the opinion of the Investigator these would interfere with the subject's safety); (no such cases)

- emesis or diarrhea within 10 hours after dosing on Day 27 or Day 34 (twice median Tmax); potential cases of emesis or diarrhea experienced within the build-up periods was to be judged on a case-by-case basis and may lead to subject withdrawal if a high likelihood of interference with steady state achievement was suspected; (no such cases)

- Investigator's decision that the withdrawal from further participation would have been in the best interest of the subject; (no such cases)

- development of discontinuation criteria; (such as the case of one subject)

- the subject left the clinical facility during the confinement period (no such cases).

Subject disposition

Out of the 52 volunteers screened, a total number of 42 were enrolled, 42 were dosed with authorized auxiliary medication during up-titration period but only 37 received the study medication (TEST or REFERENCE). 37 subjects completed the study and 41 subjects underwent the follow-up examination.

The study started on 31 January 2024 and the last follow-up was on 29 April 2024

Five subjects were considered drop-out in the present study:

• One subject was a drop-out due to personal reason (family related).

• One subject was a drop-out due to personal reason.

• One subject was a drop-out due to intake of concomitant medication for adverse event (at Investigator's decision).

• One subject was a drop-out due to personal reason.

• One subject was a drop-out due to personal reason (family related).

Data sets analyzed

For the bioequivalence assessment, guanfacine pharmacokinetic data coming from 37 subjects who received the TEST and REFERENCE treatment were statistically analysed. Five subjects were drop-outs therefore they were not included in any statistical evaluation of the pharmacokinetic parameters. The

descriptive statistic of demographic characteristics for the 42 subjects that were enrolled in the study has been performed. Screening and follow-up safety data were analyzed on 41 complete datasets.

- **Analytical methods**

Study description: An open label, two-period, two-sequences, two-way crossover, block randomized, multiple dose bioequivalence study of guanfacine 7 mg prolonged-release tablets (test formulation) vs. equal dose of intuniv prolonged release tablets (reference formulation) in healthy volunteers with administration under fasting conditions.

Sponsor of the study:

Neuraxpharm Arzneimittel GmbH

Germany

Clinical and bioanalytical center:

Romania

The analytical part of the study lasted from 17.04.2024 to 30.04.2024, the clinical part was from 06.02.2024 to 08.04.2024. longest theoretical storage was 84 days; study samples were obtained stored at a nominal temperature of -20ºC.

1707 samples from 42 subjects (46 time-points per subject, 2 periods, 37 subjects completed) were analysed, the theoretical amount of samples is 1932.

- Pre-dose on Day 1 (collected within 2 hours before dosing);
- Pre-dose on Day 21 (collected within 5 minutes before dosing);
- Pre-dose on Days 25-27 (collected within 5 minutes before dosing);
- Post-dose on Day 27 at: 0.5 (30min.); 1.0; 2.0; 3.0; 3.5 (3h 30min.); 4.0; 4.5 (4h 30min.); 5.0; 5.5 (5h 30min.); 6.0; 6.5 (6h 30min.); 7.0; 8.0; 9.0; 10.0; 12.0; 16.0; 20.0 and 24.0* hours after administration of the Day 27 morning dose. *Collected within 5 minutes **before dosing on Day 28 (detected value to be used also as $C_{D28\text{-}T0}$)**;
- Pre-dose on Days 32-34 (collected within 5 minutes before dosing);
- Post-dose on Day 34 at: 0.5 (30min.); 1.0; 2.0; 3.0; 3.5 (3h 30min.); 4.0; 4.5 (4h 30min.); 5.0; 5.5 (5h 30min.); 6.0; 6.5 (6h 30min.); 7.0; 8.0; 9.0; 10.0; 12.0; 16.0; 20.0 and 24.0* hours after administration of the Day 34 morning dose. *Collected within 5 minutes before dosing on Day 35 (detected value to be used also as $C_{D35\text{-}T0}$).

Analyses have been divided in 37 main analytical sequences containing the samples of study subjects (sequence number coinciding with the subject study number); within each sequence QC sets (a multiple of 2 for each sequence) have been analyzed after each group of 14 or 16 injections of study samples.

Three supplementary analytical sequences (sequence numbered 43, 44 and 45) have been used for drop-out subjects (15, 17. 23, 41 and 42) samples assay, for samples re-assay and for systematic samples re-assay, as follows:

Sequence number	Sequence content
43	Drop-out subjects (1 sample of P1, each) Re-assay (13 samples)
44	ISR (76 samples)
45	ISR (72 samples) Re-assay (4 samples)

Analytical methods

The analyte was guanfacine.

Internal standard was $^{13}C,^{15}N_3$-guanfacine; samples were extracted from a 0.200 mL aliquot of K_2EDTA human plasma by centrifugation, drying and reconstitution in final solvent. The extracted samples were injected into a liquid chromatograph.

The detection method used was tandem mass spectrometry detector.

Quantitation is determined by peak area ratio method. A weighted ($1/c^2$) linear regression is performed to determine the concentration of the analytes.

The validated calibration range for the assay of guanfacine is from 100.000 pg/mL to 50000.000 pg/mL.

Validation of the analytical methods

Results obtained from this validation were presented. Analytical methods were validated according to the applicable European Guidelines.

Observations and comments

Seventeen analytical samples (representing ~1.00% from the total study samples) have been reassayed. All reassays are in accordance with the presented SOP and the relevant guideline.

In order to test the accuracy of incurred samples, two samples for each subject and study period (one representing the maximum concentrations and one representing the elimination phase) have been selected for systematic incurred samples re-assay (ISR): in total 148 samples that comply with the ISR laboratory SOP and regulatory requirements (the number of samples needed for testing reproducibility will be at least 10% of the first 1000 samples of the study and at least 5% of the samples number above the first 1000 samples).

The accuracy results of incurred samples re-assay are adequate [148 out of 148 re-assayed incurred samples (100%) lie within 20% of differences from the mean] and provide sufficient confidence that the study samples concentrations obtained are accurate.

All chromatograms for all performed runs were provided.

- **Pharmacokinetic variables**

Primary PK metrics:

AUC(0-tau).ss: AUC during a dosing interval (24-h), at steady state. Integrated, by the trapezoidal rule, from blood concentrations determined between the pre-dose sample to the last sample collected during the dosing interval.

Cmax.ss: Peak drug concentration within the 24-h dosing interval, at steady state. Obtained directly from the data, without interpolation.

Ctau.ss: Last drug concentration in the dosing interval at steady state, obtained directly from the data, without interpolation.

Additional PK metrics:

Tmax.ss: Time of the maximum measured plasma concentration during the 24-h dosing interval, at steady state.

