

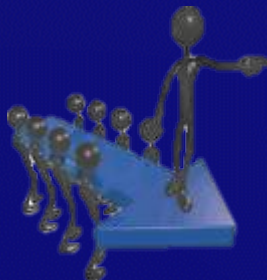
Analytical Biochemical Laboratory BV

Positive Control Group Samples in a Toxicity Study in Rabbits

NOW WHAT??

A. Muntendam

EBF

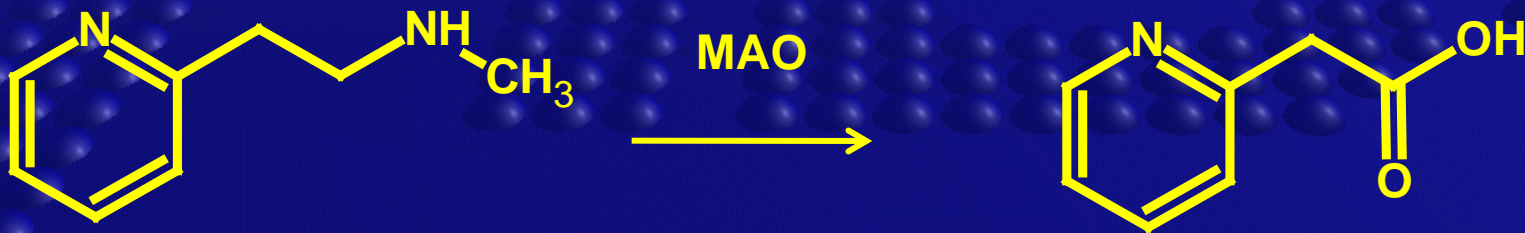


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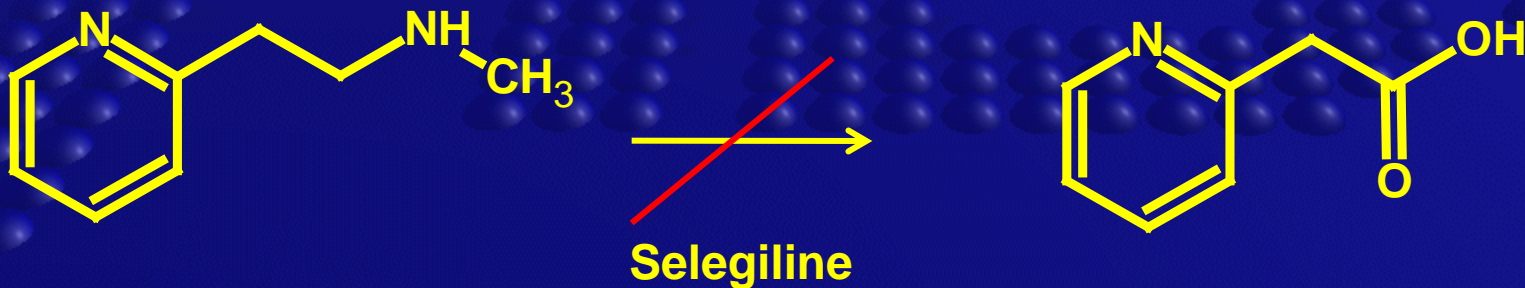
Background

- Toxicity study with Betahistine in rabbits
 - 1 control group and 3 dosed groups
 - Sampling on day 1 and day 14 of treatment
- Metabolism into 2-pyridyl acetic acid (2-PAA) by MAO
- Instable in blood - Stable in plasma
- Molecular mass is 136.2



Background

- Metabolism into 2-PAA can be stopped by:
 - Cooling and/or
 - Adding selegiline, a MAO inhibitor

