DruQuaR

UNIVERSITEIT GENT

Faculty of **Pharmaceutical Sciences**

Pharmacopeial Characterization of Asparaginase

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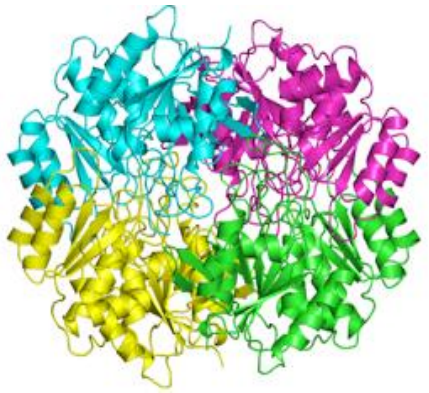
Treatment of acute lymphoblastic leukemia (ALL) - Pediatric use

- Currently only { bolus injection / pediatric use } ⇒ Stability? Compatibility with the infusion solution?
  - ⇒ Stop clinical development to fully exploit its potential
- Several products (R&D+ clinic): "biosimilar"?

⇒ **Pharmaceutical characterization is required.**

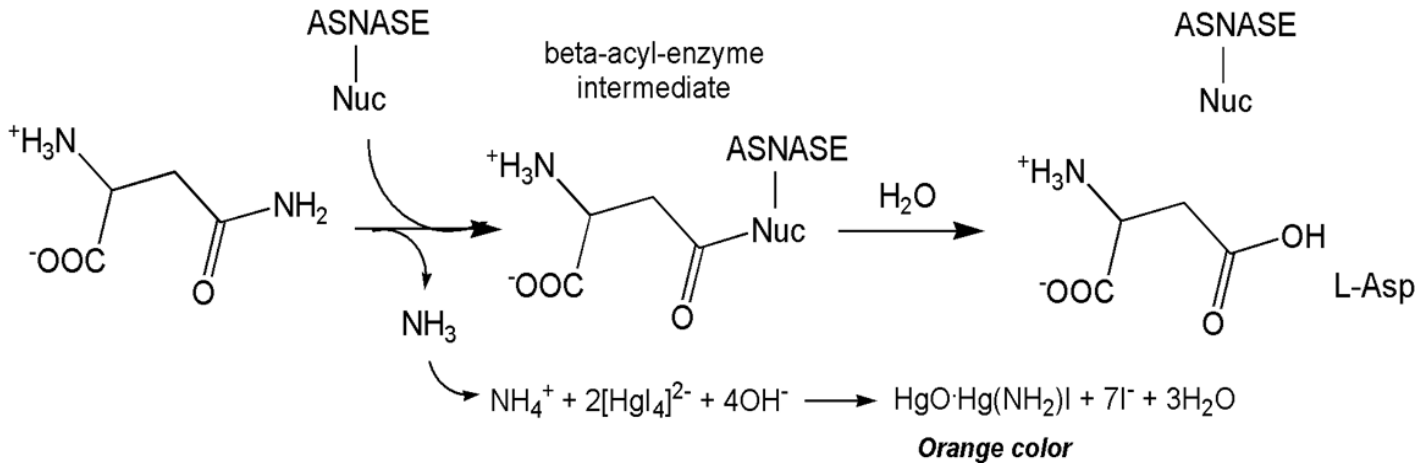


Asparaginase (ASNASE) homotetramer

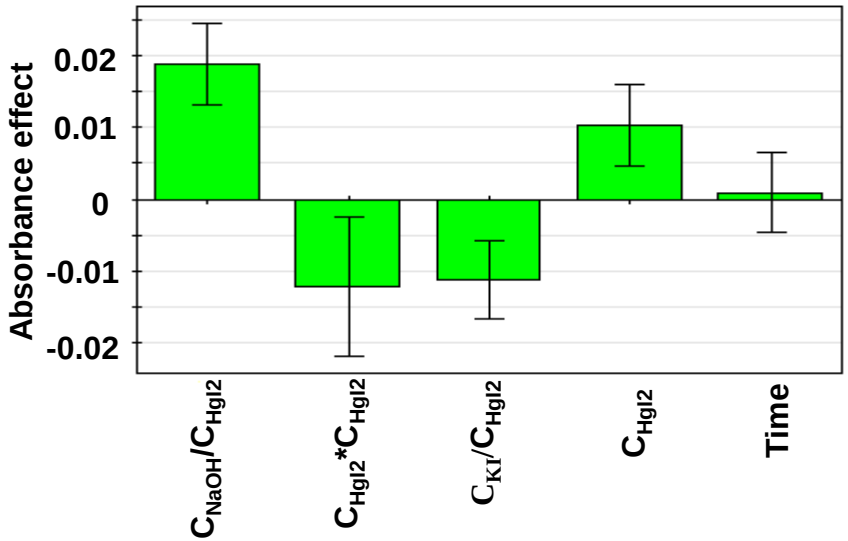
What we have done

1. Development of Nessler method for ASNASE activity
2. Pilot characterization of primary and secondary structure

1. ASNASE Activity (Nessler assay)



Mechanism of ASNASE activity and Nessler's reaction



Design of Experiment (DoE):

