

Analytical Biochemical Laboratory BV

Betahistine in human plasma by LC-MS/MS

An example of a low level analytical range, with a low molecular weight compound and its peculiarities

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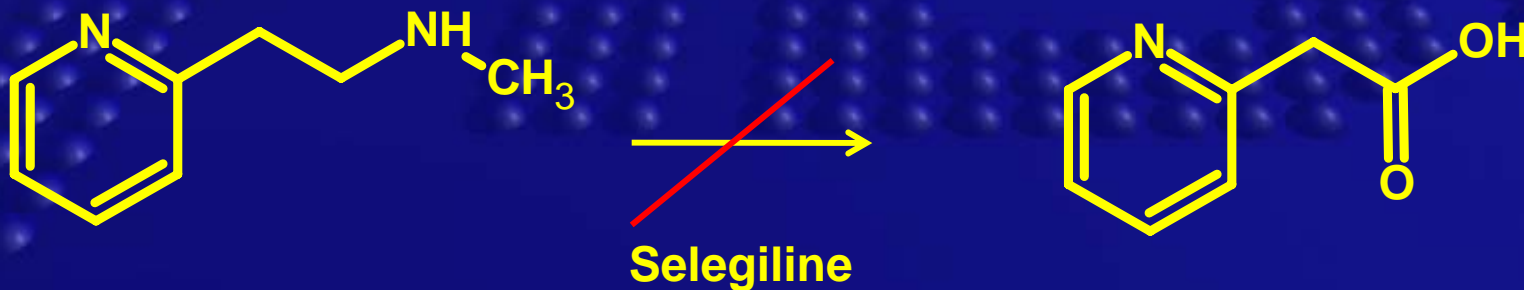
Compound characteristics

- Betahistine is marketed since 1968
- Applied against Meniere's disease
- Metabolism into 2-pyridyl acetic acid (2-PAA) by MAO
- Instable in blood - Stable in plasma
- Molecular mass is 136.2



