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Analytical QbD development of a pharmacopeial monograph for the anticancer drug asparaginase

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INTRODUCTION

Asparaginase (ASNASE) catalyzes the deamidation of asparagine and is therapeutically used for acute lymphoblastic leukemia (ALL)[1]. In order to extend its clinical development, a functional-rationalized quality monograph for ASNASE need to be established which can serve as a basis for generic ASNASE pharmacopeial purposes or in product development and stability studies. The quality attributes should also include the intrinsic stability and excipient compatibility, which are the current hurdles in the clinical applications.

OBJECTIVES

Develop Nessler method for enzyme activity of ASNASE.
Characterization of primary and secondary structure of ASNASE.

RESULTS

1. ASNASE activity

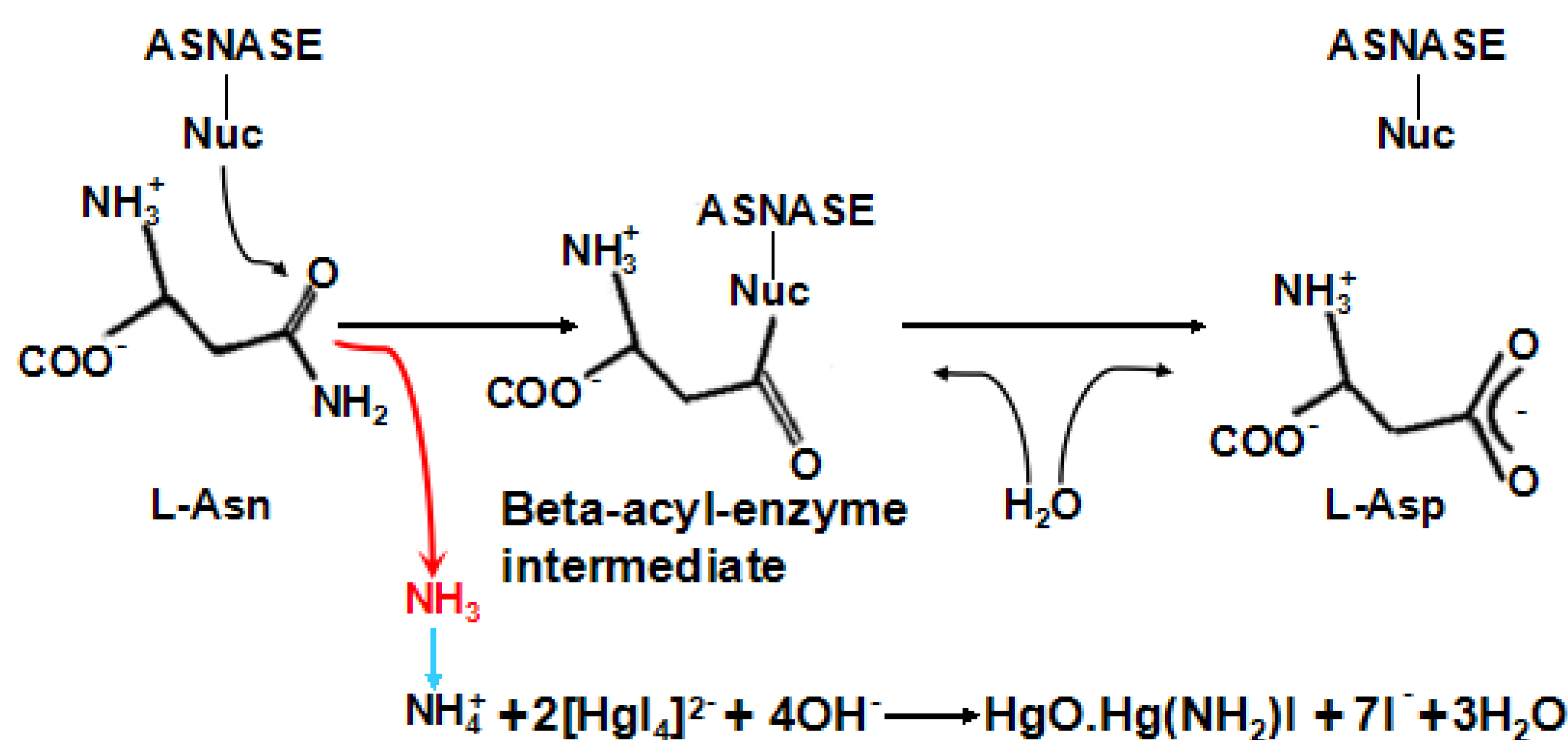

Fig 1. The mechanism of ASNASE activity and Nessler's reaction

Nessler assay

Four variables: C_{KI}/C_{HgI2}, C_{NaOH}/C_{HgI2}, $C_{HgI2\ final}$ and reaction time

Design of Experiment (DoE): D-optimal onion design (DOOD); The significance of the variables and their interactions for the response (absorbance at 425 nm) are shown at **Figure 2**.

