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Public Assessment Report

Scientific discussion

Enterol FORTE 500 mg prášok na perorálnu suspenziu
Saccharomyces boulardii (CNCM I-745)

2022/01338-REG

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This module reflects the scientific discussion for the approval of Enterol FORTE The marketing authorisation was issued on 28 September 2024. For information on changes after this date please refer to the module 'Update'.

I. INTRODUCTION

Based on the review of the quality, safety and efficacy data, the State Institute for Drug Control has granted a marketing authorisation for **Enterol FORTE 500 mg prášok na perorálnu suspenziu (hereinafter Enterol FORTE),** 500 mg, powder for oral suspension, from Biocodex, France.

The product is indicated in adults for a prevention of a diarrhoea caused by antibiotics use.

A comprehensive description of the indications and posology is given in the SmPC.

The marketing authorisation has been granted pursuant to Article 10a of Directive 2001/83/EC, which corresponds to Article 49 (1) b) of Law on medicinal products and medical devices No. 362/2011.

II. QUALITY ASPECTS

II.1 Introduction

The finished product is presented as a powder for oral suspension containing 500 mg of *Saccharomyces boulardii (*CNCM I-745) ($2x10^9$ cells per sachet) as an active substance.

The strain of yeast used for the manufacture of lyophilized *Saccharomyces boulardii* is *Saccharomyces boulardii.* The strain has been discovered during the 20th century on tropical fruits. The strain has been registered and has the following references:
- Institut Pasteur de Paris, reference I.745,
- American Type Culture Collection (ATCC), reference ATCC 74012,
- Centraalbureau voor Schimmelcultures, reference CBS 5926.

There was a synonym, *Saccharomyces cerevisiae* Hansen CBS 5926, that had been used for a certain time in Germany and Belgium.

Powder for oral suspension is packed in sachets made of polyethylene terephthalate plus aluminium foil plus polyethylene film composite and available as packs of 10, 24 and 20 sachets. Powder is light brown with characteristic fruity odour.

The other ingredients are:
fructose
silica, colloidal anhydrous
lactose monohydrate
tutti-frutti flavour

II.2 Drug Substance

***Saccharomyces boulardii (*CNCM I-745)**

Appearance: Light-brown powder with characteristic odour.

Culture characteristics: The optimum temperature for growth of *Saccharomyces boulardii* lies between 30 and 37°C.

Culture on Sabouraud agar with **chloramphenicol**: round, white and creamy colonies, frequently with raised slightly wrinkled central part.

Culture on Sabouraud agar medium with **tetrazolium**: *Saccharomyces boulardii* reduces tetrazolium yielding pink to violet-red colonies.

Culture on Sabouraud agar medium with **actidione**: no growth.

Manufacturing: The yeast is manufactured from a stock strain (slope) by serially culturing in appropriate media and in increasingly large containers to obtain a pure yeast culture. The first two cultures are conducted in glass conical flasks and subsequent cultures in fermenters.
All the equipment used for manufacture is steam sterilized. The culture media and nutrient solution are heat sterilized or sterilized by filtration. Following culture, the yeast is concentrated, then freeze-dried. Freeze-drying is conducted on a yeast suspension in lactose solution. The suspension is frozen, then sublimated. Lastly, the lyophilized yeast is graded.

Manufacturing steps are as follows:
Strain preparation and preservation
Culture in conical flasks
Culture in the fermenters
Centrifuging
Yeast suspension manufacture
Freeze-drying
Milling

Specifications: The proposed active pharmaceutical ingredient (API) specification covers tests for API identity, purity, viability of *Saccharomyces boulardii* and genotypic identification by polymerase chain reaction (PCR).

Stability: Based on provided stability data the proposed re-test period of three months when stored up to 25°C was approved.

II.3 Medicinal Product

Pharmaceutical development

The manufacturing process of drug product was developed in the sixties. The process developed is reproducible. The manufacturing process consists of the following steps:
- Mixing powders.
- Filling into sachet.

The manufacturing process does not imply special formulation manipulation.

Manufacturing of the product

The manufacturing process consists of weighing and mixing of API with excipients, and filling into sachets. Two critical process steps were identified. During mixing of several API batches homogeneity of the mixture is controlled and sachet content and tightness is then controlled. It is a standard well-established manufacturing process which has been successfully validated.

