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Experiences of Assessing Matrix Effects by Monitoring Internal Standard Response in Study Samples

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Matrix effect

From CCIII workshop report

"One phenomenon influencing mass spectrometry (MS)-based bioanalytical assays is matrix effect. Matrix effect is the suppression or enhancement of ionization of analytes by the presence of matrix components in the biological samples. Quantitative measurement of matrix effect provides useful information in validation of MS-based bioanalytical methods."

Matrix effect testing during method validation

- Determine Matrix Factor (MF)

- Influence of
  - Haemolysed samples
  - Hyperlipidemic samples
  - Co-medications

Matrix effect assessment during study sample analysis

- Not possible during method validation to investigate all possible matrix effects that can occur in "real" study samples
- One complement or alternative to matrix effect investigations during method validation is to assess possible matrix effects in individual study samples by monitoring the internal standard response

Our procedure

- We routinely monitoring the internal standard response in individual study samples to assess matrix effects in all studies
- Our criteria; the IS area of an unknown study sample must be above 50% and below 180% of the mean IS area of all CAL and QC samples in the run
- Using validated Excel spreadsheet for the evaluation
- If not fulfilled, the sample is reanalyzed

Our experience

- In a majority of studies the number of IS failures are very low
  - Last four nonclinical studies, less than 3 % of samples reanalyzed due to IS response failure
  - Last two phase I studies, less than 1% of samples reanalyzed due to IS response failure

- For two recent phase II studies, the number of IS response failures have been very high

- These two cases will be presented in more details, i.e. investigations performed, cause, impact and analytical solution

Case 1 – the background

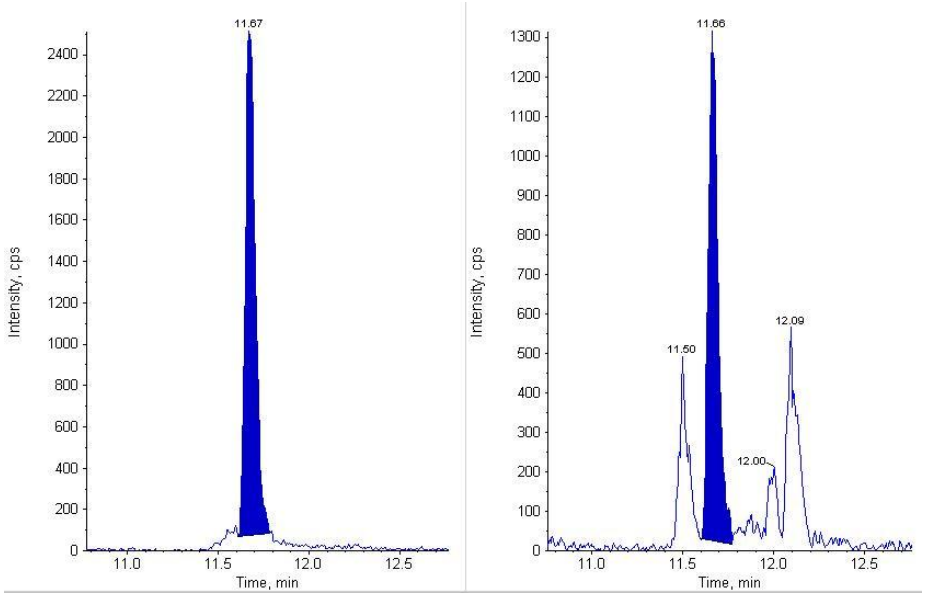
- Peptide drug (MW approximately 1000 Da)
- Very potent peptide, LLOQ 5 pg/mL
  → complex method: off line SPE (Qasis WCX) and coupled column chromatography system with API 5000
- Labelled IS ($^{2}H_{10}{}^{15}N$)
- Matrix effects tested during method validation (spiking six different sources of plasma), **acceptance criteria fulfilled**
- During FIH study, **no reanalysis (of 710 analyzed samples) due to failed IS criteria**

