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

Nessler assay

Four variables: C\textsubscript{KI}/C\textsubscript{HgI\textsubscript{2}}, C\textsubscript{NaOH}/C\textsubscript{HgI\textsubscript{2}}, C\textsubscript{HgI\textsubscript{2}}\text{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\textsubscript{KI}/C\textsubscript{HgI\textsubscript{2}} [1.9-1.95]
- C\textsubscript{NaOH}/C\textsubscript{HgI\textsubscript{2}} [17.0-18.0]
- C\textsubscript{HgI\textsubscript{2}}\text{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% \(\alpha\)-helix and 19.68% \(\beta\)-sheet (mean content).

The melting temperature (\(T\text{m}\)): Temperature range of 60-63°C: denaturation of \(\beta\)-sheet; 63-65°C: denaturation of \(\alpha\)-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