Compound characteristics

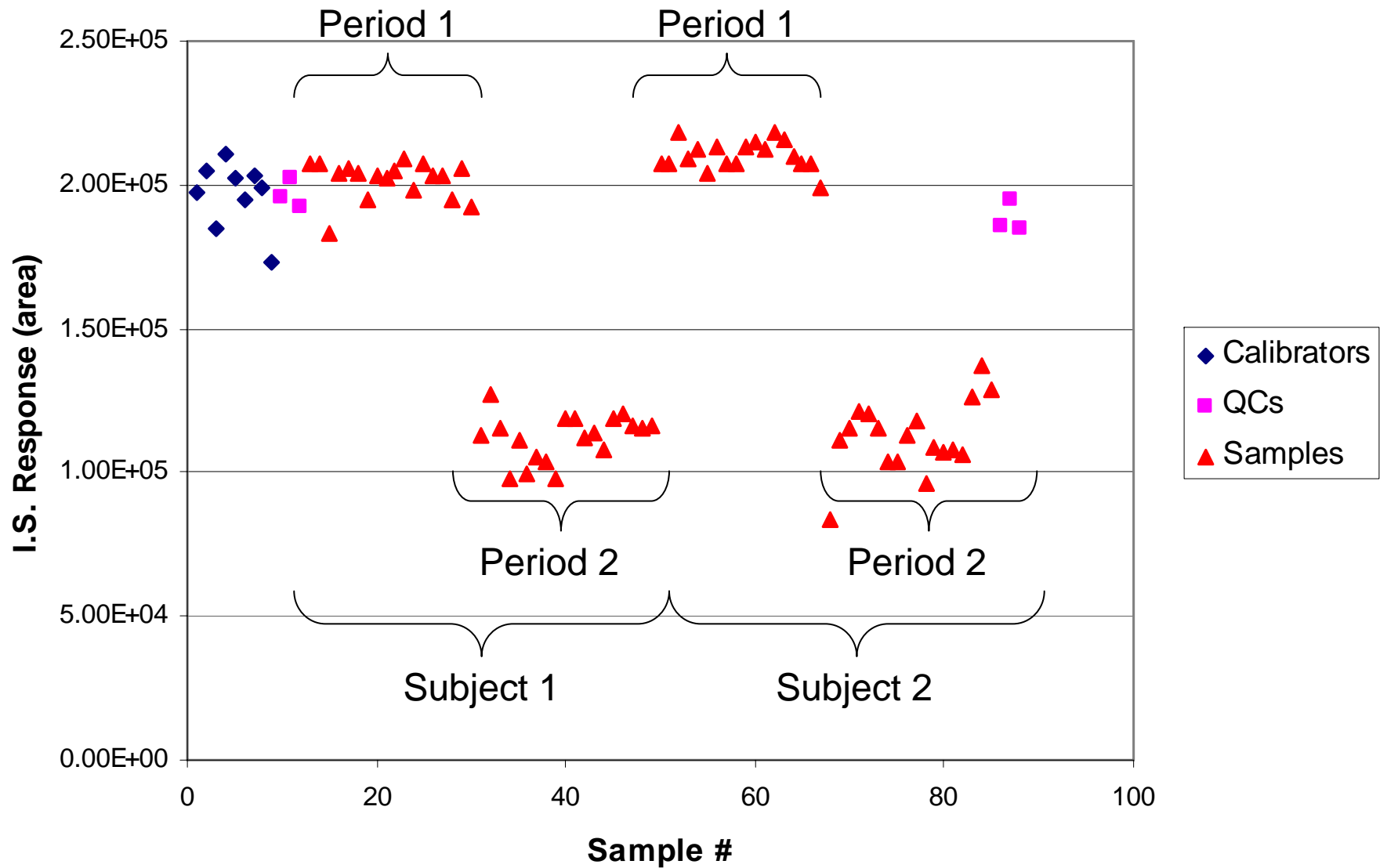
- Betahistine could not be quantified; 2-PAA was always used as surrogate PK parameter
- Nowadays measurement of betahistine is possible
- Metabolism into 2-PAA can be stopped by:
 - Cooling and/or
 - Adding selegiline, a MAO inhibitor
- C_{max} in plasma is ~ 200 pg/mL for betahistine
- C_{max} in plasma is ~ 1000 ng/mL for 2-PAA



Assay properties

- Assay was set up and validated with an analytical range of 2 – 500 pg/mL
- Deuterium labeled betahistine was used as internal standard
 - Alkaline extraction to organic phase
 - Acidic back-extraction to water phase
- Applied in a 2-way cross over clinical trial with betahistine