PTF: Peak Trough Fluctuations over the dosing interval.

PTF% = 100* $C_{max.ss} - C_{min.ss} / C_{av.ss}$, where:

$C_{min.ss}$ is the minimum plasma concentration in the dosing interval at steady state, obtained directly from the data, without interpolation..

Cav.ss is the average plasma concentration during the 24-h dosing interval, calculated as: Cav.ss = AUC(0-tau).ss/24

Actual sampling times were used in the calculation of pharmacokinetic metrics for blood sampling from the target times.

All concentration values below the lower limit of quantification were presented as "BLQ" and treated as zero concentration for the pharmacokinetic and statistical calculations.

All calculations were performed using SAS (Ver. 9.4) software.

Verification of steady state

Whether the steady-state has been achieved is assessed by comparing at least three pre-dose concentrations for each formulation.

Pre-dose concentrations CD25-T0, CD26-T0 and CD27-T0 ± CD28-T0 (pre-dose for a subsequent hypothetical dose of the formulation) was to be used for assessment of steady state in Period I.

Pre-dose concentrations CD32-T0, CD33-T0 and CD34-T0 ± CD35-T0 (pre-dose for a subsequent hypothetical dose of the formulation) was to be used for assessment of steady state in Period II.

Subject's pre-dose concentrations was to be presented as descriptive statistics, spaghetti plots and plots of the geometric mean.

- **Statistical methods**

All statistical calculations were performed using SAS version 9.4 software.

Descriptive statistics was done for all pharmacokinetic parameters (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, coefficient of variation, median, range and number of measures).

Sample size

The total number of healthy volunteers enrolled in the study will be 42. The number of 42 subjects has been estimated considering the following data provided by the Sponsor:

a) the significance level (alpha) of 0.05

b) an a priori test power of 80%

c) an intra-individual coefficient of variation for the primary PK metrics of up to 29%;

d) the expected geometric mean ratio TEST/REFERENCE for the primary PK metrics of 1.05;

e) the bioequivalence acceptance range of 80.00 – 125.00% for the 90% CIs of the geometric mean ratio TEST/REFERENCE for the primary PK metrics;

f) a potential drop-out/ withdrawal rate of up to 15% due to the long confinement period.

A study identification number (two digits) together with 4 initials will be assigned to each subject. The identification numbers will be randomly attributed at the time of enrolment in the study.

Data sets to be analysed

Pharmacokinetic data coming from all evaluable subjects will be included in the primary Per Protocol Population and considered for bioequivalence assessment. Subjects that are non-evaluable are:

- Drop out/withdrawn subjects who do not provide evaluable data for both REFERENCE and TEST. (as was the case of 5 subjects)

- Subject who experienced an adverse event and took (or not) concomitant medication, if the experienced adverse event itself and/or the concomitant medication taken, interferes in a relevant manner with his/her pharmacokinetic results (e.g. emesis or diarrhoea within 10 hours after dosing on Day 27 or Day 34 (twice median Tmax of REFERENCE). Other exclusions from pharmacokinetic and statistical analysis were made following case-by-case evaluation. (no such case)

- Subjects missing three consecutive samplings. (no such cases)

- Subjects with non-existing or aberrant concentration profiles might be excluded from the statistical analysis (applicable only if the incidence of this outlier behavior is observed with a comparable frequency in both TEST and REFERENCE products (e.g. the number of cases is not numerically higher in the TEST product). (no such cases)

Although not included in the statistical bioequivalence assessment, the pharmacokinetic metrics of non-evaluable subjects as per the criteria mentioned above were to be calculated if enough data is available and will be presented separately.

For the second criterion, decision on the non-inclusion of the subject in the pharmacokinetic analysis was to be taken between the CRO and the Sponsor prior to initiating the bio-analysis and had to be properly documented.

Samples from withdrawn/drop-out subjects were to be assayed but not to be included in the statistical analyses for bioequivalence determination and were presented separately. If enough data available, the pharmacokinetic metrics were to be calculated for withdrawal/drop-out subjects and presented separately.

Evaluation of period, sequence, subject within sequence and formulation influence on pharmacokinetic metrics considered for bioequivalence assessment

Analysis of variance (ANOVA) model was performed for ln-transformed data on the main pharmacokinetic metrics (AUC(0-tau).ss, Cmax.ss, Ctau.ss) using sequence, period, subject within sequence and treatment as fixed effects. The level of significance was set at 0.1 for the sequence effect and 0.05 for all remaining effects.

$Y = \mu + Sequence + Subject\ (Sequence) + Formulation + Period$

In case of any significant effect by period or sequence, the magnitude of the effect will be calculated in term of the ratio of both levels, using geometric means.

Bioequivalence assessment

The following PK metrics were evaluated as primary and are to be considered for bioequivalence assessment:

- AUC(0-tau).ss

- Cmax.ss

- Ctau.ss

Within-subject variability (%) for these metrics were to be calculated.

For the primary metrics considered for bioequivalence assessment (AUC(0-tau).ss, Cmax.ss, Ctau.ss) a 90% confidence interval for the geometric mean ratio (TEST/REFERENCE) was calculated. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level.

The metrics were analysed using ANOVA, after the data have been transformed (ln transformation). A confidence interval for the difference between formulations on the ln-transformed scale is obtained from the ANOVA model. This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio on the original scale.

Criteria for bioequivalence: For AUC(0-tau).ss, Cmax.ss, Ctau.ss, the 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80.00-125.00%.

In addition, descriptive statistic was done (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, coefficient of variation, median, range and number of measures).

Additional metrics and statistical analyses, other than those described in this section, may be performed if deemed appropriate.

Additional metrics

For Tmax.ss comparison, non-parametric tests was applied (Wilcoxon Signed-Rank Test) to untransformed data. Descriptive statistic was done (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, coefficient of variation, median, range and number of measures).

For the remaining additional PK metric (PTF), a descriptive statistic by treatment was performed (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, coefficient of variation, median, range and number of measures).

Statistical analysis on safety data

The safety population will include all subjects who received at least one of the investigational products under study.

Results

The results of drug concentration measurements for guanfacine for the study population (N= 37) are presented as follows:

Table 47: Pharmacokinetic parameters for guanfacine (non-transformed values)

Pharmacokinetic parameter	Test		Reference	
	arithmetic mean	SD	arithmetic mean	SD
$C_{max,ss}$ (pg/ml)	8775.166	2376.953	9203.891	2717.315

AUC0-τ (pg.h/ml)	146903.709	45025.764	155273.202	48429.260
Cτ,ss (pg/ml)	4452.482	1633.338	4762.470	1765.516
Tmax,ss * (h)	7.00	2.00-10.00	7.00	3.00-10.00
Cavg (pg/ml)	N/A	N/A	N/A	N/A
Cmin,ss (pg/ml)	N/A	N/A	N/A	N/A
% fluctuation	80.516	21.969	80.317	25.344

$C_{max,ss}$ Maximum plasma concentration at steady state.