Product specification

Specification parameters have been chosen based on Ph. Eur. monograph 3053 “Live biotherapeutic products for human use”, general Ph. Eur. monographs relating to the dosage form and guidelines 3AQ11A and ICH Q6A.
The finished product release and shelf-life specifications include appropriate tests and limits for appearance (visual), active substance identification (microscopic and study of the assimilation of sugars and other carbohydrate products, both in-house), uniformity of mass of single dose preparations (Ph.Eur.), water content (Ph.Eur.). viability determination (in-house), assay (in-house), and microbiological quality of non-aqueous preparations for oral use (Ph.Eur.).

Stability

Proposed shelf life of the finished product 3 years, with no recommended storage conditions was accepted based on compliant real time stability data covering 36 months at long term storage conditions 25°C/60% RH and 30°C/75% RH, and 6 months at accelerated storage conditions 40°C/75% RH.

II.4 Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance, and finished product was presented in a suitable manner. 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.

The quality of the product is considered acceptable when used in accordance with the conditions defined in the SmPC.

III. NON-CLINICAL ASPECTS

III.1 Introduction

Pharmacodynamic, pharmacokinetic and toxicological properties of *Saccharomyces boulardii (*CNCM I-745) are well known. As *Saccharomyces boulardii (*CNCM I-745) is a widely used, well-known active substance, the applicant has not provided additional studies, and further studies were not required. An overview based on the literature review was, thus, appropriate.

The Non-Clinical Overview on the pre-clinical pharmacology, pharmacokinetics and toxicology was adequate.

III.2 Pharmacology

Primary pharmacodynamics

The pharmacodynamic activity of *Saccharomyces boulardii* CNCM I-745 *against C. difficile* colitis in hamsters, mice, and rats; *C. rodenium* infection in mice; and *S. typhimurium* and *Shigella flexneri* infection in mice was presented. Additional data showed that *Saccharomyces boulardii* reduced the growth of *Y. enterocolitica* and its invasion into HeLa cells and that *Saccharomyces boulardii* had both preventative and therapeutic properties *in vivo* against *C. krusei* and *C. pseudotropicals* in mice.

Secondary pharmacodynamics

Saccharomyces boulardii showed beneficial effects when it improved intestinal health in piglets; inhibited irritable bowel syndrome in mice; reduced intestinal *C. albicans* colonization and inflammatory response in mice; reduced the negative gastrointestinal effects in 5-fluorouracil-induced intestinal mucositis in mice, irinotecan-induced diarrhoea and mucositis in rats, ibuprofen-induced gastric ulcer in rats; exerted anti-hypercholesterolemic properties in hamsters; improved the vaccine immune response to Bovine herpesvirus type 5 in sheep; and could prevent colon cancer in rats.

III.3 Pharmacokinetics

Based on the studies in rats and mice, *Saccharomyces boulardii* does not cross the gastrointestinal wall and is excreted in the faeces. Thus, *Saccharomyces boulardii* CNCM I-745 remains in the gastrointestinal tract till elimination, without entering the systemic circulation.
According to the rat studies by Blehaut et al., the half-life of less than 3 or 9 hours for live cells and 36 or 44 hours for dead cells was estimated.

III.4 Toxicology

In the presented two acute dose toxicity studies, after oral administration of *S. boulardii*, no death occurred in the animals, although high dose multiples were tested. Therefore, the lack of toxicity did not enable to determine the LD_{50} values in mice and rats.

No toxicological manifestations were observed in 14-day rat, 6-week dog, 6-month rat, and 6-month rabbit study after repeated dosing of *Saccharomyces boulardii* CNCM I-745.

The Applicant presented the Ames test with a complete set of bacterial strains and additional information about *S. boulardii´s* antigenotoxic and antioxidative properties.

However, the requirements for the standard test battery for genotoxicity described in ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use (EMA/CHMP/ICH/126642/2008) were not completely fulfilled. Applicant committed to perform an additional *in vitro* chromosomal aberration test and provide the results as soon as possible.