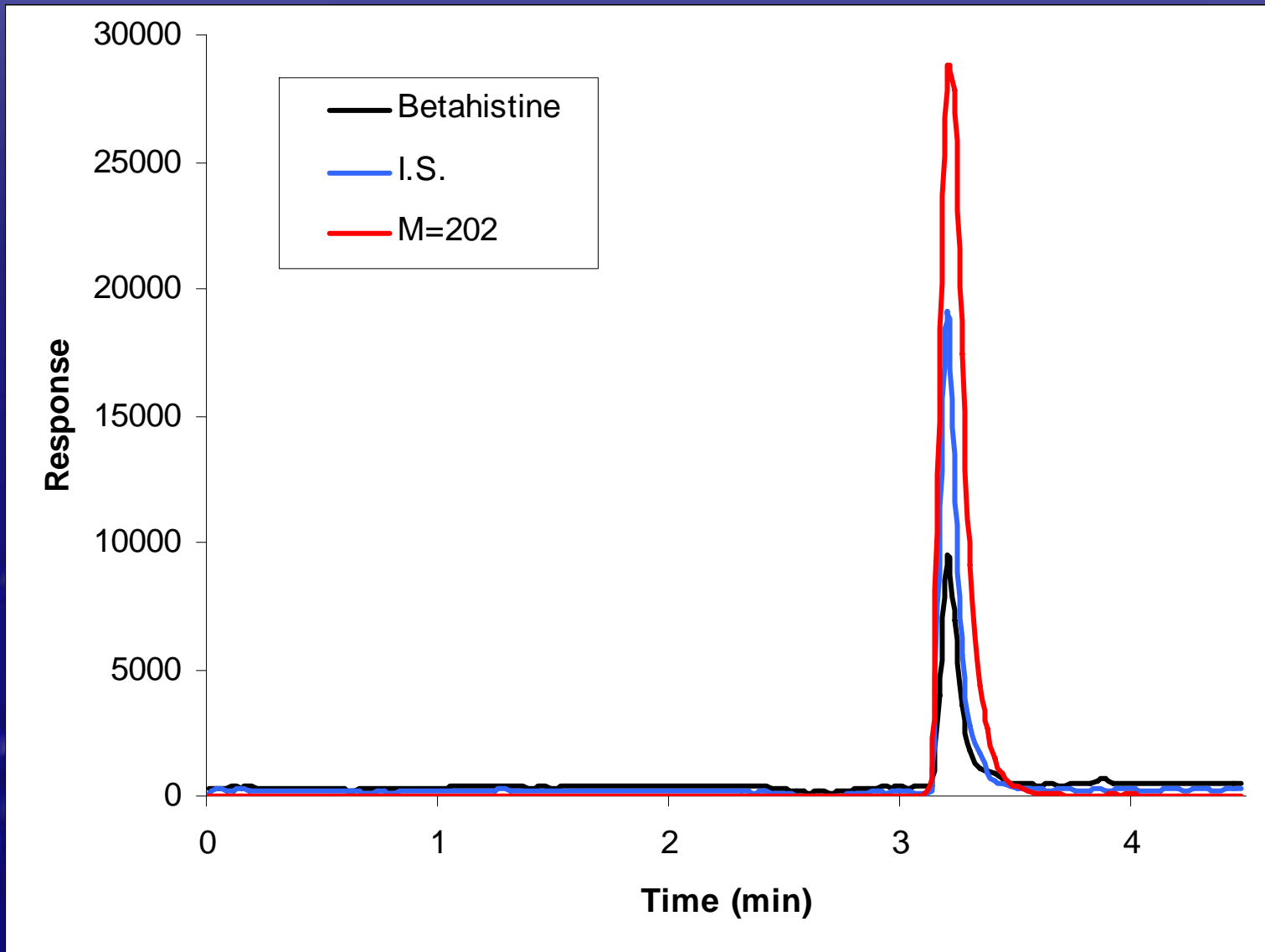
Internal Standard response run 1



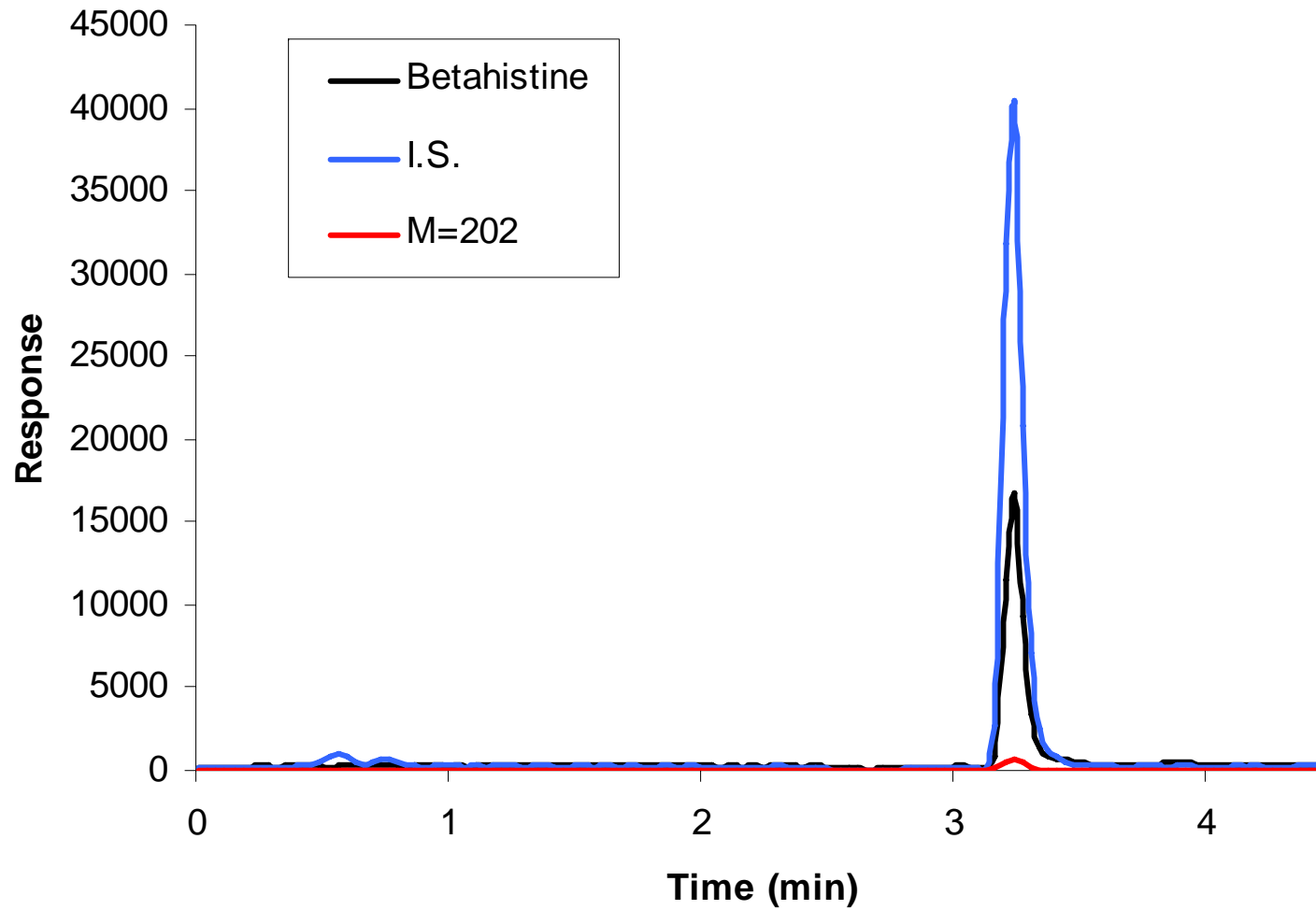
Results of first run

- All period 2 samples showed approximately $\frac{1}{2}$ the I.S. response of the calibrators, QCs and period 1 samples
- Further investigation showed an interfering compound at the RT of betahistine and the I.S. with a molecular mass of 202 (M+1)

