22 May 2025
EMA/200492/2025
Committee for Medicinal Products for Human Use (CHMP)

# Assessment report

Maapliv

International non-proprietary name/Common name: AMINO ACIDS

Procedure No. EMEA/H/C/005557/0000

## Note

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

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# List of abbreviations

| | |
|---|---|
| AGEPS | Agence Générale des Equipements et Produits de Santé, France |
| Ala | alanine |
| Arg | arginine |
| ASMF | Active Substance Master File = Drug Master File |
| Asp | aspartic acid |
| BBB | blood brain barrier |
| BCAA | branched-chain amino acids |
| BCKDH | branched chain α-ketoacid dehydrogenase |
| CEP | Certificate of Suitability of the EP |
| CFU | Colony Forming Units |
| Cys | cysteine |
| EAAs | essential amino acids |
| EDQM | European Directorate for the Quality of Medicines |
| EU | Endotoxin units |
| GC | Gas chromatography |
| Glu | glutamic acid |
| Gly | glycine |
| GMP | Good Manufacturing Practice |
| His | histidine |
| HPLC | High performance liquid chromatography |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IR | Infrared |
| IV | intravenous |
| JP | Japanese Pharmacopoeia |
| KICA | α-keto iso caproic acid |
| LAT | large neutral AA transporter |
| LDPE | Low Density Polyethylene |
| LNAAs | large neutral amino acids |
| Lys | lysine |
| MAA | marketing authorization application |
| Met | methionine |
| MO | Major objection |
| MSUD | maple syrup urine disease |
| MSUD DE | MSUD decompensation episodes |
| NMR | Nuclear Magnetic Resonance |
| non-EAAs | non-essential amino acids |
| ODD | orphan drug designation |
| OOS | Out of Specifications |
| Ph.Eur. | European Pharmacopoeia |
| Phe | phenylalanine |
| PKU | phenylketonuria |
| Pro | proline |
| QP | Qualified Person |
| RH | Relative Humidity |
| Ser | serine |
| SmPC | Summary of Product Characteristics |

| | |
|---|---|
| TAMC | Total aerobic microbial count |
| Thr | threonine |
| TLC | Thin Layer Chromatography |
| Trp | tryptophan |
| TYMC | Total yeast mold count (TYMC) |
| Tyr | tyrosine |
| UV | Ultraviolet |

# 1. Background information on the procedure

## *1.1. Submission of the dossier*

The applicant Recordati Rare Diseases submitted on 8 September 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Maapliv, through the centralised procedure falling within the Article 3(1) and point 4 of Regulation (EC) No 726/2004.

The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 30 January 2020.

Maapliv was designated as an orphan medicinal product EU/3/18/2076 on 26/10/2018 in the following condition: Treatment of maple syrup urine disease.

The applicant applied for the following indication:

*Treatment of acute decompensation episodes with maple syrup urine disease (MSUD) who are not eligible for an oral BCAA- free formulation.* Maapliv *is indicated in adults and children aged from 0 to 18 years.*

## *1.2. Legal basis, dossier content*

**The legal basis for this application refers to:**

Article 10(a) of Directive 2001/83/EC – relating to applications relying on well-established medicinal use supported by bibliographic literature

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on bibliographic literature substituting all non-clinical tests and clinical studies.

## *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. Applicant's request(s) for consideration*

### 1.5.1. Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

### 1.5.2. New active Substance status

NA

## *1.6. Steps taken for the assessment of the product*

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Ewa Balkowiec Iskra Co-Rapporteur: Jayne Crowe

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| The application was received by the EMA on | 8 September 2023 |
| The procedure started on | 28 September 2023 |
| The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on | 15 December 2023 |
| The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on | 15 December 2023 |
| The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on | 4 January 2024 |
| The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on | 25 January 2024 |
| The applicant submitted the responses to the CHMP consolidated List of Questions on | 25 October 2024 |
| The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on | 7 January 2025 |
| The CHMP agreed on a list of outstanding issues to be sent to the applicant on | 30 January 2025 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 17 April 2025 |
| The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues | 7 May 2025 |

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| to all CHMP and PRAC members on | |
| 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 MAAPLIV on | 22 May 2025 |

# 2. Scientific discussion

## *2.1. Problem statement*

### 2.1.1. Disease or condition

The Maple syrup urine disease (MSUD) is an inborn error of amino acid metabolism caused by a deficiency of the mitochondrial enzyme branched chain α-ketoacid dehydrogenase (BCKDH) complex activity, resulting in the accumulation of the branched-chain amino acids (BCAAs), leucine, isoleucine and valine as well as their metabolites (α-ketoacids). Increased BCAAs and metabolite levels in plasma may result in severe complications.

### 2.1.2. Epidemiology

The global estimated prevalence of MSUD according to Orphanet reports is 1 to 9 per one million people, corresponding to an approximate total number of patients of between 500 and 5,000. The estimated global incidence of MSUD is 1 in 185,000 live births (0.54/100,000) (Chuang and Shih 2001).

In Europe, the incidence of MSUD is approximately 0.5/100,000 births, yielding approximately 26 new cases per year (Orphanet). MSUD prevalence is higher in some populations, such as Galicia in Spain (1/52,541) (Couce et al. 2011).

Data on the incidence of decompensation episodes is lacking. It can be assumed that patients with MSUD will experience several metabolic decompensation episodes over the course of their lives.

### 2.1.3. Biologic features, Aetiology and pathogenesis

The potentially devastating consequences in MSUD derive mainly from the neurotoxic effects of leucine and its metabolite, α-ketoisocaproic acid (KICA or 2-KICA), the normal metabolism of which is blocked (Knerr et al. 2012). If leucine and 2-KICA accumulate, profound metabolic alterations and impairment of energy homeostasis occur, which are associated with severe clinical symptoms, the most serious of which are neurological and may result in permanent damage, disability, and even death. Metabolic decompensation in MSUD may occur when leucine levels rise above 500-1000 µmol/L, as a result of dietary non-compliance, infection (by far the most common cause) or other physiological or metabolic stresses (Knerr et al. 2012, Frazier et al. 2014, Strauss et al. 2010). Episodes of metabolic decompensation are acute medical emergencies and may occur at any time or any age after trauma, surgery, illness, or inappropriate dietary intake.
Failure to lower leucine levels in a timely manner may result in permanent morbid sequelae including disability and death since acute metabolic decompensation may be fatal (Muelly et al. 2013). If not appropriately diagnosed and treated, rapid elevation of circulating leucine and its ketoacid, α-ketoisocaproate, cause encephalopathy and life-threatening brain swelling. This can lead to irreversible neurological complications, including metabolic decompensation, and death (Strauss et al. 2010). Besides overt acute toxicity, children with the disease often experience subacute consequences. Many MSUD children exhibit subtle neurologic dysfunction, such as attention-deficit disorder or learning disabilities. This is true even for children who have been identified prospectively and who appear to be well controlled, making it difficult to explain these observations. One of the sources of variations may be the degree of metabolic block of the branched-chain *α* ketoacid dehydrogenase complex (Brosnan 2005). The early signs of decompensation may be subtle, including lethargy or ataxia. Vomiting is frequent and should always be taken seriously (2023). In MSUD patients of all ages, nausea and vomiting are common during crises and often necessitate hospitalisation (Morton et al. 2002).

Once the children have passed the critical period, they need a permanent reduction in dietary intake of BCAAs to maintain plasma levels close to normal. The amount of natural protein is thus highly restricted and large amounts of protein substitutes, in form of BCAAs-free amino acid mixtures are necessary. Regular close monitoring of blood BCAAs levels is essential to titrate their intake to maintain target plasma concentrations. Discontinuation of the diet, as well as febrile illnesses resulting in an increase of catabolism and amino acid release from muscle protein can precipitate severe metabolic decompensation at any age, leading to acute encephalopathy which requires emergency treatment (Simon, Wendel, and Schadewaldt 2005).

### 2.1.4. Clinical presentation, diagnosis, and stage/prognosis

There are different forms of MSUD:

- **Classic MSUD** is the most common and severe form of the disease as it corresponds to the lowest percentage from normal of BCKDH activity (0% to 2%). Soon after birth maple syrup odour is evident in cerumen, then in urine. Infants develop severe symptoms within 48 hours of delivery, when rising blood levels of leucine and its metabolites exceed critical concentrations and cause neurotoxic effects. When MSUD is first suspected, which is usually the case after 10 days of life, plasma leucine concentrations range between 2.5 and 4-5 mmol/L (normal: <150 µmol/L). There is evidence that in patients with classic MSUD, neurodevelopmental outcomes are 1) inversely related to the duration of high plasma leucine levels during the neonatal illness and 2) inversely related to the longitudinal plasma leucine concentrations in childhood, which is an indicator of the quality and compliance of dietary treatment (Simon, Wendel, and Schadewaldt 2005). In following few days symptoms include feeding difficulties, lethargy, stereotyped movements and autonomic dysregulation, bradycardia, hypothermia, axial hypotonia with episodes of distal hypertonia that could lead to cerebral oedema, coma, and central respiratory failure (Knerr et al. 2012, Morton et al. 2002, Villani et al. 2017, Strauss et al. 2010).

- **Intermediate MSUD** is a less severe form of the disease, and the onset is variable. It typically presents between the ages of 5 months and 7 years. BCKDH activity ranges from 3% to 30% of normal. Beyond the classic MSUD symptoms patients suffer developmental delays and mental retardation (Villani et al. 2017, Strauss et al. 2010).

- **Intermittent MSUD** age onset is variable. BCKDH activity ranges from 5% to 20% of normal and patients can have normal early growth and development, but then experience episodic decompensation episodes (Villani et al. 2017, Strauss et al. 2010).

- **Thiamine-responsive MSUD** has a similar clinical course to intermediate MSUD (Villani et al. 2017).

- **Dihydrolipoamide deshydrogenase deficiency,** also known as **MSUD type III** is caused by the deficiency of the E3 subunit of branched chain alpha-ketoacid dehydrogenase (BCKDH), α-ketoglutarate dehydrogenase (αKGDH), and pyruvate dehydrogenase (PDH) (Quinonez et al. 2014). The most frequent is the severe form characterized by metabolic acidosis, encephalopathy, feeding difficulties, liver failure, and early death (Blackburn et al. 2017).

### 2.1.5. Management

The long-term daily management for any form of diagnosed MSUD includes strict dietary protein restriction coupled with provision of BCAA-free amino acid supplements and caloric support. Careful dietary management reduces the incidence of episodic decompensations, improves amino acid nutrition, and greatly reduces morbidity and mortality (Strauss et al. 2010, Strauss et al. 2020).

Although some management recommendations are available (Frazier et al. 2014), there are currently no formal European guidelines for the treatment of decompensation episodes in MSUD patients. The management of decompensation episodes is intended to promptly:

(1) inhibit protein catabolism and promote anabolism by providing high calorie intake combined with a BCAA-free amino acid mixture, delivered enterally or parenterally.

(2) reduce BCAA toxic levels to achieve and maintain plasma BCAA amino acid concentrations within the targeted treatment ranges, thus preventing cerebral oedema, metabolic imbalances, neurotransmitter deficiencies and mitochondrial alterations. Targets for treatment, at which patients in crisis might be discharged from hospital are around 400 µmol/L (Servais et al. 2013, Strauss et al. 2006). The toxic accumulation of BCAA during acute metabolic decompensation is associated with the emergence of permanent neurological symptoms. Early treatment is thus essential to prevent neurotoxicity and death (Blackburn et al. 2017), and to ensure that a balance is maintained between leucine, isoleucine and valine during decompensation by giving supplements of individual amino acids as required (British Inherited Metabolic Disease Group (2017, 2020), MSUD dietetic management, USA 2017).

The management of decompensation episodes in MSUD patients faces several issues. The most critical decompensation episodes (DEs) may require extracorporeal interventions (haemodialysis or haemofiltration) combined with nutritional support through the administration of a BCAA free mixture to remove the excess BCAAs and to allow a higher plasma clearance (Aygun et al. 2019, Jouvet et al. 2001) but with all the possible complications of the procedure itself. The main complications may include vascular stenosis and venous thrombosis (Masud et al. 2018), haemodynamic instability, or infection (Aygun et al. 2019). In patients with decreased renal clearance, repeated and prolonged blood exchange transfusions, peritoneal dialysis, and haemodialysis have proved useful in removing BCAA and branched-chain keto acids (BCKAs) (Parini et al. 1993). In comparison with peritoneal dialysis, haemofiltration and transfusion, a reasonable alternative approach is to harness the forces of anabolism and protein synthesis by providing energy and a mixture of amino acids excluding BCAA, such that these accumulating amino acids are incorporated into protein, reducing levels in extracellular fluid and reversing the toxic effects.

Despite adherence to therapy, patients may suffer unpredictable metabolic decompensation episodes at any time or at any age following trauma, surgery, illness, or inappropriate dietary intake, leading to major neurological and possibly life-threatening complications or death and for which the management becomes a medical emergency. In this case, a complex therapeutic management with several treatments and several routes of administration within a limited time for ensuring a quick reduction of toxic level of BCAAs are used. A pivotal component of the therapy is the use of BCAA-free mixtures. Current commercialised mixtures are used as food supplements for the long-term dietary maintenance and not specifically designed for the treatment of decompensation episodes.
Their administration is limited to the enteral route, either by oral feeding, nasogastric intubation, or gastrostomy. In the context of metabolic decompensations, which require urgent BCAA-free administration, the enteral route is inappropriate for patients presenting clinical symptoms such as coma, seizures, gastric intolerance, nausea, vomiting, or for patients refusing nasogastric tubing (frequent for adolescents) and it is not appropriate as a preventive treatment before planned surgery (Abi-Wardé et al. 2017, de Lonlay et al. 2021, Alili et al. 2022). The intravenous (IV) administration of a BCAA-free mixture in all these circumstances has been found to be an essential alternative, potentially avoiding invasive or aggressive interventions such

as gastrostomy, which is especially relevant for the younger, vulnerable target population (Abi-Wardé et al. 2017, Frazier et al. 2014, Servais et al. 2013, Alili et al. 2022, Sanchez-Pintos et al. 2022).
In April 2021 a guideline for the management of the disease recommending the use of parenteral BCAA-free formulations was published by French Health Authorities: The French National Protocol for the Diagnosis and Care of MSUD (2021).
No medicinal product containing a BCAA-free solution, nor a medicinal product for IV administration allowing a prompt medical intervention for the treatment of DEs in MSUD patients has been approved in Europe. In Europe, BCAA-free solutions intended for IV administration in MSUD patients have been prepared locally in hospital and pharmacies; thus these were not immediately available and the pharmaceutical quality standards and the manufacturing reproducibility in terms of intra- and inter-individual subject variability were questionable.

## *2.2. About the product*

Maapliv is a BCAA-free solution for parenteral use containing 52.75 g/L of amino acids. The solution includes:

- Six essential L-amino acids (EAAs) [(Histidine (His), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp)]
- Nine non-essential L-amino acids [Alanine (Ala), Arginine (Arg), Aspartic acid (Asp), Cysteine (Cys), Glutamic acid (Glu), Glycine (Gly), Proline (Pro), Serine (Ser), Tyrosine (Tyr)]. In addition, the BCAA-free amino acid solution also includes taurine, an amino sulfonic acid with pleiotropic roles such as in biliary conjugation, brain, cardiovascular, muscular, and retinal functions.

**Composition of MAAPLIV**

| **MAAPLIV®** | **(g/L)** |
|---|---|
| L-alanine | 6.30 |
| L-arginine | 4.10 |
| L-aspartic acid | 4.10 |
| L-cysteine chloridrate monohydrate (equivalent to L-cysteine) | 1.45 (1.0) |
| L-glutamic acid | 7.10 |
| Glycine | 2.10 |
| L-histidine | 2.10 |
| L-lysine monohydrate (equivalent to L-lysine) | 6.30 (5.60) |
| L-methionine | 1.30 |
| L-phenylalanine | 2.7 |
| L-proline | 5.60 |
| L-serine | 3.80 |
| Taurine | 0.30 |
| L-threonine | 3.60 |
| L-tryptophan | 1.40 |
| L-tyrosine | 0.50 |
| Total amino acids | 52.75 |

Since 2010, the AGEPS (*Agence Générale des Equipements et Produits de Santé, France*) has manufactured and distributed a BCAA-free solution for parenteral use containing essential L-amino acids (His, Lys, Met, Phe, Trh, Trp) and non-essential L-amino acids (Ala, Arg, Asp, Cys, Glu, Gly, Pro, Ser, Tyr) as well as taurine. The follow-up of the French cohort of patients with MSUD decompensations since 2010 has been reported in three publications, reporting on this same cohort at different times and patient ages (Servais et al. 2013, de Lonlay et al. 2021, Alili et al. 2022).

Since 2017, Recordati Rare Diseases (RRD) supported by AGEPS has developed and optimised a standardised, pharmaceutical grade consistent batch quality BCAA-free product designed for intravenous use. The intention was to a provide a pre-prepared product for emergency use in Europe. This product, Maapliv, is equivalent in relative amino acid composition and quantity to the hospital prepared BCAA-free IV formulas in use in Europe since 2010.

## *2.3. Type of Application and aspects on development*

There is no specific regulatory guidance for the development of medicinal products for the treatment of MSUD or for the specific treatment of metabolic decompensation episodes in MSUD patients.

During the procedure, the applicant accepted the consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of Regulation (EC) 726/2004.

The Applicant did not seek scientific advice.

## *2.4. Quality aspects*

### 2.4.1. Introduction

Maapliv is a solution for infusion containing 16 amino acids as active substances. The finished product is presented as a solution for infusion containing 26.375 g of amino acids in 500 ml of solution.

| **Active substance** | **For 500 mL (one bottle)** |
|---|---|
| L-alanine | 3.15 g |
| L-arginine | 2.05 g |
| L-aspartic acid | 2.05 g |
| L-cysteine hydrochloride monohydrate (equivalent to L-cysteine) | 0.725 g (0.50 g) |
| L-glutamic acid | 3.55 g |
| Glycine | 1.05 g |
| L-histidine | 1.05 g |
| L-lysine monohydrate (equivalent to L-lysine) | 3.15 g (2.80 g) |
| L-methionine | 0.65 g |
| L-phenylalanine | 1.35 g |
| L-proline | 2.80 g |
| L-serine | 1.90 g |
| Taurine | 0.15 g |
| L-threonine | 1.80 g |
| L-tryptophan | 0.70 g |
| L-tyrosine | 0.25 g |

Caloric value: 206 Kcal/L

Osmolality: 475 mOsmol/Kg
pH: 5.2

Other ingredients are: acetic acid or sodium hydroxide (for pH adjustment) and water for injection.

The product is available in 500 mL colourless type II glass bottles.

## 2.4.2. Active Substance - Glycine

### *2.4.2.1. General Information*

The active substance, Glycine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1995-018-Rev 05.

### *2.4.2.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

### *2.4.2.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 614 and the R1-CEP 1995-018-Rev 05 requirements. The following additional specifications are also applied.

**Table 1: Additional specifications of Glycine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

### *2.4.2.4. Stability*

The active substance is stored in double polyethylene bags placed in a cardboard container, the retest period for Glycine is 3 years when stored in the above packaging. No special storage conditions are required.

## 2.4.3. Active Substance - L-alanine

### *2.4.3.1. General Information*

The drug substance, L-alanine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability CEP 2018-145-Rev 01.

### *2.4.3.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

### *2.4.3.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0752 and the R0-CEP 2018-145-Rev 00 requirements. The following additional specifications are also applied.

**Table 2: Additional specifications of L-Alanine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

### *2.4.3.4. Stability*

The active substance is stored in double polyethylene bags with silica gel bag in between, placed in a fibre drum. The retest period of the active substance is 48 months if stored in the above packaging. No special storage conditions are required.

## 2.4.4. Active Substance - L-arginine

### *2.4.4.1. General Information*

The drug substance, L-arginine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1998-104-Rev 04.

#### *2.4.4.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.4.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0806 and the R1-CEP 1998-104-Rev 04 requirements. The following additional specifications are also applied.

**Table 3: Additional specifications of L-Arginine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.4.4. Stability*

The active substance is stored in a polyethylene bag in a siliconized polyethylene terephthalate bag placed in a fibre drum. The re-test period of the active substance is 36 months if stored in the above packaging. No special storage conditions are required.

### 2.4.5. Active Substance - L-aspartic acid

#### *2.4.5.1. General Information*

The active substance, L-aspartic acid, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 2003-143-Rev 04.

#### *2.4.5.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.5.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0797 and the R1-CEP 2003-143-Rev 04 requirements. The following additional specifications are also applied.

**Table 4: Additional specifications of L-Aspartic Acid**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.5.4. Stability*

The active substance is stored in a polyethylene bag, in a siliconized polyethylene film container placed in a fibre drum. The retest period for Aspartic acid is 4 years when stored in the above packaging. No special storage conditions are required.

### 2.4.6. Active Substance - L-cysteine

#### *2.4.6.1. General Information*

The active substance, L-cysteine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 2003-159-Rev 03.

#### *2.4.6.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.6.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0895 and the R1-CEP 2003-159-Rev 03 requirements. The following additional specifications are also applied.

**Table 5: Additional specifications of L-Cysteine hydrochloride monohydrate**

| Test | Limit | Method |
|---|---|---|
| Endotoxin | Less than 24 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.6.4. Stability*

The active substance is stored in double polyethylene bags, with desiccant in between, placed in carton box. The re-test period of the substance is of 2 years when stored in the above packaging. No special storage conditions are required.

### 2.4.7. Active Substance - L-glutamic acid

#### *2.4.7.1. General Information*

The active substance, L-glutamic acid, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1998-066-Rev 01.

#### *2.4.7.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.7.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0750 and the R1-CEP 1998-066-Rev 01 requirements. The following additional specifications are also applied.

**Table 6: Additional specifications of L-Glutamic acid**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 26 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |

| | | |
|---|---|---|
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

The active substance is packed in a polyethylene colourless to pale white transparent bag. Compliance with Regulation 10/2011 with amendments has been confirmed. This is packed in a second bag made of polyethylene or silicone vapour deposited on a PET film, further packed into fibre drum or cardboard box.

#### *2.4.7.4. Stability*

Based on the stability data the claimed re-test period is 4 years.

### 2.4.8. Active Substance - L-histidine

#### *2.4.8.1. General Information*

The active substance, L-histidine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1998-107-Rev 04.

#### *2.4.8.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.8.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0911 and the R1-CEP 1998-107-Rev 04 requirements. The following additional specifications are also applied.

**Table 7: Additional specifications of L-Histidine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.8.4. Stability*

The active substance is stored in a polyethylene bag in a silicon oxide vapour deposited polyethylene terephthalate film, placed in a fibre drum. The retest period for histidine is 48 months when stored in the foreseen packaging. No special storage conditions are required.

### 2.4.9. Active Substance - L-lysine monohydrate

The active substance, L-lysine monohydrate, is not described in the European Pharmacopoeia. However, there are monographs for Lysine hydrochloride and Lysine acetate. L-Lysine Hydrate is described in the DAB (Deutsches Arzneibuch).

An ASMF in eCTD-format has been provided by the ASMF Holder (ref: EMEA/ASMF/01488). A letter of access in relation Maapliv, issued 15.06.2023, has been provided. For detailed assessment of the ASMF please see separate ASMF Assessment Report, and confidential annex on the Restricted Part.

The initial assessment was performed on ASMF Version 2023-7. In response to questions, an updated ASMF was provided (ASMF Version 2024-2).

#### *2.4.9.1. General Information*

The chemical name of L-Lysine Hydrate is L-2,6-diamino-caproic acid monohydrate (*S*)-2,6-diamino-hexanoic acid monohydrate corresponding to the molecular formula $C_6H_{14}N_2O_2 \bullet H_2O$. It has a relative molecular mass of 164.21 g/mol and the following structure:

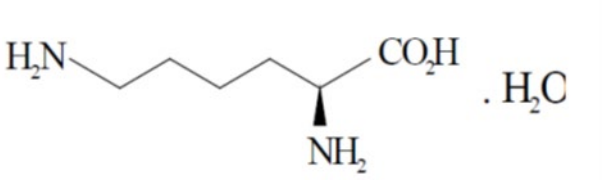



**Figure 1: Active substance structure - L-lysine monohydrate**

The chemical structure of L-Lysine hydrate was elucidated by nuclear magnetic resonance ($^1$H-NMR, $^{13}$C-NMR), mass spectrometry and elemental analysis.

L-Lysine hydrate is consists of white or yellowish crystals or crystalline powder with amine like odour, soluble in water. No polymorphism is observed.

L-Lysine hydrate is manufactured as a single stereoisomer. The drug substance has one stereogenic center. L-Lysine hydrate is from a fermentative process which is a stereospecific process, therefore the presence of the D-enantiomer is not expected. The enantiomeric purity is controlled by the specific optical rotation test.

### *2.4.9.2. Manufacture, process controls and characterisation*

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF.

During the procedure, a major objection (MO) was raised on the QP declaration as it was based on an audit performed over five years ago. A second MO was raised on the initial proposed starting material (Feed Grade L-Lysine Hydrochloride) as no synthetic steps were described in the dossier and it was considered that just ion exchange followed by purification cannot be accepted as sufficient process description. In response, the starting material was re-defined as the Working Cell bank of L-Lysine hydrochloride Feed Grade obtained by fermentation, and L-Lysine hydrochloride (Feed Grade) is now considered as an intermediate in the manufacturing of L-Lysine hydrochloride (GMP grade).

