EVALUATION OF LONG TERM BIOLOGICAL ACTIVITY OF PEGASPARGASE (ONCASPAR) AFTER DILUTION IN NACL 0.9%

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BACKGROUND

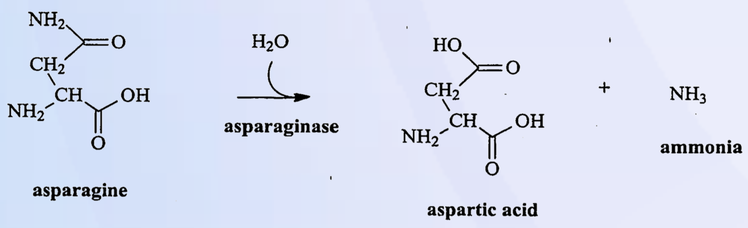
Escherichia coli asparaginase is an enzyme that depletes serum levels of asparagine. It is used to treat acute lymphoblastic leukaemia and related forms of non-Hodgkin's lymphoma. Polyethylene glycosylated–asparaginase (pegaspargase), obtained by covalently attaching polyethylene glycol to the native enzyme, has been shown to sustain similar reductions in serum asparagine concentrations compared with the native enzyme. In addition, pegaspargase has a decreased immunogenicity and a prolonged half-life. The summary of product characteristics (Oncaspar) indicates that the intravascular infusion should be given over a period of 1–2 h but nothing is known on the long term stability and activity of the enzyme after dilution.

PURPOSE

Evaluation of the biological activity of pegaspargase diluted to 16 UI/mL in NaCl 0.9% and stored up to 48 h at 4°C and at room temperature. A study of drug degradation was also carried out.

MATERIAL AND METHODS

Samples of pegaspargase solution diluted in NaCl 0.9% were stored refrigerated at 4°C and at room temperature and protected from light.



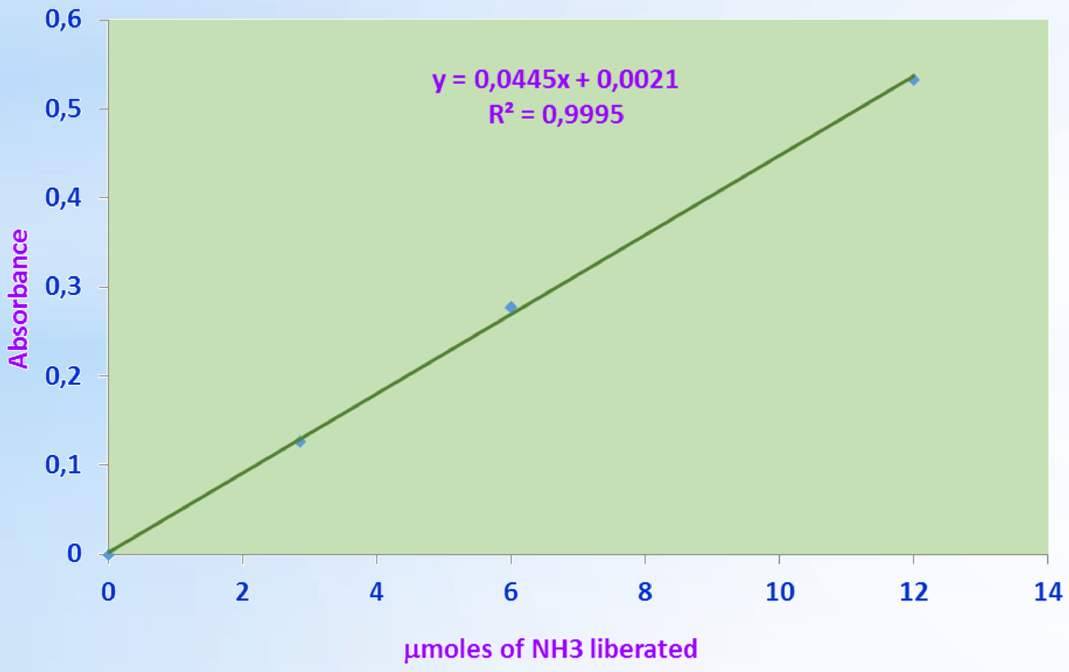
The biological activity of the two solutions was determined by measuring hydrolysis of Lasparagine, and the ammonia released by the enzyme was quantified with Nessler's reagent.