Optimal ranges of four variables:

- C_{KI}/C_{HgI2} [1.9-1.95]
- C_{NaOH}/C_{HgI2} [17.0-18.0]
- $C_{HgI2\ final}$ (mM) [27.0-35.0]
- Time (min) [10.0-13.0]

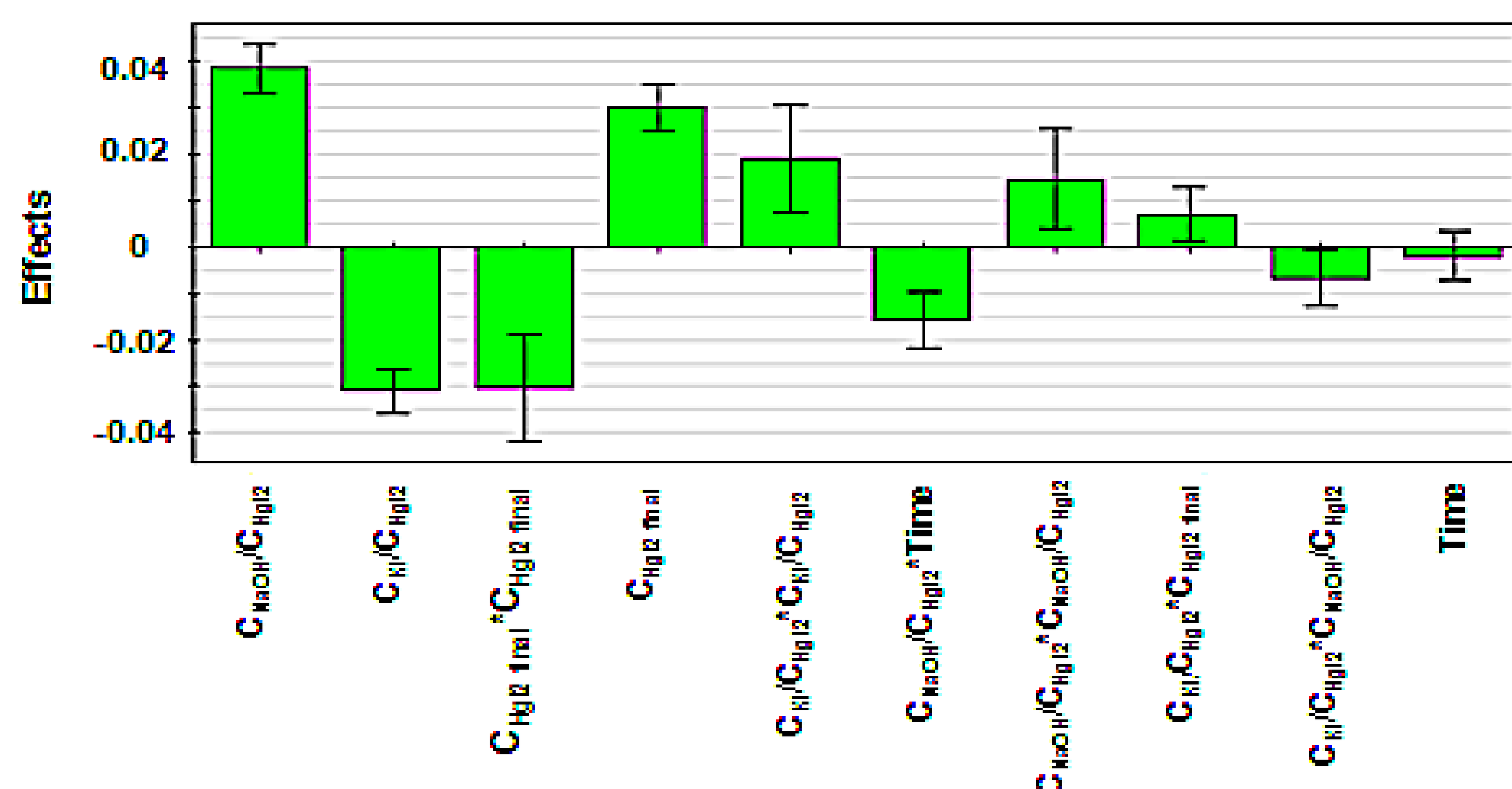

Fig 2. The effects of the four variables and their interactions for the absorbance of Nessler assay following D-optimal onion experimental design