Furthermore, the quality of L-Lysine hydrochloride (GMP) is now covered by a CEP and detailed description of the manufacturing process was assessed and approved by EDQM, (R1 CEP-2003-057 Rev 05). Information up to L-Lysine hydrochloride (GMP) was replaced by the above CEP and the QP declaration was updated accordingly to cover all sites involved in the active substance manufacturing process.

The characterisation of the active substance and its impurities is in accordance with the EU guideline on chemistry of active substances.

During the procedure, a MO was raised on the characterisation of impurities as there was limited information on genotoxic impurities and the justification based on a very short manufacturing process was not considered to be acceptable. A reassessment of the whole manufacturing process, after starting material redefinition, was requested, in particular in relation to the possible presence of mutagenic impurities, in line with the ICH M7 guideline.

In response, an assessment of potential impurities according to ICH M7 was performed; L-α-Amino-ε-caprolactam, N-ε-acetyl-Lysine and N-α-acetyl-Lysine are classified as Class 5 impurities and not mutagenic. Control of above impurities is included in the active substance specification. It was also noted that impurities in L-Lysine hydrochloride (GMP) have been evaluated and approved by EDQM in CEP procedure (CEP 2003-057). A risk assessment on nitrosamine formation has been performed by the active substance manufacturer and no risk of presence of nitrosamine impurities in Lysine hydrate has been identified. It was concluded that a sufficiently detailed impurities discussion for the new manufacturing process has been presented, potential and actual impurities (including also impurities potentially coming from the biological origin of the starting material) were well discussed with regards to their origin and characterised. The dossier now contains complete information including changes introduced to the manufacturing process from ASMF version 2023-7 to 2024-2.

The active substance is packaged in primary transparent LDPE bags. The secondary packaging is made of a polymer LDPE (polyethylene terephthalate) film coated with a thin layer of metal (aluminium). Primary packaging complies with EC 10/2011 as amended. The tertiary packaging is a fibre or plastic drum with lever-lock locking ring closure and a plastic lid. A cardboard box may be used upon request by customers.

### *2.4.9.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification includes tests for appearance, identification (IR, specific optical rotation), assay (perchloric titration), pH (Ph. Eur.), transmittance (UV), water content (Ph. Eur.), residue on ignition (Ph. Eur.), chloride (Ph. Eur.), sulfate (Ph. Eur.), ammonium (Ph. Eur.), heavy metals (Ph. Eur.), iron (Ph. Eur.), other amino acid (TLC), ethanol (GC) and related substances (HPLC).

The proposed specification is suitable to control the pharmaceutical quality of the active substance. Analytical procedures used have been described in sufficient detail. Batch analysis data from three batches have been provided. The results are within the specifications and consistent from batch to batch. Justification of specification was updated to include references to the DAB monograph for L-lysine hydrate.

The specification applied by the finished product manufacturer presented in Table 9 below, includes the same tests applied by the active substance manufacturer, with the addition of bacterial endotoxins and microbial purity (TAMC & TYMC).

**Table 9: Active substance specification valid at the finished product manufacturer – L-lysine monohydrate**

**Specification**

Specification parameters and limits applied by the drug product manufacturer are the same of drug substance manufacturer, therefore the ones foreseen by the corresponding ASMF currently in force.
For microbiological quality the following test are verified by the drug product manufacturer:

Table 1: Microbiological testing performed by drug product manufacturer

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

The corresponding test methods and validation are provided.

Batch analysis data from 3 batches tested by the active substance manufacturer were provided.

During the procedure it became apparent that batches of Lysine Hydrate with pharmaceutical grade (ASMF Version 2024-2) have not yet been tested by the finished product manufacturer. It was there for recommended that batch analysis data for L-lysine monohydrate tested by the finished product manufacturer should be provided before first batch of the finished product is released. **(REC1)**

#### *2.4.9.4. Stability*

A stability study was performed with active substance manufactured before re-definition of the starting material. The data justify a retest-date of 3 years for L-Lysine hydrate. The active substance should be stored in a refrigerator at + 2 °C – +8 °C, packed in a PE bag with external metalised bag.

The quality of L-Lysine hydrate was demonstrated to be the same before and after re-definition of the starting material, therefore, the stability data is considered to be representative.

Stability studies have been performed under accelerated (25 ± 2° C, RH = 60 ± 5 %) and long-term (5 ± 3°C) conditions. Stability studies under stress conditions (including photostability) have been also performed.

36-months stability data are available for batches manufactured in 2009-2020 –for batches manufactured from 2011 testing was performed on yearly basis. For batches manufactured in 2020, 24-months data are available, for batches form 2021, 12-months data is available. Accelerated data are available for 6 months.

Based on the available stability date, the proposed retest period of 3 years when stored in its original sealed packaging in dry, dark conditions at temperature 2-8°C, was considered acceptable.

### 2.4.10. Active Substance - L-methionine

#### *2.4.10.1. General Information*

The active substance, L-methionine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1999-136-Rev 08.

#### *2.4.10.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.10.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 1027 and the R1-CEP 1999-136-Rev 08 requirements. The following additional specifications are also applied.

**Table 10: Additional specifications of L-Methionine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

The active substance is packed in colourless to pale white transparent polyethylene bags. Compliance with Regulation 10/2011 with amendments has been confirmed. The outer packaging is a silicone oxide vapor deposited PE terephthalate bag further packed into a fibre drum or cardboard box.

#### *2.4.10.4. Stability*

Based on the stability data the claimed re-test period is 4 years.

## 2.4.11. Active Substance - L-phenylalanine

#### *2.4.11.1. General Information*

The active substance, L-phenylalanine, is described in the Ph. Eurand is the subject of EDQM Certificate of Suitability R1-CEP 2014-132-Rev 00.

#### *2.4.11.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.11.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0782 and the R1-CEP 2014-132-Rev 00 requirements. The following additional specifications are also applied.

**Table 11: Additional specifications of L-Phenylalanine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

The active substance follows the requirements of the corresponding Ph. Eur. monograph no. 0782 Limits for bacterial endotoxins and microbial purity. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.11.4. Stability*

The active substance is stored in a polyethylene bag in either a second polyethylene bag or a silicon oxide vapour deposited polyethylene terephthalate bag placed in a fibre drum. The retest period for phenylalanine is of 4 years when stored in the above packaging. No restricted storage conditions are required.

### 2.4.12. Active Substance - L-proline

#### *2.4.12.1. General Information*

The active substance, L-proline, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 1998-064-Rev 04.

#### *2.4.12.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.12.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0785 and the R1-CEP 1998-064-Rev 04 requirements. The following additional specifications are also applied.

**Table 12: Additional specifications of L-Proline**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.12.4. Stability*

The active substance is stored in a container consisting of polyethylene/polyester bags, placed in a fibre drum. The retest period for L-proline is 2 years when stored in the above packaging. No restricted storage conditions are required.

## 2.4.13. Active Substance - L-serine

### *2.4.13.1. General Information*

The active substance, L-serine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 2013-211-Rev 00 (for serine Process II).

### *2.4.13.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

### *2.4.13.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 0788 and the R1-CEP 2013-211-Rev 00 requirements. The following additional specifications are also applied.

**Table 13: Additional specifications of L-Serine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

### *2.4.13.4. Stability*

The active substance is stored in a polyethylene bag in a silicon oxide vapor deposited polyethylene terephthalate bag, placed in a fibre drum. The retest period for L-serine is 3 years when stored in the foreseen packaging. No restricted storage conditions are required.

## 2.4.14. Active Substance - L-threonine

### *2.4.14.1. General Information*

The active substance, L-threonine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability R1-CEP 2013-004-Rev 01.

#### *2.4.14.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.14.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 1049 and the R1-CEP 2013-004-Rev 01 requirements. The following additional specifications are also applied.

**Table 14: Additional specifications of L-Threonine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.14.4. Stability*

The active substance is stored a polyethylene bag in either a polyethylene bag or a siliconised polyethylene terephthalate bag, placed in a fibre drum. The retest period for L-threonine is 4 years when stored in the foreseen packaging. No restricted storage conditions are required.

### 2.4.15. Active Substance - L-tryptophan

#### *2.4.15.1. General Information*

The active substance, L-tryptophan, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability CEP 1998-137-Rev 07.

#### *2.4.15.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.15.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 1272 and the CEP 1998-137-Rev 07 requirements. The following additional specifications are also applied.

**Table 15: Additional specifications of L-Tryptophan**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.15.4. Stability*

The active substance is stored in a double polyethylene bag in a silicon oxide vapor deposited polyethylene terephthalate bag, placed in a fibre drum. The retest period for tryptophan is 4 years when stored in the foreseen packaging. No restricted storage conditions are required.

### 2.4.16. Active Substance - L-tyrosine

#### *2.4.16.1. General Information*

The active substance, L-tyrosine, is described in the Ph. Eur. and is the subject of EDQM Certificate of Suitability CEP 1999-023-Rev 03.

#### *2.4.16.2. Manufacture, process controls and characterisation*

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

#### *2.4.16.3. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification is in accordance with the current Ph. Eur. monograph no. 1161 and the CEP 1999-023-Rev 03 requirements. The following additional specifications are also applied.

**Table 16: Additional specifications of L-Tyrosine**

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 18 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

Limits for bacterial endotoxins and microbial purity have been justified. The corresponding test methods and validation are provided. Batch analysis data from three batches of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

#### *2.4.16.4. Stability*

The active substance is stored in a polyethylene bag in a polyethylene terephthalate bag, placed in a fibre drum. The retest period for tyrosine is 4 years when stored in the foreseen packaging. No restricted storage conditions are required.

### 2.4.17. Active Substance – Taurine

The active substance, Taurine, is not described in the Ph. Eur.

An ASMF in eCTD-format has been provided by the ASMF Holder (ref: EU/ ASMF /00671/0001). A letter of access in relation Maapliv, issued on 26/08/2023, has been provided. For detailed assessment of the ASMF please see the separate ASMF Assessment Report, and confidential annex on the Restricted Part.

The initial assessment was performed on ASMF Version 2023-7.

#### *2.4.17.1. General Information*

The chemical name of taurine is 2-aminoethane-1-sulfonic acid corresponding to the molecular formula $C_2H_7NO_3S$. It has a relative molecular mass of 125.15 g/mol and the following structure:

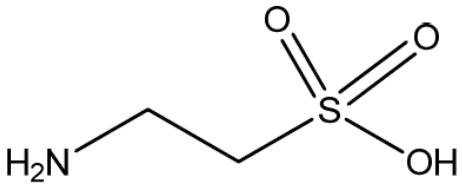



**Figure 2: Active substance structure - Taurine**

The chemical structure of taurine was elucidated by ultraviolet spectroscopy (UV), infrared absorption spectroscopy (IR), nuclear magnetic resonance spectroscopy ($^{1}$H-NMR and $^{13}$C-NMR), high resolution mass spectrometry (TOF), and elemental analysis (EA). Solid state properties were investigated by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and x-ray powder diffraction (XRPD).

Taurine presents as colourless or white crystals or a white crystalline powder, soluble in water, and practically insoluble in ethanol (99.5%).

Taurine has no chiral centres.

There is no monograph of taurine in the European Pharmacopoeia

### *2.4.17.1. Manufacture, process controls and characterisation*

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF.

The characterisation of the active substance and its impurities is in accordance with the EU guideline on chemistry of active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

The manufacturing process development history has been provided.

The active substance is packaged into transparent double low-density polyethylene bags as primary packaging and brown fibre drums as secondary packaging. Compliance of primary packaging with current Ph. Eur. requirements and regulation (EU) No. 10/2011 as amended has been confirmed.

### *2.4.17.2. Specification, analytical procedures, reference standards, batch analysis, and container closure*

The active substance specification includes tests for appearance, solubility, identification (IR), HPLC), clarity and color of solution (JP), pH (JP), chorides (JP), sulfates (JP), heavy metals (JP), iron (JP), ammonium (JP), related substances (HPLC), loss on drying (JP), residue on ignition (Ph. Eur.), microbial purity (Ph. Eur.) and bacterial endotoxins (Ph. Eur.).

The proposed specification is mainly based on Japanese Pharmacopoeia (JP) requirements.

During the procedure, a MO was raised on the initial proposal to control impurities via a semi quantitative TLC method and due to the absence of limits for unidentified impurities and total impurities. In response, the applicant updated the test for related substances from TLC to HPLC and included limits for identified impurities, unidentified impurities and total impurities. The proposed limits for identified impurities have been tightened to qualification threshold in line with guideline ICH Q3A (R2).

Appropriate information on the analytical procedures and their validation was provided.

Batch analysis data for eleven batches have been presented.

The specification applied by the finished product manufacturer is presented in Table 18 below and includes the same tests applied by the active substance manufacturer, with tighter limits for bacterial endotoxins and microbial purity (TAMC & TYMC).

**Table 18: Specifications of Taurine valid at the drug product manufacturer**

**Specification**

Specification parameters and limits applied by the drug product manufacturer are the same of drug substance manufacturer, therefore the ones foreseen by the corresponding ASMF currently in force.
For microbiological quality the following test are verified by the drug product manufacturer:

Table 1: Microbiological testing performed by drug product manufacturer

| Test | Limit | Method |
|---|---|---|
| Bacterial Endotoxins | Less than 6.0 EU/g | Ph.Eur. 2.6.14, current edition |
| TAMC | Not more than 100 CFU/g | Ph.Eur. 2.6.12, current edition |
| TYMC | Not more than 10 CFU/g | Ph.Eur. 2.6.12, current edition |

During the procedure it became apparent that batches of taurine manufactured by the active substance manufacturer had not yet been tested by finished product manufacturer. It was therefore recommended that batch analysis data for taurine tested by the finished product manufacturer should be provided before the first batch of finished product is released. **(REC2)**

### *2.4.17.3. Stability*

Stability studies have been performed for three production scale batches under long term conditions (25 ± 2 ºC/ 60 ± 5% RH) for up to 24 months and under accelerated conditions (40 ±2 ºC/75 ± 5% RH) for up to 6 months, as well as under stressed conditions. All results are within the defined limits.

Following the addition of the HPLC related substances method and tightening of the limits for related substances, the stability data was re-evaluated. It was confirmed that all batches complied with the revised specification limit at the last available stability timepoint.

Therefore, the proposed retest period of 3 years with storage conditions "preserve in a well-closed container" are considered acceptable.

## 2.4.18. Finished Medicinal Product

### *2 . 4 . 1 8 . 1 . Description of the product and Pharmaceutical Development*

Maapliv is a solution for infusion containing 52.75 g/L of amino acids. Detailed qualitative and quantitative composition of the finished product is presented, per 1 ml and per primary container in Table 19.

The finished product is supplied in a 500ml type II glass bottle sealed with a rubber stopper.

**Table 19: Finished product composition**

| Names of ingredients | Formula (mg/mL) | Quantity for 500 mL (one bottle) | Function | Quality Standard |
|---|---|---|---|---|
| Alanine | 6.30 | 3.15 g | Drug substance | Ph. Eur. curr. ed. |
| Arginine | 4.10 | 2.05 g | Drug substance | Ph. Eur. curr. ed. |
| Aspartic acid | 4.10 | 2.05 g | Drug substance | Ph. Eur. curr. ed. |
| Cysteine hydrochloride monohydrate | 1.45 | 0.725 g | Drug substance | Ph. Eur. curr. ed. |
| Glutamic acid | 7.10 | 3.55 g | Drug substance | Ph. Eur. curr. ed. |
| Glycine | 2.10 | 1.05 g | Drug substance | Ph. Eur. curr. ed. |
| Histidine | 2.10 | 1.05 g | Drug substance | Ph. Eur. curr. ed. |
| Lysine hydrate | 6.30 | 3.15 g | Drug substance | Internal monograph |
| Methionine | 1.30 | 0.65 g | Drug substance | Ph. Eur. curr. ed. |
| Phenylalanine | 2.70 | 1.35 g | Drug substance | Ph. Eur. curr. ed. |
| Proline | 5.60 | 2.80 g | Drug substance | Ph. Eur. curr. ed. |
| Serine | 3.80 | 1.90 g | Drug substance | Ph. Eur. curr. ed. |
| Taurine | 0.30 | 0.15 g | Drug substance | Internal monograph |
| Threonine | 3.60 | 1.80 g | Drug substance | Ph. Eur. curr. ed. |
| Tryptophan | 1.40 | 0.70 g | Drug substance | Ph. Eur. curr. ed. |
| Tyrosine | 0.50 | 0.25 g | Drug substance | Ph. Eur. curr. ed. |
| Acetic acid or Sodium hydroxide* | q.s. | q.s. | pH adjusters | Ph. Eur. curr. ed. |
| Water for injection | q.s | q.s. | Vehicle | Ph. Eur. curr. ed. |
| Nitrogen** | q.s. | q.s. | Processing aid | Ph. Eur. curr. ed. |

* Acetic acid or sodium hydroxide are used, if necessary, as pH adjuster during preparation to reach a pH between 5.1 and 5.2

** Nitrogen is used during the manufacturing process to prevent oxidation and maintain an inert atmosphere.

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 2.1.1 of this report.

The active substances are sufficiently described, and the choice of excipients is justified. The compatibility of active substances with each other has been demonstrated.

Maapliv sterile solution for infusion is a pharmaceutical preparation containing 16 amino acids. This combination of amino acids has been used in Europe for at least 10 years, with consistent evidence of efficacy and safety, as confirmed by data from European studies and additional data from countries outside Europe. The applicant was asked to align the dossier with the requirements for well-established use application.

Taking into account conditions laid out in Annex I of Directive 2001/83/EC, Part II, point 1d in relation to well-established use applications, the applicant was asked to substantiate that "the product studied [in bibliographic referenced literature] can be considered as similar to the product, for which application for a marketing authorisation has been made in spite of the existing differences." For this purpose, the quality aspects such as composition, pH and osmolality were discussed in the development section of the dossier, including comparison with a similar product already authorised and marketed in EEA - Vaminolact.

The chosen manufacturing process involves compounding, filtration, filling and terminal sterilisation. Manufacturing process development has been sufficiently described. The control of oxygen levels is defined as a critical process parameter in all the manufacturing steps between the start of manufacturing (compounding) until the capping of the unsterilised bottle. Manufacturing process development data is in line with ICH Q8. It is described in the dossier how the process development studies provide the basis for control strategy.

The selection of the sterilisation process (steam sterilisation via autoclave, 118 °C; $F_0$ >15 mins), is justified and in line with the EMA guideline on sterilisation and relevant decision trees.

It was observed that the content of L-glutamic acid decreases as a result of terminal sterilisation whereas the content of the other amino acids remains stable. L-Glutamic acid content was also found to decrease further during stability studies on these batches, more so under accelerated conditions (40°C/75% RH). Several out of specification (OOS) or near OOS results were observed. As a result, an overage of 5.5% L-glutamic acid is applied. Process validation studies conducted with the overage demonstrate that the proposed overage is justified.

A risk assessment for extractables and leachables for the product contact materials used in the manufacturing process was provided. Overall, the development of the container closure system is considered adequately described. The compatibility of the finished product with the primary packaging material is proven by an ICH compliant stability study. The extractable study has been performed with different media. The most representative media are water and $H_3PO_4$ solution in water for injections (pH 3), because the finished product is a mixture of amino acids with an aqueous nature with a pH in the range of 5.1 – 5.4, due to the addition of acetic acid or sodium hydroxide as pH adjuster. Several analytical chromatography methods were used. Volatile, semi volatile, non-volatile compound and metal analysis was performed. A complete profile of extractables in all media was summarised. Based on the extractable study results, no leachable study was deemed to be necessary since there are no extractables in water and pH 3 solutions, that are most representative of the finished product.

Compatibility of the finished product with administration devices has been addressed and substantiated with relevant data.

The finished product is supplied in glass bottles, nominal capacity 500 mL of colourless, type II (Ph. Eur.) glass. The bottles are closed with an elastomeric rubber closure (chlorobutyl rubber) compliant with Ph. Eur chapter 3.2.9 and sealed with an aluminum cap with a center tear-off seal. The choice of container is justified. The testing of the packaging components is in accordance with Ph. Eur.

### *2.4.18.2. Manufacture of the product and process controls*

The name, address and responsibility of each manufacturer involved in the manufacturing and testing of the finished product was provided, with proof of their GMP compliance.

The manufacturing process for Maapliv solution for infusion includes five main phases; compounding, filtration, filling, terminal sterilisation and packaging.

The manufacturing process is a standard manufacturing process. There are no process intermediates. The standard bottle fill volume is 500 ml, and batch size is 600 L. This corresponds to 1,165 theoretical bottles since a 3% overfill is applied.

The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

During process validation, it was shown that in process control and release data for three consecutive batches manufactured with the proposed commercial process and scale meet the acceptance criteria. The holding time between addition of raw materials and start of filling (48 hrs) and the standing time between the end of filling and start of sterilization (36 hrs) were determined.

Validation of the sterilisation process is in line with EMA guideline on sterilisation EMA/CHMP/CVMP/QWP/850374/2015. Confirmation of SAL is accomplished using physical measurements and the response from bioindicators. The minimum and maximum load of 500 ml bottles in the autoclave have been validated with compliant results. Filters used in the process (non-sterilizing, pre-filtration and filtration filters) were found to be compatible with the bulk solution and no issues were identified with extractables and leachables.

Overall, the applicant has provided data to assure that the manufacturing process is appropriately validated and can produce finished product batches of consistent quality.

### *2.4.18.3. Product specification, analytical procedures, batch analysis*

The finished product specification includes appropriate tests for this type of dosage form; appearance, colour of solution (Ph. Eur.), pH (Ph. Eur.), osmolality (Ph. Eur.), amino acid identification (HPLC-UV), amino acid assays (HPLC-UV), purity (HPLC-UV), extractable volume (Ph. Eur.), endotoxins (Ph. Eur.), sterility (Ph. Eur.) and sub-visible particles (Ph. Eur.).

The specification is considered appropriate and is in line with ICH Q6A and Ph. Eur.

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 purity testing has been presented.

Batch analysis results are provided for three commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

### *2.4.18.4. Stability of the product*

Stability studies were performed in line with ICH guidance. The parameters tested and used methods were in line with the finished product specification.

Stability data under long term conditions (25ºC ± 2ºC & 40% ± 5%) are available for 18 months for 3 finished product batches manufactured and packaged as per the commercial process.

Stability data under intermediate conditions (30ºC ± 2ºC & 65% ± 5%) and under accelerated conditions (40ºC ± 2ºC & 75% ± 5%) are available for 6 months for 3 finished product batches.

Results don't show any significant change over the time under long term, intermediate and accelerated conditions for all batches and results are all within the limits of the proposed specifications.

The finished product has been subjected to the following conditions of forced degradation: temperature (80 °C for 24h), acid degradation, basic degradation, oxidative degradation (3% $H_2O_2$ for 1, 4 and 8 hours at room temperature), photodegradation (1 x ICH cycle and 2 x ICH cycles). The results obtained show that the amino acids are particularly sensitive to oxidation.

The product information includes a warning to protect from light, not to refrigerate or freeze and information that product is for single use only.

The proposed shelf-life of 24 months (without specific storage conditions) can therefore be accepted.

### *2.4.18.5. Post approval change management protocol(s)*

Not applicable.

#### *2.4.18.6. Adventitious agents*

Not applicable.

#### *2.4.18.7. GMO*

Not applicable.

## 2.4.19. Discussion on chemical, pharmaceutical and Rapporteur assessment

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner.

The product contains 16 amino acids as active substances. The information provided for 14 amino acids followed the CEP procedure. For these active substances no Major Objections (MOs) were raised.

For two active substances (L-lysine monohydrate and taurine) the ASMF procedure was used. During the procedure, 3 MOs were raised relating to L-lysine monohydrate ASMF (relating to QP declaration, starting materials, genotoxic impurities) and 1 MO was raised relating to the taurine ASMF (relating impurity test method and limits). Following satisfactory answers from the applicant and respective ASMF holders, the MOs were considered to be resolved.

No MOs were raised on the finished product. A number of other concerns were raised during the procedure and these were satisfactorily answered by the applicant.

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.

## 2.4.20. 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.

## 2.4.21. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

*L-lysine monohydrate*

1. Batch analysis data for L-lysine monohydrate tested by the finished product manufacturer should be provided before first batch of the drug product is released.

*Taurine*

2. Batch analysis data for taurine tested by the finished product manufacturer should be provided before first batch of the drug product is released.

## *2.5. Non-clinical aspects*

### 2.5.1. Introduction

Maapliv is a BCAA-free solution for parenteral use containing 52.75 g/L of amino acids. No non-clinical studies have been performed with Maapliv as this is a bibliographical application.

### 2.5.2. Pharmacology

No pharmacology studies (primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology) have been performed with Maapliv.

The mechanism of action of this drug is to alter the metabolism of branched chain amino acids and thus restrict metabolic decompensation due to MSUD. Amino acids are used in the synthesis of hormones and bioactive molecules and some amino acids may act as signalling molecule.

The applicant has summarised signalling functions in mammals for 6 of the 16 amino acids in Maapliv, from a book by Dmello *et al.* (2003).

The summary is provided in tabular format (Table 2.4.3 below).

