

INVESTIGATION OF CHIRAL ACTIVE SUBSTANCES

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INVESTIGATION OF CHIRAL ACTIVE SUBSTANCES

PREAMBLE

This note for guidance considers active substances with one or more stereogenic centres which display chirality but not constitutional isomerism or cis/trans isomerism. It should be read in conjunction with Directive 65/65/EEC as amended and Directive 75/318/EEC as amended (The Rules Governing Medicinal Products in the European Union, Vol. I). Its contents are additional to the notes for guidance on quality, safety and efficacy which, where relevant, should be read in conjunction with this note.

1 INTRODUCTION

Constituents of living organisms are predominantly constructed from chiral building blocks: e.g. L-amino acids and D-carbohydrates. Life processes therefore take place in an asymmetric environment and involve molecules of defined chirality (defined stereogenic centres). Pharmacotherapeutically active substances therefore act in an asymmetric environment and may themselves be chiral or may be brought in a chiral conformation at the receptor surface (induced fit).

In screening promising active substances stereochemically defined active substances should be studied where possible. New active substances should be checked for stereogenic centres and chirality. If a new racemate appears promising, both enantiomers should be studied separately as early as possible to assess the relevance of stereoisomerism for effects and fate "in vivo".

The decision to submit a marketing authorisation application for a single enantiomer or for a racemate is one for the applicant/manufacturer. It is therefore not the intention of this note for guidance to prescribe a selection strategy, but rather to describe and explain the requirements for studies justifying such strategy and the medicinal product resulting from it. It is the task of the authority to evaluate the balance of benefit versus risk and to make decisions accordingly.

This document, *mutatis mutandis*, applies also to non-equimolar mixtures of enantiomers.

2. DEFINITIONS AND NOMENCLATURE

To prevent confusion during the preparation and evaluation of a submission file it is imperative to use consistent terminology based upon accepted definitions and nomenclature.

2.1 Definitions

Stereoisomers: have the same constitution (same molecular formula, same atomic bonding) but differ in the spatial orientation of the atoms or groups of atoms within the molecule.

Enantiomers: are stereoisomers which are non-superimposable mirror images; outside chiral environments they possess identical physico-chemical properties, except that they rotate the plane of polarised light in opposite directions and by equal amounts.

Diastereoisomers: Stereoisomers that are not enantiomeric of each other are termed diastereoisomers.

Racemate: equimolar mixture of enantiomers.

Stereogenic centre: an atom where the interchange of two ligands or substituents creates a new stereoisomer

2.2 Nomenclature

Unambiguous nomenclature should be used throughout the dossier to describe each stereoisomer; it may be desirable to give major components unique trivial names; if there is a mixture of stereoisomers this should have a clearly designated name. For the correct nomenclature the applicant must consult the IUPAC recommendations on stereochemistry (R and S nomenclature).

3. SYNTHESIS

3.1 Data

For the exact data, which have to be supplied for the description of the manufacturing procedure, the applicant should refer to the note for guidance on *Chemistry of Active Substances*.

It is important that the step where the stereogenic centre is formed be described in detail, including the relevant reagents used, the in-process controls and other factors critical to the process, together with data validating the ability of the process to provide the desired stereochemical control. The resulting product must be sufficiently characterised as to identity, stereoisomeric purity etc.

The precautions which have been taken to maintain the desired configuration during the following stages of the synthesis must be shown.

3.2 Several cases are possible:

3.2.1 The starting material already possesses a stereogenic centre (e.g. racemate or enantiomer)

It is essential that the starting material is fully characterised: identity, stereoisomeric purity etc., including specifications and validated stereoselective test procedures.

3.2.2 Formation of a racemate

If not obvious from the synthesis (e.g. the formation of a racemate from racemic or achiral starting material under achiral conditions), evidence confirming that a racemate, or any other intended stereochemical mixture, is formed should be provided.

3.2.3 Formation of an enantiomer (stereoselective step)

Evidence, including if possible the mechanism, confirming the formation of the enantiomer, should be provided.

3.2.4 Isolation of the preferred enantiomer

The resolution step producing the enantiomer will also be considered as a part of the overall manufacturing procedure. The number of resolution cycles typically used to achieve complete separation should be indicated.