AUC0-τ AUC during a dosage interval at steady state, as calculated by the linear trapezoidal method.

Cτ,ss Concentration at the end of the dosing interval at steady state.

Tmax,ss Time until $C_{max,ss}$ is reached (*median and range).

Cavg Average concentration during a dosing interval (AUC0-τ / τ).

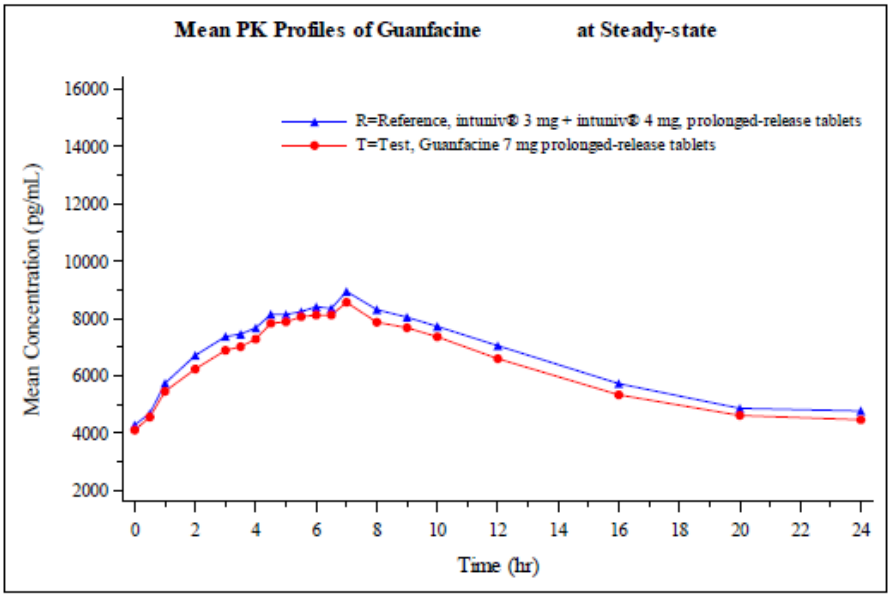
Cmin,ss : Minimum plasma concentration at steady state.

% fluctuation [(C_{max} -C_{min})/Cavg] %.

The 90% confidence intervals of the ratio T/R for $AUC_{0\text{-}tau.ss}$, $C_{max.ss}$ and $C_{tau.ss}$ were within the accepted bioequivalence range of 80.00-125.00%.

Table 48: Statistical analysis for guanfacine (ln-transformed values)

Pharmacokinetic parameter	**Geometric Mean Ratio Test/Reference %**	**Confidence Intervals 90%**	**CV%***
$C_{max,ss}$	96.18	89.81-103.01	17.593
$C_{τ,ss}$	93.79	87.28-100.79	18.455
AUC0-τ	95.06	88.56-102.04	18.168
* estimated from the Residual Mean Squares			



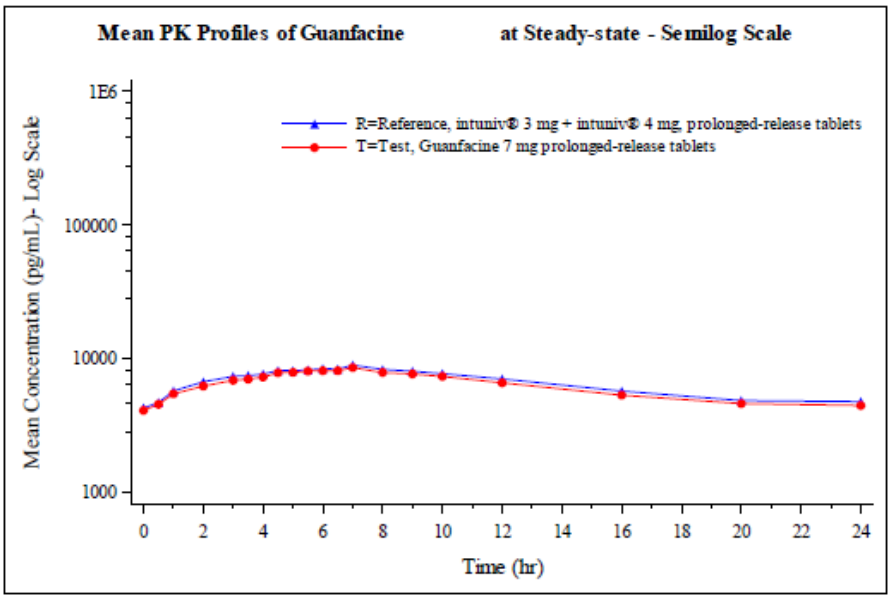


Figure 25: Mean PK profiles of guanfacine

The results obtained from the ANOVA comparisons of the main Guanfacine pharmacokinetic data considered for bioequivalence assessment (N=37, primary analysis) were as follows:

Source of error	Significance level	Probability (p)		
		$AUC_{0\text{-}tau.ss}$	$C_{max.ss}$	$C_{tau.ss}$
SEQUENCE	0.10000	0.50089	0.56385	0.39817
SUBJECT(SEQUENCE)	0.05000	0.00000*	0.00001*	0.00000*
PERIOD	0.05000	0.02378*	0.00554*	0.02849*
TREATMENT	0.05000	0.23480	0.34461	0.14118

The ANOVA model implemented by GLM procedure in SAS for checking the sequence and treatment effect, showed no statistically significant influence on the primary pharmacokinetic parameters.

Statistically significant period effect was identified for all primary parameters. Statistically significant subject within sequence fixed effects were identified for all primary parameters. It is noteworthy to mention that subject effects are frequently observed due to interindividual variations, without affecting the validity of the study.

A significant period effect may potentially arise from differences between the periods, in the physiological status of subjects and/or changes in the environmental conditions. Specifically, a significant period effect could possibly reflect different positioning, timing and degree of physical activity, timing and composition of food/beverages ingested, or the temperature of the water administered in the two periods. However, during this study, the conditions were maintained similar for both periods; therefore, it seems none of these are applicable in this study. Moreover, the plasma samples of each subject of both periods were analysed all together and in a sequence in which the plasma samples were collected excluding any chance of analytical error.

As a result, the statistically significant period effect should not affect the bioequivalence conclusions.