**Table 2.4. 3 Signalling functions of some amino acids (DMello 2003)**

| *Amino acid* | *Anabolic Products* | *Function/s* |
|---|---|---|
| *Methionine* | ➢ Formylmethionine | ➢ Initiation of protein synthesis |
| *Tryptophan* | ➢ Serotonin | ➢ Neurotransmitter |
| *Tyrosine* | ➢ Dopamine | ➢ Neurotransmitter |
| | ➢ Noradrenaline | ➢ Neurotransmitter |
| | ➢ Adrenaline | ➢ Hormone |
| | ➢ Thyroxine | ➢ Hormone |
| *Arginine* | ➢ Nitric oxide | ➢ Involved in vasorelaxation; ➢ neurotransmission; male reproductive ➢ performance; gut motility |
| | ➢ Polyamines | ➢ Regulation of RNA synthesis; ➢ maintenance of membrane stability |
| *Histidine* | ➢ Histamine | ➢ Potent vasodilator |
| *Glutamate* | ➢ Glutathione | ➢ Maintenance of reduced cysteine residues of blood proteins |
| | ➢ Aminobutyrate | ➢ Neurotransmitter |

In addition, a range of pharmacological actions are listed for taurine from literature studies by DeLuca *et al.* (2015) and Huxtable *et al.* (1992) (Table 2.4.4 below).

**Table 2.4.4 Pharmacological actions are ascribed to taurine (De Luca, Pierno, and Camerino 2015, Huxtable 1992).**

| Main target organ | Effect |
|---|---|
| *Central nervous system* | ➢ Inhibitory effects |
| *Heart* | ➢ ↑Inotropism<br>➢ Antiarrhythmic action |
| *Vascular system* | ➢ Vasorelaxation<br>➢ ↓Blood pressure<br>➢ ↓Atherosclerotic plaque |
| *Immune system* | ➢ Immunostimulant |
| *Liver* | ➢ Bile salt synthesis |
| *Skeletal muscle* | ➢ ↑Performance<br>➢ Effects on membrane permeability |
| *Genitourinary system* | ➢ ↑Fertility |
| *Pancreas* | ➢ ↑Insulin secretion |

The applicant discussed that methionine initiates muscle protein synthesis through its anabolic product formylmethionine. The applicant has listed polyamines, as the anabolic product of arginine, which regulate RNA synthesis, and conceivably have a role in protein synthesis. No discussion on anabolic effects of other mono-components in Maapliv has been provided by the applicant. Tyrosine's anabolic products are listed in the non-clinical overview as having roles in neurotransmission and hormone function.

The applicant provided a literature evidence and discussion on primary pharmacology of Maapliv to address the following aspects:

a) Proof-of-concept for the use of Maapliv, in a relevant non-clinical disease model of MSUD, to address primary pharmacology and demonstrate reduction of brain leucine levels following administration of non-BCAAs.

b) Transport of the individual amino acids mono-components across BBB in situations of hyperleucinaemia/leucinosis, where the LAT-1 transporter could be saturated with BCAAs, and therefore may prevent further transport of other large neutral amino acids, including phenylalanine, tyrosine, methionine, histidine and tryptophan, across the BBB.

c) The relevance of plasma leucine levels as a representative biomarker of CNS leucine levels.

Zinnanti et al. demonstrated that consumption of a low-BCAA diet by iMSUD mice resulted in reduced plasma and brain BCAA accumulation, increased survival and neuronal protection. This study supports the concept that BCAA-free solutions may have beneficial effects in MSUD. Zinnanti et al. demonstrated the protective effect of a low BCAA diet to reduce brain leucine accumulation through dietary manipulation. The applicant also refers to the review by Yudkoff et al. and two clinical studies (Strauss et al. and Sánchez-Pintos et al.) to further provide evidence that BCAA-free interventions can effectively reduce brain leucine levels and improve neurological outcomes in MSUD patients.

The applicant provided literature evidence that in MSUD, particularly during hyperleucinaemia, the transport of other large neutral amino acids across the BBB is impaired due to competition with elevated BCAAs for the

LAT1 transporter. It is acknowledged that this supports the rationale for using BCAA-free solutions, like Maapliv, to help restore the balance of amino acid transport across the BBB. Accumulation of leucine, in particular, in addition to other branched-chain amino acids (BCAAs), can result in exclusion of other LNAAs from the brain (Vogel *et al* 2014, PMID: 24886632). Of note, Vogel *et al* have stated that exclusion of LNAAs due to LAT-1 saturation has not been rigorously evaluated in either patients or in an animal model of MSUD. In the same study using a murine model of branched chain ketoacid dehydrogenase deficiency MSUD, assessment of metabolic control using serum leucine levels was determined as a poor surrogate for disturbances of amino acid homeostasis in the brain. The literature evidence exists that plasma levels may not always accurately reflect brain amino acid status. This evidence is based on chronic amino acid depletion models in laboratory animals and is only indirectly relevant to the acute administration of Maapliv. Nevertheless, continuous monitoring of BCAAs as clinical biomarkers of efficacy of the treatment, as well as clinical signs suggestive of excessive levels of BCAAs in the brain is necessary. It is acknowledged that relevance of change in plasma leucine levels was demonstrated in the supporting clinical studies (see clinical section).

The non-clinical efficacy of BCAA-free amino acid supplementation provides the theoretical basis for the efficacy of formulations such as Maapliv, designed to balance blood and brain levels of amino acids. Therefore, the rationale for using BCAA-free solutions like Maapliv is to help restore the balance of amino acid transport across the BBB.

#### *2.5.2.1. Pharmacodynamic drug interactions*

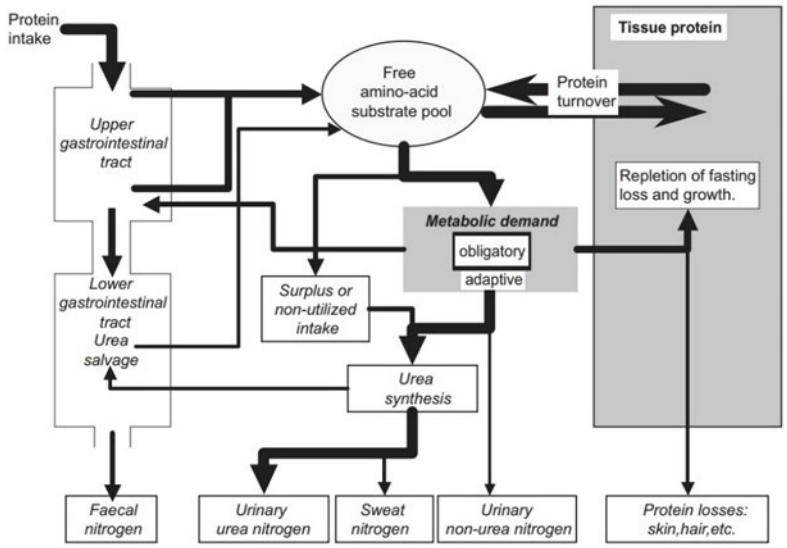
No non-clinical drug interaction studies have been performed. However, the body does not store free amino acids, which are rapidly anabolized or catabolized in the systemic circulation or tissues, (DMello 2003) and are therefore unlikely to be responsible for drug interactions.

## 2.5.3. Pharmacokinetics

No ADME studies have been conducted with Maapliv. However, since Maapliv is administered by peripheral or intravenous continuous infusion, 100% of the product is absorbed systemically.

The metabolic demand for amino acids is well characterized, and the main routes are summarized in Figure 3.2.3.1.

**Figure 3.2.3.1.** Metabolic demands for amino acids




Absorption/reabsorption of amino acids from digested proteins into the systemic circulation occurs in the intestine and kidney. Once absorbed, circulating and tissues levels of amino acid are tightly regulated, even under conditions of protein restriction. Absorbed amino acids are rapidly distributed to tissues and have different metabolic fates depending on the organ involved:

- *Amino acid biosynthesis (by transamination etc.), and degradation*: All organs
- *Urea synthesis*: principally the liver
- *Protein biosynthesis and degradation*: mainly in muscle tissue
- *Regulation of protein intake and metabolism*: the central nervous system and metabolic hormones.

Cells constantly turnover proteins and recycle most amino acids; a net loss of amino acid results only from their oxidation.

Homeostasis is achieved by exchange of essential amino acids with non-essential amino acids and the transfer of amino groups from amino acid oxidation pathways to amino acid biosynthetic pathways. Homeostasis is maintained by *mammalian target of rapamycin complex 1* (mTORC1) causing an increase in the breakdown of cellular proteins and a reduction in protein biosynthesis. Moreover, the non-derepressable *activating transcription factor 4* pathway may be activated resulting in the transcription of genes involved in amino acid transport and the biosynthesis of non-essential amino acids. Food intake briefly increases plasma amino acid levels, stimulates insulin release and *mammalian target of rapamycin* (m-TOR)-dependent protein synthesis in muscle.

Excess amino acids are metabolized (oxidized), resulting in increased urea production. As half of all amino acids are essential, reduction in protein synthesis and amino acid oxidation are the two key measures to reduce amino acid demand. Long-term malnutrition causes depletion of plasma amino acids. The central nervous system appears to generate a protein-specific response upon amino acid depletion, resulting in avoidance of an inadequate diet. High protein levels, in contrast, contribute together with other nutrients to a reduction in food intake.

Amino acids are also used by many organs to produce energy and as a result amino groups are released either as ammonia or amino acids. Free ammonia from the circulation or locally produced ammonia is

converted to urea, in the urea cycle, prior to excretion in the urine. The activity of the urea cycle is regulated by amino acids levels.

The liver is the major site of nitrogen metabolism in the body. When amino acids are catabolized, their potentially toxic nitrogen moiety is eliminated by transamination, deamination, or urea formation. The carbon skeleton of amino acids is generally conserved as carbohydrate, via gluconeogenesis, or as fatty acids via fatty acid synthesis pathways.
In this respect amino acids fall into three categories: glucogenic, ketogenic, or glucogenic and ketogenic. Glucogenic amino acids are those that give rise to a net production of pyruvate or tricarboxylic acid cycle intermediates, such as 2-oxoglutarate or oxaloacetate, all of which are precursors to glucose via gluconeogenesis. All amino acids except lysine and leucine are at least partly glucogenic. Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetyl-CoA or acetoacetyl-CoA, neither of which can bring about net glucose production. Isoleucine, phenylalanine, threonine, tryptophan and tyrosine give rise to both glucose and fatty acid precursors and are thus characterized as being glucogenic and ketogenic. A third catabolic route is activated during episodes of diet restriction/starvation; the reduced carbon skeleton of amino acids is oxidized to carbon dioxide and water for ATP production.

There are two sources of taurine in the body: dietary and endogenous. The primary route of taurine biosynthesis is from methionine and cysteine, which are converted to cysteine sulfinate, which is then decarboxylated to hypotaurine and oxidized to taurine. Some animals (cats, humans, and certain monkeys) are unable to synthesize significant quantities of taurine and rely on a dietary taurine to meet their metabolic needs. As a result, in most species, cell, organ and whole-body taurine concentrations are regulated by transport, and biosynthesis from metabolism has only a minor contribution.

In mammals, while taurine is synthesised in many tissues, the main sites of synthesis are the liver, brain, and pancreas. For example, an adult rat consuming standard laboratory food produces about 80 % of its total body taurine and obtains the remainder from the diet. However, when needed rats can meet their taurine needs from biosynthesis. Taurine is highly polar and lipophobic, thus passive diffusion of taurine through the cell wall is limited, making the primary route of absorption into enterocytes via transporters.

In all vertebrates except mammals, taurine is the sole amino acid conjugated to form bile salts. High concentrations of taurine are present in retina, liver, pancreas, central nervous system, and white blood cells. The largest pools of taurine are found in skeletal and cardiac muscles.

The rate of elimination of intracellular taurine is dependent on the rate of turnover of the intracellular pool for that particular tissue. The brain has the lowest rate of taurine turnover.

The majority of taurine is excreted as the unchanged amino acid. In all vertebrates except for mammals, taurine is the sole amino acid conjugated with cholesterol derivatives to form bile salts.

## 2.5.4. Toxicology

The applicant has provided non-clinical bibliographic data relating to toxicity studies with individual amino acid mono-components of Maapliv. For some amino acids, No Observed Effect Level (NOEL) and/or No Observed Adverse Effect Level (NOAEL) and/or a Maximum Tolerated Dose (MTD) have been estimated. A comprehensive review of the available literature found no systematic pattern of toxicity findings for any amino acid. Adverse events due to amino acid supplementation occur only at high doses (in the order of grams/kg body weight per day).

#### *2.5.4.1. Genotoxicity*

The absence of genotoxicity studies is considered acceptable for Maapliv due to the nature of mono-components as naturally occurring amino acids, which are not anticipated to interact directly with DNA, or other chromosomal material.

#### *2.5.4.2. Carcinogenicity*

The absence of carcinogenicity studies is considered acceptable due to proposed acute administration schedule and nature of Maapliv's mono-components as naturally occurring amino acids.

#### *2.5.4.3. Reproductive and developmental toxicity*

The applicant has provided a table of non-clinical bibliographic data relating to toxicity studies with individual amino acid mono-components of Maapliv. Effects on reproduction were only examined for L- alanine (weanling rats), L-lysine (rat), L-methionine (rat), L-tryptophan (rat), L-tyrosine (rat) and taurine (rat and mouse).

The following findings of possible concern are reported:

a) L-alanine administered to weanling rats (in-diet for 26 weeks) increased urinary alanine, serum alanine and blood ammonia levels. In addition, it decreased plasma pyruvate, triglycerides, cholesterol (males) and plasma lactate (females) levels and decreased weight gain at 20%.

b) L-lysine (dose not specified) administered to pregnant rats increased foetal mortality and decreased maternal and foetal body and brain weights.

b) L-methionine (4% in diet) administered to pregnant rats decreased placental and foetal weights.

f) L-tryptophan (1.4 to 6% in-diet) administered to pregnant rats decreased maternal and foetal weight gain. In addition, rats (males and females) administered 1% of L-tryptophan in-diet beginning 2 weeks before mating, resulted in decreased brain weights and over three successive generations.

e) L-tyrosine (2.64% in-diet) administered to rats for 2-weeks prior to mating and continually for three generations) had no effect on brain weight in all three generations except at days 15 and 20 postpartum in the F2 generation (92 and 95% of control). Increased serum concentration of tyrosine of F3 generation rats at post-natal day 5 was also reported.

Taurine administered to rats in an EFD study up to 4 g/kg/day did not demonstrate any maternal or foetal adverse outcomes. No adverse events were observed in a PPND study administering 7g/kg of taurine to pregnant mice and monitoring offspring post-partum. Although another PPND study demonstrated maternal effects of lipogenesis, hepatic gene expression related to potential inflammation and hepatotoxicity, no adverse effects were observed in offspring. Hence, there are no findings of concern regarding use of taurine in pregnancy.

The applicant conducted an additional literature review in order to include updated toxicology data, particularly focusing on glutamic acid, tryptophan, and tyrosine. Based on the additional literature review, glutamic acid and tryptophan, at the levels provided in Maapliv and on an acute basis, are not expected to pose any significant toxicity concerns. The literature search performed by the applicant did not reveal any recent studies or reviews about tyrosine toxicity**.**

### *2.5.4.4. Other toxicity studies*

The applicant has provided a table of non-clinical bibliographic data relating to toxicity studies in rodents for each amino acid mono-component of Maapliv. The applicant has also listed the data in non-rodents for L-glutamic acid, L-phenylalanine, tryptophan, taurine.

***Toxicology studies with individual amino acids (excluding taurine)***

The *Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Food and Nutrition Board, Institute of Medicine of the National Academies* has reviewed data from toxicology studies with amino acids (excluding taurine) and set reference values for dietary energy and macronutrients for healthy individuals and populations. The studies reported for amino acids are not an evolving systematic ICH-compliant program of non-clinical toxicology studies. In many studies dosing was in-diet and toxicokinetic data or dose estimates, based on food consumption, are not reported. As a result, a systematic comparison of dose and effect between studies and between amino acids is difficult.

For some amino acids, NOEL and/or NOAEL and/or an MTD was estimated. In general, adverse effects due to amino acids is low, occurring only at high doses (for most in the order of grams/kg body weight/day), which is consistent with a low toxicity profile. In the few studies which incorporated reversibility periods (alanine (rat), arginine (rat), lysine (rat)), any treatment related effects were completely reversed by the end of the reversibility phase. Moreover, there is no systematic pattern of toxicity findings for any amino acid and in most instances only a variable range of changes occurred in studies typical of those which often occur in regulatory toxicology studies. There were no increases in tumour types or rates in chronic studies (i.e., of 2-year duration) which were conducted for glutamic acid (rat and mouse), phenylalanine (monkey) or tryptophan (rat and mouse).

In studies which examined effects on reproduction (alanine (weanling rats), aspartic acid (mouse), glutamic acid (rat and mouse), lysine (rat), methionine (rat), tryptophan (rat), tyrosine rat)) there were minor findings of toxicity, but only at very high doses.

***Toxicology studies with taurine***

Acute toxicity data for taurine is summarized in Table 2.4.5.

**Table 2.4.5** Acute toxicity of taurine

| ***Species*** | ***Route*** | ***$LD_{50}$*** |
|---|---|---|
| ***Rat*** | Oral | >5 gm/kg |
| | *iv* | >7 gm/kg |
| ***Mouse*** | Oral | >7 gm/kg |
| | *ip* | 6630 mg/kg |
| | *sc* | 6 gm/kg |
| | *iv* | >7 gm/kg |
| | *im* | >7 gm/kg |
| ***Dog*** | *iv* | >2 gm/kg |
| ***Rabbit*** | *iv* | >1 gm/kg |

im: intramuscular; iv: intravenous; ip: intraperitoneal; sc: subcutaneous

Taurine has extremely low acute toxicity in rodents and non-rodents when administered by different routes.

Based on (limited) data from studies in cats, fish, dogs, poultry, pigs and ruminants, the *European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed* has released a scientific opinion on the safety and efficacy of taurine as a feed additive for all animal species. Additional non-clinical toxicity data from the open scientific literature is available from the GRAS submission for taurine.

The European Food Safety Authority Panel states in its report that "Taurine is not genotoxic, teratogenic or carcinogenic" and estimates that the observed safe level in humans for taurine is 6 g/person per day (equal to about 100 mg/kg body weight per day). This conclusion is consistent with the data supplied in the GRAS submission; toxicity studies were conducted in rat, mouse, and guinea pig for other amino acids found that toxicity only occurred at high doses and was generally non-specific. Moreover, in genotoxicity studies taurine was not mutagenic and did not induce chromatid exchange or chromosome aberrations.

It is acknowledged that effects observed following exposure to amino acids occurred mainly following exposure to high doses.

The following findings of possible concern were noted:

(a) Mice dosed with L-arginine (60-240 mg/kg in drinking water) demonstrated increased thymus weight, spleen mitogenesis and inducible natural killer cell activity. No duration of dosing is reported. The maximum effect was observed at 60 mg/kg. It is noted that the aforementioned toxicological findings were not replicated in rats in a more recent study in 2004 carried out by Tsubuku *et al*.

(b) In adult rats, L-aspartic acid had effects on renal function in a 90 day repeat dose toxicity study attributed to the accumulation of $\alpha$2u-globulin. An increase in kidney weight and tubular dilation in renal tubules was observed at potentially clinically relevant doses. Aspartic acid was also administered to juvenile animals in two non-clinical studies.

(c) L-cysteine administered to SD rats by IV infusion demonstrated necrosis of the Purkinje cells and granular layer of cerebellum at 1000 mg/kg. Slight tubular basophilia with blood or hyaline casts in the kidney was also observed.

(d) L-methionine was administered to 5-week-old rats in a study by Chin *et al.* Adverse effects of decreased food intake and growth rate, increases in haemolysis, splenic and hepatic accumulation of hemosiderin and haemolytic anaemia were observed. Thymus atrophy, and other histological abnormalities were reported in both adrenal gland and testis implying effects of methionine on male fertility.

(e) While a 26-week study in rats fed with 20% L-alanine in diet, did not demonstrate any adverse effects, a NOAEL was not defined. In contrast, 2000 mg/kg of alanine administered directly to SD rats resulted in squamous cell hyperplasia of the stomach in a 4-week rodent toxicity study at potentially clinically relevant doses.

(f) In a study by Ikezaki *et al*, haematological findings are reported with effects observed on haemoglobin volume and haematocrit in addition to alterations in creatinine levels. In this study effects on male fertility are suggested with documentation of sperm granulomas in epididymis at 5.0% in diet. Effects on serum chemistry are also reported (increase in haemoglobin, and haematocrit, increase in creatinine). However, no discussion of the effects of hepatomegaly and hypercholesterolaemia observed with histidine and the potential relevance to hepatic findings in clinic were provided.

(g) Irreversible brain damage was observed in neonatal monkeys treated with phenylalanine at 3g/kg from first few days after birth to 2-3 years of age.

(h) Tyrosine was administered to rats as 3-8% dietary intake for three weeks. Keratopathy and corneal changes were reported, speculated due to an increase in tyrosine in the aqueous humour.

Additionally, the following adverse effects are reported from juvenile animal studies:

a) L-aspartic acid (2 g/kg, sc) administered to mice (24h postpartum) and followed for 7 months, increased hypothalamic lesions, obesity, skeletal stunting and decreased reproductive organ size. In addition, mice administered with escalating doses (2.2 to 4.4 g/kg, sc) of L-aspartic acid on days 2 to 11 post-birth reduced litter sizes and fewer pregnancies in females and reduced fertility and significant reductions in activity and exploratory behaviour. Aspartic acid administered to 8-day-old mice at 650 mg/kg increased hypothalamic lesions. It is noted that these effects were not observed in neonatal monkeys after single dose administration of L-aspartic acid.

b) L-glutamic acid (2.2 to 4.2 g/kg) administered to mice (1-10 days of life) increased "female sterility". However, no adverse effects on fertility and survival of the young were noted in rats following L-glutamic acid exposure in diet at concentration 0.1 or 0.4%.

In addition, the applicant identified two narrative reviews, one focused on the recent human studies on amino acids to determine tolerable upper intake levels (UL) for amino acids (Elango, 2023), and another one where human amino acid upper intake levels were calculated based on animal data (Blachier et al. 2021). Based on the available data, the applicant provided a comparison for those amino acids used in Maapliv and their relative total doses for a 70 kg individual at the recommended doses of 1 to 2 g/kg per 24h. Two out of nine amino acids for which there were available data (methionine and lysine) exceeded their safety limits at the maximum Maapliv dose when compared to data available from two reviews. The available data support that in acute use and at recommended doses, there is no particular toxicological risk for the amino acids: arginine, cysteine, glutamic acid, histidine, phenyalanine, serine, threonine and tryptophan. Methionine and lysine are provided at higher than recommended doses, but the applicant mentions that in acute use, no clinically significant adverse effects are expected. It is acknowledged that Maapliv is intended for short-term use in a specific clinical context (acute decomposition in MSUD patients) under expert medical supervision and that the risk-benefit profile in this specific clinical situation is considered different from general supplementation scenarios. The applicant mentioned that knowledge about safe levels of enteral amino acid intakes is sparse, and little is known about human parenteral amino acid toxicity beyond hyperammonaemia, acidosis, and seizures. Recent literature reviews and toxicology studies support the safety profile of Maapliv 's amino acid components at recommended doses for acute use. The amino acid doses proposed in Maapliv are comparable to other approved parenteral amino acid products.

## 2.5.5. Ecotoxicity/environmental risk assessment

Maapliv is constituted by a mixture of amino acids administrated for few days in the treatment of decompensation episodes in Maple Syrup Urinary Disease (MSUD) and does not pose a risk to the environment because the components of the mixture – the amino acids themselves - are metabolized/catabolized and cleared as physiological excretion products of very common presence in the environment, whose concentrations cannot be altered by Maapliv intake. Therefore, according to Q&A guidance on "Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use" (EMA/CHMP/SWP/44609/2010 Rev. 1) and EMEA guidance EMEA/CHMP/SWP/4447/00 corr 2 on "The environmental Risk Assessment of Medicinal Products for Human Use", no further environmental risk assessment is deemed necessary for Maapliv.

## 2.5.6. Discussion on non-clinical aspects

Based on the available literature data and the fact that all the amino acids in Maapliv are naturally occurring and integral to a normal human diet, toxicity from repeat dosing of any of the ingredients at their approved dose is not expected.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, Maapliv, which is a mixture of amino acids, is not expected to pose a risk to the environment.

## 2.5.7. Conclusion on the non-clinical aspects

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, this mixture of amino acids within is not expected to pose a risk to the environment.

Maapliv is considered approvable from a non-clinical perspective.

## *2.6. Clinical aspects*

## 2.6.1. Introduction

- **Tabular overview of clinical studies**

**Table 3.3.1.** List of publications documenting the use of parenteral BCAA-free solutions in MSUD patients

| Author | Year | Country | Study design | Sample size |
|---|---|---|---|---|
| **Main studies** | | | | |
| Servais A. | 2013 | France | Retrospective registry | 4 |
| Blanco Dorado S. | 2017 | Spain | Case report | 1 |
| de Lonlay P. | 2021 | France | Retrospective observational | 54 |
| Alili JM. | 2022 | France | Prospective observational | 24 |
| Sanchez-Pintos | 2022 | Spain | Retrospective observational | 5 |
| **Supporting studies** | | | | |
| Townsend I. | 1982 | USA | Case report | 1 |
| Berry G.T. | 1991 | USA | Case reports | 5 |
| Morton D.H. | 2002 | USA | Case reports and follow-up | 36 |
| Couce Pico M.L. | 2007 | Spain | Case reports | 3 |

## 2.6.2. Clinical pharmacology

No pharmacokinetic studies have been conducted with Maapliv. Very few pharmacological studies have considered the pharmacokinetics of an amino acid mixture solution administered by the IV route.