The resolution step (among others) can consist of:

- a) diastereoisomers crystallisation;
- b) kinetic resolution: chemical and enzymatic;
- c) preferential crystallisation;
- d) chromatography resolution.

3.2.5 Non-equimolar mixtures of enantiomers

In some cases the procedure may yield a product which intentionally is an intermediate between a racemate and a single enantiomer, either due to the reaction mechanism itself (partial stereoselective reaction) or due to the resolution process yielding partial resolution. It is then very important to define the exact conditions (validation of the manufacturing process), which will guarantee an active substance of reproducible range – within specifications – of stereoisomeric composition.

A defined mixture can also be prepared by mixing the pure enantiomers in stated but different proportions.

3.3 Synthesis or isolation

When the synthesis or isolation of the preferred enantiomer is not practicable (difficulties in scale-up from laboratory synthesis to manufacturing procedure) the methods attempted to achieve resolution must be adequately described and the reasons for failure should be explained.

In the case of an application for a racemate where the manufacturer claims that he has been unable to perform the required preclinical and clinical tests on each enantiomer, due to failure to resolve or prepare the separate enantiomers, the efforts made should be described and the failure discussed.

4. QUALITY ASPECTS

Active substances with one or more stereogenic centres can be produced as:

- a racemate;
- in case of one stereogenic centre: a single enantiomer;
- in case of multiple centres: a single enantiomer or a mixture of enantiomers and/or diastereoisomers;

- a non-equimolar mixture of enantiomers.

In the case where only a single enantiomer is selected, then the other enantiomer will be considered as an Impurity.

For all these cases, the note for guidance *Chemistry of Active Substances* is applicable. The test procedures must be fully described and validated.

In addition, special attention must be given to the identity and the stereoisomeric purity of the active substance. Many methods and test procedures are available to fulfil these requirements; they range from the more accessible methods such as optical rotation, melting point, liquid chromatography with a chiral stationary phase etc., to the more sophisticated methods such as optical rotary dispersion, circular dichroism, NMR using chiral shift reagents, etc.

Depending upon the intended use of the active substance and upon the specification which define the quality, it is for the applicant to choose those methods which will adequately demonstrate the stereoisomeric purity of his active substance. Validation studies should address the control limits for stereoisomeric impurities. Reference should be made to the note for guidance on *Analytical Validation* in this regard.

The monograph for a racemate should include a method suitable for showing that the active substance is a racemate.

Chemical development

The proof (elucidation) of the structure and the configuration in space of the active substances with a chiral centre should be fully established; it should be demonstrated (validated) that the methods used at release guarantee the identity and the specified stereoisomeric purity or stereoisomeric composition of the active substance.

Special attention must be given to the physico-chemical properties of the active substance (enantiomer, racemate): crystallinity, polymorphism, rate of dissolution etc. If a racemate is developed, it should be determined whether it is a true racemate (homogeneous solid phase) or a conglomerate of two enantiomers (e.g. a mixture of two crystalline enantiomers) by investigating its melting point, solubility, crystal properties etc.

Finished product

It should be demonstrated and supported by validated test procedures, that during the manufacturing process of the finished product no unacceptable change in stereochemical purity or ratio of the active substance occurs.

Stability

It should be demonstrated for both the raw material (active substance) and finished product that, within the specifications, no unacceptable change in stereochemical purity or ratio occurs during the proposed shelf-life.

Quality of reference material

The reference material necessary for the test procedures must be fully characterised. This includes mainly – among others (see notes for guidance on *Analytical Validation*) – the

identity (configuration) and stereochemical purity. For the stereochemical purity, a value as assay determination must be given.

5. PRE-CLINICAL STUDIES

5.1 Development of a single enantiomer as a new active substance

An application for marketing authorisation of a single enantiomer as a new active substance should be considered and documented in the same way as any application for a new active substance.

Studies should be carried out with the single enantiomer. However, where development studies commence with a racemate, the studies conducted up to the decision to develop the enantiomer may be taken into account to determine the necessity for further studies.

The possibility of the formation of the other enantiomer "in vivo" should be considered in relation to the chemical structure at an early stage in order to justify the need for any enantiospecific bioanalysis. If the other enantiomer is formed "in vivo" it should be evaluated with its biotransformation products in the same way as for other biotransformation products.