Tmax.ss values were compared separately for TEST vs. REFERENCE (N=37) using a nonparametric test (Wilcoxon Signed-Rank Test). The statistical test used presented no statistically significant difference between the formulations for the Tmax.ss parameter.

For TEST and REFERENCE treatments steady state for guanfacine was concluded as achieved for all 37 subjects after a descriptive comparison of the last three pre-dose concentrations in Period I (pre-dose concentrations for doses of Days 28, 26 and 27 (CD25-T0, CD26-T0 and CD27-T0) and for doses of Days 32, 33 and 34 (CD32-T0, CD33-T0 and CD34-T0).

- ***Safety data***

Adverse events

Thirty-four (34) adverse events of mild and moderate intensity occurred in twenty (20) subjects in the present study. These were not serious adverse events. All the subjects recovered before the end of the study.

From the total number of subjects having experienced Adverse Events (10 subjects) after TEST and REFERENCE study drug administration, 30% experienced adverse events (AEs) after treatment with TEST (3 subjects) and 80% experienced adverse events (AEs) after treatment with REFERENCE (8 subjects). The statistical test applied (single sample proportion test) did not put in evidence any statistically significant difference between the REFERENCE and TEST treatments, for the incidence of subjects having experienced AEs.

From the total number of adverse events that occurred in the present study after TEST and REFERENCE study drug administration (12 AEs), 25% of adverse events occurred after the administration of TEST (3 AEs) and 75% of adverse events occurred after the administration of REFERENCE (9 AEs). The statistical test applied (single sample proportion test) did not put in evidence any statistically significant difference between the REFERENCE and the TEST treatments, for the incidence of AEs.

No serious adverse events occurred in the present study. No deaths occurred in the present study.

Table 49: Display of adverse events by SOC (System Organ Classes) and according to MedDRA version 26.0

Study treatment: REFERENCE **N = 37**

Adverse Events	Mild		Moderate		Severe		Total		Total
	Related	NR	Related*	NR#	Related	NR	Related	NR	R (Related + NR (Non-related)
SOC: Nervous system disorders									
Headache *MedDRA code:* 10019211			2 (5.4%)				**2 (5.4%)**		**2 (5.4%)**
SOC: Gastrointestinal disorders									
Diarrhoea *MedDRA code:* 10012735			2 (5.4%)				**2 (5.4%)**		**2 (5.4%)**
Abdominal pain *MedDRA code:* 10000081	1 (2.7%)		1 (2.7%)				**2 (5.4%)**		**2 (5.4%)**
Abdominal colic *MedDRA code:* 10000055			1 (2.7%)				**1 (2.7%)**		**1 (2.7%)**
Constipation *MedDRA code:* 10010774			1 (2.7%)				**1 (2.7%)**		**1 (2.7%)**
Tooth pain *MedDRA code:* 10059723				1 (2.7%)				**1 (2.7%)**	**1 (2.7%)**

*Related = plausible relationship with drug administration; #NR = non plausible relationship with drug administration
** Subject identification number.

Study treatment: TEST **N = 37**

Adverse Events	Mild		Moderate		Severe		Total		Total
	Related	NR	Related*	NR#	Related	NR	Related	NR	R (Related + NR (Non-related)
SOC: Nervous system disorders									
Headache *MedDRA code:* 10019211			1 (2.7%)				**1 (2.7%)**		**1 (2.7%)**

Adverse Events	Mild		Moderate		Severe		Total		Total
	Related	NR	Related*	NR#	Related	NR	Related	NR	R (Related) + NR (Non-related)
SOC: Gastrointestinal disorders									
Constipation *MedDRA code:* 10010774			1 (2.7%)				**1 (2.7%)**		**1 (2.7%)**
Tooth pain *MedDRA code:* 10059723				1 (2.7%)				**1 (2.7%)**	**1 (2.7%)**

*Related = plausible relationship with drug administration; #NR = non plausible relationship with drug administration
** Subject identification number.

Study treatment: Auxiliary treatment (up-titration period)

Adverse Events	Mild		Moderate		Severe		Total		Total
	Related	NR	Related*	NR#	Related	NR	Related	NR	R (Related) + NR (Non-related)
SOC: Nervous system disorders									
Headache *MedDRA code:* 10019211	1		2				**3**		**3**
Dizziness *MedDRA code:* 10013573	1						**1**		**1**
Neuralgia (dental) *MedDRA code:* 10029223				1				**1**	**1**
Psychomotor agitation *MedDRA code:* 10056436	1						**1**		**1**
Tremor of hands *MedDRA code:* 10044577			1				**1**		**1**
SOC: Ear and labyrinth disorder									
Vertigo *MedDRA code:* 10047340			1				**1**		**1**
SOC: Gastrointestinal disorders									
Diarrhoea *MedDRA code:* 10012735	1		1				**2**		**2**

Adverse Events	Mild		Moderate		Severe		Total		Total
	Related	NR	Related*	NR#	Related	NR	Related	NR	R (Related) + NR (Non-related)
Abdominal pain *MedDRA code:* 10000081			1				1		1
Abdominal cramps *MedDRA code:* 10000057			1				1		1
Tooth pain *MedDRA code:* 10059723				6				6	6
Dental abscess (vestibular) *MedDRA code:* 10012314				1				1	1
Constipation *MedDRA code:* 10010774			1				1		1

*Related = plausible relationship with drug administration; #NR = non plausible relationship with drug administration

Clinical laboratory evaluation

Before entering the trial, subjects were checked for the haematological and biochemical parameters (red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), white blood cells (WBC), platelets (PLT), leukocyte formula (neutrophils, eosinophils, basophils, lymphocytes, monocytes), uric acid (UAC), urea, glucose (GLU), creatinine (CRE), total bilirubin (TBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), total cholesterol (TCOL), triglycerides (TRIG); for urine a complete examination has been done (including pH).

The same laboratory parameters were tested again at the end of the study (follow-up examination).

Table 50: Vital Signs

N = 42		*SAP (mmHg)*	*DAP (mmHg)*	*HR (beats/min.)*	*Body Temp. (°C)*	*Resp. Rate (breaths/min)*
Screening supine position	MEAN	129.38	81.60	72.83	36.48	15.67
	SD	9.76	5.93	7.31	0.14	0.57
upright position	MEAN	130.40	82.62			
	SD	9.29	6.79			
Follow-up supine position	MEAN	127.76	77.95	77.80	36.43	15.88
	SD	5.83	7.38	9.12	0.16	0.68
upright position	MEAN	132.73	80.80			
	SD	4.03	5.88			

ECG

12 leads ECGs were performed at screening and at the end of the study (follow-up) and also at about 5 hours after each first administration of a new dose level or formulation conducted at the clinic (5h post-dose on Day 1, Day 5, Day 9, Day 13, Day 17, Day 21, Day 28, Day 35 and Day 38). An ECG was also performed before discharge (at about 5h post-dose on Day 38).