### *2.6.2.1. Pharmacokinetics*

#### *Absorption*

Not applicable due to a parenteral way of administration.

#### *Bioavailability*

During the parenteral administration of free amino acid mixtures, there is a complete systemic bioavailability of amino acids and the variations of plasma amino acid concentrations depend on the maintenance of amino acid homeostasis and on the interorgan transport, organ uptake and utilization.

The required amount of amino acids is known to be lower in parenterally-fed infants and children than in enterally-fed infants because the supply bypasses the intestine. No clear-cut human data is available, but animal studies suggest that 30% to 50% of the enteral protein intake is used by the intestine in neonates. However, according to the available literature data, there are huge differences in intestinal utilisation of specific amino acid related to age. In addition, a number of amino acids (such as lysine or threonine) are also metabolised and converted into other amino acids in the intestine or liver. Therefore, bypassing the intestine through IV administration may at some point lower the bioavailability of these amino acids and may increase their parenteral requirements.

It is noted that the data to support differences in bioavailability of amino acids administered parenterally and enterally are lacking. This is of particular importance in paediatric patients.

***Distribution***

Studies describing the distribution of amino acids when given in a combination similar to Maapliv have not been found in the available literature.
Initial distribution of most amino acids takes place via the central vascular compartment and extravascular water, and they are transported into cells where incorporation into proteins, conversion to other amino acids, degradation for fuel or deamination occurs. The primary accessible pool of amino acids is thus the venous circulation, which contains only a tiny fraction of the amino acids present in the entire system. Circulation is the main route of amino acid movement between organs, but it is not the location of amino acid metabolic events. Other inaccessible compartments contain the vast majority of amino acids and proteins and are the locations of metabolic transformations.

The body does not store amino acids. Human proteins are synthesized for their functional properties, not for storing. Dietary amino acids exceeding those required for protein synthesis are rapidly catabolised. The appreciable function of interorgan amino acid traffic is homeostatic, leading to the maintenance of the relatively constant extracellular amino acid concentrations in which tissues are bathed.

Bürger *et al*. showed that the infusion into 19 healthy adults of a mixture of amino acids - different from the Maapliv composition as it included BCAAs and with a higher concentration of amino acid (except for aspartic acid, L-cysteine, glutamic acid and serine) - produced a mono-exponential decay up to about 15-20 minutes after the end of the infusion, with glutamic acid and aspartic acid reaching baseline values even earlier. The half-life of most amino acids was between 7 and 10 minutes. Valine and histidine were metabolised somewhat slower, whereas the half-lives of glutamic acid and aspartic acid were substantially shorter. Broad variations in individual values were noted in all specific pharmacokinetic parameters.

In another study (Berard et al., 2001), a mixture of amino acids was continuously administered by infusion to 8 healthy adult volunteers. This mixture did not contain glutamine and taurine and was generally more concentrated in terms of amino acid content than Maapliv. All plasma amino acid concentrations increased rapidly, except for tyrosine and cysteine. Glycine and lysine reached their steady state within 6 hours, whereas the other amino acids reached it within 3 hours. When the parenteral nutrition was stopped, plasma levels of infused amino acids decreased, with most of them returning to their basal levels within three hours, or significantly quicker for lysine ($p < 0.05$), alanine ($p < 0.05$) and proline ($p < 0.01$). The plasma concentrations of the infused amino acids during parenteral nutrition were significantly correlated with intakes, resulting from the rate of appearance (i.e., exogenous source, protein degradation and de novo synthesis) and the rate of disappearance (i.e., protein synthesis, metabolic processing and irreversible oxidation). The rate of appearance of EAAs mostly results from the exogenous source. The rate of appearance of non-EAAs supplied by parenteral nutrition, results from both the exogenous intake and the endogenous synthesis.

In MSUD patients, the elevation of plasma leucine, valine, isoleucine and their respective alpha-keto acids results in rapid neurological deterioration. The transport of EAAs from blood to intracellular spaces in the brain involves movement of amino acids through two biological membranes in series: the blood brain barrier (BBB) and the plasma membrane of brain cells. The transport of large neutral amino acids across the BBB is mediated by a large neutral amino acid transporter (LAT) analogous to the leucine-preferring system in peripheral tissues. This system has a much higher affinity for amino acids as compared with the transport systems in peripheral tissues. The high affinity of large neutral amino acid transport across the BBB provides the physical basis for the selective vulnerability of the brain to the pathological effects of

hyperaminoacidaemia. Amino acid supply to the brain is altered by the increase in plasma concentration of a single large neutral amino acid, for instance leucine in MSUD and phenylalanine in phenylketonuria (PKU), which causes selective competition effects at the BBB relative to peripheral tissue. Tryptophan, phenylalanine, and histidine compete with leucine at the LAT1 transporter site on the BBB.

***Elimination***

***Excretion***

There are no studies describing the excretion of amino acids when given in a combination similar to Maapliv available. In one of the available published studies after administration of amino acid mixture different in composition compared to Maapliv, plasma concentrations of all amino acids reached a maximum at the end of the infusion period. Reduction of concentrations was observed 30 minutes after the end of the infusion. Increase in absolute renal excretion of all amino acids measured was reported.

***Metabolism***

The distribution and metabolism of intravenously infused amino acids is similar to that of dietary proteins. However, there are significant differences, one of which is that when dietary proteins are metabolised, the liver is exposed to high concentrations of amino acids as the proteins enter circulation via the portal vein into the liver. With slow infusion of mixtures like Maapliv, amino acids enter blood circulation directly and the excessive elevation of plasma levels and urinary loss is avoided. The liver is the major organ of amino acid disposal. It is the only organ with the enzymatic armamentarium to metabolize all the amino acids, although its capacity to metabolize BCAAs is limited. The carbon skeletons of these amino acids are not completely oxidized in the liver, even in the fed state, but are largely converted to glucose. Amino acids, arising primarily from muscle proteolysis are the principal substrates for this process.

The kidney plays a major role in the inter-organ metabolism of citrulline, arginine, glycine, serine, and glutamine. Circulating citrulline is converted to arginine, which is released. The enzymes that accomplish this, argininosuccinate synthetase and argininosuccinate lyase, are found in the cells of the proximal tubule. Renal phenylalanine uptake is followed by stoichiometric tyrosine release. The kidney takes up glycine and releases serine. Rather more serine is released than can be accounted for by glycine uptake so serine may also be synthesized from glycolytic intermediates. Quantitatively, for several important amino acids, the kidneys are as important as the gut in intermediary metabolism.

Overall, inter-organ amino acid transport is a major metabolic process by which amino acids are distributed to the different tissues to accomplish their physiological functions and it plays a major role in amino acid homeostasis.

**Amino acid Anabolism**

Most amino acids are used for protein biosynthesis. In addition, many amino acids are synthesised from other available amino acids, whether essential or not.

- Alanine, aspartate, and glutamate play a central role in the metabolism of amino acids. Their metabolism is closely associated with the intermediates of the Krebs cycle. They are produced from their respective

α-ketoacids, pyruvate, oxaloacetate, and α-ketoglutarate, and can be converted back into them via a reversible transamination reaction. Glutamate is synthesised in the mitochondria from α-ketoglutarate and free ammonia. It provides amine groups essential for the biosynthesis of alanine and aspartate. Aspartate may produce alanine through decarboxylation.

- Proline, the only proteinogenic amino acid, is synthesised from glutamate. After phosphorylation of glutamate, proline is synthesized from semi-aldehyde glutamate in three steps.
- The two amides, glutamine and asparagine are produced from their respective acids (Glu, Asp). The reaction of glutamine synthetase, which synthesises irreversible glutamine from glutamate, serves to fix ammoniacal nitrogen produced during amino acid catabolism. Asparagine is produced during a reaction of transamination, in which glutamate gives up its amino group to aspartate.
- The biosynthesis of serine is related to an intermediate of glycolysis. Serine is produced from 3-phosphoglycerate in three steps. The serine itself serves as a starting point for the synthesis of glycine and cysteine. By elimination of the hydroxymethyl group, serine can be converted to glycine. This reaction is reversible, so serine can also be produced from glycine. In general metabolism, serine is used in the synthesis of phospholipids, purines, and cysteine.
- Arginine, the last non-essential amino acid is produced from ornithine. Ornithine is one of the two most important non-proteinogenic amino acids, the other is citrulline.
- Glycine: is synthesised from serine.
- Histidine is converted from L-phenylalanine by an irreversible oxidation by phenylalanine 4-monooxygenase, mostly found in the liver and kidneys.
- Taurine: this sulphur-containing amino acid is a normal constituent of the human diet and is synthesised from methionine and cysteine. Studies have shown that prolonged parenteral nutrition in children with a cysteine- and taurine-free parenteral solution resulted in reduced plasma taurine levels. Taurine deficiency may increase the concentration of glyco-conjugates in bile acids and result in cholestasis. Although the cause of neonatal cholestasis is probably multifactorial, there are data indicating that adequate levels of taurine may prevent cholestasis in neonates. Taurine deficiency may also result in retina dysfunction. Normalisation of plasma and blood cell taurine concentrations has been obtained in adult patients undergoing long-term parenteral nutrition supplemented with intravenous taurine.

In MSUD patients, providing amino acids without BCAAs in combination with carbohydrate and lipid supplementation inhibits protein catabolism and promotes protein anabolism, resulting in decreased leucine levels as a result of anabolic consumption. This process can be considered to be an "endogenic purification".

**Amino acid catabolism**

Amino acids from degraded proteins, from dietary sources, or from supplementation can be used to promote the biosynthesis of new proteins. During starvation, proteins are degraded to amino acids to favour glucose formation.

Amino acid catabolism usually begins with a transamination reaction. The amine group ($-NH^2$) is removed and transferred onto the glutamate (Glu), forming glutamine (Gln), and the corresponding α-ketoacid (α-CA) is formed. The catabolism of amino acids can take place in each cell, but the major organ for this metabolic process is the liver. The liver is responsible for the consistency of amino acid concentrations in the blood and adjusts the rate of synthesis to the body's needs. Amino acids used in the production of glucose are called glycogens. Amino acids whose catabolism provides acetyl-CoA and acetoacetate are called ketogens (Lys, Leu).

Some amino acids not only break down into ketogenic compounds, but also into glucogenic compounds (Phe, Tyr, Thr, Ile, Trp).

There are no studies describing the metabolism of amino acids when given in a combination similar to Maapliv available. However, metabolism of amino acids can be considered established.

***Special populations***

There are literature data on the pharmacokinetics of BCAA-free mixtures in patients with hepatic insufficiency. The available data do not allow any conclusions regarding effects of age on amino acids metabolism. Very scarce literature data on amino acid solution PK in patients with renal insufficiency suggest PK differences between patients with predominantly glomerular and those with predominantly tubular disease chronic renal disease. However, the study was conducted with 10% L-amino acid solution including BCAA.

### *2.6.2.2. Pharmacodynamics*

***Mechanism of action***

***Primary and Secondary pharmacology***

The objective of Maapliv is to provide a mixture of EAAs and non-EAAs free of leucine, isoleucine, and valine in MSUD patients undergoing acute metabolic decompensation. Acute elevation of BCAAs results in rapid neurological deterioration and the reduction in plasma BCAA levels is generally achieved by increasing the net deposition of BCAAs into proteins, which is the main metabolic fate of BCAAs. Providing a source of immediately available amino acids and precursors for amino acid synthesis promotes protein anabolism and inhibits protein catabolism. The administration of BCAA-free solution reduces BCAAs by removing excess BCAAs and metabolites, which are used for protein anabolism.

Acute episodes of metabolic decompensation require prompt medical intervention to counteract the organ and metabolic distress and to prevent subsequent neurological damage. Several therapeutic strategies have been considered to reduce leucine plasma levels, promote net anabolic processes, inhibit catabolism of skeletal muscle, maintain electrolytic and plasma amino acid homeostasis, and balance nitrogen fluxes effectively and rapidly. The literature has produced a large range of amino acid mixture preparations designed for this purpose. The Maapliv solution meets the proposed objectives with a high concentration of alanine as a substrate and for the transamination of a-keto-acids that accumulate during catabolic processes. Moreover, elevated plasma concentrations of alanine counteract the increased leucine plasma concentration. Neutral amino acids are useful to maintain amino acid homeostasis in plasma and are needed as fuel in order to compete with the degradation of BCAAs released by skeletal muscles. Tyrosine, tryptophan, phenylalanine, and histidine compete with leucine at the LAT1 transporter site on the BBB. Cysteine and methionine provide sulphur. It has been demonstrated that taurine plays an important role in growth, the development of the brain and maturation of retinal function, and is involved in hepatic biliary conjugation, especially in the neonates, where bile acids are conjugated almost exclusively with taurine until sometime after birth. Taurine should be included in amino acid solutions for infants and children as its deficiency may induce cholestasis. Glutamic acid is included as the glutamine system is the foundation of nitrogen exchange, intervening in an essential way during anabolic and catabolic processes. It is involved in the synthesis of non-EAAs during anabolism and in the neutralisation and elimination of ammonia during catabolism. Requirements for arginine rise with increases in metabolic activity and stressful events. The parenteral administration of arginine thus constitutes a stimulus for the release of growth hormone in humans.

It is noteworthy that when a single essential amino acid is deficient in the diet, the amount of protein that can be synthesized is limited. Since the limiting amino acid also limits the use of all other dietary amino acids for protein synthesis, the body must oxidize excess amounts of these amino acids. If one increases the dietary amount of the limiting amino acid, protein synthesis will increase and so will the utilization of the other dietary amino acids, which in turn inhibits their oxidation. Once the requirement for the limiting amino acid is reached, further increases in its dietary intake will cause no further increase in protein synthesis, inhibit the oxidation of the other essential amino acids.

## 2.6.3. Discussion on clinical pharmacology

Overall, available clinical pharmacology data is rather limited. There is a complete systemic bioavailability of amino acids after a parenteral administration. No literature data on the distribution and excretion of a combination of amino acids comparable to Maapliv is available. No differences in the distribution and metabolism of intravenously infused amino acids compared to dietary proteins should be expected.

The aim of the administration of the essential amino acids mixture free of leucine, isoleucine, and valine in MSUD patients undergoing acute metabolic decompensation is to prevent acute elevation of BCAAs which would result in rapid neurological deterioration.

The following mechanism of action is being stated in the SmPC: *In patients with MSUD decompensation, Maapliv in combination with carbohydrate and lipid supplementation allows blocking protein catabolism and promotes protein anabolism, reducing the corresponding alpha-keto acid levels.*

## 2.6.4. Conclusions on clinical pharmacology

No major issues are identified in respect to the clinical pharmacology. Although only limited literature data regarding clinical pharmacology of amino acids administered parenterally is available, it is considered sufficient to support this MAA.

Maapliv is approvable from a clinical pharmacology perspective.

## 2.6.5. Clinical efficacy

The Applicant conducted the literature search which consisted of an automated search across several medical literature databases, including PubMed, The Cochrane Library and Embase, up until September 2022, without any language restrictions.

Keywords were selected using the index list and focussed on the targeted patient population and the parenteral administration of BCAA-free amino acid mixtures.
The selection of publications was a 2-step process, initially screening titles, and abstracts (step 1), and subsequently reviewing the full text (step 2) to ensure the text was relevant, with active substances similar to those in Maapliv, including publications in the targeted patient population in the European Union. Any type of study design was considered as well as 'named patient use' reports.

Publications on the management of MSUD with BCAA-free mixtures or solutions were limited but were considered to be relevant given the rarity of the disease.

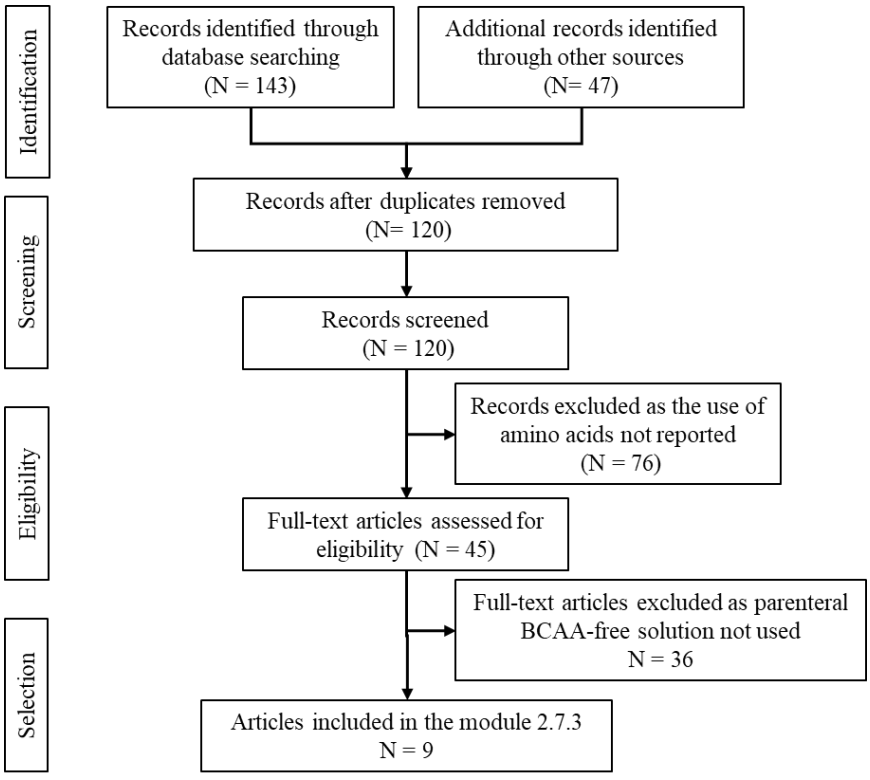



**Figure 3.1.1.** PRISMA diagram of the literature review

Of 120 publications screened on step 1, a total 45 publications were assessed for eligibility.

Out of 45 eligible publications, 36 were excluded as they did not document the parenteral use of a BCAA-free solution in MSUD patients undergoing decompensation episodes. The final selection included 9 publications reporting the parenteral use of BCAA-free mixtures in patients with MSUD undergoing decompensation episodes (Table 3.3.4.1.):

- Five publications report on the parenteral use in Europe of a BCAA-free mixture the same as the Maapliv formulation (Servais et al. 2013, Abi-Wardé et al. 2017, de Lonlay et al. 2021, Alili et al. 2022, Blanco Dorado et al. 2017, Sanchez-Pintos et al. 2022), here designated as "main studies";

- Four published studies reported the parenteral use of BCAA-free mixtures different from Maapliv (Townsend and Kerr 1982, Berry et al. 1991, Morton et al. 2002, Couce Pico et al. 2007). These studies are designated as "supporting studies".

**Table 3.3.4.1.** List of publications documenting the use of parenteral BCAA-free solutions in MSUD patients

| Author | Year | Country | Study design | Sample size |
|---|---|---|---|---|
| **Main studies** | | | | |
| Servais A. | 2013 | France | Retrospective registry | 4 |
| Blanco Dorado S. | 2017 | Spain | Case report | 1 |
| de Lonlay P. | 2021 | France | Retrospective observational | 54 |
| Alili JM. | 2022 | France | Prospective observational | 24 |
| Sanchez-Pintos | 2022 | Spain | Retrospective observational | 5 |
| **Supporting studies** | | | | |
| Townsend I. | 1982 | USA | Case report | 1 |
| Berry G.T. | 1991 | USA | Case reports | 5 |
| Morton D.H. | 2002 | USA | Case reports and follow-up | 36 |
| Couce Pico M.L. | 2007 | Spain | Case reports | 3 |

Given the scarcity of individuals with MSUD, these observational studies and registries offer a comprehensive overview of the combined clinical experience associated with the administration of an intravenous BCAA-free solution equivalent to Maapliv for managing episodes of acute decompensation. These studies encompassed a significant proportion of the total MSUD populations in France and Spain. Furthermore, patients who received the intravenous BCAA-free solution equivalent to Maapliv were subjected to long-term follow-up, with some cases spanning over a decade. Although limited by the inherent ethical and practical challenges encountered in conducting treatment studies involving rare diseases, this collective body of literature serves as valuable evidence pertaining to the efficacy, safety, and clinical utility of this intervention.

### *2.6.5.1. Dose response study(ies)*

The rationale for this dosing range was based on WHO daily protein requirements for adults and children (WHO 2007) and increased by approximately 20 (van Wegberg et al. 2017). Further, the German 2009 parenteral nutrition guidelines for basic amino acid requirements for adult patients were used. According to this guideline, the starting dose is 0.8 g/kg/day which could be increased with the maximum dose of 2.5 g/kg/day in exceptional cases. Additional references are also being made to the phenylketonuria treatment guidelines which are recommending: 2 to 3 g/kg per day of protein in infancy, to 1.2–2 g/kg per day by the age of 10 years and all but one centre gave 1–1.5 g/kg per day of protein equivalent to patients >10 years of age. The justification for the dose as provided by the applicant is acknowledged.

It is important to note that the response to treatment should be monitored, and the dose could be adjusted depending on many factors.

### *2.6.5.2. Main study(ies)*

Five publications report on the parenteral use in Europe of a BCAA-free mixture the same as the Maapliv formulation (Servais et al. 2013, Abi-Wardé et al. 2017, de Lonlay et al. 2021, Alili et al. 2022, Blanco Dorado et al. 2017, Sanchez-Pintos et al. 2022), here designated as "main studies".

**Servais et al., (2013)**

**Study Title:** Treatment of acute decompensation of maple syrup urine disease in adult patients with a new parenteral amino-acid mixture

This study was designed to compare the efficacy and tolerability of a new parenteral treatment with those of standard enteral feeding via nasogastric tube in MSUD patients suffering from metabolic decompensation. The study profile is displayed in Table 3.3.4.2.1.

**Table 3.3.4.2.1.** Study profile

| | | |
|---|---|---|
| Objectives | To compare the efficacy and tolerance of a new parenteral treatment with standard enteral feeding via nasogastric tube in MSUD patients suffering from metabolic decompensation | |
| Methods | Design | Observational study of the prospective use of parenteral BCAA-free solution in patients previously treated with standard enteral feeding. Seventeen decompensation episodes in four adult patients with MSUD treated with a parenteral amino-acid mixture in 2010 (group P, enteral feeding refused) were compared to 18 previous episodes in the same patients treated by enteral feeding between 2005 and 2010 (group E). |
| | Patients | Adult patients with classic MSUD with neonatal onset, without neurological symptoms and leucine plasma concentration between 763 µmol/L (10mg/dL) and 1525 µmol/L (20 mg/dL). |
| | Treatments | Enteral feeding through a nasogastric tube at the dose of 1 to 2 g per kilogram of body weight per day of one of the following BCAA-free amino-acid mixtures: MSUD 2 secunda® (Milupa), or Maxamum MSUD® (SHS Nutricia), containing 290 kcal and 84 g of amino acids per litre.<br>Parenteral BCAA-free mixture corresponding to the MAAPLIV formulation in 500 mL bags, containing 200 kcal and 52 g of amino acids per litre to be infused through a peripheral intravenous line, at the dose of 1.5 g/kg/day.<br>All patients received oral valine and isoleucine supplementation adjusted to plasma concentrations determined daily and additional energy provided by glucose and lipid solutions. |
| | Statistical analyses | - Chi-square test and the Fisher's exact test applied for dichotomous and categorical data, respectively, and the unpaired t test used to compare continuous variables.<br>- Repeated measures two-way ANOVA used to compare the curves of mean leucine concentration decrease in the two groups.<br>- Two-tailed p values <0.05 were regarded as statistically significant. |
| Evaluation criteria | Primary efficacy | Mean decrease in plasma leucine concentration |
| | Secondary efficacy | Mean duration of hospitalisation |

**Results**

Seventeen decompensation episodes in four adult patients with MSUD treated with a parenteral amino-acid mixture (group P) were compared to 18 previous episodes in the same four patients treated by enteral feeding (group E). All four patients had subsequently refused enteral feeding.
Patient characteristics and episode evolutions are summarised in Table 3.3.4.2.2.

**Table 3.3.4.2.2.** Patient characteristics and evolution

| | Group E | Group P |
|---|---|---|
| Age, years ± SD | 25.2±5.1 | |
| Male/female | 3/1 | |
| Decompensation episodes (N) | 18 | 17 |
| Cause of decompensation episodes | | |
| Diet deviation | 10 | 6 |
| Infection | 3 | 3 |
| Anorexia | 2 | 5 |
| Other | 3 | 3 |
| Leucine concentration on D0 (µmol/L) | 1196.9±312.6 | 1196.9±289.7 |
| Time to normalisation of Leu conc. (days) | 3.4±1.0 | 3.8±1.8 |
| Duration of hospitalisation (days) | 3.7±0.9 | 4.1±1.9 |
| Leucine concentration at discharge (mmol/L) | 343.1±236.3 | 320.2±129,6 |
| Leucine normalisation at discharge n (%) | 11/18 (61.1) | 13/17 (76.5) |
| Enteral feeding refusal | 4 | - |
| Dialysis (N) | 1 | 0 |
| Calories (kcal/day) | 4812±994 | 4687±364 |
| Amino acids (g/day) | 78.3±26.4 | 73.4±10.2 |

Leucine plasma concentration was normalised (<381 µmol/L) 4 days after initiation of the parenteral administration of the BCAA-free mixture. However, in 7 cases in the E group and 4 cases in the P group, patients were discharged before leucine normalisation against the attending physicians' advice. The decrease in the plasma Leu concentration was significantly greater in group P than in group E (p = 0.0026, by two-way ANOVA). Furthermore, the treatment was better tolerated and accepted than nasogastric tube insertion.