In the case of endogenous human chiral compounds, it is considered that data on disposition (ADME) should not necessarily be based upon enantiospecific methods.

The enantiomeric purity of the active substance used in pre-clinical studies should be defined.

5.2 Development of a racemate as a new active substance

An application for marketing authorisation of a new racemic active substance should be considered and documented in the same way as any application for a new active substance.

The choice of the racemate instead of a single enantiomer should be explained.

In practice one of the following two situations may occur:

- Rapid interconversion "in vivo"
if the interconversion rate "in vivo" is appreciably higher than the apparent distribution and elimination rates of the enantiomers, only the racemate should be studied as the active substance.
- No or slow interconversion "in vivo", allowing for separate enantiomer effects on and fate in the organism
in most situations, the following studies, in principle, will be needed for evaluation.

Pharmacodynamics

The profile of the effects related to the therapeutic use should be provided for the racemate and each enantiomer. The racemate data related to the general pharmacodynamic properties should be extended with enantiomer data if necessary from the point of view of safety.

Pharmacokinetics

The effective exposure to the enantiomers after administration of the racemate in pivotal studies should be measured by enantiospecific analytical methods allowing extrapolation to the human exposure.

Toxicology

It is ordinarily sufficient to carry out toxicity studies on the racemate. If toxicity other than that predicted from the pharmacological properties of the medicinal product occurs at relatively low multiples of the exposure planned for clinical trials, relevant toxicity studies should be repeated with the individual enantiomers when possible.

5.3 Development of a new single enantiomer from an approved racemate

In principle this concerns the development of a new active substance requiring a complete new application. The decision to develop an enantiomer should be explained.

It would be counterproductive in this case not to use data on the corresponding racemate as far as is applicable to the enantiomer. It is thereby assumed that the applicant has the contents of the full dossier on the corresponding racemate at his disposal. Suitable “bridging” studies should be carried out to link the complete racemate data to the incomplete data on the selected enantiomer. The use of racemate results should be explained. The extent of bridging studies should be defined on a case-by-case basis. Considerations are:

Pharmacodynamics

The profile of the selected enantiomer should be compared with that of the racemate. It may be appropriate in addition to study the other enantiomer to correct for interactions.

Pharmacokinetics

The profile of the selected enantiomer should be compared with that of the racemate. It may be appropriate to additionally study the other enantiomer to correct for interactions.

Toxicology

A suitable program of “bridging” studies may consist of:

- an acute toxicity study of the selected enantiomer using the racemate as positive control,
- a repeated dose study (up to 3 months) in a single most appropriate species and a study for effects on pre- and post-natal development (including maternal function) with the modification of starting treatment at conception, not at implantation, in a single most appropriate species, with the selected enantiomer and with the racemate as a positive control at least one effective dose level.

Results should be compared with corresponding previous racemate studies. If unexpected results are found, further studies on a case-by-case basis will be necessary. In the worst case, the enantiomer should be fully investigated.

5.4 Development of a new racemate from an approved single enantiomer

The choice of the racemate to be developed should be explained.

In this case a completely new application will have to be submitted in analogy with para 5.2. Useful data on the approved enantiomer may be added to support the new application.

5.5 Development of a non-racemic mixture from an approved racemate or single enantiomer

In principle a tailored (non-racemic, non equimolar) mixture of enantiomers can be viewed as an approach towards the optimisation of a pharmacotherapeutic profile.

In this case the application concerns a fixed combination of which one or both of the components may be unknown. So all necessary data on the unknown enantiomer(s) should be provided, as well as those necessary for the justification of this fixed combination, according to the note for guidance *Fixed-Combination Medicinal Products*.

6. CLINICAL STUDIES

6.1 Development of a single enantiomer as a new active substance

An application for marketing authorisation of a single enantiomer as a new active substance should be considered and documented in the same way as any application for a new active substance. All pivotal studies should be carried out with the single enantiomer.

The possibility of the formation of the other enantiomer "in vivo" should be considered in relation to the chemical structure. If the other enantiomer is formed "in vivo" it should be evaluated with its biotransformation products in the same way as for other biotransformation products.

In the case of endogenous human chiral compounds, it is considered that data on disposition (ADME) should not necessarily be based upon enantiospecific methods.