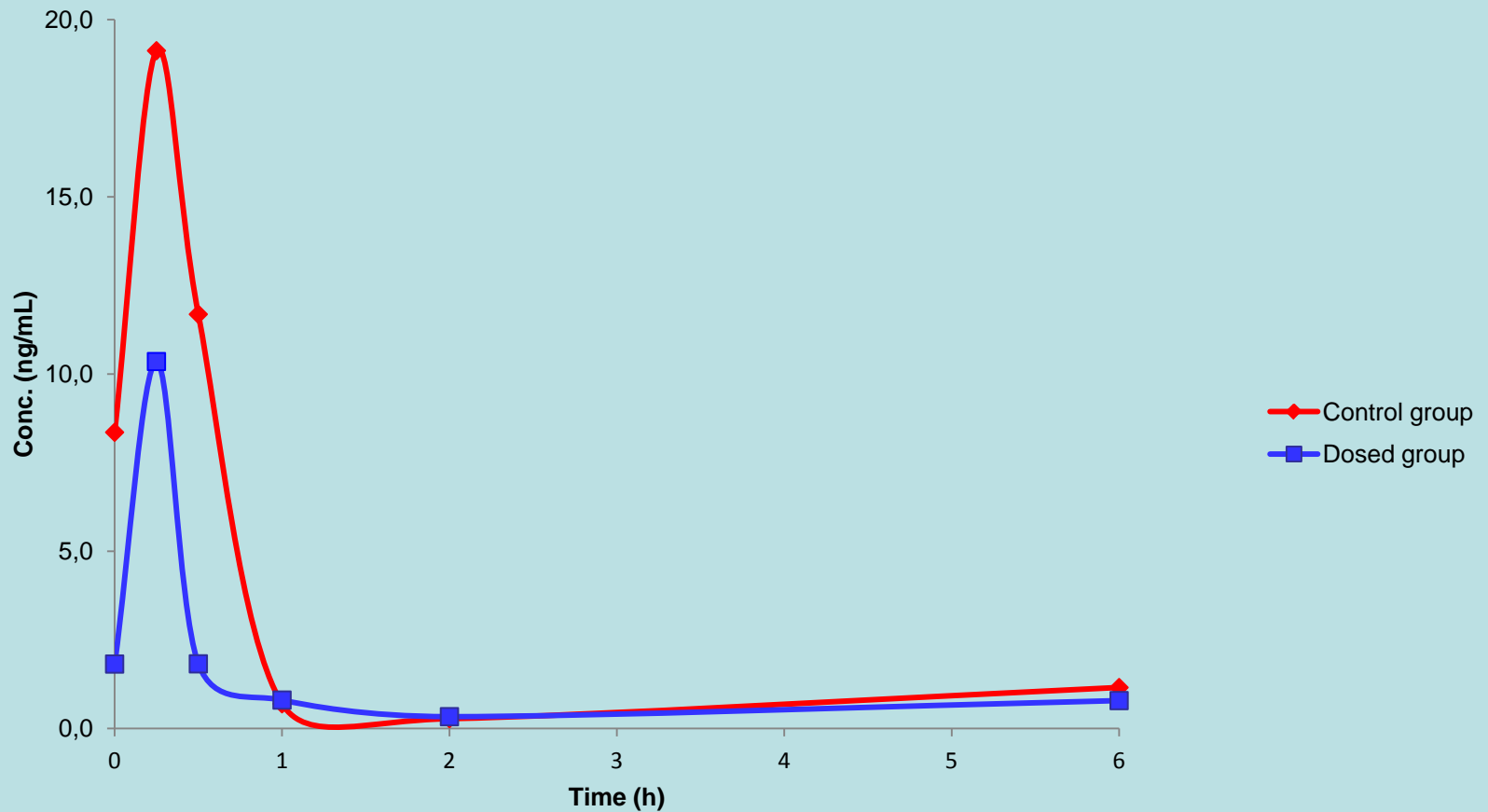


Assay properties

- **Betahistin assay was set up and validated with an analytical range of 0.100 – 100 ng/mL**
- **Deuterium labeled betahistine was used as internal standard**
 - **Alkaline extraction to organic phase**
 - **Acidic back-extraction to water phase**
- **Analysis on a AB Sciex API 4000 MS/MS**