There was no carcinogenicity study presented because the expected clinical use should be less than 6 months.

No data on relevant studies related to reproductive and developmental toxicity of *Saccharomyces boulardii* were available.

III.5 Ecotoxicity/environmental risk assessment (ERA)

Yeasts belonging to the *Saccharomyces* genera, and especially *Saccharomyces cerevisiae* to which species *Saccharomyces boulardii* CNCM I-745 closest taxonomically relates to, have been used in food fermentation and brewery for millennia, worldwide.

The applicant declared that measurement of the partition coefficient of *Saccharomyces boulardii* is not feasible due to its complex composition. This statement also supports the provided *Saccharomyces cerevisiae* data (ECHA). Moreover, it is anticipated that the high solubility of these components will result in a low log Kow, which leads to the conclusion that bioaccumulation poses no concern.

Based on the arguments that *Saccharomyces boulardii* obtained from therapeutic products does not contribute to harmful environmental exposure, it can be said that when used as recommended, this product is not likely to pose an environmental concern.

III.6 Discussion on the non-clinical aspects

No new non-clinical studies were performed by the applicant. An overview based on literature review was appropriate for a chosen legal basis, i.e. bibliographic application. This was also considered acceptable in view and the nature of the active substance, *Saccharomyces boulardii* and decades of experience with the clinical use of this active substance.

This information about summary of non-clinical data is adequately presented in section 5.3 of the SmPC.

IV. CLINICAL ASPECTS

IV.1 Introduction

The proposed medicinal product is produced in the form of powder for oral suspension, intended for oral administration, with *Saccharomyces boulardii* CNCM I-745 as an active substance. The medicinal product belongs to the pharmacotherapeutic group: antidiarrheal microorganisms with Anatomical Therapeutic Chemical (ATC) code: A07FA02.

Saccharomyces boulardii CNCM I-745 is a non-pathogenic yeast with an unusual optimum growth temperature of 37°C (i.e., human body temperature) (Surawicz 1989a) and has been used for years in the prevention and treatment of diarrhoea of various origins.

This marketing authorisation application (MAA) was submitted based on scientific bibliographical evidence supporting the risk-benefit profile for *Saccharomyces boulardii* and provides adequate justification for the marketing approval of the product. A literature review was undertaken to identify and retrieve relevant research articles describing the clinical pharmacology, efficacy and the safety profile of *Saccharomyces boulardii*.

IV.2 Pharmacokinetics

The first investigations in human were conducted by Blehaut in 1989 on 8 healthy volunteers receiving *Saccharomyces boulardii* 1,000 mg daily (two 250 mg capsules, bid) for two weeks. The yeast concentrations in the stool samples increase rapidly over the first 2 days and then reach a steady state always achieved by day 3. Cessation of dosing with *Saccharomyces boulardii* led to a rapid decrease in the concentration of the yeast in the faeces with none detected between 2 to 5 days after the last dose. Globally, less than 1% of the administered dose was recovered in feces.

Recovery and elimination of *Saccharomyces boulardii* was also investigated in a randomized, cross-over study with 3 escalated doses given to 8 healthy volunteers for one week (100, 500 and 1,500 mg bid) separated by a one week wash-out period (Klein 1993). Steady-state fecal levels of *Saccharomyces boulardii* were achieved by 72 hours for each dose. There was a significant trend of increased *Saccharomyces boulardii* levels in the stool as the administered dose was increased. The dose recovery at steady-state concentrations was significantly higher when comparing the administration of 200 mg/day to 1,000 or 3,000 mg/day but not between 1,000 and 3,000 mg/day.

Overall, the mean recovery in stool in this study was found to be < 5%, which was independent of the dose administered. This relative low recovery is in agreement with the findings of Blehaut (1989) who reported a global recovery of less than 1%. It was also demonstrated that exposure to *Saccharomyces boulardii* did not significantly alter the quantitative populations of tested normal flora of the gut. The only substantive increases observed were for total aerobes and total coliforms but these increases were not significant.

IV.3 Pharmacodynamics

Primary pharmacology

Anti-microbial action
Within the intestinal luminal *Saccharomyces boulardii* exerts several anti-microbial activities that could be divided in two groups: direct anti-toxin effects and inhibition of growth and invasion of pathogens (Canani 2011).