The enantiomeric purity of the active substance used in the clinical studies should be defined.

6.2 Development of a racemate as a new active substance

An application for marketing authorisation of a new racemic active substance should be considered and documented in the same way as any application for a new active substance.

The choice of a racemate Instead of a single enantiomer should be explained.

In practice one of the following two situations may occur:

- Rapid interconversion "in vivo"

If the interconversion rate "in vivo" is appreciably higher than the apparent distribution and elimination rates of the enantiomers, only the racemate should be studied as the active substance.

- No or slow inversion “in vivo”, allowing for separate enantiomer effects on and fate in the organism.

In most situations the following studies will be needed, in principle, for evaluation:

Human pharmacodynamics and tolerance: The main effect should be studied with the racemate. The results should be compared with those obtained in animal studies with racemate and enantiomers. Where deemed necessary from a point of view of safety the separate enantiomers should be studied.

Human pharmacokinetics: Studies on healthy volunteers, patients and risk groups should be carried out with enantiospecific methods, unless it is shown that there is no qualitative and quantitative difference in the fate of both enantiomers.

Pharmacotherapeutics: The clinical studies necessary to demonstrate safety and efficacy should be carried out with the racemate. It may be helpful to monitor these studies pharmacokinetically, where necessary with enantiospecific methods.

6.3 Development of a new single enantiomer from an approved racemate

In principle this concerns the development of a new active substance requiring a complete new application.

However, data on the corresponding racemate can be used as far as applicable to the enantiomer. It is assumed that the applicant can provide the full dossier of the racemate to the authorities, or an equivalent to the scientific content of the full dossier. In this case suitable “bridging” studies may help to reduce the amount of new clinical studies to be carried out with the selected enantiomer.

The choice to develop an enantiomer should be explained. The extent of bridging studies should be defined on a case-by-case basis. Considerations are:

Human pharmacodynamics and tolerance

The pharmacodynamic profile of the selected enantiomer should be compared with that of the racemate and with the results of animal studies. It may be appropriate to study the other enantiomer to correct for possible racemate interactions.

Human pharmacokinetics

The pharmacokinetic profile of the selected enantiomer should be compared with that of the racemate and with the results of animal studies. It may be appropriate to study the other enantiomer to correct for possible racemate interactions.

Pharmacotherapeutics

In principle the usual pharmacotherapeutic studies should all be carried out.

Using data from animal pharmacodynamic, pharmacokinetic and toxicity studies with racemate and the selected enantiomer, as well as from therapeutic trials with the racemate it may be possible to extrapolate to the situation with the single enantiomer. This may reduce the number of additional clinical studies needed.

The expert report should reflect this situation and provide justification for the inference and give additional arguments to support extrapolation from one indication studied with the single enantiomer to other indications.

6.4 Development of a racemate from an approved single enantiomer

The choice of the racemate to be developed should be explained.

In this case a completely new application will have to be submitted in analogy with para 6.2. Useful data on the approved enantiomer may be added to support the new application.

6.5 Development of a non-racemic mixture from an approved racemate or single enantiomer

In principle a tailored (non-racemic, non-equimolar) mixture of enantiomers can be viewed as an approach towards optimisation of the pharmacotherapeutic profile of an active enantiomer.

In this case the application concerns a fixed combination product, of which one or both of the components may be unknown. Therefore, all necessary data on the unknown enantiomer should be provided as well as those necessary for the justification of this fixed combination according to the note for guidance *Fixed-Combination Medicinal Products*.

6.6 Generic applications of chiral medicinal products

Bioequivalence studies supporting generic applications of chiral medicinal products should be based upon enantiospecific bio-analytical methods, unless:

- 1) both products contain the same, stable single enantiomer as the active substance, or
- 2) both products contain the racemate and both enantiomers show linear pharmacokinetics.

7. STATUS OF APPROVED RACEMATE

The safety and efficacy of marketed medicinal products, the active substance of which is a racemate, is generally considered to be well established. There is no intention in this note for guidance to formulate requirements for further data on such products, unless new evidence emerges indicating a relationship between one enantiomer and a safety or efficacy issue.

If new claims related to the chiral nature of the active substance are put forward, supporting studies with the separate enantiomers will be required.