- **D-optimal onion design**

Optimal ranges of four variables:

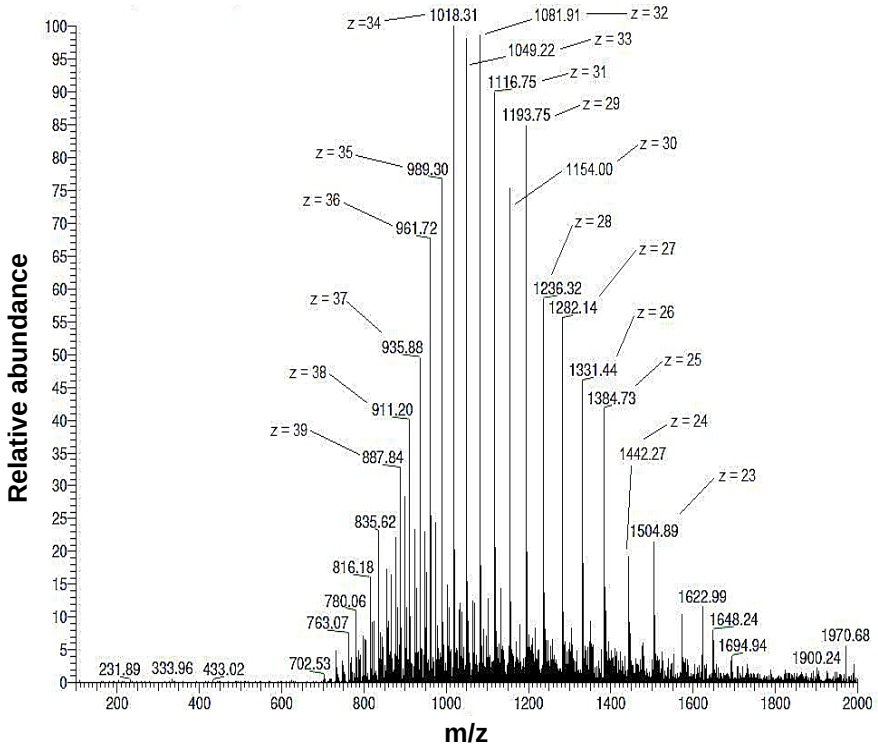
- C_{KI}/C_{HgI2} [1.9-1.95]
- C_{NaOH}/C_{HgI2} [17.0-18.0]
- $C_{HgI2\ final}$ **(mM)** [20.0-40.0]
- **Time (min)** [10.0-40.0]

2. Pilot Structure Characterization (*E. coli* ASNASE)

2.1 Primary structure

LC-MS methods

Calculated MW of *E. coli* ASNASE: 34590±2 Da.

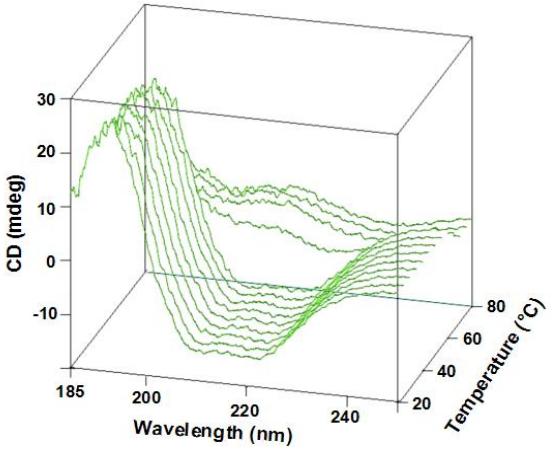


Conclusion

Stability-Robustness Evaluation Methods

2.2 Secondary structure

2.2.1 Circular dichroism (CD)



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

Melting temperature: 60-63°C: β-sheet; 63-65°C: α-helix.

2.2.2 Fourier transform infrared (FTIR)