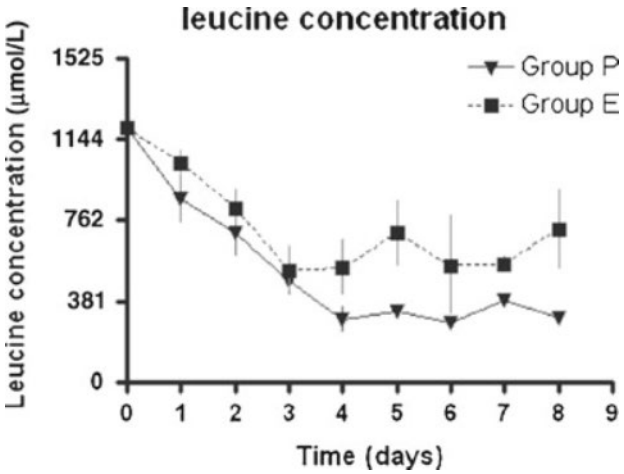



**Figure 3.3.4.2.1.** Comparison between the decrease in mean leucine concentration in the enteral (E) and in the parenteral (P) groups (p = 0.0026 by two-way ANOVA). Mean ± SEM

Leucine levels reduction or normalisation in all patients and this effect was associated with clinical improvement and resolution of the acute episodes in all cases.

**Blanco Dorado et al., (2017)**

**Study title:** Case - report of the parenteral use of branched-chain-free amino acids mixture for the urgent management of severe MSUD decompensation.

This patient was admitted to neonate intensive care unit at the age of 7 days. The diagnosis of MSUD was confirmed with a leucine plasma level of 29.6 mg/dL (2258.5 µmol/L).
Treatment included a dietary restriction of BCAAs with supplementation in vitamins, valine, isoleucine, and thiamine. Enteral feeding with a BCAA-free formulation (MSUD Anamix Infant®) was implemented.
At the age of 2 years and 3 months the patient was admitted to the Emergency Department and diagnosed with metabolic decompensation in a context of adenovirus infection.

**Results**

**Table 3.3.4.2.3.** Summary of the management of the MSUD decompensation episodes

| Days | Events | Key treatment/feeding measures |
|---|---|---|
| 1 | Hospital admission for adenovirus respiratory infection | Protein-free diet |
| 2 | Transfer to intensive care unit for metabolic decompensation | Continuous haemofiltration |
| 3 | Enteral feeding via nasogastric tube | MSUD Anamix Infant®<br>Protein-free parenteral feeding<br>Tailored supplementation of isoleucine, valine, and alanine |
| 5 | Vomiting<br>Increase in BCAA plasma levels | Enteral feeding discontinued<br>Protein-free parenteral feeding |
| 6-14 | Episodes of metabolic acidosis, vomiting.<br>Implementation of a transpyloric tube to improve enteral feeding tolerability.<br>Persistence of elevated leucine plasma levels. | Enteral feeding with MSUD Anamix Infant®, protein-free parenteral.<br>Reduced supplementation of isoleucine and valine. |
| 15 | Elevated leucine plasma levels. | Initiation of parenteral feeding with the BCAA-free mixture corresponding to the MAAPLIV® formulation (140 kcal/kg daily).<br>Enteral feeding discontinued. |
| 18 | Major reduction in leucine plasma levels and normalisation (Figure). | Reduced supplementation of valine and isoleucine. |
| 20 | Persistence of low leucine plasma levels. | Addition of enteral feeding and reduction in parenteral feeding. |
| 24 | | Supplements of valine, alanine, isoleucine discontinued. |
| 26-56 | Enteral feeding well tolerated.<br>Absence of vomiting, significant improvement in neurologic symptoms and haemodynamic condition. | Low-doses supplementation in isoleucine.<br>Oral feeding with natural yogurt. |
| 58-67 | Good oral feeding tolerability and discharge from hospital on day 67. | |

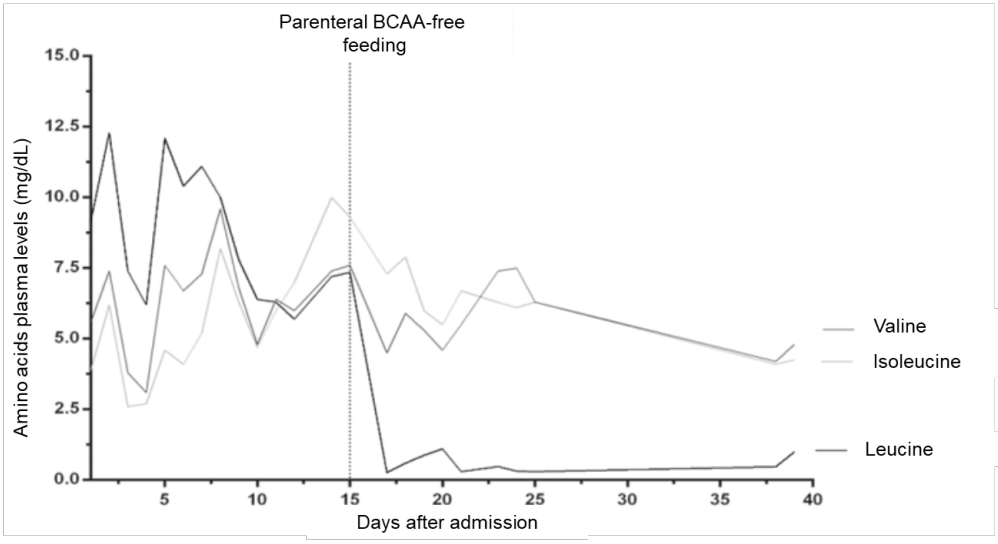



**Figure 3.3.4.2.2.** Evolution of branched-chain amino acids plasma levels (*Plasma levels of 10.0 mg/dL correspond to 763 µmol/L. Leucine levels were normalised two days after initiation of parenteral feeding with the BCAA-free mixture).*

**De Lonlay et al., (2021)**

**Study title:** Real-world management of maple syrup urine disease metabolic decompensations with BCAA-free supplements in France and Germany: a retrospective observational study

This retrospective observational study of 126 decompensation episodes from 54 MSUD patients treated at five centres in France and Germany from 2010 to 2016, describes profiles of episodes and compares decompensation outcomes for patients stratified into those who received enteral/oral or intravenous (IV) BCAA-free formula, and by age.

The study profile is displayed in Table 3.3.4.2.4

**Table 3.3.4.2.4.** Study profile

| | |
|---|---|
| Objectives | 1) to describe the real-world management of metabolic decompensation episodes in a large cohort of MSUD patients hospitalized in five centres in France and Germany, and 2) to explore the management of decompensation episodes with oral/enteral or IV BCAA-free formulas in terms of laboratory and clinical outcomes. |
| Methods | |
| Design | Observational, non-interventional retrospective cohort study. Medical records of MSUD patients hospitalized for decompensation episodes between January 1, 2010 and December 31, 2016 were collected at four rare-disease reference centres in France and one in Germany. |
| Patients | Patients with biochemically confirmed diagnosis of MSUD before the onset of the decompensation episode defined by plasma leucine levels above 381 µmol/L (5.0 mg/dL) at hospital admission. Patients having experienced at least 1 decompensation episode that required at least 1 day (>24 hours) of hospitalisation and with a plasma leucine >381 µmol/L at hospital admission were included in the study.<br>'Episode resolution' was defined according to the physician's judgement based on biological and clinical outcomes and assumed a normalisation or pseudo normalisation of leucine levels (<381 µmol/L), together with resolution of symptoms. |

| | |
|---|---|
| Treatments | Oral/enteral BCAA-free formulas (brand names not recorded) and since 2010 use of a mixture of BCAA-free amino acid delivered IV equivalent to the MAAPLIV® formulation. |
| Statistics | Exploratory analyses by subgroups of route of administration of BCAA-free formula (oral/enteral and IV) and by age (paediatrics versus adult). Median survival times and the corresponding 95% confidence intervals (CI) were estimated by the Kaplan Meier method using a two-sided log-rank test. A P-value of <0.05 was considered significant. |
| Evaluation criteria | Normalisation of plasma leucine levels.<br>Duration of hospitalisation. |

**Results**

A total of 55 patients having experienced 129 decompensation episodes were included in the study. One patient and 3 episodes were excluded from the analysis due to leucine plasma levels < 381 µmol/L. Baseline data were available for 126 decompensation episodes. Stratification by treatment route was not available for 5 decompensation episodes. A total of 121 decompensation episodes in 54 patients were retrospectively stratified to either the "oral/enteral" route (n=69, 57.0%) or the intravenous (IV) administration route (n=36, 29.8%). Decompensation episodes were also stratified at the time of episode onset to a "paediatric" (aged <18 years, n=79) or an "adult" group (aged ≥18 years, n=26).

**Table 3.3.4.2.5.** Patients and decompensation episodes characteristics

| | |
|---|---|
| **Gender n (%)** | |
| Male | 27 (50) |
| Female | 27 (50) |
| **MSUD diagnosis <2 month of age, n patients (%)** | 49 (90.7) |
| **Signs of decompensation episodes at hospital admission** | 43/126 (34.1) |
| Gastrointestinal disorders n (%) | 40/126 (31.8) |
| Nervous system disorders n (%) | |
| **Main causes of metabolic decompensation n (%)** | |
| Febrile illness/infection | 51/126 (40.5) |
| Non-compliance to dietary restrictions | 28/126 (22.2) |
| Surgery or planned surgery | 8/126 (6.3) |
| **Elevated leucine levels at baseline n (%)** | |
| Mildly <762 µmol/L | 51/126 (40.5) |
| Moderately <1144 µmol/L | 47/126 (37.3) |
| Severely <1525 µmol/L | 13/126 (10.3) |
| Critically ≥1525 µmol/L | 15/126 (11.9) |

*BCAA-free administration*

The most common route of administration (as percentage of total administrations) for BCAA-free formula was nasogastric tube (n = 69, 55.2%), followed by oral (n = 60, 48.0%), IV (n = 51, 40.8%), and gastrostomy (n = 2, 1.6%). Reasons for administration of IV BCAA-free formula included gastric intolerance (n = 17, 33.3%), refusal to undergo nasogastric tubing (n = 16, 31.4%), "emergency" (n = 7, 13.7%) or coma patients (n = 4, 7.8%), and as prophylaxis before a planned surgery (n = 3, 5.9%). Valine followed by leucine and isoleucine were re-introduced a mean 4.2 days after onset of the decompensation episode to maintain the BCAA balance.
Enteral or oral BCAA-free formula was more commonly used in children than in adults (94% and 44.4%, respectively) while the IV formulation was more commonly used in adults than in children (85.2% and 21.7%, respectively).

*Clinical outcomes*

The mean (±SD) time to first leucine normalization (<381 µmol/L) was 68.4 hours (±52.9) in the oral/enteral group and 95.6 hours (±79.2) in the IV group. Leucine plasma concentrations at the 7th day were 279.8 (197.9) µmol/L and 293.7 (121.9) µmol/L in the oral/enteral and the IV groups, respectively. Overall, leucine normalization at discharge was achieved in 76.8% (n=53) of episodes in the oral/enteral group and in 80.6% (n=29) in the IV group. At discharge, leucine levels had decreased by a mean of 548.5 µmol/L in the oral/enteral group and by 657.2 µmol/L in the IV group.
Except for one patient per group, all decompensations were resolved at time of discharge. Mean (±SD) time to episode resolution from baseline was 9.2 days (±6.2) in the oral/enteral group and 7.4 days (±4.6) in the IV group. The mean (±SD) length of hospital stay was 6.6 days (±4.2) days for the oral/enteral group and 5.4 days (±2.7) for the IV group. The mean (±SD) length of Intensive Care Unit (ICU) stay was 3.2 days (±2.1) for the oral/enteral group and 2.5 days (±1.3) for the IV group.

**Table 3.3.4.2.6.** Clinical outcomes of decompensation episodes treated with BCAA-free mixtures

| Outcomes | Oral/enteral 69/126 | Intravenous 36/126 |
|---|---|---|
| Leucine levels at baseline, µmol/L, mean (SD) | 791.4 (267.7) | 921.1 (333.5) |
| Leucine category at admission, µmol/L: | | |
| Mild (381 to 761) | 36 (52.2%) | 10 (27.8%) |
| Moderate (762 to 1143) | 28 (40.6%) | 17 (47.2%) |
| Severe (1144 to 1524) | 3 (4.3%) | 8 (22.2%) |
| Critical (≥ 1525) | 2 (2.9%) | 1 (2.8%) |
| Time to 1st leucine normalisation, hours, mean (SD) | 68.4 (±52.9) | 95.6 (79.2) |
| Leucine normalisation at discharge n (%) | 53 (76.8) | 29 (80.6) |
| Leucine decrease from baseline at discharge, µmol/L | 548.5 | 657.2 |
| Time to DE resolution, mean, days (SD) | 9.2 (6.2) | 7.4 (4.6) |
| Length of hospital stay, days (SD) | 6.6 (4.2) | 5.4 (2.7) |
| Need for intensive care unit (ICU), n (%) | 53/69 (76.8) | 29/36 (80.6) |
| Length of stay in ICU, mean, days (SD) | 6/69 (8.7) | 4/36 (11.1) |
| Overall | 3.2 (2.1) | 2.5 (1.3) |
| In paediatric patients | 8.8 (6.0) | 6.8 (3.6) |
| In adult patients | 15.8 (4.3) | 7.7 (5.2) |

The changes in leucine levels in the oral/enteral group compared with those of the IV group during the first seven days of treatment are shown in Figure 3.3.4.2.3.

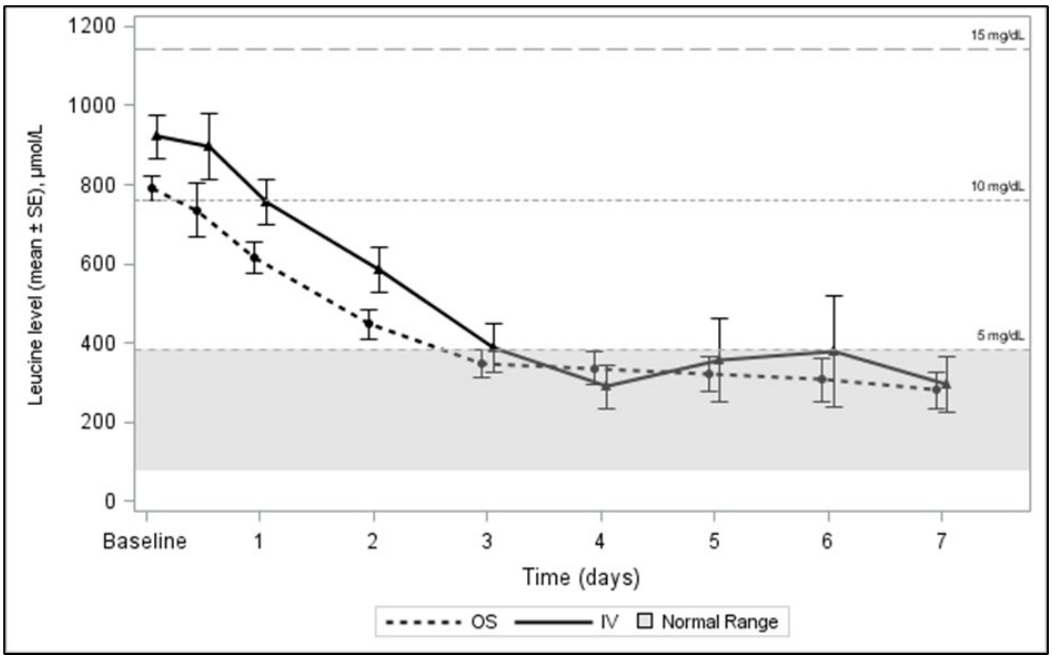



**Figure 3.3.4.2.3.** Changes in leucine plasma levels over the first 7 days, by nutrition route

*OS = oral/enteral nutrition; IV = parenteral nutrition. Leucine plasma levels are normalised by day 4.*

*Safety*

Seven serious adverse events were reported in 2 patients receiving oral/enteral nutrition during hospitalisation. These included fever, soreness, nausea, headache, abdominal pain, and vomiting. Only nausea and vomiting were reported as treatment-related, due to poorly tolerated enteral BCAA-free formulation. No SAE was reported the intravenous group. All adverse events were resolved.

**Alili et al., (2022)**

**Study title:** Intravenous administration of a specific amino-acid mixture in children and adults with acute decompensation of maple syrup urine disease: a prospective multicentre observational study.

This prospective study reports the use of an IV BCAA-free solution equivalent to Maapliv for the management of MSUD decompensation episodes in patients admitted in 6 metabolic disease referent centres in France. The study profile is reported in Table 3.3.4.2.7.

**Table 3.3.4.2.7.** Study profile

| | |
|---|---|
| Objectives | To evaluate the effectiveness and safety of an IV BCAA-free formula equivalent to MAAPLIV® in the management MSUD metabolic decompensation episodes between 2010 and 2016.<br>Additional analyses included corroboration of a revised treatment algorithm (de Lonlay et al. 2013) in terms of time to normalise leucine levels and to explore rates of decrease in leucine levels in terms of disease aetiology at admission. |
| Methods | |
| Design | Prospective observational study |

| | |
|---|---|
| Patients | MSUD patients experiencing metabolic decompensation episodes treated with an intravenous BCAA-free solution at 6 specialist referent centres in France. |
| Treatment | BCAA-free amino acid delivered IV and corresponding to the MAAPLIV® formulation at the dose of 1.0 to 2.0 g/kg/day. |
| Statistics | Dichotomous data are provided in percentages, continuous data are provided as means ± standard deviation (SD). Dichotomous variables were analysed using the chi-square test categorical data were compared using Fisher's exact test. Repeated measures of two-way ANOVA were used to compared leucine levels. Two-tailed p values of <0.05 were considered statistically significant. |
| Efficacy criteria | Leucine normalisation < 380 µmol/L |

**Results**

*Patients/decompensation episodes characteristics*

A total of 24 patients diagnosed MSUD were admitted to hospital from March 2010 to March 2016 having experienced 126 decompensation episodes. Eleven patients (52%) were children aged below 15 years and 10 patients (48%) were adults while 8 (38%) were female and 13 (62%) were male.
The safety set of 126 episodes corresponded to all episodes treated with IV BCAA-free solution during the study. The efficacy set (n = 102 episodes, 22 in children and 80 in adults, 16 patients) included all episodes treated with the IV BCAA-free solution which did not involve exogenous epuration, and with a documented leucine concentration at admission ≥ 381 µmol/L (5 mg/dL), and with at least one documented leucine level post-admission. Multiple episodes were reported in one patient analysed as both a child and an adult.
MSUD was mostly neonatal, firstly diagnosed at the median age of 10.5 days. Of the 126 decompensation episodes which involved admittance to hospital during the study follow-up, 31% (n = 39) occurred in children at the mean/median age of 7.8/8 years (11 episodes occurred before 2 years of age) and 69% (n=87) occurred in adults at the mean/median age of 26.5/27 years. At presentation, mean leucine plasma concentration was ≥ 381 µmol/L in 113/126 (89.7%) episodes.
In the efficacy set of 102 analysable episodes mean (SD) leucine was 985 (279) µmol/L with 80% of episodes above 760 µmol/L at admission [(900 (267) in children and 1008 (279) in adults]. In the subgroup of infants <2 years, the mean was 698 (307) µmol/L.

**Table 3.3.4.2.8.** Clinical and biochemical characteristics of decompensation episodes at admission

| | **Children** N = 39 | **Ado/adults** N = 87 | **All** N = 126 |
|---|---|---|---|
| **Sex** | | | |
| Male | 10 (7.9%) | 52 (41.3%) | 62 (49.2%) |
| Female | 29 (23.0%) | 35 (27.8%) | 64 (50.80%) |
| **Age at admission, years** | | | |
| N | 39 | 87 | 126 |
| Mean (SD) | 7.3 (5.1) | 26.5 (6.2) | 20.6 (10.7) |
| Median | 8.0 | 27.0 | 21.0 |
| Min - Max | 0.0-14.0 | 17.0-37.0 | 0.0 - 37.0 |
| **Main precipitating factors** | | | |
| Infectious episodes | 16 (41.0%) | 25 (28.7%) | 41 (32.5%) |
| Dietary non-adherence | 7 (17.9%) | 30 (34.5%) | 37 (29.4%) |
| Neonatal/ First diagnosis | 2 (5.1%) | 0 (0.0%) | 2 (1.6%) |
| Vaccination | 0 (0.0%) | 2 (2.3%) | 2 (1.6%) |
| Stress | 0 (0.0%) | 2 (2.3%) | 2 (1.6%) |
| Other (no detail) | 2 (5.1%) | 17 (19.5%) | 19 (15.1%) |
| **Neurological disorders** | 19 (48.7%) | 8 (9.2%) | 27 (21.4%) |

| | Children<br>N = 39 | Ado/adults<br>N = 87 | All<br>N = 126 |
|---|---|---|---|
| **Clinical signs** | 27 (69.2%) | 51 (58.6%) | 78 (61.9%) |
| Nausea/vomiting/GE | 23 (59.0%) | 26 (29.9%) | 49 (38.9%) |
| Anorexia | 5 (12.8%) | 11 (12.6%) | 16 (12.7%) |
| Digestive troubles inc. diarrhoea | 3 (7.7%) | 5 (5.7%) | 8 (6.3%) |
| Gastroenteritis | 3 (7.7%) | 4 (4.6%) | 7 (5.6%) |
| **Leucine category at admission** | | | |
| < 380 µmol/L | 6 (15.4%) | 2 (2.3%) | 8 (6.3%) |
| [380 - 760] | 8 (20.5%) | 13 (14.9%) | 21 (16.7%) |
| > 760 | 22 (56.4%) | 70 (80.5%) | 92 (73.0%) |
| Missing | 3 (7.7%) | 2 (2.3%) | 5 (4.0%) |

*Management of decompensation episodes*

Reasons to initiate the administration of the IV BCAA-free solution were plasma leucine levels >760 µmol/L in 95/126 episodes, between 380 and 760 µmol/L in 17/126 episodes, and presence of neurological disorders or vomiting in 9 episodes while data for the remaining 5 episodes were missing. Children were treated with continuous IV BCAA-free solution at doses of 0.8 to 2.0 g/kg/day, for 4.8 days and adults for 3.8 days at doses of 0.5 to 2.6 g/kg/day. Median doses were 1.1 g/kg/day in adults and 2.0 g/kg/ day in children, shown in Table 3.3.4.2.9.

**Table 3.3.4.2.9.** Treatment of decompensation episodes during hospitalisation

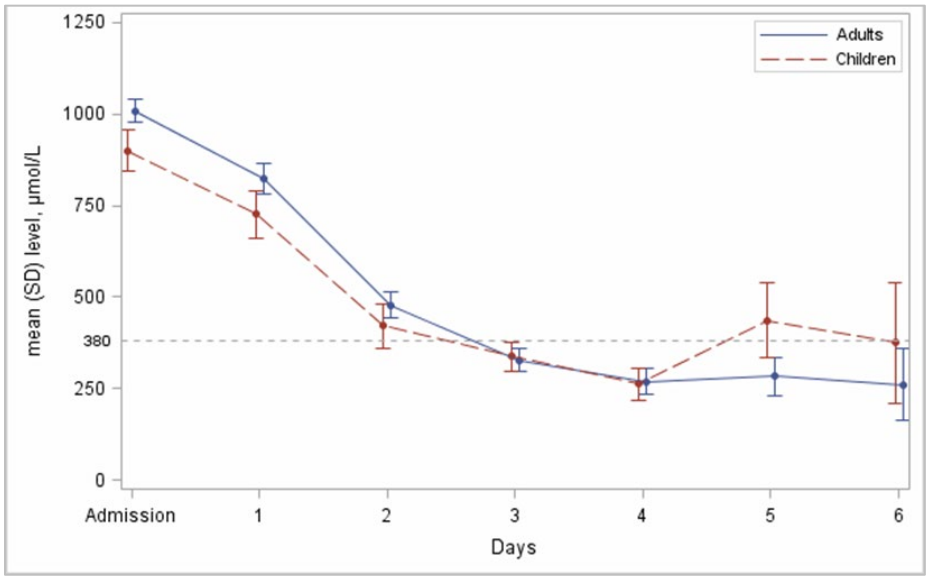
| | Children<br>N = 39 | Adults<br>N = 87 | All<br>N = 126 |
|---|---|---|---|
| **Decision to start IV BCAA** | | | |
| Leucine > 760 µmol/L | 23 (59.0%) | 72 (82.8%) | 95 (75.4%) |
| Leucine between 380 and 760 µmol/L | 6 (15.4%) | 11 (12.6%) | 17 (13.5%) |
| Neurological disorders/vomiting | 6 (15.4%) | 3 (3.4%) | 9 (7.1%) |
| Missing | 4 | 1 | 5 |
| **IV BCAA treatment duration, days** | | | |
| N | 30 | 75 | 105 |
| Mean (SD) | 4.8 (5.4) | 3.8 (1.4) | 4.1 (3.1) |
| Median | 3.0 | 4.0 | 4.0 |
| Min - Max | 1.0 - 30.0 | 1.0 - 8.0 | 1.0 - 30.0 |
| **IV BCAA treatment duration w/o HF/HD, days** | | | |
| N | 23 | 74 | 97 |
| Mean (SD) | 3.4 (2.0) | 3.8 (1.4) | 3.7 (1.6) |
| Median | 3.0 | 4.0 | 4.0 |
| Min - Max | 1.0 - 8.0 | 1.0 - 8.0 | 1.0 - 8.0 |
| **Dose (g/kg/day)** | | | |
| N | 26 | 40 | 66 |
| Mean (SD) | 1.9 (0.3) | 1.3 (0.5) | 1.5 (0.5) |
| Median | 2.0 | 1.1 | 1.5 |
| Min - Max | 0.8 - 2.0 | 0.5 - 2.6 | 0.5 - 2.6 |
| **Extracorporeal epuration (HF/HD)** | 7 (17.9%) | 1 (1.1%) | 8 (6.3%) |
| **Continuous enteral feeding** (during or after IV BCAA-free) | 19 (48.7%) | 4 (4.6%) | 23 (18.3%) |

*HF/HD = haemofiltration/haemodialysis*

*Efficacy*

Eighty-three percent (n = 85) of the efficacy set of 102 episodes with fully documented leucine levels were normalised (to below 381 µmol/L) in a median duration of 3 days (2 days in children and 3 days in adults) (Figure 3.3.4.2.4.).