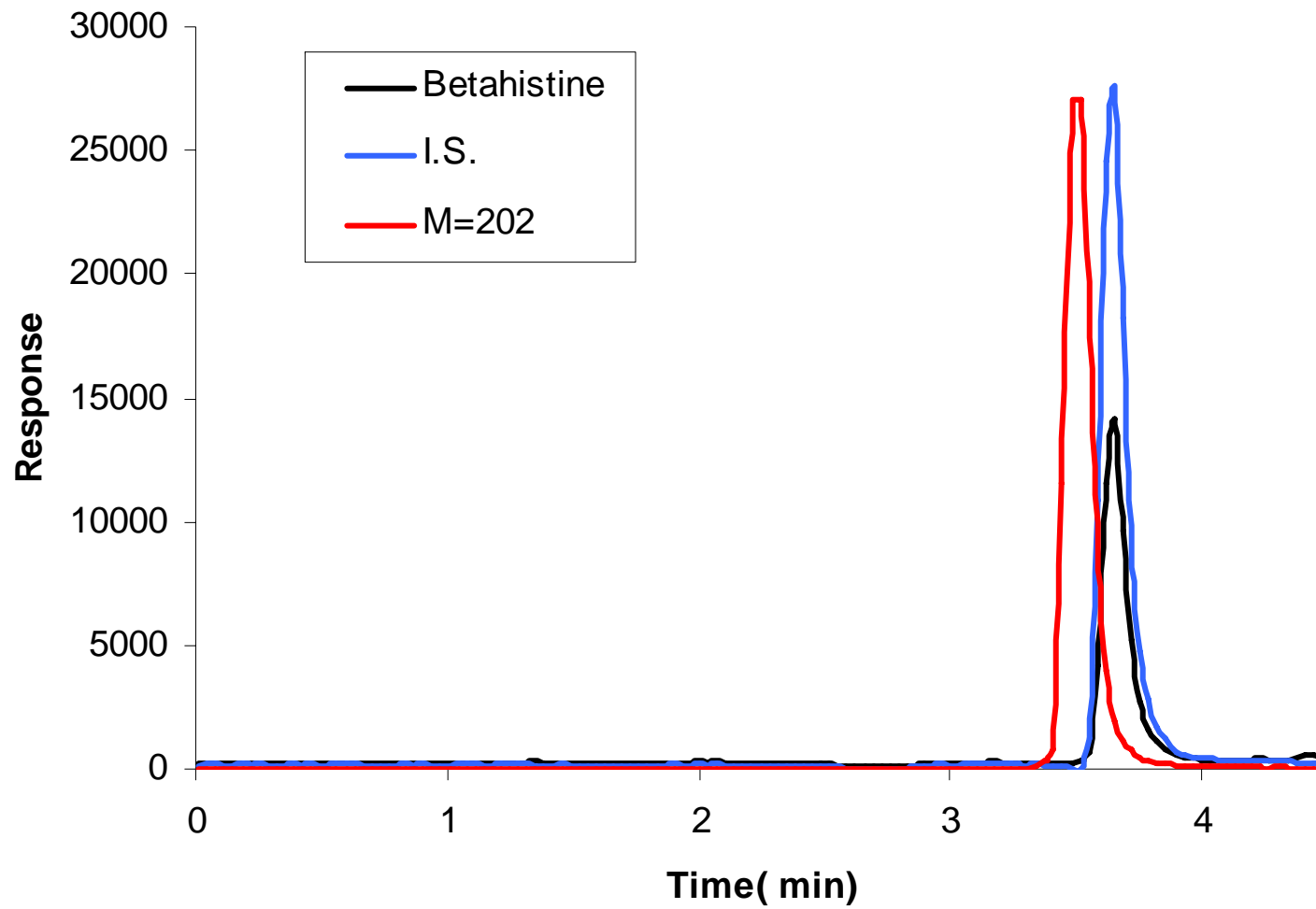
Example chromatogram period 2



Example chromatogram period 1



Applying a new HPLC column



Solutions and further observations

- Applying a new HPLC column separated the interfering compound from betahistine and the I.S
- The I.S. response was consistent throughout the study
- ISR amply met the criteria
- The interfering compound was monitored throughout the study

Solutions and further observations

- The interfering compound was present in either Tx period 1, Tx period 2, in both periods or in none of the periods
- The interfering compound was only present in samples drawn in a certain timeframe
- Most probably the interfering compound was introduced during sample collection (in relation to a certain batch of sampling tubes used in this study)

Unanswered questions

WHAT COMPOUND ARE WE DEALING WITH?

WHAT IS THE ORIGIN OF THIS COMPOUND?