2.4.3.2. Pharmacokinetic conclusion

Please refer to "Conclusions on clinical aspects".

2.4.3.3. Pharmacodynamics

No new pharmacodynamic studies were presented and no such studies are required for this application.

2.4.4. Clinical safety

2.4.4.1. Post marketing experience

No post-marketing data are available. The medicinal product has not been marketed in any country.

2.4.5. Discussion on clinical aspects

To support the application, the applicant had initially submitted 8 bioequivalence studies but the clinical program did not align with guideline recommendations as no multiple-dose study for the higher strength product group was submitted and a major objection was raised. Hence, an additional study was submitted upon request with the responses to the Day 120 LoQ.

The overall study design of submitted clinical bioequivalence trials, (comparative randomised, single dose / multiple dose, two-way crossover), primary PK variables and statistical methods are in line with recommendations stated in the guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **). For 8 studies the wash-out period of at least 10 days is more than 5 elimination half-lives (18 hours according to the SmPC of Intuniv) and thus, considered sufficient to avoid carry over effects. For study GFC-BEMD-01-NXP/23 (NXPGUAN/23/BQ-11), there was no formal wash-out without treatment between period I and period II. However, as the build-up period during the investigational cross-over period lasted 6 days (i.e. 8 half-lives; elimination half-life of guanfacine approx. 18 hours) and thus, was sufficiently long, this is acceptable. The Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **) outlines that at least 5 times the terminal half-life is required in such instances. Further, the PK of guanfacine appears to be dose proportional between 1 and up to the maximal recommended daily dose of 7 mg in the target population and is considered similar between children (aged 6 to 12) and adolescent (aged 13 to 17) ADHD patients, and healthy adult volunteers. The same pertains to the overlap of the wash-out from the up-titration dosing with Intuniv and the build-up phase upon dosing with either the test or reference (i.e. Intuniv) formulation in period I. Of note, study GUA-0722-87 was conducted to repeat bioequivalence evaluation for the guanfacine 4 mg prolonged-release tablets under fasted conditions, after a preceding study (GUA-T1221/118) did not demonstrate bioequivalence for the 4 mg strength under fasted conditions. The applicant attributed the result to small sample size and the observed intrasubject variability of roughly 30%. To address whether the 4 mg guanfacine formulation can be considered a highly variable drug product (HVDP), a full replicate cross-over (four period, two sequence) design was applied in the repeated study. This study design is in principle acceptable and aligns with current recommendations in above mentioned guidance on the investigation of bioequivalence pertaining to study design for potential HVDP.

Lower strengths 1 mg and 2 mg

Concerning the 1 mg strength (single dose under fasted conditions) (study GUA-T1221/117), the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}) were contained within the pre-specified BE margin of 80%-125%. The 90% CIs were 97.33%-120.35% for AUC_{0-t}, 97.08%-122.10% for $AUC_{0-\infty}$, and 94.12%-106.91% for C_{max}, indicating bioequivalence between reference and test product. However, the term subject was included as random effect in the ANOVA model, while the Guideline on the Investigation of Bioequivalence recommends that fixed effects should be applied but since there is information on both periods for each subject both analyses are equivalent and no concern is raised.

Regarding the 2 mg strength (single dose under fasted conditions) (study GUA-1122-133), the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}) were contained within the pre-specified BE margin of 80 %-125 %. The 90% CIs were 94.09%-107.83% for AUC_{0-t}, 93.30%-107.26% for $AUC_{0-\infty}$, and 101.51%-115.52% for C_{max}, which is indicative for bioequivalence between reference and test product. A statistically significant subject (sequence) effect was observed for all primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ and, additionally, a period and drug effect for C_{max}, and a sequence effect for AUC_{0-t} and $AUC_{0-\infty}$.

For the 2 mg strength (single dose under fed conditions) (study GUA-1022-120), the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}) were contained within the pre-specified BE margin of 80%-125 %. The 90% CIs were 97.02%-113.79% for AUC_{0-t}, 96.11%-113.38% for $AUC_{0-\infty}$, and 91.32%-103.57% for C_{max}, indicating bioequivalence between reference and test product. A statistically significant subject (sequence) effect was observed for all primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ and, in addition, a period effect for C_{max}.

With regard to the 2 mg strength (multiple dose under fasted conditions) (study GUA-0123-6), the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables ($AUC_{0-\tau}$, $C_{\tau,ss}$, and $C_{max,ss}$) were contained within the pre-specified BE margin of 80 %-125 %. The 90% CIs were 97.37%-114.48% for $AUC_{0-\tau}$, 96.52%-124.16% for $C_{\tau,ss}$, and 100.35%-116.18% for $C_{max,ss}$, indicating bioequivalence between reference and test product. A statistically significant subject (sequence) effect was observed from the ANOVA for the logarithmically transformed primary PK parameters $C_{max,ss}$, $C_{\tau,ss}$ and $AUC_{0-\tau}$ for guanfacine.

Higher strengths 3 mg, 4 mg and 7 mg

Regarding the 3 mg strength (single dose, under fasted conditions) (study GUA-1122-134), the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were contained within the pre-specified BE margin of 80%-125%. The 90% CIs were 105.34%-123.37% for C_{max}, 103.15%-122.66% for AUC0-t and 103.25%-123.30% for $AUC_{0-\infty}$. These results indicate an increased maximum concentration, and also an increased exposure upon administration of the 3 mg test product under fasted conditions. However, since the 90% CIs of the geometric mean ratio for all primary PK variables were entirely contained within the BE acceptance criteria, no concern is raised. Notably, there was a statistically significant drug and subject(sequence) effect observed in the ANOVA model for all primary PK parameters. A significant drug effect *per se* does not raise concerns on BE conclusions, as long as the BE acceptance range is contained, which was the case in this study.

Bioequivalence for the 4 mg test product single dose, under fed conditions (study GUA-1122-135) was formally demonstrated. The 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were contained within the pre-specified BE acceptance range of 80%-125%. It is though noted that the lower bound of the 90% CI of the AUC variables is at the edge of the BE acceptance criteria (80.09%-100.78% AUC0-t and 80.52%-

101.73% $AUC_{0-\infty}$) and thus, suggesting a decreased exposure upon administration of 4 mg test product under fed conditions, but within the pre-defined BE acceptance range.