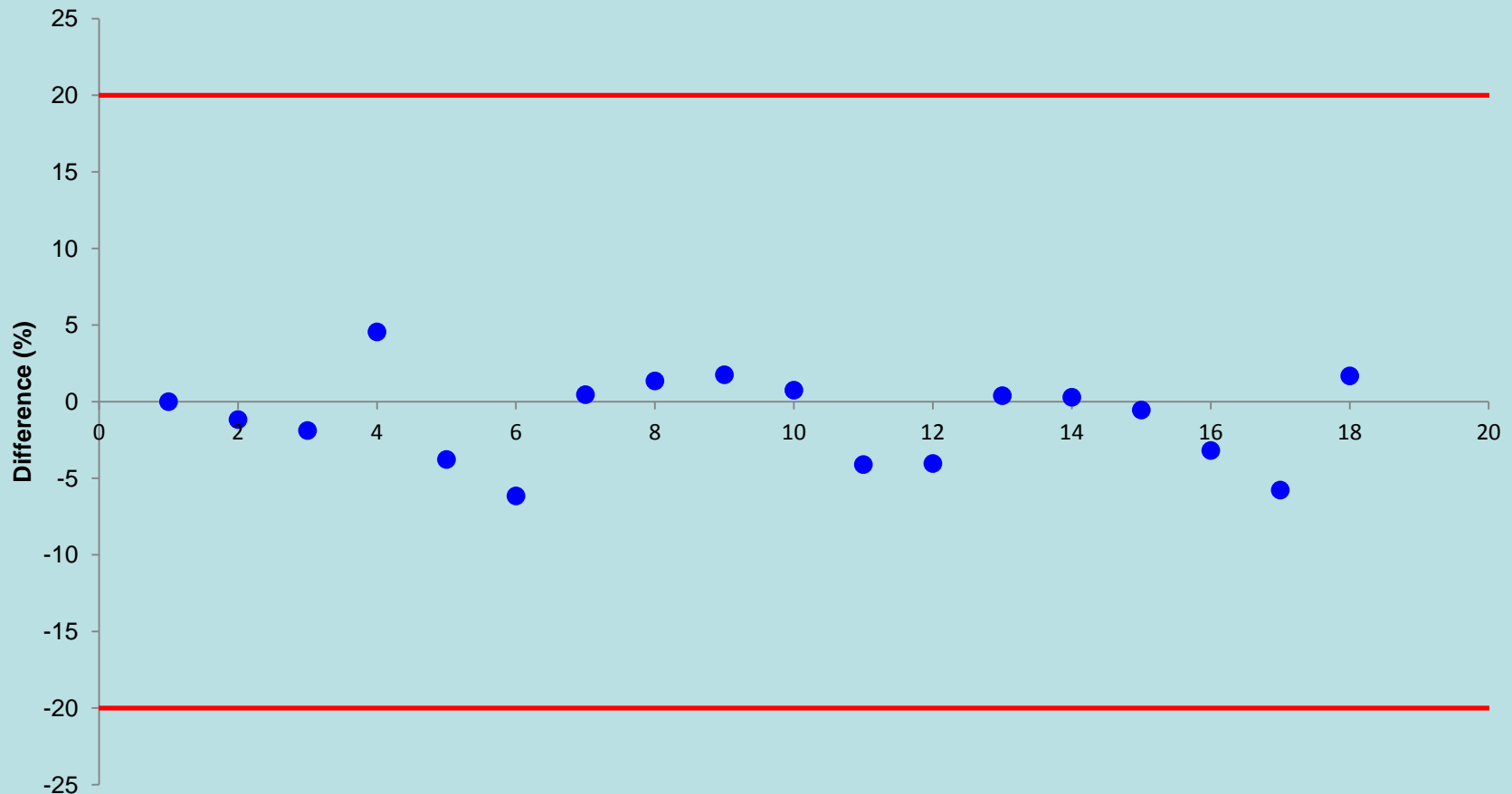
Results

- PK curves in control samples (D14) were observed



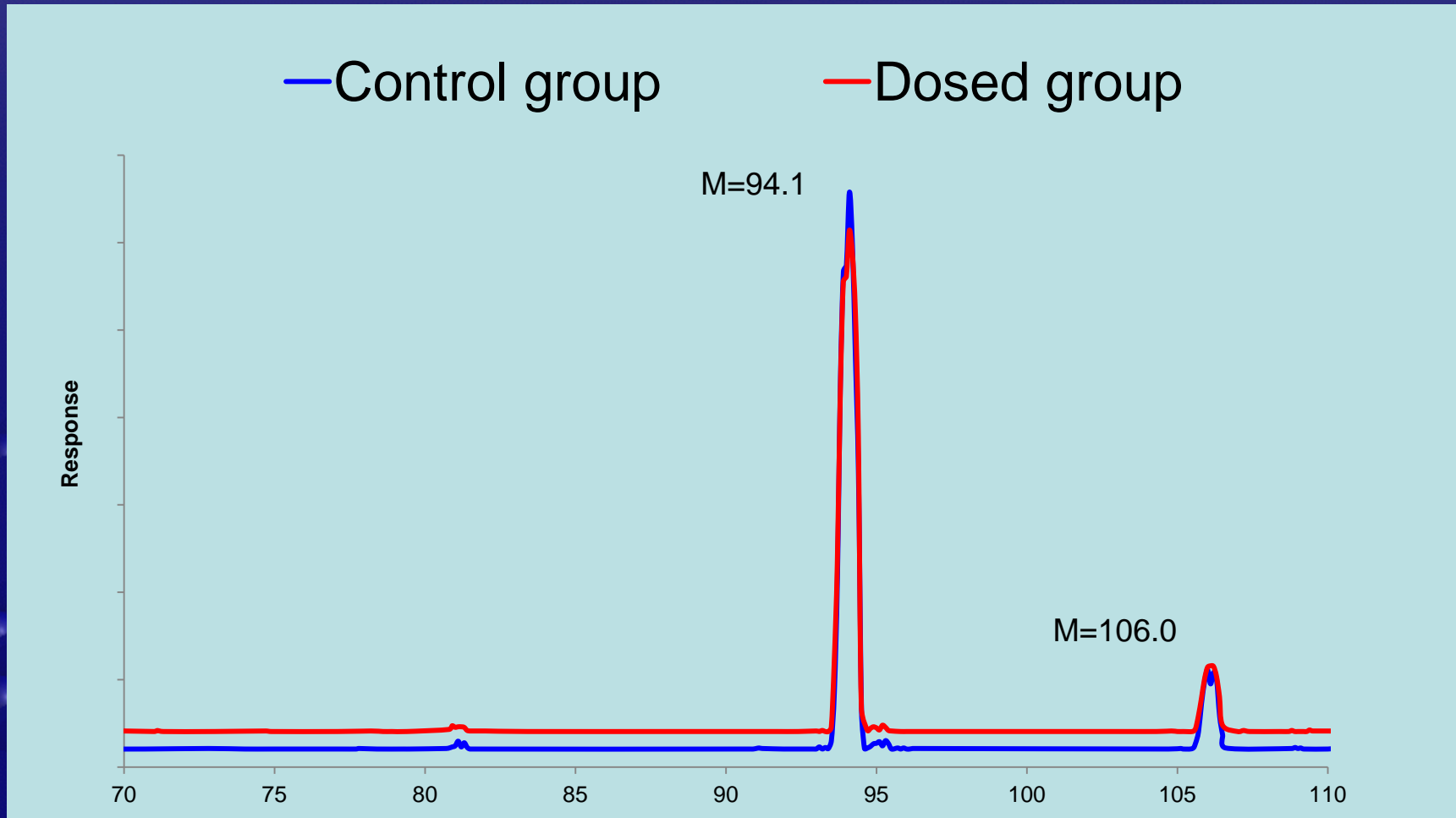
Investigation

- Suggestion that control animals were dosed
 - Confirmation of initial result