**Figure 3.3.4.2.4.** Leucine plasma levels overtime for adults and children (n = 102)




A total of 17 episodes did not achieve leucine plasma levels normalisation (<380 µmol/L) while leucine levels decreased from the >760 µmol/L to the [380-760] µmol/L category in 14 of these episodes. In one episode leucine levels remained above 760 µmol/L despite a 48% reduction from admission of leucine plasma levels. In each case discharge was approved by the attending physician even though leucine levels had not been normalised to below 380 µmol/L.

**Table 3.3.4.2.10.** Changes in leucine plasma levels between admission and normalisation or last measurement by leucine level category

| | | **Leucine at admission** | | |
|---|---|---|---|---|
| | | [380-760] µmol/L | >760 µmol/L | **Total** |
| **Leucine at normalisation or last measurement** | < 380 µmol/L | 18 | 67 | **85** |
| | [380-760] µmol/L | 2 | 14 | **16** |
| | >760 µmol/L | 0 | 1 | **1** |
| | **Total** | **20** | **82** | **102** |

At the time of normalisation mean leucine levels were 216 µmol/L in children and 217 µmol/L adults. The mean/median reduction from admission was 75/77% and the observed average rate of decrease was 274 µmol/L/day, which was similar for both children and adult groups.

**Table 3.3.4.2.11.** Summary of normalisation of leucine levels in analysable episodes (n = 102)

| | | Children N = 22 | Adults N = 80 | All N = 102 |
|---|---|---|---|---|
| **Leucine at admission (µmol/L)** | Mean (SD) | 900 (267) | 1008 (279) | 985 (279) |
| **Normalisation of leucine** | < 380 µmol/L | 18 (81.8%) | 67 (83.8%) | 85 (83.3%) |
| **Leucine at normalisation (µmol/L)** | N | 18 | 67 | 85 |
| | Mean (SD) | 216 (116) | 217 (105) | 217 (107) |
| | Median | 248 | 213 | 221 |
| | Min - Max | 30 - 374 | 23 - 381 | 23 - 381 |
| **% change from admission** | N | 18 | 67 | 85 |
| | Mean (SD) | -71 (20.9) | -76 (14.8) | -75 (16.2) |
| | Median | -75 | -78 | -77 |
| | Min - Max | -97 - 22 | -97 - 31 | -97 - 22 |
| **Rate of decrease (µmol/L/day)** | N | 18 | 67 | 85 |
| | Mean (SD) | -267 (146) | -276 (117) | -274 (123) |
| | Median | -264 | -267 | -267 |
| | Min - Max | -526 - 53 | -724 - 50 | -724 - 50 |

*N = number of decompensation episodes with fully documented leucine levels*

Overall, clinical improvement was reported in 98% (124/126) of episodes, with 38 (99%) in children and 86 (99%) in adults respectively.
At discharge, considering all decompensation episodes, the median/mean leucine level was 264 µmol/L (275 µmol/L in children 261 µmol/L in adults). Patients were discharged from hospital after mean/median of 5.8/5 days, with 7.1/6.0 days in children and 5.2/5 days in adults. In 2 episodes patients requested to be discharged prematurely against the advice of the physician.

In episodes without extracorporeal detoxification/epuration mean/median time to discharge was 5.4/5 days, compared with 10.9/8.5 days for those who underwent extracorporeal detoxification/epuration.

**Table 3.3.4.2.12.** Hospitalisation duration

| | | | |
|---|---|---|---|
| **Hospitalisation duration, days** | | | |
| N episodes | 39 | 86 | 125 |
| Mean (SD) | 7.1 (4.9) | 5.2 (3.0) | 5.8 (3.8) |
| Median | 6.0 | 5.0 | 5.0 |
| Min - Max | 1.0 - 30.0 | 2.0 - 27.0 | 1.0 - 30.0 |
| **Hospitalisation duration, days w/o HF/HD*** | | | |
| N episodes | 32 | 85 | 117 |
| Mean (SD) | 6.0 (3.1) | 5.2 (3.0) | 5.4 (3.0) |
| Median | 5.0 | 5.0 | 5.0 |
| Min - Max | 1.0 - 13.0 | 2.0 - 27.0 | 1.0 - 27.0 |

**HF/HD = haemofiltration/haemodialysis*

*Safety*

No adverse events were reported as specifically related to the IV BCAA-free solution. Seven adverse events considered unrelated to the solution were reported in 4 patients (3 adults and 1 infant) for 6 episodes. These included 2 discontinuations of perfusion in adult patients due to discharge from hospital contrary to the physician's agreement: one discontinuation of perfusion in an adult due to replacement with central catheter, and one as the adult patient died. The death was not considered be to be related to the use of IV BCAA-free solution. The patient had severe pre-existing neurological disorders and experienced an acute worsening in general condition leading to death. In one episode sepsis occurred in an adult patient after the decompensation episode resolution and the transfer to the Cardiology ward. In one episode in a child younger

than 1 year old there was a worsening of neurological symptoms with sequelae after being transferred to another ward following normalisation of the leucine levels.

**Additional analyses**

*Comparison of predicted vs observed time to normalisation*

The comparison with the predicted time needed for leucine normalisation was done on the 85 normalized episodes. According to the treatment algorithm, the predicted mean time to leucine normalisation was 5.1 days: 3.6 in children and 5.5 in adults. In practice, with IV BCAA mean time to leucine normalisation was found to be 2.9 days (p<0.001): 2.7 days in children (p = 0.048) and 3.0 days in adults (p <0.001).

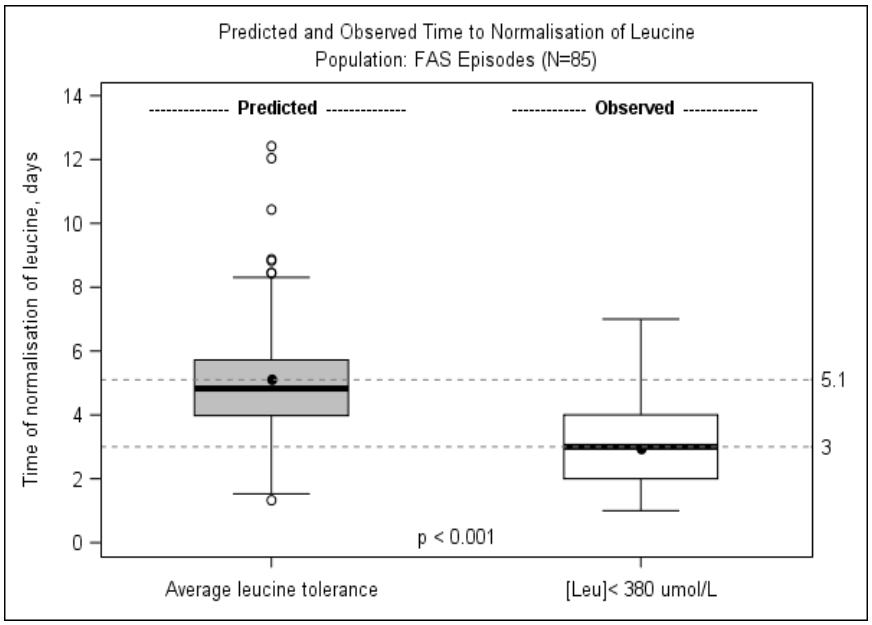



**Figure 3.3.4.2.5.** Comparison of predicted and observed time to leucine normalisation

*Influence of aetiology at admission on efficacy*

The status of leucine plasma levels was analysed according to the aetiology of decompensation episodes: dietary non-adherence, infection, or nausea/vomiting. No significant differences were observed in leucine level categories at admission, normalisation of leucine levels, percentage change in leucine levels from admission, rate of decrease, treatment duration and hospitalisation duration of decompensation episodes treated with the IV BCAA-free solution.

**Sanchez-Pintos et al., (2022)**

**Study title:** Intravenous branched-chain amino-acid-free solution for the treatment of metabolic decompensation episodes in Spanish paediatric patients with maple syrup urine disease.

This retrospective observational study reports the parenteral use an IV BCAA-free solution equivalent to Maapliv for the management of MSUD decompensation episodes in 5 patients admitted to 2 metabolic disease centres in Spain.

**Table 3.3.4.2.13.** Sanchez-Pintos study profile

| | |
|---|---|
| Objectives | To evaluate the efficacy and safety of an IV BCAA-free formula corresponding to MAAPLIV® in the management MSUD metabolic decompensation episodes between 2012 and 2020. |
| Methods<br>Design | Retrospective observational study |
| Patients | MSUD patients experiencing metabolic decompensation episodes defined as plasma leucine levels >381 µmol/L (5.0 mg/dL) treated with an intravenous BCAA-free solution at 3 specialist centres in Spain. |
| Treatment | BCAA-free amino acid delivered IV and equivalent to the MAAPLIV® formulation at the dose of 2-3 g/kg daily for neonates and infants (<2 years) and 1-2 g/kg/daily for children (>2 years). The BCAA-free solution was initially obtained from France for compassionate use, and subsequently was produced in Spain by local pharmacies following the French composition. |
| Statistics | Descriptive statistics |
| Efficacy criteria | Leucine normalisation < 380 µmol/L and symptom resolution |

**Results**

*Patient characteristics*

The series in this publication consists of 5 cases of metabolic decompensation episodes in 5 MSUD patients that required hospital admission. All patients were diagnosed by new-born screening. The series included 2 cases of decompensation episodes associated with neonatal clinical onset (1 complicated with intestinal perforation), 3 cases of decompensation episodes with concomitant infection and vomiting, and 1 case of risk of a decompensation episode due to surgery (in patient 1, who had a history of intestinal perforation).

**Table 3.3.4.2.14.** Patient characteristics at admission

| Patient | Case | Date | Age | Trigger | Symptoms |
|---|---|---|---|---|---|
| 1 female | 1 | 02/2020 | 1 week | Neonatal | Neurological disorders (boxing movements) |
| | 2 | 07/2020 | 5 months | Surgery | None (previous neurological injury on MRI) |
| 2 male | 3 | 02/2016 | 7 days | Neonatal | Irritability; coma with cerebral oedema |
| 3 male | 4 | 05/2015 | 2 y 3 m | Infection | Vomiting, nausea, respiratory distress |
| 4 male | 5 | 12/2011 | 9 years | Infection | Vomiting, nausea, fever |
| 5 male | 6 | 02/2017 | 16 years | Infection | Vomiting, nausea |

*Leucine levels, treatment and clinical outcomes*

The reason for treatment of all patients was an elevated leucine plasma level. In all decompensation episodes leucine levels at admission were above the decompensation threshold (669–3296 µmol/L). In patient 1 enteral feeding was impossible at the first admission due to intestinal perforation. In this patient the BCAA-free formulation was administered prophylactically at the second admission in view of the planned surgery. Patients 1, 2, and 3 received only the IV formulation, patients 4 and 5 received a combined IV + enteral BCAA-free formulation as the rapid improvement and vomiting resolution allowed lower doses of the IV formulation and oral feeding.

**Table 3.3.4.2.15.** Leucine plasma levels, treatments, and outcomes

| Patient | Age | Leucine levels (µmol/L) | | | IV BCAA-free treatment | | Hospitalisation (days) |
|---|---|---|---|---|---|---|---|
| | | Admission | End of BCAA-free IV treatment | Discharge | Duration (days) | Dose (g/kg/daily) | |
| 1 | 7 days | 3296 | 199 | 38 | 8 | 2.75 | 30 |
| 2 | 5 months | 2218 | 198 | 236 | 3 | 0.5-1 | 42 |
| 3 | 7 days | 699 | 46 | 23 | 20 | 1.5 | 65 |
| 4 | 2 y 6 months | 938 | 320 | 282 | 7 | 0.5 | 13 |
| 5 | 16 years | 953 | 343 | 175 | 3 | 0.7 | 14 |

Leucine plasma levels were normalised in all patients. Hospitalisation of patient 2 was longer (42 days) due to poor enteral tolerance and frequent vomiting. The patient was discharged without sequelae. Patient 3's discharge was also delayed due to persistent oral intolerance and vomiting, forcing repeated discontinuation of enteral feeding. This patient was finally discharged with progressive symptom resolution. Patients 4 and 5 were discharged after symptom resolution without sequelae.

*Safety*
In this case series, no adverse events were detected that were associated with either the BCAA-free solution or its administration.

### *2.6.5.3. Supportive study(ies)*

In supportive studies, parenteral BCAA-free solutions of composition other that Maapliv used.

**Townsend and Kerr (1982)**

**Study title:** Total parenteral nutrition therapy of toxic maple syrup urine disease.

This study is a case report on one neonate patient with MSUD who was unable to continue nasogastric feeding and peritoneal dialysis had failed due to the patient's hypotonia.

**Table 3.3.4.7.1.** Study profile

| | |
|---|---|
| Objective | To assess the usefulness of parenteral nutrition with a BCAA-free solution as an alternative to multiple exchange transfusions or peritoneal dialysis |
| Methods | Case report |
| Patients | One male patient presenting with a MSUD decompensation episode at the age of 7 days with symptoms including poor feeding, weight loss, generalised hypotonia, and seizures. Elevated branched-chain keto acids in blood and urine. Admitted to children hospital at the age of 9 days. |

| | |
|---|---|
| Treatments | Initially enteral feeding by nasogastric tube with a mixture of all amino acids except leucine, isoleucine, and valine, and 120 g/L of a protein-free base. Parenteral nutrition fluid of MSUD amino acid mixture lacking leucine, isoleucine, and valine (BCAA-free) and composed (in g/kg) of L-alanine 39, L-aspartic acid 89, L-glutamic acid 250, L-cystine 56, L-histidine 39, L-proline 89, L-phenylalanine 56, L-tyrosine 56, L-serine 61, L-threonine 61, L-arginine 50, L-lysine HCI 72, L-tryptophan 22, L-methionine 28, and glycine 33. |
| Statistics | Descriptive |

**Results**

*On admission:* BCAA plasma levels: leucine 58 mg/dL (normal 1.7 to 4.0), isoleucine 7.0 mg/dL (normal 1.2 to 2.7), and valine 8 mg/dL (normal 2.7 to 5.8). Gasping respiration and lack of spontaneous movements leading to placement on a ventilator. Plasma isoleucine and valine levels but not leucine levels decreased below normal in the first 24 hours. Isoleucine and valine were added to the formula to ensure protein synthesis necessary for removal of leucine.
*By 11 days of age:* transient improvement and extubated but dietary therapy interrupted by vomiting, bradycardia, and aspiration pneumonitis necessitating reintubation thereby making feeding by mouth or nasogastric tube impossible. Two attempts of dialysis failed due to extreme hypotonia.
*By 14 days of age:* parenteral nutrition initiated with the BCAA-free mixture and evidence of improvement.
*From age 19 to 30 days of total parenteral nutrition:* decrease of plasma leucine levels to normal at the age of 27 days (Figure 3.3.4.7.1.)

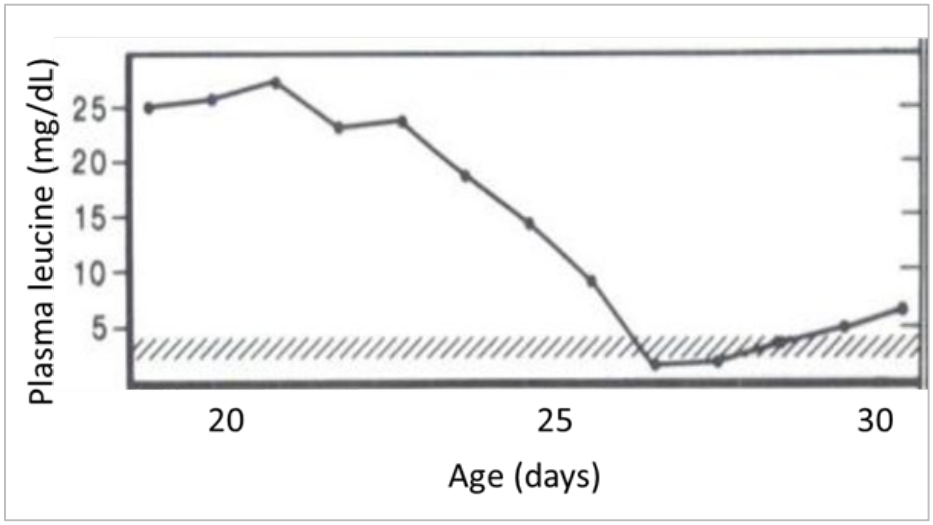



**Figure 3.3.4.7.1.** Leucine plasma levels following the administration of a parenteral BCAA-free mixture at 14 days of age.

**Berry et al., (1991)**

**Study title:** Branched-chain amino acid-free parenteral nutrition in the treatment of acute metabolic decompensation in patients with maple syrup urine disease

**Table 3.3.4.7.2.** Study profile

| | |
|---|---|
| Objectives | To demonstrate that administering a BCAA-free regimen of modified parenteral nutrition can effectively reduce plasma BCAA levels in acutely ill patients. |
| Methods | Cases report |
| Patients | Patients with classic MSUD |
| Treatments | Enteral therapy with a formula prepared from Maple Syrup Urine Disease Diet Powder (2.5 g of amino acids, 13.4g of carbohydrate, and 4.2g of fat per 100 kcal, Mead-Johnson, Evansville, Ind., USA).<br>Parenteral solution based on the composition of Aminosyn® II (Abbott, North Chicago, Ill., USA), except with BCAAs omitted. A 9 percent solution of the BCAA-free amino acids was mixed with 20 to 25 percent glucose, vitamins, and minerals and administered to the patient through a central venous catheter. Alternatively, the amino acid solution was mixed with 10% glucose and administered with 10 to 20% Intralipid (KabiVitrum, Clayton, N.C.) through a peripheral venous catheter.<br>Regimen of parenteral nutrition instituted if patients failed to tolerate the enteral formula by mouth or nasogastric tube and if the plasma leucine level was ≥1.0 mmol/L (1000 µmol/L). If plasma leucine level was below 1.0 mmol/L decision was based on the presence of lethargy or ketoacidosis.<br>Patients with leucine plasma levels above 2.5 mmol/L were to be treated with haemodialysis.<br>The goal of the parenteral regimen was to reduce the plasma leucine concentration to normal or to a level at which the signs of metabolic decompensation abated and the patient could resume enteral nutrition therapy.<br>Parenteral therapy at a lower rate in patients who were unable to consume an adequate amount of oral formula or in whom nasogastric feeding was deemed inappropriate. Patients to be discharged when plasma leucine levels were below 1.0 mmol/L. |
| Statistics | Descriptive |
| Evaluation criteria | Clinical and laboratory assessments with focus on leucine plasma levels. |

**Results**

Five patients (three girls and two boys) with classic MSUD decompensation episodes were treated intravenously with BCAA-free solution between July 1988 and May 1990.
Three patients were between 2 and 6 years of age, one was a 6-day-old neonate, and one was 11 and half years old. A total of 9 decompensation episodes were reported at different ages.
Infection was usually the cause of metabolic imbalance.

**Table 3.3.4.7.3.** Illness description during decompensation episodes

| Patient N° | Age | Clinical features |
|---|---|---|
| 1 | 6 days | Neonatal coma, ketosis |
| 2 | 2 years | Gastroenteritis, coma, ketoacidosis |
| | 3 years, 5 months | Upper respiratory tract infection |
| | 3 years, 6 months | Streptococcal pharyngitis, acidosis |
| 3 | 2 years | Upper respiratory tract infection, ketoacidosis |
| 4 | 11 years, 1 month | Upper respiratory tract infection, ketosis |
| 2 | 3 years, 10 months | Gastroenteritis and dietary noncompliance, ketosis |
| 3 | 2 years, 4 months | Upper respiratory tract infection, ketosis |
| 5 | 6 years, 1 month | Hyperleucinaemia, ketoacidosis after orthopaedic surgery |

Parenteral nutrition including BCAA-free Aminosyn® II was used alone in 6 occasions in 4 patients whereas both the parenteral formulation and the enteral formulation (MSUD Diet Powder) were used in 3 occasions in 3 patients. The composition of the parenteral BCAA-free Aminosyn® II is displayed in Table 3.3.4.7.4.

**Table 3.3.4.7.4.** Composition of parenteral BCAA-free Aminosyn® II compared with MAAPLIV

| Amino acid | Aminosyn II g/500 mL* | MAAPLIV® g/500mL* |
|---|---|---|
| L-alanine | 4.95 | 3.15 |
| L-arginine | 5.1 | 2.05 |
| L-aspartic acid | 3.5 | 2.05 |
| L-cysteine | | 0.50 |
| L-glutamic acid | 3.7 | 3.55 |
| Glycine | 2.5 | 1.05 |
| L-histidine | 1.5 | 1.05 |
| L-lysine | 5.25 | 2.80 |
| L-methionine | 0.86 | 0.65 |
| L-phenylalanine | 1.5 | 1.35 |
| L-proline | 3.6 | 2.80 |
| L-serine | 2.65 | 1.90 |
| Taurine | | 0.15 |
| L-threonine | 2.0 | 1.80 |
| L-tryptophan | 1.0 | 0.70 |
| L-tyrosine | 1.35 | 0.25 |

**Different amino acids concentrations are available. Aminosyn® at 10% based on product monograph.*

BCCA-free regimens effectively reduced leucine plasma levels in all patients and to below 1 mmol/L in 7 out of 9 decompensation episodes. This reduction was associated with cessation of vomiting, resolution of illness and a return to oral feeding in all patients.

**Table 3.3.4.7.5.** Effect of BCAA-free nutritional therapy on plasma leucine concentrations in MSUD patients

| Patient No | Age | Calories administered | Amino acids administered | Plasma leucine | |
|---|---|---|---|---|---|
| | | | | Baseline | After treatment |
| | | Kcal/kg/24 hr | g/kg/24 hr | µmol/L (hours)* | |
| Modified parenteral nutrition only | | | | | |
| 1 | 6 days | 64 | 1.20 | 2250 | 1770 (11.5) |
| 2 | 2 years | 90 | 1.25 | 1930 | 660 (42) |
| | 3 years, 5 months | 90 | 1.49 | 1900 | 680 (62.5) |
| | 3 years, 6 months | 90 | 1.49 | 2090 | 1550 (13.2) |
| 3 | 2 years | 126 | 1.92 | 920 | 20 (72) |
| 4 | 11 years, 1 month | 95 | 2.00 | 1540 | 980 (24) |
| Modified parenteral nutrition plus enteral formula | | | | | |
| 2 | 3 years, 10 months | 90 | 2.65 | 2290 | 740 (131.5) |
| 3 | 2 years, 4 months | 128 | 2.70 | 1510 | 760 (33) |
| 5 | 6 years, 1 month | 129 | 3.20 | 840 | 420 (16) |

**Hours of treatment*

**Morton et al., (2002)**

**Study title:** Diagnosis and treatment of maple syrup disease: a study of 36 patients.

The treatment protocol emphasized the enhancement of protein anabolism and dietary correction of imbalances in plasma amino acids rather than removal of leucine by dialysis or haemofiltration.

**Table 3.3.4.7.6.** Study profile

| | |
|---|---|
| Objectives | To evaluate an approach to the diagnosis and treatment of maple syrup urine disease (MSUD). |
| Methods | Observational follow-up study |
| Patients | Neonates with classic MSUD either sick or at high risk of MSUD |
| Treatments | The treatment protocol emphasized the enhancement of protein anabolism and dietary correction of imbalances in plasma amino acids rather than removal of leucine by dialysis or haemofiltration. It included:<br>• Inhibiting endogenous protein catabolism<br>• Sustaining protein synthesis<br>• Preventing deficiencies of essential amino acids<br>• Maintaining normal serum osmolarity |
| Statistics | Descriptive |
| Evaluation criteria | Clinical and laboratory assessments with focus on leucine plasma levels. |

**Results**

Of the 36 enrolled infants (numbered 1 to 36) in the study diagnosed or at risk of MSUD, 18 were sick with dystonic posturing and markedly abnormal serum leucine to tyrosine (Leu/Tyr) molar ratios (22–140; normal: 0.5–3.5). Five neonates received enteral and parenteral amino acid mixture free of leucine, isoleucine, and valine. Neonates were diagnosed MSUD within their first 16 days of life, three of them because of illness and two from positive newborn screening test. All patients experienced poor feeding, lethargy, irritability, and dystonia while 3 of them had seizures during the initial illness. All patients had elevated leucine plasma levels > 2000 µmol/L. Leucine plasma levels were normalised in all patients in 3.5 to 8.0 days while patients remained in hospital during 5 to 21 days.