2. Structure characterization

2.1 Primary structure: LC-MS methods, *e.g.* whole protein mass analysis

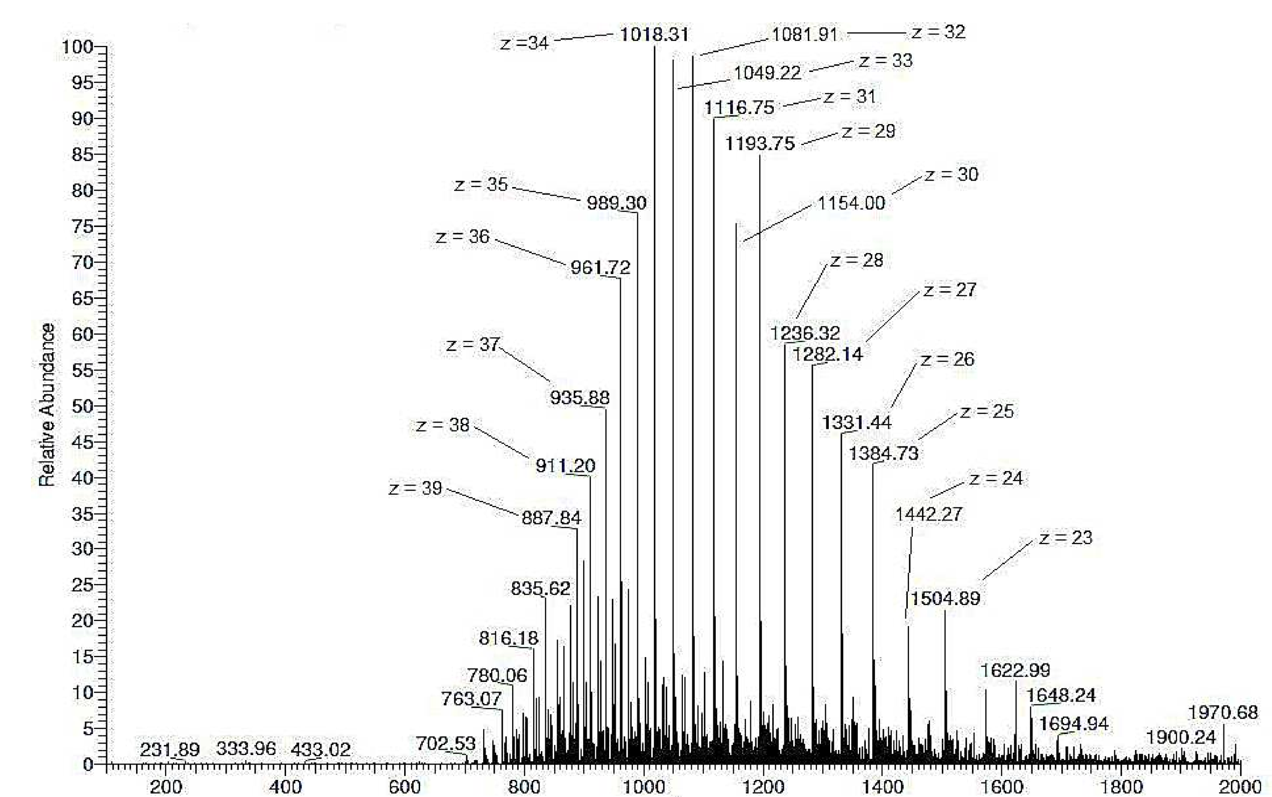

Fig 3. The mass spectrum of *E. coli* ASNASE

Calculated MW of *E. coli* ASNASE : 34590 ± 2 (**Fig. 3**).