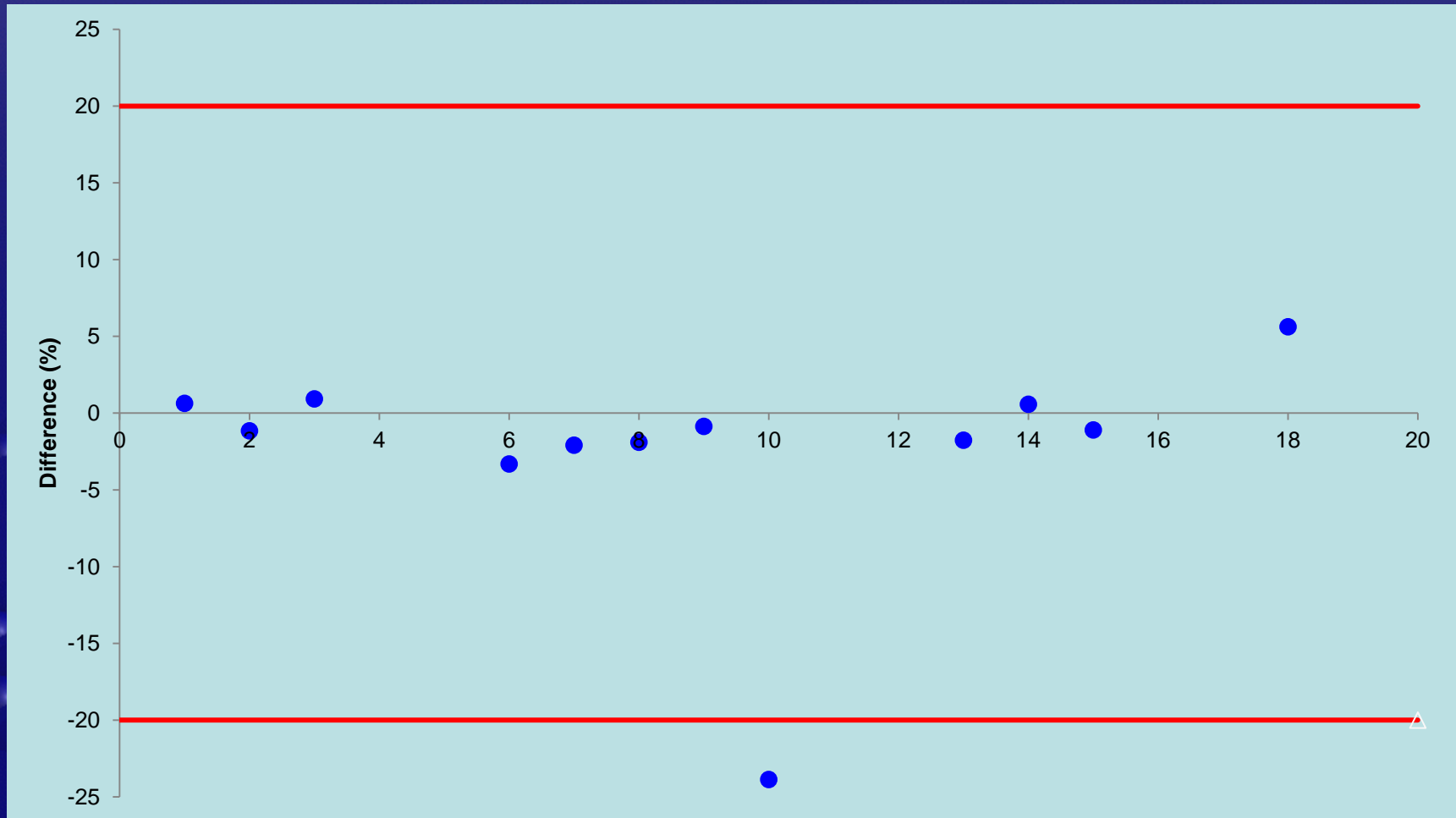
Investigation

- Confirmation of the identity of the analyte



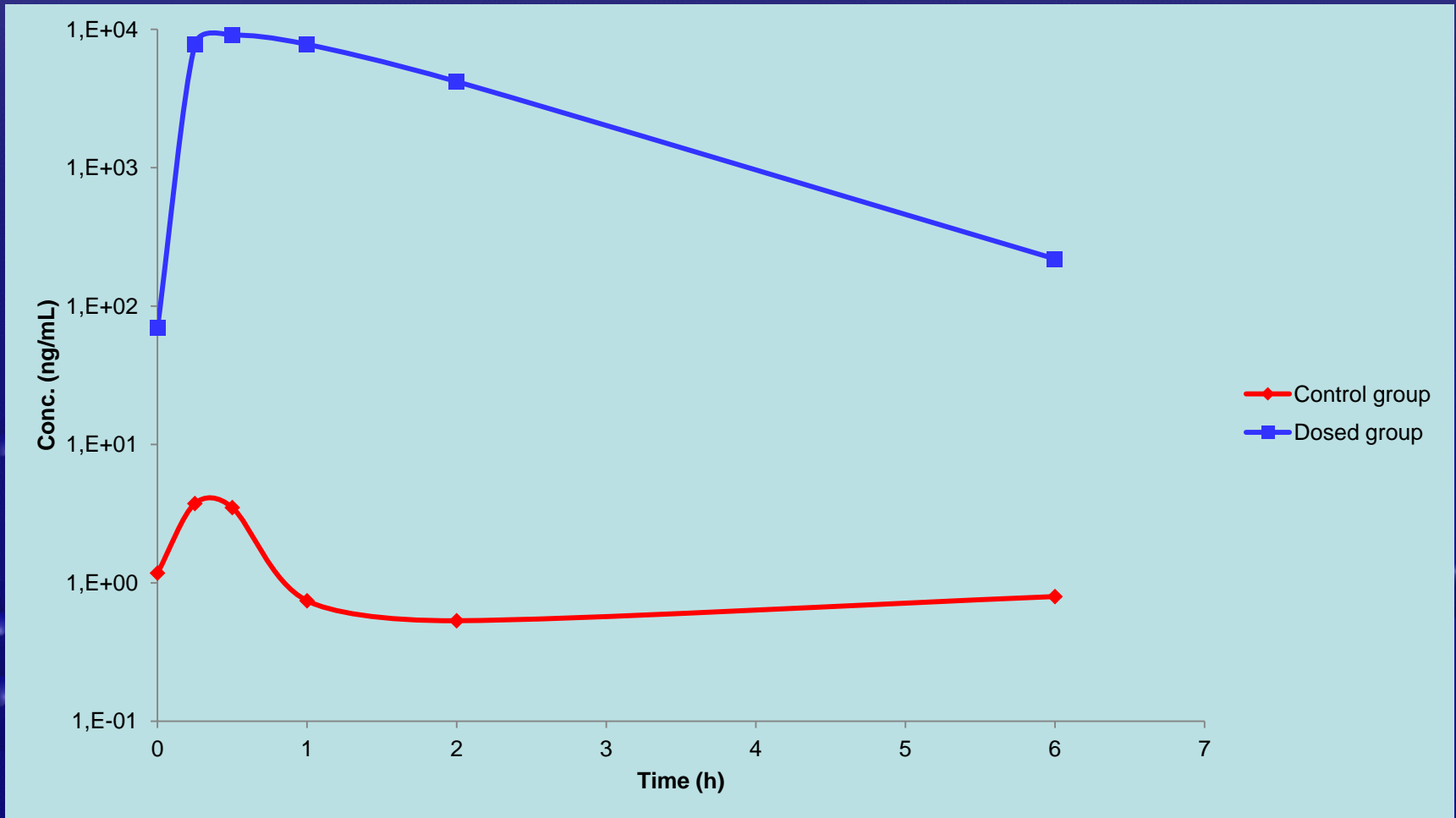
Investigation

- Comparison of results using a different mass transition



Investigation

- Measurement of the metabolite 2-pyridyl acetic acid



Conclusions

- **The initial analysis results are confirmed**
- **It was confirmed that betahistine was found in control samples**
- **Minor amounts of the metabolite were present in the control samples**

Conclusions

- **Control animals have not been dosed**
- **Betahistine must have been introduced to the control samples during blood collection, otherwise no 2-PAA would have been measured in the control samples.**

Unanswered question

- Why are PK curves observed in the control samples?