**Table 3.3.4.7.7.** Clinical and biochemical profiles of 5 neonates with MSD treated with enteral and parenteral BCAA-free mixtures

| Patient number | Age at diagnosis (days) | Reason for diagnosis | Poor feeding, lethargic, irritable | Dystonia | Seizures during initial illness | Plasma leucine (µmol/L) | Days until [leucine] <400 µmol/L | Days in hospital |
|---|---|---|---|---|---|---|---|---|
| 20 | 4 | Illness | + | + | + | 2366 | 4.0 | 7 |
| 22 | 6 | Screening | + | + | + | 2440 | 3.5 | 5 |
| 32 | 16 | Illness | + | + | + | 2443 | 4.0 | 21 |
| 33 | 6 | Illness | + | + | – | 2290 | 7.0 | 10 |
| 34 | 7 | Screening | + | + | – | 2595 | 8.0 | 10 |

**Couce Pico et al., (2007)**

**Study title:** Advances in the diagnosis and treatment of maple syrup urine disease: experience in Galicia, Spain

**Table 3.3.4.7.8.** Study profile

| | |
|---|---|
| Objective | To describe the clinical and biochemical details at confirmation of diagnosis and the long-term outcome of three patients with MSUD. |
| Methods | Case reports |
| Patients | Patients with diagnosis of MSUD based on neonatal screening. |
| Treatments | Enteral feeding (BCAA-free mixture)<br>Parenteral BCAA-free solution |
| Statistics | Descriptive |
| Evaluation criteria | Delay for reduction of toxic metabolites measured in hours to reach a leucine plasma concentration below 1,000 µmol/L. |

**Results**

Three children (two male and one female) aged 3 to 7 days at admission diagnosed with neonatal MSUD between July 2000 and July 2006 experienced a total of 11 hospitalisations for metabolic decompensation with plasma leucine levels > 1000 µmol/L.

**Table 3.3.4.7.9.** Patient characteristics

| Patient | Age (days) | Leucine concentration at diagnosis (µmol/L) | Days with Leu >1,000 µmol/L | Clinical symptoms at admission |
|---|---|---|---|---|
| 1 | 14 | 1682 | 2 | Loss of weight |
| 2 | 7 | 2500 | 2 | Encephalopathy |
| 3 | 7 | 2269 | 2 | Encephalopathy |

Due to an episode of gastroenteritis, one patient received a parenteral BCAA-free mixture prepared by the Pharmacy Hospital of München-Bogenhausen in Germany.
Enteral or parenteral nutrition with BCAA-free mixtures aimed at decreasing BCAA plasma levels were successfully used in these children without extracorporeal elimination techniques.
During the 5-year follow-up, metabolic decompensations of moderate severity occurred, most frequently related to respiratory infections and otitis. Leucine plasma levels sporadically exceeded 1000 µmol/L but generally remained within the normal range as shown in Table 3.3.4.7.10.

**Table 3.3.4.7.10.** Mean annual leucine concentrations (µmol/L) during follow-up [mean (± SD)]

| Patient | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|
| 1 | 201 (± 109) | 211 (± 119) | 237 (± 81,8) | 300 (± 174) | 267 (± 204) |
| 2 | 356 (± 303,6) | 131 (± 107) | 193 (± 123,8) | 187 (± 99) | 244 (± 96) |
| 3 | 156 (± 95) | 197 (± 114) | - | - | - |

## 2.6.6. Discussion on clinical efficacy

This marketing authorisation application is for Maapliv solution for infusion, submitted as under the "well established medicinal use" legal basis.

The proposed indication for Maapliv is the treatment of maple syrup urine disease (MSUD) presenting with an acute decompensation episode in patients from birth who are not eligible for an oral and enteral branched-chain amino acids free (BCAA- free) formulation.
Maple syrup urine disease is an orphan disease. The studies provided by the applicant for this application referenced the use of the product for more 10 years in EU.

The main literature data provided in support of this application consists of 5 publications (Servais et al. 2013, de Lonlay et al. 2021, Alili et al. 2022, Sanchez-Pintos et al. 2022, Blanco Dorado et al. 2017) reporting the parenteral use in Europe of a BCAA-free mixture equivalent to the Maapliv.
Further, four publications reported the parenteral use of BCAA-free mixtures different from Maapliv (Townsend and Kerr 1982, Berry et al. 1991, Morton et al. 2002, Couce Pico et al. 2007) and therefore these publications are considered, 'supportive-only'.

The composition of Maapliv is very similar to the composition of products used in studies discussed by the applicant. The types and number of amino-acids are the same; in relation to a general amino-acid content there were small differences (52.75g in Maapliv versus 52g in studies), however this is considered as insignificant. Further, differences in osmolarity (i.e 110 mOsm/L difference) is unlikely to contribute to any clinical effect, as both are well within the osmolarity safety limits for parenteral nutrition.

**Dose finding studies**
As highlighted by the applicant, the number of amino-acids in Maapliv (except isoleucine, L-leucine, L-valine) are the same as in Vaminolact (approved in the EU for paediatric patients as IV supply of proteins). The doses proposed for Maapliv are very similar as the one used in Vaminolact, with only a small difference for children aged 0-2 years.
The applicant clarified that the rationale for the proposed dosing range is based on WHO daily protein requirements for adults and children (WHO 2007) increased by approximately 20% as this formulation contains amino acids and not proteins (van Wegberg et al. 2017). Further, the applicant referenced the German 2009 parenteral nutrition guidelines for basic amino acid requirements for adult patients. According to this guideline the starting dose is 0.8 g/kg/day which could be increased to the maximum dose of 2.5 g/kg/day in exceptional cases. Additional references are also being made to the phenylketonuria treatment guideline which is recommending: 2 to 3 g/kg per day of protein in infancy, to 1.2–2 g/kg per day by the age of 10 years and all but one centre gave 1–1.5 g/kg per day of protein equivalent to patients >10 years of age. The justification for the dose as provided by the applicant are acceptable.

**Mechanism of action**
The mechanism of action of Maapliv as proposed by the Applicant is stated in section 5.1 of the SmPC as follows:

*"In patients with MSUD decompensation, BCAA-free solution in combination with carbohydrate and lipid supplementation prevents or reverses protein catabolism and promotes anabolism, reducing the corresponding alpha-keto acid levels".*

This is considered acceptable.

**Design, conduct of clinical studies and efficacy data**

***Design and conduct of clinical studies***

The main literature data comes from 5 publications : Servais et al. 2013, de Lonlay et al. 2021, Alili et al. 2022, Sanchez-Pintos et al. 2022, Blanco Dorado et al. 2017. These studies are either observational (retrospective or prospective) or case reports.
Of note, studies by Servais et al. 2013, de Lonlay et al. 2021, Alili et al. 2022 were performed in overlapping study centres in France by the same group of authors.

**Study population**
All studies enrolled patients with confirmed MSUD diagnosis who were hospitalised due to metabolic decompensation episode.

There was some variability in the inclusion criteria used across studies and how decompensation episodes were defined. The study performed by Servais included patients who were admitted for clinical deterioration with anorexia and/or gastric intolerance, diarrhoea, infection, or if Leu concentration levels were above 763 µmol/L (10 mg/dL). In other studies (de Lonlay, Alili, Sanchez-Pintos) Leu concentration level >381 µmol/L (5.0 mg/dL) was required for enrolment. Further, it seems that in some of these studies, decompensation episode could be also defined clinically (based on the presence of clinical symptoms of metabolic decompensation) without the need for the confirmed increase in the blood Leu levels. For example, in study performed by Alili for 9 decompensation episodes treated with IV BCAA-free mixture, the leucine levels were below 381 µmol/L (which is considered as normal level).

Therefore, the applicant was requested to discuss if there is a widely agreed definition on how a decompensation episode in MSUD is defined. The applicant clarified that in general, metabolic decompensations episodes in MSUD are defined by using plasma leucine level >380 µmol/L.

A small number of patients in the studies provided were treated with the IV BCAA-free solution despite the fact that in these patients plasma leucine was <380 µmol/L (as clarified by the applicant these patients were at risk of development of decompensations episodes). These patients, however, are not considered by the applicant to be a target population for the use of Maapliv.

Maapliv MAAPLIV is to be used in patients who are not eligible for an oral BCAA-free formulation.
As requested, the applicant provided the list of cases where patients could be considered as not eligible for an oral and enteral branched-chain amino acids free (BCAA- free) formulations including when:
-patients cannot tolerate oral/enteral feeding due to issues like vomiting, nausea;
-patients in whom the sequential intakes of an oral formulation would be less effective than a continuous IV formulation (e.g. during a severe decompensation with neurological symptoms such as coma);
-patients who refuse enteral feeding for whatever reason;
-patients for whom oral/enteral feeding is inappropriate, such as those undergoing surgery and have to fast.

In their response, the applicant also clarified that in the studies presented, IV BCAA-free formulation was given not only to patients who cannot tolerate oral formulations, for example as prophylaxis before surgery. However, as these episodes were very rare, the impact on the study results was minimal.

**Study treatment**

In all the "main" studies, patients received BCAA-free mixture essentially equivalent to the Maapliv (see comments above).

**Dose used in studies**

In Servais et al. study the recommended dose (for adult patients) was 1 to 2 g per kilogram of body weight per day. In the study performed by de Lonlay et al. 2021, the dose of BCAA-free mixture solution was 1-2 g/kg of body weight per day for adults and children including those younger than 1 years of age.

In Alili et al. 2022 study, the recommended dose was very similar but not identical to the dose as proposed for the SmPC. In relation to adults the dose was ranging from 0.5 to 2.6g/kg and therefore some adult patients received doses higher than this recommended by the SmPC.

Although, there were some small differences in the doses used in studies, it can be agreed that most patients received the dose which is in line with this recommended for the SmPC.

**Adjustment of the dose**

The applicant provided a detailed discussion on the requirements for monitoring and dose adjustments during the treatment with parenteral BCAA-A free amino acid solution.

**Comparator**

As no data from randomised controlled comparative trial is available, no conclusion could be made that the IV BCAA-free solution is providing any additional benefit as compared oral solution. Only in two studies (Servais, de Lonlay) the comparator (oral BCAA-free formula) was used. In the study performed by Servais enteral feeding through a nasogastric tube involved 1 to 2 g per kilogram of body weight with the following products: MSUD secunda (Milupa) or Maxamum MSUD (SHSNutricia). In de Lonlay study commercially available oral/enteral BCAA-free products were used but the names and compositions of these products were not recorded. The review of the limited comparative data available do not indicate that the treatment effect was significantly different between those treated with IV BCAA-free solution or oral solution. Further, this comparison has a limited relevance as the proposed indication for Maapliv is for patients who are not eligible to the oral solution.

**Other concomitant therapies**

**Supplementation with isoleucine and valine**

As indicated by the applicant, failure to provide sufficient level of isoleucine, valine, and other essential amino acids for protein synthesis slows the rate of Leu depletion.

Maapliv is a solution for parenteral use free of branched chain L-amino acids such as isoleucine, leucine and valine. Therefore, the treatment with Maapliv should be supplemented with isoleucine and valine solution.

Indeed, in all studies provided valine and isoleucine supplementation was introduced but approaches used varied across studies. For example, in some studies there was delay in starting this supplementation. This supplementation was guided by the isoleucine and valine plasma level however, different reference values were used.

The importance of valine and isoleucine supplementation is highlighted in the SmPC as follows:

"Careful supplementation with isoleucine and valine should also be consistently added; if no solution for infusion is available with these 2 amino acids they can be administered by oral or enteral route".

Nevertheless, the lack of availability of IV valine and isoleucine formulations was questioned.

In responses it was clarified that the lack of availability of IV valine and isoleucine formulations may have a limited impact as valine and isoleucine are not mandatory during the first 48 hours of a MSUD decompensation. This is supported by the literature, where in the de Lonlay study, [oral] valine and isoleucine were re-introduced in a median time of 3.0 days after onset of the decompensation episode, followed by leucine (median time: 5.5 days).

**Supplementation with other amino-acids**
It is noted that in studies patients were supplemented with other amino-acids, depending on their plasma levels. This is particularly relevant for amino acids that compete with BCAAs for the LAT1 transporter, such as tyrosine; histidine, methionine, glutamine, tryptophan, but also alanine.

The applicant discussed recommendations for monitoring and/or supplementation with other amino-acids, while on treatment. No update to the SmPC is required in this regard.

**Haemofiltration/haemodialysis**

Many patients enrolled in studies were also treated with haemofiltration/haemodialysis. In study performed by Alili et al 8 (6.3%) of decompensation episodes required such treatment. The need for haemofiltration/haemodialysis in most severe cases is highlighted in the SmPC as follows: "In severe/critical acute depensation episodes the treatment with IV BCAA-free amino acid formulation could be insufficient and other treatments (for example haemodialysis/haemofiltration) may need to be implemented.".

Of note, this additional treatment confounded the efficacy results of the IV BCAA-free solution. In the most severe population of patents, it is not clear as to what extent the positive effect of the treatment was due to haemofiltration/haemodialysis and what extent was due to IV BCAA-free solution.

**Study endpoints**

In most studies the level of leucine was used as a surrogate marker of efficacy. As the clinical presentation of MSUD is toxic levels of leucine, plasma leucine is a clinical marker of the effectiveness of the treatment approach. It was also highlighted that leucine levels respond quickly to interventions and therefore it could be easily monitored.

Time to normalise plasma leucine concentrations (defined as a plasma leucine concentration of < 381 µmol/L) when treated with IV BCAA free solution was the most frequently reported outcome measure (in studies Servais, de Lonlay and Alili).

In studies, other endpoints were also investigated (such as length of hospital stay, duration of hospitalisation, length of ICU stay) but these endpoints are not considered as informative as they are depending on many external factors including local practices (availability of intermediate care words), preference of patients or doctors.

***Efficacy data and additional analyses***

30 unique patients were included, and 132 decompensation episodes were treated with BCAA-free mixture equivalent to the Maapliv. Of note, some patients had multiple decompensation episodes.

Three studies (de Lonlay et al. 2021, Alili et al. 2022, Sanchez-Pintos et al. 2022, Blanco Dorado et al. 2017) enrolled children and 20 children were treated with iv BCAA- free mixture.
In de Lonlay et al. study, BCAA-free mixture was used during 12 decompensation episodes in children however, the number of children and age was not specified. Some children seem to be younger than 1 years of age. Alili et al. 2022 study enrolled 13 children and 39 decompensation episodes were treated in this study including 17 episodes in children aged 1-10 years and 14 episodes in children aged 11–15 years. Sanchez-Pintos et al. 2022 enrolled 6 children aged 1 week-16 years, Blanco Dorado provided a case report for one child.

**Baseline characterises- Leucine concentration at baseline**.

Most episodes of decompensation treated with IV BCAA-free mixture had mild or moderate increase in the plasma Leu concentration at baseline. Small percentage of episodes had concentration below 381 µmol/L (5.0 mg/dL). On the other hand, some patients included in these studies had severe or critical Leu concentration at admission. In the study performed by Servais the maximum Leu concentration was 1684 µmol/L but the numbers of parents in each severity category is not provided. In de Lonlay study 8 (22%) episodes treated with IV solution were classified as severe due to an increase in the plasma Leu concentration ranging from 15.0 mg/dL (1144 µmol/L) to 20.0 mg/dL (1525 µmol/L). In one case in this study an increase was critical > 20.0 mg/dL (1525 µmol/L). In Alili study, the majority of patients had the plasma Leu concentration at admission higher than > 762 µmol/L (10 mg/100 mL) but it is not clear how many patients had severe or critical leucine concentration at admission. In Sanchez-Pintos study two patients had the Leucine plasma concentration at admission i.e higher then > 20.0 mg/dL (1525 µmol/L).

As requested, the applicant performed the analysis of the data depending on the severity of the disease (i.e level of leucine at baseline). The data analysed indicated that the majority of patients enrolled to studies and treated with the IV BCAA-free formulation had mild to moderate disease. Patients with severe elevation of leucine levels accounted for less than 27 % of a total population. There was only 1 patient with critical elevation of leucine levels who was treated with the IV BCAA-free formulation.

In relation to the response to treatment, no significant differences depending on the baseline leucine level values were noted. Nevertheless, taking into consideration a limited number of patients investigated there is still uncertainty regarding the treatment response in particular in patients with severe or critical elevation in leucine level.

**Efficacy results**

Time to normalise plasma leucine concentrations (defined as a plasma leucine concentration of < 381 µmol/L) when treated with IV BCAA free solution was the most frequently reported endpoint.

In Servais et al., observational study, aimed to compare the efficacy and tolerance of a parenteral treatment with standard enteral feeding via nasogastric tube in MSUD patients with metabolic decompensation, in total four adult patients were included. The patients experienced in total 17 decompensation episodes in 2010 and in total 18 decompensation episodes (DE) between 2005 and 2010.
Decompensation episodes were treated with the parenteral BCAA-A free amino acid mixture, produced by AGEPS. The recommended dose was 1 to 2 g per kilogram of body weight per day for both enteral feeding and parenteral treatment. In group P (receiving IV solution), mean plasma Leu concentrations decreased, and in most cases, normalised (<381 µmol/L) within 3.5 days.

In publication by Blanco Dorado, after administration of parenteral protein solution without branch chain amino acids a rapid and significant descent in plasma leucine levels was observed on day 15 of hospitalization in patient with MSUD with a metabolic decompensation due to infection.

Study by de Lonlay et al., was a retrospective observational noninterventional study of 126 decompensation episodes from 54 MSUD patients treated at 5 centres in France and Germany. In in total 51 (40.8%) of patients IV BCAA-free formula was administered. Mean time to decompensation episode resolution was 9.2 days in the oral/enteral group and 7.4 days in the IV group. The mean length of hospital stay was 6.6 days for the oral/enteral group and 5.4 days for the IV group. The mean length of ICU stay was 3.2 days for the oral/enteral group and 2.5 days for the IV group.

Overall, leucine normalization was achieved at discharge in 76.8% of episodes in the oral/enteral group and in 80.6% in the IV group. The mean (±SD) time to first leucine normalization (<381 µmol/L) was 68.4 hours

(±52.9) in the oral/ enteral group and 95.6 hours (±79.2) in the IV group. At discharge, leucine levels had decreased by a mean of 548.5 µmol/L in the oral/enteral group and by 657.2 µmol/L in the IV group.

In an observational, prospective study by Alili et al., patients with MSUD who were hospitalized between 2010 and 2016 due to decompensation episodes in France were included. In total 17 episodes in subjects between 1-10 years and 14 episodes in subjects between 11-15 years were included in the analysis.
In the efficacy set (n = 102), leucine plasma concentrations were normalised in 83.3% (85/102) of episodes in a mean (SD) duration of 3.0 days (1.2). In children the observed time to leucine normalisation was a mean of 2.7 (1.1) days, and for adults a mean time of 3.0 (1.3) days.
Reduction in time compared with predicted time to leucine normalisation was 41% and 25% in children and adults, respectively.

Study by Sanchez-Pintos is a retrospective observational study of 5 cases of metabolic DE in paediatric MSUD patients, treated with IV BCAA-free 5% solution. Moreover, 1 case of risk of DE due to fasting for intestinal surgery was included in the Study. However, it is noted that 2 patients with DE were treated with proteins both parenteral and enteral and only 3 patients received only IV BCAA-free solution. 3 patients were treated with HD/HF.
All patients responded to treatment, discharge leucine level ranged from 23 to 282 µmol/L however, only limited information were provided. Duration of IV BCAA-free treatment was different for each included patient, ranging from 3 days to 20 days.

Of note, these results were confounded by the fact not all patients were treated with IV BCAA-free solution continuously, some received additional treatment with oral solution. In de Lonlay study, approximately one-third of episodes (37.6%) multiple administration routes for BCAA-free formula were employed (also within the same episode).

**Rebound effect/lack of response.**
Although in most patients (based on the limited information provided), improved while in studies, some did not respond or even worsen their neurological status while on treatment. For example, in the study performed by Alili, an infant had worsening neurological symptoms while on treatment. Also, concerning the patient described by Blanco Dorado et al. 2017, after initial improvement with normalisation of plasma leucine concentration, a significant deterioration was seen three days later i.e this patient was re-admitted to ICU due to cranial hypertension caused by persisting cerebral oedema. After re-admission, the patient's leucine levels remained low, which is concerning as it may indicate that the plasma leucine level monitoring is insufficient in respect to monitoring of response.

The applicant provided the list of circumstances when patient's leucine levels do not drop sufficiently with the IV BCAA-free solution including:

- very severe status of the disease. These patients may need to undergo haemodialysis and/or hemofiltration. This is clearly highlighted in the SmPC as follows: "In severe/critical acute depensation episodes the treatment with IV BCAA-free amino acid formulation could be insufficient and other treatments (for example haemodialysis/haemofiltration) may need to be implemented".
- patients eating food by mistake that contains natural proteins, which should be avoided during acute illness. As this is linked to the general management of patients, no updates to the SmPC are necessary for the group of patients.
- inadequate doses of oral valine and isoleucine, insufficient caloric or BCAA-free formulation (IV or oral) intakes. It is stated in the SmPC that dose adjustments could be necessary.

Further, it is considered that non-responses should be monitored post-marketing in annual reassessment.

**Additional efficacy data needed in the context of a MA under exceptional circumstances**

As the level of evidence available is limited (the studies were observational, without a priori defined criteria for success in the form of primary (or secondary) outcomes, or statistical hypotheses), it was agreed that further data addressing some uncertainties post-MA will be needed. The Applicant agreed to initiate a post-authorisation study enrolling patients who have experienced an acute decompensation episode treated with MAPPLIV. The analysis of responders and non-responders, the assessment of the effect of MAPPLIV on symptom resolution, description of outcomes across different MSUD sub-types and collection of safety data will be performed.

As part of this procedure, the Applicant submitted the synopsis of the proposed non-interventional, multicentre, observational, prospective, post-authorisation study. The final protocol will be submitted in Q1 2026.

## 2.6.7. Conclusions on the clinical efficacy

Maapliv is considered approvable from a clinical perspective.

## 2.6.8. Clinical safety

The safety profile is based on data available from the literature search.

### *2.6.8.1. Patient exposure*

Safety database is composed by patients included in the identified publications, considered as main publications. In total safety data are available from 30 evaluable patients exposed to the BCAA-free solution equivalent to Maapliv administered intravenously for the treatment of 132 metabolic decompensation episodes. The overall size of the safety database is considered rather limited, which makes the evaluation of safety difficult. It cannot be fully excluded that less common adverse events have not been detected.

The main studies selected to support this MAA show the effects of parenteral administration of BCAA-free solutions in patients with MSUD, including 30 evaluable patients exposed to the BCAA-free solution equivalent to Maapliv administered intravenously for the treatment of 132 metabolic decompensation episodes.

In 4 supporting studies, 14 patients who experienced 26 decompensation episodes were exposed to a parenteral BCCA-free mixture other than Maapliv.

### *2.6.8.2. Adverse events*

**Adverse drug reactions from MAIN efficacy publications**

Sanchez Pintos et al (2022), Delonlay et al (2021), Abi Warde (2017), Blanco Dorado et al 2017 and Servais et al (2013) do not report any adverse events as part of their studies.

In the submitted studies, the treatment is administered in a population who are potentially critically unwell, requiring ICU admission. None of the study reports include data on any medical issues, clinical signs or

laboratory findings. It is unclear whether the adverse events were not captures in the population, or whether there were actually no adverse events experienced.

In the de Lonlay study, in total seven adverse events were reported. However, two events (nausea and vomiting) were considered to be associated with the oral/enteral BCAA-free formulation.

In the Sanchez-Pintos study, no BCAA-free solution -related adverse events were reported.

**Adverse drug reactions with other parenteral amino acid products**

Although the use of the BCAA-free solution equivalent to Maapliv did not raise any safety concerns in main publications discussed in this overview and given the absence of post-marketing or pharmacovigilance follow-up studies for Maapliv specifically, the adverse drug reaction information available derives from the post-marketing experience of other amino acid solutions for parenteral use.

The description of potential undesirable effects is summarized in Table 4.3.1., based on the information available in product monographs or summaries of product characteristics of other amino acid solutions on the market for parenteral use (ProSol®, Aminosyn®-PF 10%, Vaminolact®). The incidence of potential safety effects is unknown. Moreover, these products were used for different indications, and on a long-term basis, thus their adverse event profiles may only be indirectly relevant to that of Maapliv.

**Table 4.3.1.** Summary of undesirable effects with marketed amino acid solutions for parenteral use as mentioned in product monographs or SmPCs

| MedDRA standard system organ class | Adverse reactions |
|---|---|
| General disorders and administration site conditions | Infusion site thrombophlebitis |
| | Venous irritation |
| Immune system disorders | Anaphylactic / anaphylactoid reactions |
| Hepatobiliary disorders | Hepatic failure* |
| | Hepatic cirrhosis* |
| | Hepatic steatosis* |
| | Hepatic fibrosis* |
| | Hepatic enzyme increased |
| | Cholestasis |
| | Cholecystitis |
| | Cholelithiasis |
| | Blood bilirubin increased |
| Metabolism and nutrition disorders | Hyperammonaemia |
| Renal and urinary disorders | Azotaemia |
| Skin and tissue disorders | Erythema |
| Vascular disorders | Phlebitis |
| | Pulmonary vascular precipitate |

**Some events occurred with long-term usage.*

Besides cholestasis, liver disease or hepatic dysfunction (hepatic steatosis/fibrosis/cirrhosis) have been described in adults, neonates/infants/pre-term infants/children with long-term parenteral nutrition (several months or years). As an example, these findings were observed in patients with underlying intestinal failure, in which the likelihood of liver disease may be greater in patients with the least amount of residual intestine.