2.2 Secondary structure: Circular dichroism (CD)

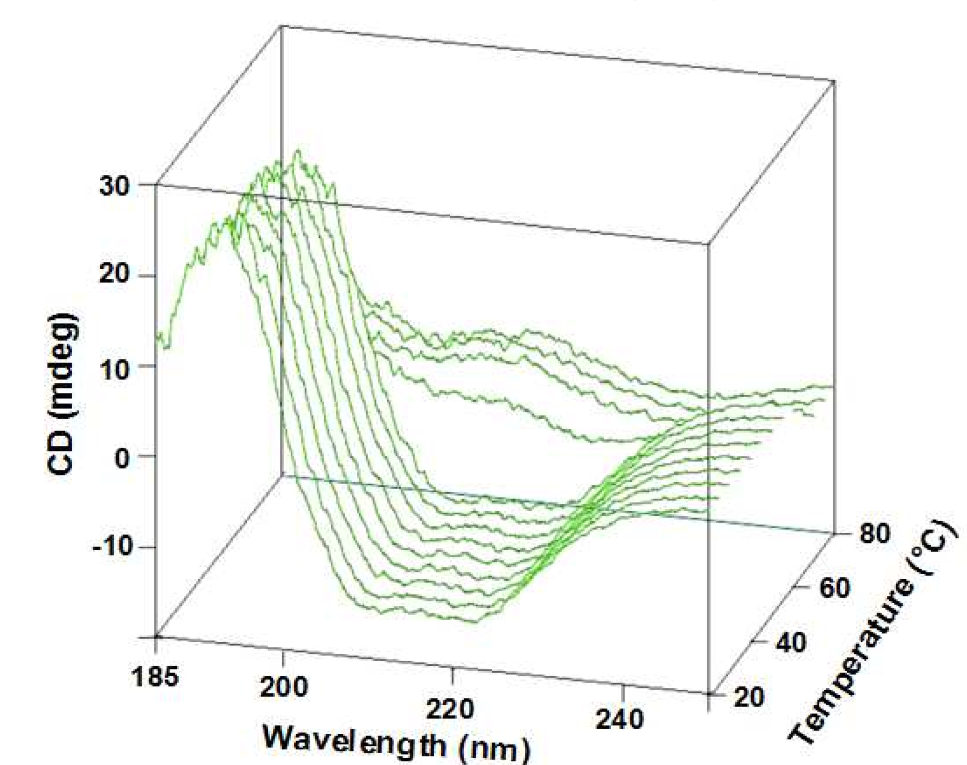

Fig 4. Temperature-stability CD spectra of E. coli ASNASE

Secondary structure content (K2D, K2D2 and Raussens): 29.26% α-helix and 19.68% β-sheet (mean content).

The melting temperature (T_m): Temperature range of 60-63℃: denaturation of β-sheet; 63-65℃: denaturation of α-helix **(Fig 4.)**.

2.3 Secondary structure: FTIR

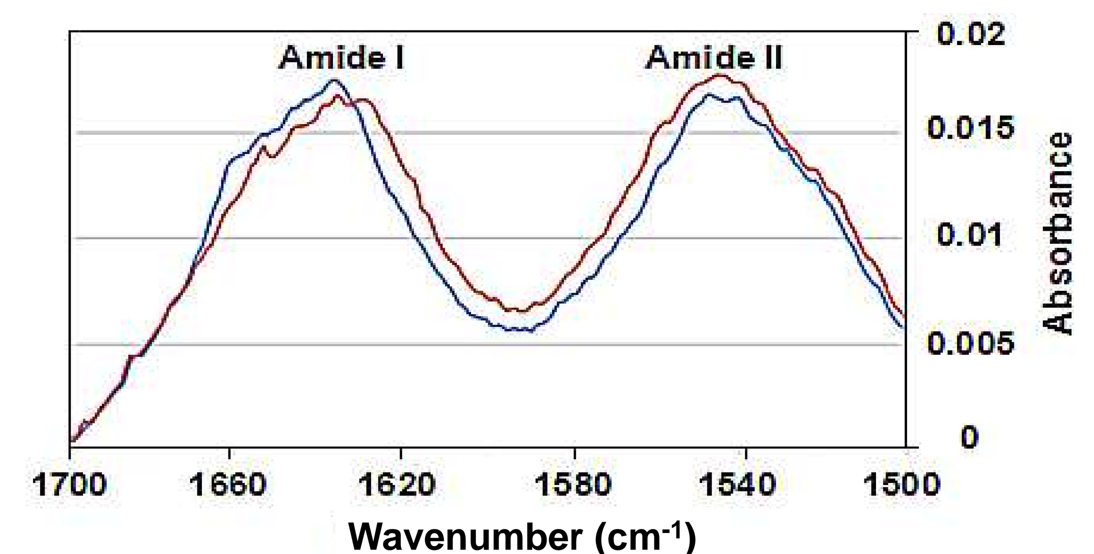

Fig 5. Typical part of FTIR spectra of E. coli ASNASE: amide I and II bands (native ASNASE (blue) and heat-stressed ASNASE (red)

Amide I and II infrared bands allow stability-indicating secondary structure estimation.

CONCLUSIONS

Enzymatic activity by ammonia Nessler assay, primary identification by LC-MS methods and secondary structure characterization by FTIR techniques have been shown in these preliminary experiments to be suitable stability-indicating quality attributes and methods, which will be further developed.

REFERENCES

[1] Verma Neelam et al. L-asparaginase: A promising chemotherapeutic agent. Critical reviews in biotechnology (2007), 27, 45-62.