Pharmaceutical qualification of BIOINVERT® 200, an enzyme substitute to SUCRAID®

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Objectives

Enzyme replacement therapy with SUCRAID® offers a pharmacologic alternative to sucrose-free diets to treat symptoms in Congenital Sucrase-Isomaltase Deficiency (CSID), but recent shortages and cases of intolerance lead to find another medication for treated patients.

Invertase, present in Kerry's BIOINVERT® 200 solution, is an enzyme which hydrolyses sucrose like the sucrase-isomaltase enzyme, with closed characteristics. BIOINVERT® is already used in England as a substitute to SUCRAID® with empiric dosages. However this alimentary grade solution required a pharmaceutical qualification, according to the European Pharmacopoeia.

The purpose of this poster is to provide a method for pharmaceutical qualification of BIOINVERT® 200 based on our experience and, especially, the enzyme activity dosage.

Methods

According to the European Pharmacopoeia, the pharmaceutical qualification required several tests or measures, such as density (calculated by gravimetric method), pH, osmolality (measurement by freezing point), residual solvent (search by gas chromatography coupled with flame ionization detector), sterility test (by liquid cultures) and abnormal toxicity (tested on mice).

Enzyme activity dosages was performed by an enzymatic method with the Sigma-Aldrich MAK118® test set.
The set is designed to determined low invertase activities in soil samples so, BIOINVERT® solution had to be diluted to the 4 millionth. The experience was run three times, few days apart : one at 37°C, to evaluate the expected physiological activity and compared it with SUCRAID®, and two at 55°C, to compare to the BIOINVERT® specifications.

Results

Parameters	Results
Osmolality	9188 ± 203 mOsm/L
pH (BIOINVERT® solution)	5.15 ± 0.02
Enzyme activity physiological conditions	2822 ± 22 IU/mL (37°C, pH = 4.5)
Enzyme activity in qualification conditions - Test N°1	6307 ± 77 IU/mL (55°C, pH = 4.5)
Enzyme activity in qualification conditions - Test N°2	6372 ± 93 IU/mL (55°C, pH = 4.5)
Sterility	No bacteria or fungus after 10 days of culture
Residual solvents	Ethanol (4 mg/L), no solvent under C8
Abnormal toxicity	No lethality in mice according to the European Pharmacopeia

Discussion

Osmolality and pH were conformed to the specifications.

Sterility test, residual solvents research and abnormal toxicity assay were conformed to the European Pharmacopeia.

Enzyme dosages :
At 37°C, the enzyme activity was 2822 ± 22 IU/mL, 3 times lower than SUCRAID®'s (8500IU/ml).
At 55°C, the invertase activity was 2 times lower than Kerry's BIOINVERT® specifications (11 020–13 340 IU/mL at 55°C).

In order to exclude a reaction pH problem, we have tested it. The result was 4.5 like expected.
Reproducibility of the glucose calibration range (test 1 : r^2=0,9999 and test 2 : r^2=0,9988) seemed to excluded problems with glucose standard or coloring reagents.

Several limitations led to moderate this results:
Firstly, the highly dilution of the BIOINVERT® samples brought experimental bias.
Secondly, there was no invertase positive control in the test set. So, we were not able to evaluated the accuracy of the dosages.
For these reasons, we will use a positive control made from pure invertase powder for the next experiences.

Conclusions

Although the enzymatic activity need to be retested, the BIOINVERT® 200 meets the criteria for a pharmaceutical qualification, according to the 9th European Pharmacopoeia.
Now, clinical effectiveness and tolerance have to be tested, based on English experience

Acknowledgements

We address special thanks to Dr Mélanie Bonnet, for her time and the loan of the microplate reader, and the association "La vie sans sucre"
No conflict of interest