Regarding the 4 mg strength when evaluated as single dose, under fasted conditions, the first conducted fasted study (GUA-T1221/118) did not formally demonstrate bioequivalence, as the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were not contained within the pre-specified BE margin of 80%-125%. The point estimates of the geometric mean ratio were around 115% for all PK variables, suggesting an increased maximum concentration as well as an increased exposure upon administration of the 4 mg test product under fasted conditions. Thus, the applicant's explanation for non-demonstration of bioequivalence solely by the small sample size and insufficient power may or may not be the true underlying reason.

Noteworthy, study GUA-T1221/118 is referred to as pilot study in modul 3. However, this is neither stated in the protocol, nor the clinical study report, or the clinical overview. The repeated study evaluating the 4 mg strength under fasted condition (GUA-0722-87) demonstrated bioequivalence with different design (replicate design) and different batch of the test product than in study GUA-T1221/118. In addition, while in this failed study GUA-T1221/118, an increased maximal concentration and exposure was observed upon test product administration, the statistical analysis of PK variables in the repeated study GUA-0722-87 clearly indicates comparable maximal concentration and drug exposure between 4 mg test and reference product under fasted conditions, both from the perspective of point estimates, as well as from more narrow confidence intervals. Of note, under fed conditions, a trend towards decreased exposure upon 4 mg test administration was observed in study GUA-1122-135, which employed the same test and reference product batches as study GUA-0722-87. Hence, the totality of the data from the two fasted studies is considered relevant for the overall bioequivalence assessment pertaining to the 4 mg strength in the fasted condition. Consequently, a combined analysis of both studies (GUA-T1221/118 and GUA-0722-87) was requested to support a potential conclusion on bioequivalence concerning the 4 mg strength due to reasons outlined above. The applicant provided a pooled analysis with data from both studies. The analysis however included a study*treatment interaction, which was only requested as an additional analysis to assess potential differences in the effect between studies. The requested main model was a model that does not include a study*treatment interaction and the *EMA Clinical pharmacology and pharmacokinetics: questions and answers (EMA/618604/2008 Rev. 13), Question 11* explicitly discourages using a study*treatment interaction, noting difficulties in the interpretation of the output of such a model. Upon further request the applicant provided estimates from the model without a study*treatment interaction. In addition, as the first trial was intended to provide confirmatory evidence for PK equivalence (as stated by the applicant, see below), but failed to do so, the two trials investigating the 4 mg strength in the fasting state resulted in inconsistent conclusions. Hence, and to further assess the sufficiency of the level of evidence available, a tipping point analysis was performed for this pooled analysis providing the largest possible (nominal) coverage probability of the confidence interval that would still entirely lie within the bioequivalence acceptance range. In this tipping point analysis the confidence intervals up to a confidence level of 99% were entirely contained in the equivalence margins.

As noted above, study GUA-T1221/118 was not planned as a pilot study, but was in fact planned and used as a pivotal study. The applicant argued that the study was underpowered to detect the observed effect. The intrasubject coefficient of variation (ISCV) observed in the later study GUA-0722-87 was larger than the ISCV assumed when planning of study GUA-T1221/118 and in line with the ISCV observed in the earlier study. Further, the applicant ascribed also the observed difference between the failed (over-exposure with the test product) and the successful BE study (comparable exposure between test and reference product) to the smaller sample size - thereby insufficient power - of study GUA-T1221/118 (n= 18). While this is a post-hoc power argumentation and the trial was planned to be

pivotal, considering the larger sample size of the successful study GUA-0722-87 (n = 38), the inconsistent conclusions between the studies can however indeed be seen in the context of sample size. Therefore, study GUA-T1221/118 can indeed be seen as not sufficiently powered based on the ISCV estimate from data external to study GUA-T1221/118. Nevertheless, since the study was otherwise suitable - and was in fact used - to conclude on bioequivalence, the data generated in the unsuccessful study should not be disregarded, but be included when taking into account the totality of evidence. Hence, the results of the provided pooled analysis of the two studies investigating the 4 mg strength in the fasting condition and the tipping point analysis investigating the robustness of the result of the pooled analysis are important for the assessment of robustness of the bioequivalence conclusion of this strength. In this respect the provided tipping point analysis – where the confidence intervals up to a confidence level of 99% are entirely contained in the equivalence margins – provides reassurance that the results from the second study GUA-0722-87 are in line with the results from the totality of PK data and it is therefore agreed that bioequivalence of the 4 mg strength in the fasting condition can be concluded. Regarding aspects on product quality, it can be agreed presented dissolution profiles of test product batches used in both studies indicate similarity between both batches.

With regard to the 7 mg strength (multiple dose under fasted conditions), which was provided in the Day 120 responses, the 90% CIs of the geometric mean ratio of ln-transformed values (Test/Reference) for all primary PK variables (AUC0-$_{tau.ss}$, $C_{max,ss}$ and $_{Ctau,ss}$) were contained within the pre-specified BE margin of 80%-125%. The point estimates and corresponding [90% CIs] were 96.18% [89.81%-103.01%] for $C_{max,ss}$, 93.79% [87.28%-100.79%] for $C_{tau,ss}$ and 95.06% [88.56%-102.04%] for $AUC_{0\text{-}tau.ss}$. Thus, bioequivalence for the 7 mg test product multiple doses, under fasted conditions was formally demonstrated. Notably, an additional sampling time point between 7.0 and 8.0 hours would have been desirable to estimate $C_{max,ss}$ as the observed median t_{max} was 7.0 hours upon both treatments. However, the course of the guanfacine plasma concentration curve appears comparable between treatments and peak levels are accompanied by a rather flat rise and fall. Although this poses a slight source of uncertainty, it is considered unlikely that the "sampling gap" between mentioned time points would significantly affect bioequivalence conclusions on $C_{max.ss}$. Thus, no concern is raised. Further, there was a statistically significant period effect. However, the study was sufficiently standardized although the up-titration period occurred only prior to period I of the cross-over study part and importantly, potential carry-over effects from the up-titration period are considered not applicable as the build-up period was sufficiently long (> 5 half-lives) in this study (see above). Thus, the statistically significant period effect does not raise a concern pertaining to bioequivalence conclusions.