The anti-toxin action elicited by *Saccharomyces boulardii* is mainly due to small peptides produced by the yeast. A 54kDa serine protease can inhibit enterotoxin and cytotoxic activities of *Clostridium difficile* by degradation of toxin A and B and receptors sites of toxin A on the enterocyte cell surface and directly degrades C. difficile toxins A and B (Canani 2011, Szajewska 2015a). A 120kDa protein

that has a nonproteolytic activity competes specifically with the hyper-secretion caused by the toxins of *Vibrio cholerae* decreasing cyclic adenosine monophosphate in the enterocytes. Finally, *Saccharomyces boulardii* produces a phosphatase able to dephosphorylate endotoxins (such as lipopolysaccharide of E. coli 055B5) and inactivates its cytotoxic effects. This mechanism may account for the protection afforded in cases of sepsis (Canani 2011).

Trophic action
When *Saccharomyces boulardii* is given to patients with diarrhoea, normal microbiota is re-established more rapidly. On the contrary, *Saccharomyces boulardii* has no effect on microbiota composition in healthy humans (Swidsinski 2008, Canani 2011). This effect is tightly linked to a stimulation of short chain fatty acids (SCFA) production, especially butyrate. The production of SCFAs is significantly decreased in patients receiving antibiotics. The effect on butyrate production is particularly relevant considering the important role of this compound for the regulation of many intestinal functions including, the stimulation of enterocytes growth and differentiation, fluid absorption, immune stimulation, anti-inflammatory effects, enteric neurons growth and differentiation.

Immunoregulation
The toxins elaborated by *Clostridium difficile* and pathogenic bacteria, such as enteropathogenic and enterohaemorragic *E. coli*, activate the mitogen activated protein (MAP) extracellular signal-regulated kinase 1 and 2 (ERK 1/2) and p38 as well as the nuclear factor κB (NF-κB) (p65/p50) system leading to transcription of pro-inflammatory genes such as interleukin 8 (IL-8) that promote inflammation. *Saccharomyces boulardii* inhibits MAP kinase and NF-κB signal transduction pathways and decreases the secretion of IL-8 and reducing inflammatory diarrhea (Canani 2011).

Secondary pharmacology

A mechanism indirectly involved in the immune-regulation effect exerted by *Saccharomyces boulardii* is the modulation of intestinal permeability. Increased intestinal permeability is frequently observed in different situations such as following shock, burn injury, obstructive jaundice, intestinal resection, hepatic transplant, or intestinal obstruction. Animal studies showed that oral pretreatment with viable or heat killed cells of *Saccharomyces boulardii* preserves intestinal integrity and modulates inflammation, preventing bacterial translocation and intestinal lesions (Canani 2011).
Finally, it has been demonstrated that *Saccharomyces boulardii* modulates the nitrogen oxide pathway through the inhibition of the iNOS, contributing to a general down-regulation of intestinal inflammation and an anti-secretory stimuli on transepithelial ion transport (Canani 2011).

IV.4 Clinical efficacy

Several data on conducted clinical trials supporting the use of *Saccharomyces boulardii* was submitted. For a final indication: prevention of antibiotics assotiated diarhoea (AAD), following metaanalysis was of most importance supporting approved indication of AAD. A meta-analysis was conducted by H. Szajewska & M. Kotodziej (Szajewska et al. 2015) on 6 randomized controlled trials, including 1,653 patients. It shows that *Saccharomyces boulardii* reduced the risk from 20.9% to 8.8% corresponding to a relative risk of 0.43 ([95% CI: 0.3–0.6]) and a protective efficacy was evaluated around 57%. The authors concluded that if the use of probiotics for preventing AAD is considered, the ESPGHAN Working Group for Probiotics Prebiotics recommends (strong recommendation) using *Saccharomyces boulardii.*

IV.5 Clinical safety

Applicant submitted data on serious adverse events (SAE), which have been collected in most recent three clinical trials sponsored by them and data from the last PSUR cycle.

The last PSUR for *Saccharomyces boulardii* had been submitted in May 2020 to the EMA and safety data covered the period of the 07 February 2017 to the 07 February 2020.