The absence of degradation products or aggregates in the two solutions was verified using size exclusion fast protein liquid chromatography (SEC-FPLC) under the following condition: Superdex 200 10/300 column; Tris buffer pH=8.6; 0.5 mL/min flow rate; 280 nm UV detection; 100 µL injection volume.

RESULTS

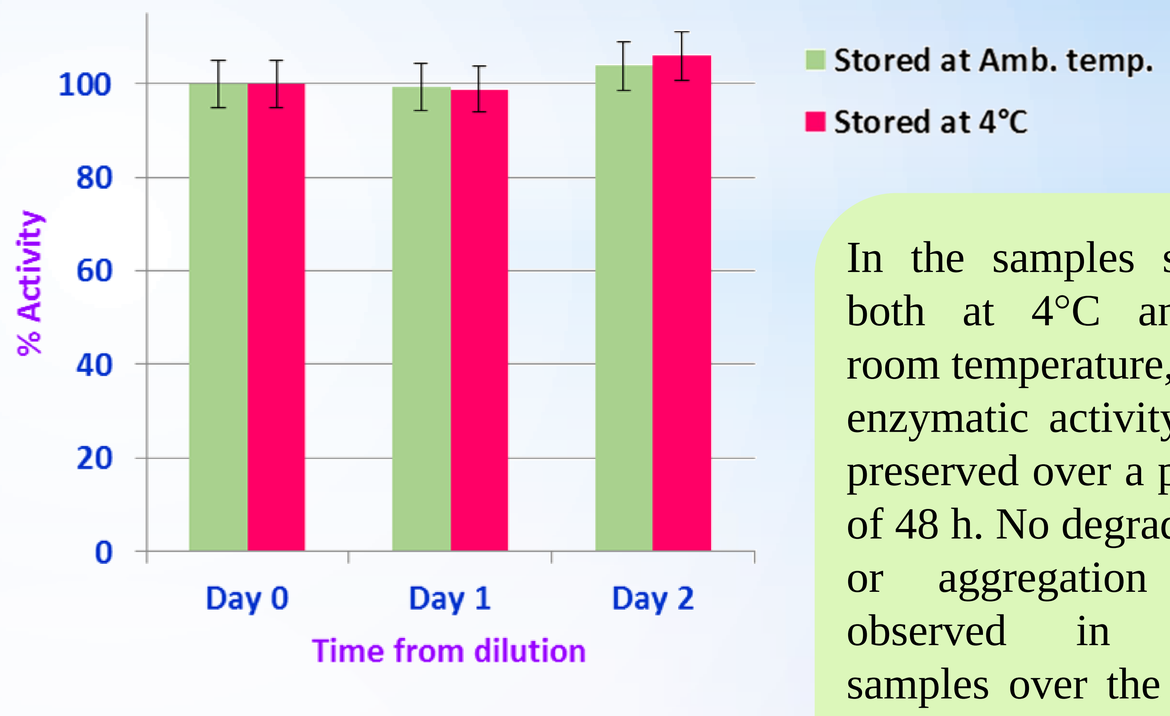
CALIBRATION CURVE

The assay is based on direct reaction of ammonia released with Nessler's reagent; µmoles of ammonia are estimated by detecting the optical density of the reaction's solution at 436nm.