Case 1 – the background

- Phase II study, septic shock patients at intensive care units
- All patients on noradrenaline as standard treatment
- Additional method validation performed to investigate possible matrix effects from noradrenaline, **acceptance criteria fulfilled**
- Some patients had received up to 80 other medications + under dialysis
  → not possible to test all these during method validation, thus IS response of study samples only way to assess possible matrix effects

Case 1 – the problem

- Approximately 60 % of the samples from the first cohort of this study did not fulfil the IS response criteria
- Despite a very complex analytical procedure, interferences were seen in the chromatograms

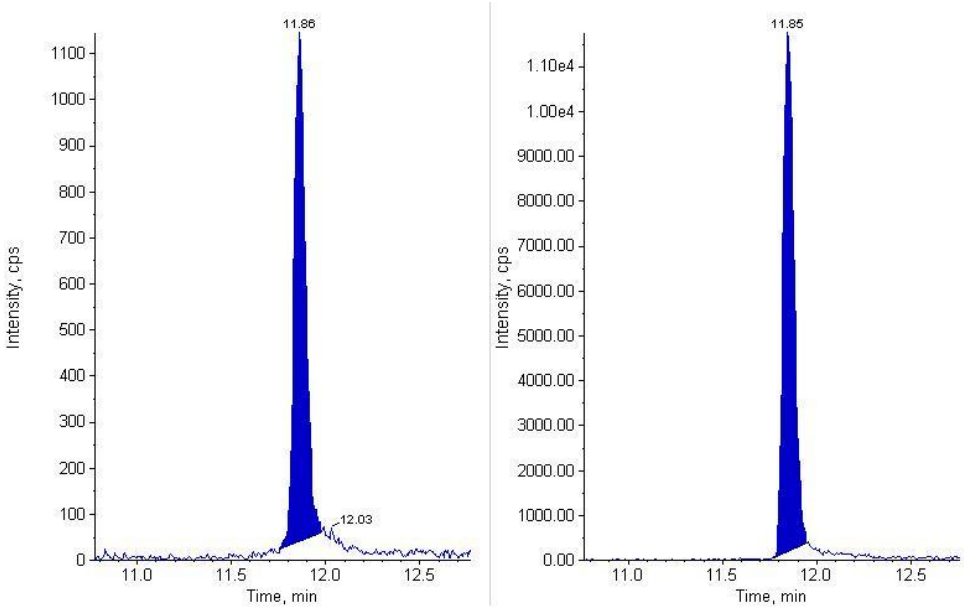


Case 1 – the solution

- Fairly high plasma concentrations at steady state
- Dilution with blank plasma tested during method validation
- A 10 x dilution with blank plasma was applied to these samples

Case 1 – the solution

- After dilution, 80 samples (out of 82) fulfilled the IS criteria
- Chromatogram for same sample as before, but after tenfold dilution with blank plasma



Case 1 – comparison

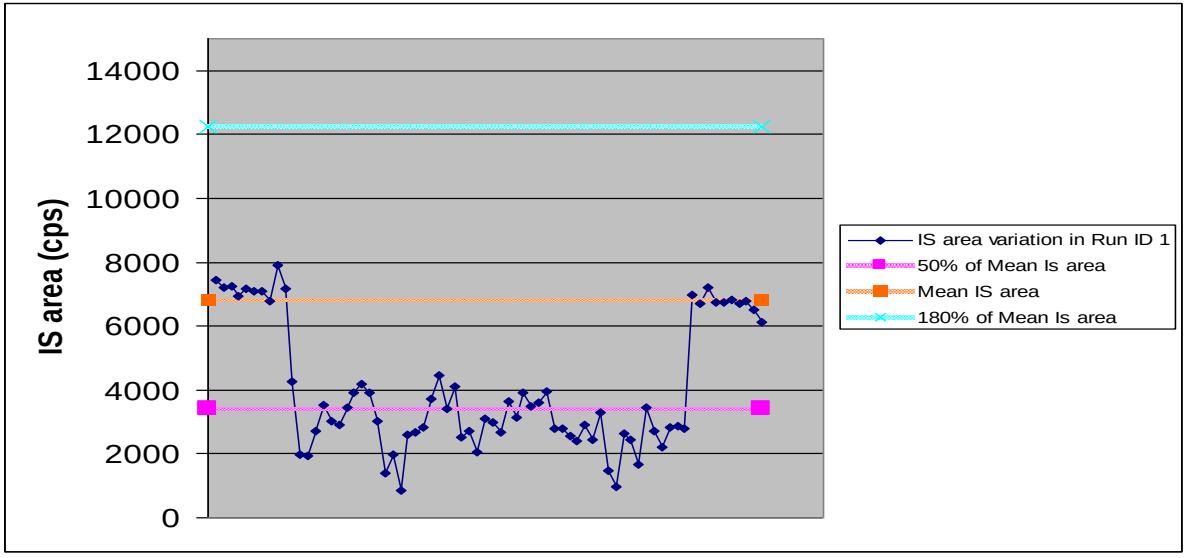
- The results for the analysis with the IS criteria fulfilled (diluted) gives in average 15% lower concentrations compared with the results from the original analysis (undiluted), (expressed as % difference of original)
- For 71 % of the samples, the difference between the results were within 20% of original results (undiluted)
- Maximum difference was 63%