Across the initially submitted 8 bioequivalence trials, the nature of reported adverse events (AEs) did not raise concerns, although the number of AEs appeared rather low with in total 10 AEs in 10 subjects, of which 8 occurred in the 2 mg multiple-dose study (study GUA-0123-6). It is noted that according to the applicant's information, the study site was inspected multiple times. The last two inspections were conducted by the WHO in 2022 and two EU national authorities in 2018. In addition, the AEs reporting procedure, as outlined in the protocol, seemed adequate. Regarding the requested 7 mg multiple-dose study GFC-BEMD-01-NXP/23, 20 out of 42 dosed subjects reported 34 AEs in the whole study period (up-titration and cross-over period). During the investigational cross-over period, which encompassed 37 subjects, 10 subjects experienced 12 AEs out of which 9 occurred during test treatment in 8 subjects and 3 during reference treatment in 3 subjects. The nature and frequency of AEs do not raise a safety concern *per se* and all subjects were considered recovered at the end of the study and reported AEs are predominantly linked to the pharmacological action of guanfacine (alpha2–adrenergic agonist). Although this observation may have been a chance finding due to the small sample size and/or rather low event occurrence, the imbalance in AEs (9 vs 3 AEs upon reference and test treatment, respectively) was considered apparent and further clarification was requested.

However, the trial setting does not allow a conclusive safety assessment in a meaningful way, which is also not the primary scope of a bioequivalence trial. Ultimately, as bioequivalence was demonstrated, due to the small number of subjects and reported events, observations regarding AEs between reference and test treatment are probably a chance finding.

Initially, the applicant requested a strength biowaiver for the additional higher strength 5 mg, 6 mg and 7 mg, which was deemed unacceptable and raised a major objection. Additionally, another critical issue was the absence of multiple dose data for all the higher strengths (3-7 mg) concerning thus both, the generic strengths 3 and 4 mg and the hybrid strengths 5, 6, and 7 mg; since the lower and higher strengths have a different formulation, each strength range requires its own multiple dose examination as per the Guideline on the pharmacokinetic and clinical evaluation of modified-release dosage forms (EMA/CHMP/EWP/280/96 Rev.1). Both issues were addressed by the applicant by conducting a new bioequivalence multiple-dose study (study GFC-BEMD-01-NXP/23) with the highest strength of 7 mg under fasted conditions. Notably, the study protocol was already finalized prior the start of the MAA procedure indicating that the applicant foresaw the potential need to clinically investigate the highest dose strength.

2.4.6. Conclusions on clinical aspects

Based on the presented bioequivalence studies Paxneury 1 mg, 2 mg, 3 mg, and 4 mg prolonged release tablets are considered bioequivalent with Intuniv 1 mg, 2 mg, 3 mg, and 4 mg prolonged release tablets.

The results of study GUA-T1221/118, study GUA-0722-87, and study GUA-1122-135 with 4 mg formulation and study GFC-BEMD-01-NXP/23 with the 7 mg formulation can be extrapolated to the strengths 5 mg and 6 mg, according to conditions in the Guideline on the Investigation of Bioequivalence CPMP/EWP/QWP/1401/98 Rev.1, section 4.1.6.

2.5. Risk Management Plan

2.5.1. Safety concerns

Table 51: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	QT prolongation
Missing information	• Use in pregnant or breastfeeding women. • Use in patients with hepatic or renal impairment. • Long-term safety (neurocognition in particular, but also effects on growth, sexual maturation).

2.5.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.5.3. Risk minimisation measures

Table 52: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
QT prolongation	Routine risk minimisation measures: *SmPC section 4.2.* *SmPC section 4.4.* *SmPC section 4.5.* *Legal status: prescription only medicine.* Additional risk minimisation measures: *None.*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: None.
Use in pregnant or breastfeeding women	Routine risk minimisation measures: *SmPC section 4.6.* *PL section 2.* *Legal status: prescription only medicine.* Additional risk minimisation measures: *None.*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: None.
Use in patients with hepatic or renal impairment	Routine risk minimisation measures: *SmPC section 4.2.* *Legal status: prescription only medicine.* Additional risk minimisation measures: *None.*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: None.
Long-term safety (neurocognition in particular, but also effects on growth, sexual maturation)	Routine risk minimisation measures: *SmPC section 4.2.* *Legal status: prescription only medicine.* Additional risk minimisation measures: *None.*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: None.

2.5.4. Conclusion

The CHMP and PRAC considered that the risk management plan version 0.3 is acceptable.

2.6. Pharmacovigilance

2.6.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.6.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.7. Product information

2.7.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-risk balance

This application concerns a generic version for the strengths 1 mg, 2 mg, 3 mg, and 4 mg and a hybrid version for the strengths 5 mg, 6 mg, and 7 mg of guanfacine prolonged-release tablets. The reference product Intuniv is "indicated for the treatment of attention deficit hyperactivity disorder (ADHD) in children and adolescents 6-17 years old for whom stimulants are not suitable, not tolerated or have been shown to be ineffective. Intuniv must be used as a part of a comprehensive ADHD treatment programme, typically including psychological, educational and social measures".

From a quality point of view, the application is approvable.

Regarding non-clinical aspects, no new nonclinical studies have been submitted for this application, which is adequate for a generic and hybrid application. The provided comprehensive literature-based review is considered sufficient.

From a clinical perspective, this application does not contain new data on pharmacodynamics as well as the efficacy and safety of the guanfacine, and contains new data only on pharmacokinetics in the context of bioequivalence; the applicant's clinical overview on these clinical aspects based on information from published literature is considered sufficient.

To support the application, the applicant had submitted 8 bioequivalence studies, and an additional upon request. It is noteworthy that not only the formulations of Intuniv tablets and Paxneury tablets differ but also the formulations within some of Paxneury's (and also within some of Intuniv's) strengths differ (i.e., the compositions of the strengths are not quantitatively proportional). The applicant developed a differing formulation for the strengths 1 mg and 2 mg (termed lower strengths) and the strengths 3 mg, 4 mg, 5 mg, 6 mg, and 7 mg (termed higher strengths) as with a unique formulation for all strengths, tablets of 5, 6 and 7 mg strength would be extremely big and not suitable for administration to children. However, this approach entails the requirement for a different set of

bioequivalence studies for the lower strengths and higher strengths according to the BE-guideline (CPMP/EWP/QWP/1401/98 Rev.1).

To fulfil this requirement, the applicant submitted 4 studies for the lower strengths: study GUA-T1221/117 examining 1 mg fasted single dose, study GUA-1122-133 (NXPGUAN/22/BQ-12) examining 2 mg fasted single dose, study GUA-1022-120 (NXPGUAN/22/BQ-9) examining 2 mg fed single dose and study GUA-0123-6 (NXPGUAN/23/BQ-1) examining 2 mg fasted multiple dose), which is, in principle, adequate.