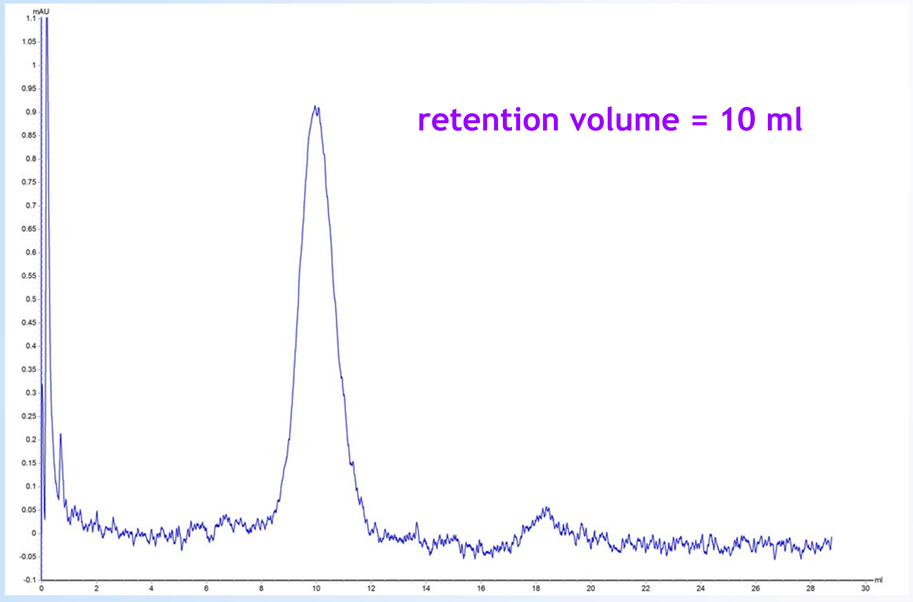
ACTIVITY STUDY

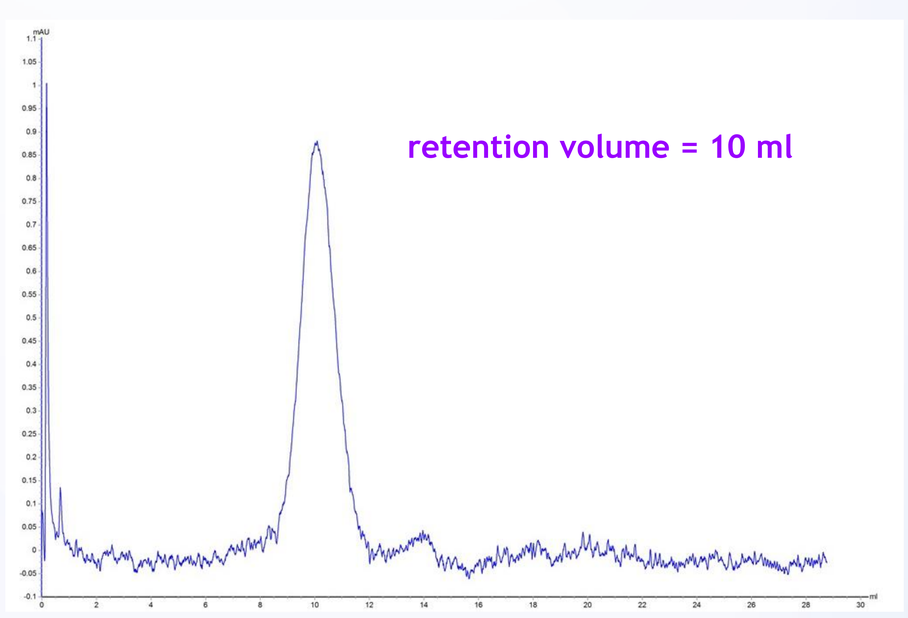
Activity of pegaspargase samples 16UI/ml stored at ambient temperature and 4°C, protected from light.



In the samples stored both at 4°C and at room temperature, enzymatic activity was preserved over a period of 48 h. No degradation or aggregation was observed in these samples over the same period.

DEGRADATION STUDY





Chromatograms of pegaspargase samples 16UI/ml after 48 h stored at 4°C (left) and at ambient temperature (right).

CONCLUSION

The variation in enzymatic activity of the diluted pegaspargase solutions compared with the fresh solution was less than 5% after 48 h, with no significant differences between storage at 4°C or at room temperature. Preservation of the enzymatic activity and the stability of the solutions evaluated will allow us to store pegaspargase for up to 48 h with costs savings and an improvement in patient compliance. A microbiological study is in progress to validate the aseptic manufacturing process in order to guarantee the sterility of the stored solutions.

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No conflict of interest.