Case 2 – the background

- Biomarker (testosterone), MW 288 Da
- LLE using Isolute SLE+
- UPLC-MS/MS
- Labeled IS ($^{2}H_{3}$)
- Equine (gelding) serum used for preparation of calibration curves
- During method validation matrix effects was investigated by comparing slopes of calibration curve in serum from six females with slope for calibration curve in equine (gelding) serum, **acceptance criteria fulfilled**
- Cross-validated against method used by CRO, **good agreement**
- Used in several late phase studies without any problems

Case 2 – the problem

- Problem occurred when method was used in a new study for another indication
- Run ID 1: IS response criteria not fulfilled for 2/3 of study samples
- The analysis was put on hold and an investigation was started



Case 2 – the investigation

- LC-MS full scan showed that ions with *m/z* values of 531, 539 and 553 were co-eluting with the analyte of interest
- These ions were identical to those of a sulfoxide oxidation product of didodecyl 3,3'-thiodipropionate (DDTDP) as published by Xia et al (Identification of a new source of interference leached from polypropylene tubes in mass-selective analysis). J Am Soc Mass Spectrom. 2005:16:417-421
- **Sarstedt Cryovials**
  - Used for CAL, QC and study samples.
  - Vial from batches used at clinical sites (NA and EU) and our lab were tested
  - None of them showed the plastic related interferences
- **Plastic pipettes** used for transfer of samples
  - No plastic related interference

Case 2 – the cause

- **BD Vacutainers® Plus SST™ Advance tubes** were used
  - In this study, 8.5 mL tubes were used
  - In other studies, 5.0 mL tubes were used
  - Same tube material and additives in both tubes
  - Only difference, the closure material of the cap
    - 8.5 mL tubes have **polypropylene cap**
    - 5.0 mL tubes have **low density polyethylene resin cap**

- From a logistic point of view, hard to change the collection tubes in the on-going study, thus an analytical solution preferred

Case 2 – the analytical solution

- Transfer of the method to a more sensitive MS instrument and 5-fold less volume injected
- Method partially re-validated with new set-up
- When reanalyzing the samples from Run ID 1 with the new method set-up, all samples fulfilled the IS response criteria

Case 2 – comparison

- The results for the analysis with the IS criteria fulfilled gives in average 16% higher concentrations compared with the results for the original analysis (expressed as % difference of original)
- For 63 % of the samples, the difference between the results were within 20% of original results
- Maximum difference was 45%

Conclusion

- Monitoring of internal standard response of study samples to assess matrix effects could be a complement or alternative to the investigation performed during method validations
- Especially in late phase studies it might be hard to predict all possible matrix effects in the method validation
- In majority of our studies, there are very few samples failing the IS response criteria
- Our “two worst cases studies” gave differences of approximately 15% in results between IS response OK resp. not OK, using labeled IS

Future at Ferring?

- On-going internal discussion, based on our experience presented
- How to investigate matrix effects in the future?
  - During method validation only
  - IS response assessment of study samples only
  - Combination of both, e.g. in minimum package in method validation + IS response assessment of study samples in late phases

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