Cumulatively, a total of 4,372 cases were retrieved for *Saccharomyces boulardii* in the pharmacovigilance database, including 423 serious cases ((9,7%) and 3,949 non-serious cases. 31 cases had led to death of patients.
The conclusion of this PSUR is the following: Analysis of all cases received by Biocodex between 08 February 2017 and 07 February 2020 did not identify any new safety information that could alter the benefit-risk assessment of *Saccharomyces boulardii* CNCM I-745, compared to the previous reports. Based on the data presented in this PSUR, it may be concluded that the overall benefit-risk balance of *Saccharomyces boulardii* CNCM I-745 containing products used according to the reference information remains positive.

Changes requested in the PSUR assessment report (PSUSA/00009284/202002), for the product information were implemented as follows:
Section 4.4: addition of cases of sepsis in patients with central venous catheter, critically ill or immunocompromised patients, most often resulting in pyrexia.
Section 4.8: addition of sepsis in critically ill or immunocompromised patients (frequency unknown).

IV.6 Risk Management Plan

The MAH has submitted a risk management plan, in accordance with the requirements of Directive 2001/83/EC as amended, describing the pharmacovigilance activities and interventions designed to identify, characterise, prevent or minimise risks relating to Enterol FORTE.

- Summary table of safety concerns as approved in RMP

Important identified risks	Fungemia and sepsis in patients with a central venous catheter (CVC), in critically ill patients or in immunocompromised patients
Important potential risks	None
Missing information	None

IV.7 Discussion on the clinical aspects

No new clinical studies were performed by the applicant. An overview based on literature review was appropriate for a chosen legal basis, i.e. bibliographic application. Scientific bibliographical evidence supported the risk-benefit profile for *Saccharomyces boulardii* and provided adequate justification for the marketing approval of the product. Information about summary of clinical data is adequately presented in sections 4 and 5 of the SmPC.

V. USER CONSULTATION

The package leaflet has been evaluated via a user consultation study in accordance with the requirements of Articles 59(3) and 61(1) of Directive 2001/83/EC. The language used for the purpose of user testing the PL was English.
The results 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.

VI. OVERALL CONCLUSION, BENEFIT/RISK ASSESSMENT AND RECOMMENDATION

This was a MAA of medicinal product for human and use as it is defined in Article 10(a) (bibliographic application) of the European Directive 2001/83/EC as amended corresponding to Article 49 (1) b) of Law on medicinal products and medical devices No. 362/2011 as amended. MAA

was submitted via national procedure according to Article 52 of Law on medicinal products and medical devices as amended.

From a (non-)clinical point of view, the application contained adequate (non-)clinical data that supported chosen legal basis – bibliographic application.

Quality aspects of the dossier were adequately described.

Based on submitted data referring to published scientific literature applicant adequately addressed the requirements for a bibliographical application and sufficiently demonstrated the quality of a medicinal product in the scope of this MAA. Therefore, the State Institute for Drug Control granted the marketing authorisation on 28 September 2024.

Literature references (mentioned in PAR)

Blehaut H, Massot J, Elmer GW, Levy RH. Disposition kinetics of Saccharomyces boulardii in man and rat. Biopharm Drug Dispos 1989, 10:353-364

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Klein SM, Elmer GW, McFarland LV, Surawicz CM, Levy RH. Recovery and elimination of the biotherapeutic agent, Saccharomyces boulardii, in healthy human volunteers. Pharm Res 1993, 10(11):1615-1619

Canani RB, et al., Saccharomyces boulardii: a summary of the evidence for gastroenterology clinical practice in adults and children, European Review for Medical and Pharmacological Sciences, 2011; 15:808-822

Szajewska H, Horvath A, Kolodziej M. Systematic review with meta-analysis: Saccharomyces boulardii supplementation and eradication of Helicobacter pylori infection. Aliment Pharmacol Ther 2015a, 41:1237-1245

Swidsinski A, Loening-Baucke V, Verstralen H, Osowska S, Doerffel Y. Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. Gastroenterology 2008, 135(2):568- 579

Szajewska H, Kolodziej M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhea. Aliment Pharmacol Ther 2015b, 42:793-801