Concerning the higher strengths, the applicant had initially submitted 4 studies: study GUA-1122-134 (NXPGUAN/22/BQ-11) examining 3 mg fasted single dose, study GUA-T1221/118 examining 4 mg fasted single dose, study GUA-0722-87 examining 4 mg fasted single dose, and study GUA-1122-135 examining 4 mg fed single dose. The 4 mg fasted single dose was investigated in two studies; study GUA-0722-87 was conducted to repeat bioequivalence evaluation for 4 mg single dose under fasted conditions after the preceding study GUA-T1221/118 failed to demonstrate bioequivalence. The applicant attributed this result partially to the observed intrasubject variability of roughly 30%. To address whether the 4 mg guanfacine formulation can be considered a highly variable drug product (HVDP), a full replicate cross-over (four period, two sequence) design was adopted, which is acceptable and aligns with current recommendations stated in the guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr**) pertaining to study design for potential HVDP.

Initially, the applicant requested a strength biowaiver for the additional higher strength 5 mg, 6 mg and 7 mg, which was deemed unacceptable and raised a major objection. Additionally, another critical issue was the absence of multiple dose data for all the higher strengths (3-7 mg) concerning thus both, the generic strengths 3 and 4 mg and the hybrid strengths 5, 6, and 7 mg. Both issues were addressed by the applicant by providing data of a new bioequivalence multiple-dose study (study GFC-BEMD-01-NXP/23) with the highest strength of 7 mg under fasted conditions. That study's protocol was already finalized prior to the start of the MAA procedure indicating that the applicant foresaw the potential need to clinically investigate the highest dose strength.

The bioequivalence studies form the pivotal basis with an open-label, randomized, two-period (in one instance four-period, full-replicate due to reasons outlined above) two-sequence, crossover design, and either single- or multiple oral dose due to guideline requirements as stated above. The study designs are considered adequate to evaluate the bioequivalence of the formulations and were in line with the respective European requirements. Choice of dose, sampling points, overall sampling time as well as wash-out period were adequate. The analytical method was validated. Pharmacokinetic and statistical methods applied were in general adequate.

The test formulation of guanfacine 1 mg prolonged-release tablets met the protocol-defined criteria for bioequivalence when compared with Intuniv guanfacine 1 mg prolonged-release tablets in a single dose setting under fasted conditions. The 90% confidence intervals for the parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were all contained within the protocol-defined acceptance range of 80.00% to 125.00%. Bioequivalence of the two formulations was demonstrated.

The test formulation of guanfacine 2 mg prolonged-release tablets met the protocol-defined criteria for bioequivalence when compared with Intuniv guanfacine 2 mg prolonged-release tablets in a single dose setting under fasted and fed conditions and multiple dose setting under fasted conditions. The 90% confidence intervals for the parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for single dose and $C_{max,ss}$, $C_{\tau,ss}$ and AUC0-τ for multiple dose were all contained within the protocol-defined acceptance range of 80.00% to 125.00%. Bioequivalence of the two formulations was demonstrated.

The test formulation of guanfacine 3 mg prolonged-release tablets met the protocol-defined criteria for bioequivalence when compared with Intuniv guanfacine 3 mg prolonged-release tablets in a single dose setting under fasted conditions. The 90% confidence intervals for the parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were all contained within the protocol-defined acceptance range of 80.00% to 125.00%. Bioequivalence of the two formulations was demonstrated.

The test formulation of guanfacine 4 mg prolonged-release tablets meet the protocol-defined criteria for bioequivalence when compared with Intuniv guanfacine 4 mg prolonged-release tablets in a single dose setting under fed conditions. The 90% confidence intervals for the parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were all contained (though borderline regarding AUC_{0-t} and $AUC_{0-\infty}$) within the protocol-defined acceptance range of 80.00% to 125.00%. However, regarding the 4 mg strength, it must be kept in mind that one of the two 4 mg single dose study under fasted conditions (GUA-T1221/118) did not demonstrate bioequivalence for all three primary PK variables, where an increased maximum concentration as well as an increased exposure upon administration of the 4 mg test product under fasted conditions was observed. However, in the repeated 4 mg single dose study under fasted conditions (GUA-0722-87) the test formulation of guanfacine 4 mg prolonged-release tablets met the protocol-defined criteria for bioequivalence (i.e., the 90% confidence intervals for the parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were all contained within the protocol-defined acceptance range of 80.00% to 125.00%) when compared with Intuniv guanfacine 4 mg prolonged-release tablets in a single dose setting. Of note, the PK results differed also in location between the two 4 mg fasted single dose studies, where the successful larger study was centered close to unity and had narrower 90% CIs.

A discussion on these aspects, as well as a justification on why results from study GUA-T1221/118 were not to be considered for the bioequivalence assessment of the 4 mg strength, and a pooled analysis for study GUA-T1221/118 and study GUA-0722-87 was requested initially. A pooled analysis was provided according to the requested methodology as well as a tipping point analysis for this pooled analysis.

Concerns pertaining to batch selection could be resolved. The applicant argued that the study GUA-T1221/118 was underpowered to detect the observed effect based on the small sample size and the high variability observed in both studies. Considering the larger sample size of the successful study GUA-0722-87 and that the observed intrasubject coefficient of variation (ISCV) in both studies was larger than the ISCV assumed when planning, study GUA-T1221/118 can indeed be seen as not sufficiently powered based on the ISCV estimate from data external to study GUA-T1221/118 and the inconsistent conclusions between the studies can indeed be seen in the context of sample size. Nevertheless, since the study was otherwise suited - and in fact used - to support a bioequivalence claim the data generated in the failed study should not be disregarded but be included when taking into account the totality of evidence. Hence, the results of the provided pooled analysis of the two studies investigating the 4 mg strength in the fasting condition are important for the assessment of robustness of the bioequivalence conclusion of this strength. In this respect the provided tipping point analysis provides reassurance that the results from the second study GUA-0722-87 are in line with the results from the totality of the PK data and indeed bioequivalence of the 4 mg strength in the fasting condition can be concluded.

The test formulation of guanfacine 7 mg prolonged-release tablets met the protocol-defined criteria for bioequivalence when compared with Intuniv guanfacine 3 mg + 4 mg prolonged-release tablets in a multiple dose setting under fasted and fed conditions and multiple dose setting under fasted conditions. The 90% confidence intervals for the parameters $C_{max,ss}$, $C_{\tau,ss}$ and AUC0-τ were all contained within the protocol-defined acceptance range of 80.00% to 125.00%. Bioequivalence of the two formulations was demonstrated.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Paxneury is favourable in the following indication:

Paxneury is indicated for the treatment of attention deficit hyperactivity disorder (ADHD) in children and adolescents 6-17 years old for whom stimulants are not suitable, not tolerated or have been shown to be ineffective.

Paxneury must be used as a part of a comprehensive ADHD treatment programme, typically including psychological, educational and social measures.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- ***Periodic Safety Update Reports***

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.