The aetiology of parenteral nutrition associated liver disease is unknown and likely multifactorial. The causal role of amino acids solution in such hepatic disorders has never been demonstrated.

In MSUD decompensation episodes, the duration of parenteral nutrition and amino acid solution infusion ranges between several days to a maximum of 1 month in the available study publications (see Summary of Clinical Efficacy). It is therefore unlikely that hepatic steatosis/fibrosis/cirrhosis could occur.

Hepatobiliary disorders are inconstantly reported as possible adverse reactions in amino acid solutions SmPCs and are listed for example, in Clinisol 15%, Primene 10%, Prosol 20%, and Synthamin 17 product information, principally because they are associated with parenteral nutrition known side effects to which the amino acid component may play a causal or contributory role. In the case of MSUD patients treated with Maapliv, the occurrence of hepatic disorders would rather be due to other parenteral nutrition components (lipids and carbohydrates) and the duration of parenteral nutrition rather than to Maapliv content, whose maximum dose remains at 3g/kg/day in new-borns up to 2-year-old infants. The short duration of amino acid supplementation during MSUD decompensation episodes is unlikely to produce hepatic steatosis, fibrosis, or cirrhosis.

#### *2.6.8.3. Serious adverse event/deaths/other significant events*

**Deaths**

No deaths related to the parenteral use of the BCAA-free solution, equivalent to Maapliv, have been reported. One death was reported in Alili study in patient who had severe pre-existing neurological disorders and experienced an acute worsening of general condition leading to death. This death was considered not related to the parenteral use of the BCAA-free solution.

**Serious adverse events**

No serious adverse drug reactions have been reported in the main studies.

#### *2.6.8.4. Laboratory findings*

No laboratory abnormalities related to the parenteral use of the BCAA-free solution, equivalent to Maapliv, have been reported.

#### *2.6.8.5. Safety in special populations*

**Data in children and adolescents**

Most data presented in this submission were collected in paediatric populations as classic MSUD is an inborn disease usually diagnosed during the neonatal period. Patients included in selected studies were followed from the early days of life into adulthood. There was no evidence of safety concerns with the use of IV BCAA-free solutions equivalent to Maapliv in children and adolescents.

**Use during pregnancy and lactation**

Only few cases of pregnancies in MSUD patients have been reported, with overall favourable outcomes. There is no data regarding the use of a parenteral BCAA-free mixture during pregnancy. Long-term treatment included a low-protein diet with enteral supplementation with a BCAA-free amino acid mixture. Pregnancy can put patients at risk of decompensation due to possible hyperemesis gravidarum, catabolic stress during

delivery and excessive protein turnover during the postpartum period. Successful management of pregnancy in classical MSUD is possible and requires careful prevention of metabolic decompensations by an individually tailored treatment plan, including protein restriction, adequate caloric supply, and prevention of catabolism.

**Patients with hepatic impairment**

No data are available in patients with hepatic impairment.

**Patients with renal impairment**

No data are available in patients with renal impairment.

#### *2.6.8.6. Discontinuation due to adverse events*

No cases of discontinuation due to adverse events related to the parenteral use of the BCAA-free solution, equivalent to Maapliv, have been reported in the main studies.

#### *2.6.8.7. Post marketing experience*

Post-marketing studies for Maapliv are not currently available.

### 2.6.9. Discussion on clinical safety

The overall size of the safety database is considered limited but overall acceptable taking into account rarity of the disease and the knowledge of the safety profile of the active substance (endogenous amino acids) used in other available formulations.

The main studies selected to show the safety of parenteral administration of BCAA-free solutions in patients with MSUD include 30 evaluable patients exposed to the BCAA-free solution equivalent to Maapliv, as per the applicant. This was administered intravenously for the treatment of 132 metabolic decompensation episodes (Servais et al. 2013, de Lonlay et al. 2021, Sanchez-Pintos et al. 2022, Alili et al. 2022, Blanco Dorado et al. 2017, Abi-Wardé et al. 2017). No serious adverse events were reported in the main publications.

It is agreed that of a poor leucine level response or rebound effect can be attributed to non-adherence to dietary protocols, inadequate dosing, insufficient protein intake, and specific patient circumstances such as switching from parenteral to enteral nutrition. The complex management of MSUD patients with multiple parallel co-variates preclude attribution of these occurrences to any single factor.

Only Alili et al reported adverse events (7), none of which were attributed to IV BCAA free treatment. The applicant provided discussion around the adverse event relating to the worsening of neurological symptoms in a child <1 year old and it can be agreed that no update to the SmPC is necessary based on this case. Also, the case of death reported in Alili et al. study in patient who had severe pre-existing neurological disorders and experienced an acute worsening of general condition leading to death was considered not related to the parenteral use of the BCAA-free solution

No laboratory abnormalities related to the parenteral use of the BCAA-free solution, equivalent to Maapliv, have been reported. There is no data regarding the use of a parenteral BCAA-free mixture during pregnancy, in patients with hepatic impairment and in patients with renal impairment.

It is noted that the majority of patients treated in studies had a classical form of MSUD. The applicant clarified that during an acute decompensation, recommended treatment approaches do not differ by MSUD sub-type and patients with very rare MSUD sub-types undergoing a decompensation episode would be treated using the same approach (with gold-standard oral or enteral BCAA-free solution) as the classic neonatal disease subtype. Although it is likely that the response to treatment in other forms of MSUD will be similar (taking into consideration pathomechanism of decompensation episodes) it is imported this this is confirmed post-marketing through a post-authorisation study.

Looking into the data provided and also into the SmPCs for other amino-acid solutions it seems that these amino-acid solutions products are well tolerated. Nevertheless, it is considered that additional safety data should be generated post-marketing through a post-authorisation study.

## 2.6.10. Conclusions on the clinical safety

The overall size of the safety database is considered limited but overall acceptable taking into account rarity of the disease and the knowledge of the safety profile of the active substance (endogenous amino acids) used in other available formulations. The available safety data do not raise any concern.

Nevertheless, post-marketing safety data should be collected through a post-authorisation study.

# *2.7. Risk Management Plan*

The applicant has submitted an updated version of the RMP (version 1.3, dated 15 May 2025).

## 2.7.1. Safety concerns

**Summary of safety concerns**

The applicant identified the following safety concerns in the RMP version 1.3:

| **Summary of safety concerns** | |
|---|---|
| Important identified risks | None |
| Important potential risks | None |
| Missing information | Long-term safety |

## 2.7.2. Pharmacovigilance plan

### *2.7.2.1.* Routine pharmacovigilance activities

Routine pharmacovigilance activities are considered sufficient to identify and monitor any safety concerns associated with Maapliv.

#### *2.7.2.2.* Summary of additional PhV activities

No additional pharmacovigilance activity is proposed. The applicant will conduct a post-authorisation study, as a specific obligation for this MA under Exceptional circumstances, in patients with MSUD treated with Maapliv during acute metabolic decompensation episodes to characterise efficacy and safety outcomes.

## 2.7.3. Plans for post-authorisation efficacy studies

Planned and Ongoing Post-authorization Efficacy Studies that Are Conditions of the Marketing Authorization or that Are Specific Obligations

| Study/Status | Summary of Objectives | Efficacy Uncertainties Addressed | Milestones | Due Dates |
|---|---|---|---|---|
| Efficacy studies which are Specific Obligations in the context of a conditional marketing authorization or a marketing authorization under exceptional circumstances | | | | |
| A registry of patients with maple syrup urine disease (MSUD) treated with MAAPLIV<br><br>Planned | **Primary Objectives:**<br>Characterize safety and efficacy outcomes on MAAPLIV during acute metabolic decompensation episodes.<br><br>**Secondary objectives:**<br>1. Describe the association between plasma leucine levels and clinical symptom resolution<br>2. Describe the characteristics of responders/non-responders patients per episode to MAAPLIV<br>3. Describe the acute decompensation episode outcomes per MSUD subtypes, or per disease severity (i.e. leucine level at baseline)<br>4. Describe therapeutic management of acute decompensation episodes: hospitalization (duration, unit), decompensation episode treatments (including emergency diet management, MAAPLIV and other concomitant treatments).<br>5. Evaluate real-world treatment healthcare resource utilization (HRU).<br>6. Measure the health-related quality of life outcomes for patients and caregivers. | Long term safety and efficacy<br><br>Describe efficacy profile per MSUD subtype and per severity of the decompensation episode based on leucine level at baseline | Protocol submission<br><br>Annual interim report | First quarter of 2026<br><br>Interim reports will be submitted upon periodic annual re-assessment |

## 2.7.4. Risk minimisation measures

### *2.7.4.1. Routine Risk Minimisation Measures*

Routine risk minimisation measures are considered sufficient to manage the risks related to Maapliv.

### *2.7.4.2. Summary of additional risk minimisation measures*

No additional risk minimisation measures are warranted.

**Description of Routine Risk Minimization Measures by Safety Concern**

| | **Routine Risk Minimization Activities** |
|---|---|
| **Missing information** | |
| Long term safety | Routine risk communication<br>• SmPC 4.4 and section 2 of the PI mentions that there is limited information on the repeated administration of the intravenous BCAA-free formula<br><br>Routine risk minimization activities recommending specific clinical measures to address the risk: None<br><br>Other routine risk minimization measures beyond the Product Information:<br>• Special medical prescription |

## 2.7.5. Conclusion

The CHMP considers that the risk management plan version 1.3 is acceptable.

## *2.8. Pharmacovigilance*

## 2.8.1. 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.

### 2.8.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required.

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.

## *2.9. Product information*

The attached product information is considered acceptable.

### 2.9.1. 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.*

### 2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Maapliv (amino acids) is included in the additional monitoring list as it is approved under exceptional circumstances [REG Art 14(8), DIR Art (22)].

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

# 3. Benefit-Risk Balance

## *3.1. Therapeutic Context*

### 3.1.1. Disease or condition

Maple syrup urine disease is an inborn error of amino acid metabolism caused by a deficiency of the mitochondrial enzyme branched chain α-ketoacid dehydrogenase (BCKDH) complex activity, resulting in the accumulation of the branched-chain amino acids (BCAAs), leucine, isoleucine and valine as well as their metabolites (α-ketoacids). Increased BCAAs and metabolite levels in plasma may result in severe complications. The potentially devastating consequences in MSUD derive mainly from the neurotoxic effects of leucine and its metabolite, α-ketoisocaproic acid (KICA or 2-KICA), the normal metabolism of which is blocked. If leucine and 2-KICA accumulate, profound metabolic alterations and impairment of energy homeostasis occur, which are associated with severe clinical symptoms, the most serious of which are neurological and may result in permanent damage, disability, and even death.

Metabolic decompensation in MSUD may occur when leucine levels rise above 500-1000 µmol/L, as a result of dietary non-compliance, infection (by far the most common cause) or other physiological or metabolic stresses. Episodes of metabolic decompensation are acute medical emergencies and may occur at any time or any age after trauma, surgery, illness, or inappropriate dietary intake. Failure to lower leucine levels in a timely manner may result in permanent morbid sequelae including disability and death since acute metabolic decompensation may be fatal. If not appropriately diagnosed and treated, rapid elevation of circulating leucine and its ketoacid, α-ketoisocaproate, cause encephalopathy and life-threatening brain swelling. This can lead to irreversible neurological complications, including metabolic decompensation, and death.

## 3.1.2. Available therapies and unmet medical need

The management of decompensation episodes (DEs) in MSUD patients faces several issues. Despite adherence to therapy, patients may suffer unpredictable metabolic decompensation episodes at any time or at any age following trauma, surgery, illness, or inappropriate dietary intake, leading to major neurological and possibly life-threatening complications or death and for which the management becomes a medical emergency. In this case, a complex therapeutic management with several treatments and several routes of administration within a limited time for ensuring a quick reduction of toxic level of BCAAs are used. The most severe episodes may also require extracorporeal interventions (haemodialysis or haemofiltration) to remove the excess of BCAAs and to allow a high plasma clearance but with all the possible complications of the procedure itself. The main complications may include vascular stenosis and venous thrombosis, haemodynamic instability, or infection.
A pivotal component of the therapy is the use of BCAA-free mixtures. Current commercialised mixtures are used as food supplements for the long-term dietary maintenance and not specifically designed for the treatment of decompensation episodes.
Their administration is limited to the enteral route, either by oral feeding, nasogastric intubation, or gastrostomy. In the context of metabolic decompensations, which require urgent BCAA-free administration, the enteral route is inappropriate for patients presenting clinical symptoms such as coma, seizures, gastric intolerance, nausea, vomiting, or for patients refusing nasogastric tubing (frequent for adolescents) and it is not appropriate as a preventive treatment before planned surgery. The intravenous (IV) administration of a BCAA-free mixture in all these circumstances has been found to be an essential alternative, potentially avoiding invasive or aggressive interventions such as gastrostomy, which is especially relevant for the younger, vulnerable target population.
In April 2021 a guideline for the management of the disease recommending the use of parenteral BCAA-free formulations was published by French Health Authorities: The French National Protocol for the Diagnosis and Care of MSUD.
No medicinal product containing a BCAA-free solution, nor a medicinal product for IV administration allowing a prompt medical intervention for the treatment of DEs in MSUD patients has been approved in Europe. In Europe, BCAA-free solutions intended for IV administration in MSUD patients have been prepared locally in hospital's pharmacies and thus were not immediately available and the pharmaceutical quality standards and the manufacturing reproducibility in terms of intra- and inter-individual subject variability were questionable.

## 3.1.3. Main clinical studies

The applicant submitted a MA application based on scientific data from publications. The applicant conducted the literature search which consisted of an automated search across several medical literature databases, including PubMed, The Cochrane Library and Embase, up until September 2022, without any language restrictions.

Keywords were selected using the index list and focussed on the targeted patient population and the parenteral administration of BCAA-free amino acid mixtures.

The selection of publications was a 2-step process, initially screening titles, and abstracts (step 1), and subsequently reviewing the full text (step 2) to ensure the text was relevant, with active substances similar to those in Maapliv, including publications in the targeted patient population and in the European Union.
All type of study designs were considered as well as 'named patient use' reports.
Five publications report on the parenteral use in Europe of a BCAA-free mixture the same as the Maapliv formulation were identified as main studies :Servais et al. 2013, Abi-Wardé et al. 2017, de Lonlay et al. 2021, Alili et al. 2022, Blanco Dorado et al. 2017, Sanchez-Pintos et al. 2022.

| Author | Year | Country | Study design | Sample size |
|---|---|---|---|---|
| **Main studies** | | | | |
| Servais A. | 2013 | France | Retrospective registry | 4 |
| Blanco Dorado S. | 2017 | Spain | Case report | 1 |
| de Lonlay P. | 2021 | France | Retrospective observational | 54 |
| Alili JM. | 2022 | France | Prospective observational | 24 |
| Sanchez-Pintos | 2022 | Spain | Retrospective observational | 5 |

## *3.2. Favourable effects*

In presented studies that plasma leucine concentrations were decreasing in patients but as to what extent IV BCAA free solution is contributing to this decrease is not clear.

Time to normalise plasma leucine concentrations (defined as a plasma leucine concentration of < 381 µmol/L) when treated with IV BCAA free solution was the most frequently reported endpoint.

In Servais et al., in total four adult patients were included, who experienced 17 decompensation episodes in 2010 and in total 18 decompensation episodes between 2005 and 2010, mean plasma Leu concentrations decrease, and in most cases, normalisation (<381 µmol/L) within 3.5 days was reported.
In publication by Blanco Dorado, after administration of parenteral protein solution without branch chain amino acids a rapid and significant descent in plasma leucine levels was observed on day 15 of hospitalization in patient with MSUD with a metabolic decompensation due to infection.

In Study by de Lonlay et al., in total 51 (40.8%) of patients received IV BCAA-free formula. Mean time to decompensation episode resolution was 9.2 days in the oral/enteral group and 7.4 days in the IV group. The mean length of hospital stay was 6.6 days for the oral/enteral group and 5.4 days for the IV group. The mean length of ICU stay was 3.2 days for the oral/enteral group and 2.5 days for the IV group.

Overall, leucine normalization was achieved at discharge in 76.8% of episodes in the oral/enteral group and in 80.6% in the IV group. The mean (±SD) time to first leucine normalization (<381 µmol/L) was 68.4 hours (±52.9) in the oral/ enteral group and 95.6 hours (±79.2) in the IV group. At discharge, leucine levels had decreased by a mean of 548.5 µmol/L in the oral/enteral group and by 657.2 µmol/L in the IV group.

In an observational, prospective study by Alili et al., in patients with MSUD who were hospitalized due to decompensation episodes, leucine plasma concentrations were normalised in 83.3% (85/102) of episodes in a mean (SD) duration of 3.0 days (1.2), In children the observed time to leucine normalisation was a mean of 2.7 (1.1) days, and for adults a mean time of 3.0 (1.3) days.
Reduction in time compared with predicted time to leucine normalisation was 41% and 25% in children and adults, respectively.

Study by Sanchez-Pintos all patients responded to treatment, discharge leucine level ranged from 23 to 282 µmol/L. However, duration of IV BCAA-free treatment was different for each included patient, ranging from 3 days to 20 days.

In some studies, it was stated that symptoms improved once on treatment however, an improvement of symptoms associated with decompensation was not systematically investigated.

In the context of metabolic decompensations, which require urgent BCAA-free administration, the enteral route is inappropriate for patients presenting clinical symptoms such as coma, seizures, gastric intolerance, nausea, vomiting, or for patients refusing nasogastric tubing (frequent for adolescents) and it is not appropriate as a preventive treatment before planned surgery. No medicinal product containing a BCAA-free solution, nor a medicinal product for IV administration allowing a prompt medical intervention for the treatment of DEs in MSUD patients is approved in Europe.
The intravenous (IV) administration of a BCAA-free mixture in all these circumstances has been found to be an essential alternative, potentially avoiding invasive or aggressive interventions such as gastrostomy, which is especially relevant for the younger, vulnerable target population.

## *3.3. Uncertainties and limitations about favourable effects*

There are some uncertainties and limitations to the favourable effects. The quality of evidence provided in support of this application was limited and the studies presented by the applicant were observational (in most cases retrospective).
There is uncertainty as to what extent the population of patients investigated in the studies presented, was representative of the target population in the claimed indication. Indeed, the majority of patients treated in studies had a classical form of MSUD. The applicant clarified that during an acute decompensation, recommended treatment approaches do not differ by MSUD sub-type and patients with very rare MSUD subtypes undergoing a decompensation episode would be treated using the same approach (with gold-standard oral or enteral BCAA-free solution) as the classic neonatal disease subtype. Nevertheless, it is considered that the data on treatment of other sub-types should be collected post marketing in a post-authorisation study. Further, the SmPC, section 5.1, highlights that there is no experience with other sub-types of MSUD.

There is also uncertainty on the efficacy results in sub-groups of patients with severe or critical increase in the plasma Leu concentration as the number of these patients was very small in the provided studies.

In severe/critical acute depensation episodes the treatment with IV BCAA-free amino acid formulation could be insufficient and other treatments, e.g haemodialysis/ haemofiltration may need to be considered/ implemented. This is reflected in the SmPC

Another uncertainty relates to how treatment with IV BCAA-free mixture contributed to improvement of symptoms in patients treated, as clinical endpoints were not systematically investigated across studies. This would especially be important in the context of the improvement of neurological symptoms as the central nervous system is most affected in MSUD.

Further, it is important to note that the pathophysiology of the brain damage in MSUD is not only dependent on accumulation of leucine and α-KIC (Chuang et al., 2019) but also on imbalances in other amino-acids, leading potentially to deficient cerebral protein and neurotransmitter synthesis.

The pre-clinical data are indicating that blood amino acid analysis may be a poor surrogate for assessing the outcomes of protein restriction and amino-acid levels in brain. Therefore, it is considered that data to further characterise safety and efficacy outcomes on Maapliv during acute metabolic decompensation episodes should be collected post marketing in an appropriately designed post-authorisation study.

## 3.4. Unfavourable effects

There were no common, serious adverse events reported in the main publications.

The data available do not raise any safety concern.

## 3.5. Uncertainties and limitations about unfavourable effects

The overall size of the safety data base, consisting of 30 patients exposed to the BCAA-free solution equivalent to Maapliv administered intravenously for the treatment of 132 metabolic decompensation episodes is limited. Therefore, additional safety data should be generated post-marketing through a post-authorisation study.

## 3.6. Effects Table

**Table 5.6.1. Effects Table for MAAPLIV**

| Effect | Short Description | Unit | Parenteral | Oral/enteral | Uncertainties/ Strength of evidence | References |
|---|---|---|---|---|---|---|
| **Favourable Effects** | | | | | | |
| Leucine plasma level at discharge | Normalisation of leucine level was considered a surrogate PD endpoint for efficacy | µmol/L | 320.2±129,6 (Group treated parenterally) | 343.1±236.3 (Group treated enterally) | Plasma leucine normalisation is <381 µmol/L | Servais et al. |
| Leucine normalisation at discharge n | | n | 13/17 (76.5) | 11/18 (61.1) | In 7 cases in the E group and 4 cases in the P group, patients were discharged before leucine normalisation against the attending physicians' advice. | Servais et al. |
| Time to normalisation of Leu conc. | | days | 3.8±1.8 | 3.4±1.0 | | Servais et al. |
| Leucine normalisation at discharge | | N (%) | 29 (80.6) | 53 (76.8) | Unclear criteria for decompensation episodes selection | de Lonlay et al. |
| Leucine decrease from baseline at discharge | | µmol/L | 657.2 | 548.5 | | de Lonlay et al. |
| Time to DE resolution, mea | | days | 7.4 (4.6) | 9.2 (6.2) | | de Lonlay et al. |

## *3.7. Benefit-risk assessment and discussion*

### 3.7.1. Importance of favourable and unfavourable effects

Overall, leucine normalization, which is considered a surrogate for efficacy in decompensation episodes, was achieved at discharge in most of decompensation episodes described in the main studies. In the context of metabolic decompensations, which require urgent BCAA-free administration, the enteral route is inappropriate for patients presenting clinical symptoms such as coma, seizures, gastric intolerance, nausea, vomiting, or for patients refusing nasogastric tubing (frequent for adolescents) and it is not appropriate as a preventive treatment before planned surgery. The availability of a ready-made BCAA free IV formulation is an important benefit in the management of decompensation episodes for patients who are not eligible for oral and enteral formulations.
Treatment with Maapliv was well tolerated with no serious adverse events reported; based on the data available, the safety profile of Maapliv does not raise any concern.

### 3.7.2. Balance of benefits and risks

The overall benefit /risk balance of Maapliv is considered positive.

### 3.7.3. Additional considerations on the benefit-risk balance

***Marketing authorisation under exceptional circumstances***

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was proposed by the CHMP during the assessment, after having consulted the applicant.

The CHMP considers that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence. Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

Indeed, the estimated global incidence of MSUD is 1 in 185,000 live births [with some variability currently identified across countries (Italy 1:240.000 newborns; France 1:150.000 newborns; Germany 1:175.000 newborns)].

As of today, there are no medicinal products or devices approved specifically for the treatment of either MSUD or acute decompensation episodes. The unmet medical need is acknowledged. The treatment is based on early implementation of emergency treatment, diet, close monitoring, and even dialysis in the setting of acute metabolic decompensation. Liver transplantation in pediatric patients with classic MSUD is also adopted as long-term treatment.

The low incidence of the disease, accompanied by the currently used options implemented to treat MSUD especially in emergency settings, reduces the number of patients that could have access to Maapliv, limiting thus the capability to provide comprehensive and exhaustive data.

As a specific obligation for this MA under exceptional circumstances, the applicant commits to initiate a non-interventional post-authorisation study in order to further characterise the efficacy and safety of Maapliv in the treatment of patients with MSUD presenting with acute decompensation episodes.

### *3.8. Conclusions*

The overall benefit /risk balance of Maapliv is considered positive.

## 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 Maapliv is favourable in the following indication(s):

Maapliv is indicated for the treatment of maple syrup urine disease (MSUD) presenting with an acute decompensation episode in patients from birth who are not eligible for an oral and enteral branched-chain amino acids free (BCAA- free) formulation.

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances 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.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

***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.

***Obligation to conduct post-authorisation measures***

- **Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances**

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

| **Description** | **Due date** |
|---|---|
| Non-interventional post-authorisation study:<br>In order to further characterise the efficacy and safety of Maapliv in the treatment of patients with maple syrup urine disease (MSUD) presenting with acute decompensation episodes, the MAH should conduct and submit the results of a non-interventional post-authorisation study, according to an agreed protocol | Protocol submission:<br>Q1 2026<br><br>Annual reports to be submitted with annual reassessment |
| In order to ensure adequate monitoring of safety and efficacy of Maapliv in the treatment of patients with maple syrup urine disease (MSUD) presenting with acute decompensation episodes, the MAH should provide yearly updates on any new information concerning the safety and efficacy of Maapliv. | Annually (with annual reassessment) |