Anomalous results case studies

presented at the EBF's 3rd open symposium Barcelona 2010

M. Brudny-Klöppel, Bayer Schering Pharma on behalf of EBF

Provided by EBF companies
Observed in small and large molecule assays
During assay validation and sample measurement
Decribed as:

- Basic facts (assay characteristics)
- Observations
- Resolution (technically and/or scientific and regulatory)

Anomalous results case studies: #1

Basic facts:

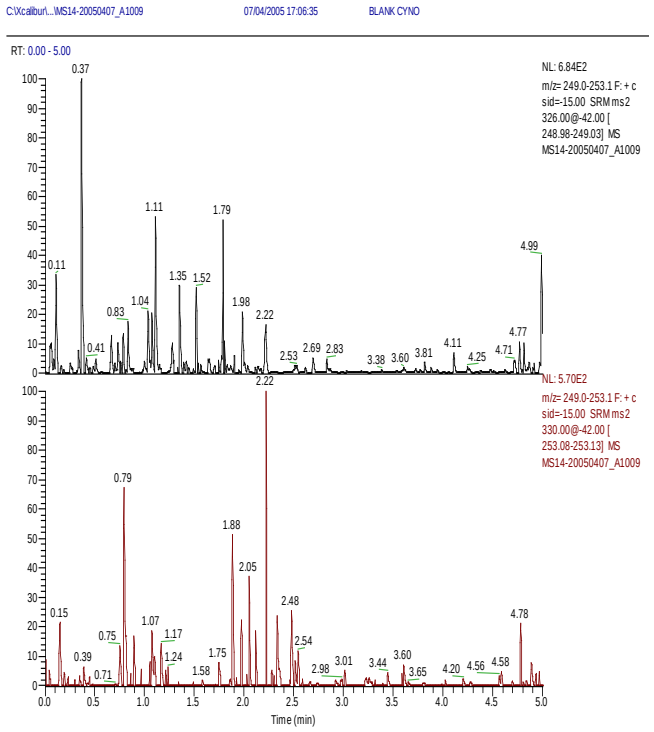
- Fully validated LC-MS/MS assay for cyno & rat (2005)
- Drug MW 325, $^{2}H_{3}{}^{13}C$-IS used,
- Assay range 1 to 5000 ng/mL, 50 µL plasma
- Sample prep – protein precipitation, on-line SPE
- LC-MS/MS
- 10 µL plasma equivalents inj. on TSQ Quantum Ultra
- A&P (99.0-113.1% & 2.3-12.2%)
- Blank XIC of a cyno samples and the LLOQ didn't show any interference

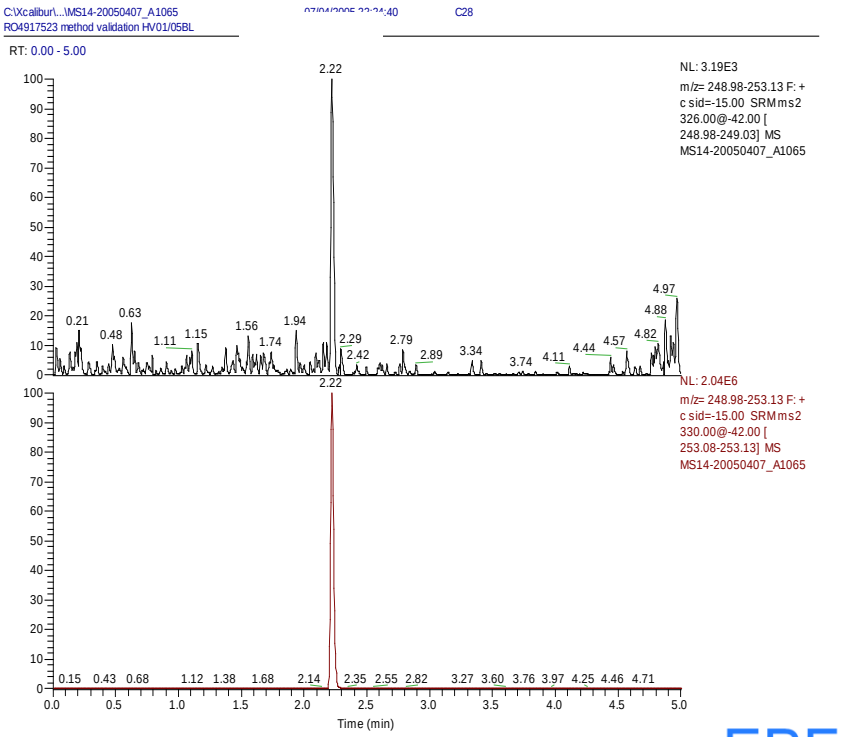
EBF

Anomalous results case studies: #1

Basic facts:

0-cyno plasma and LLOQ cal standard sample





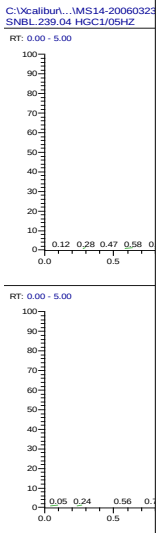
Anomalous results case studies: #1

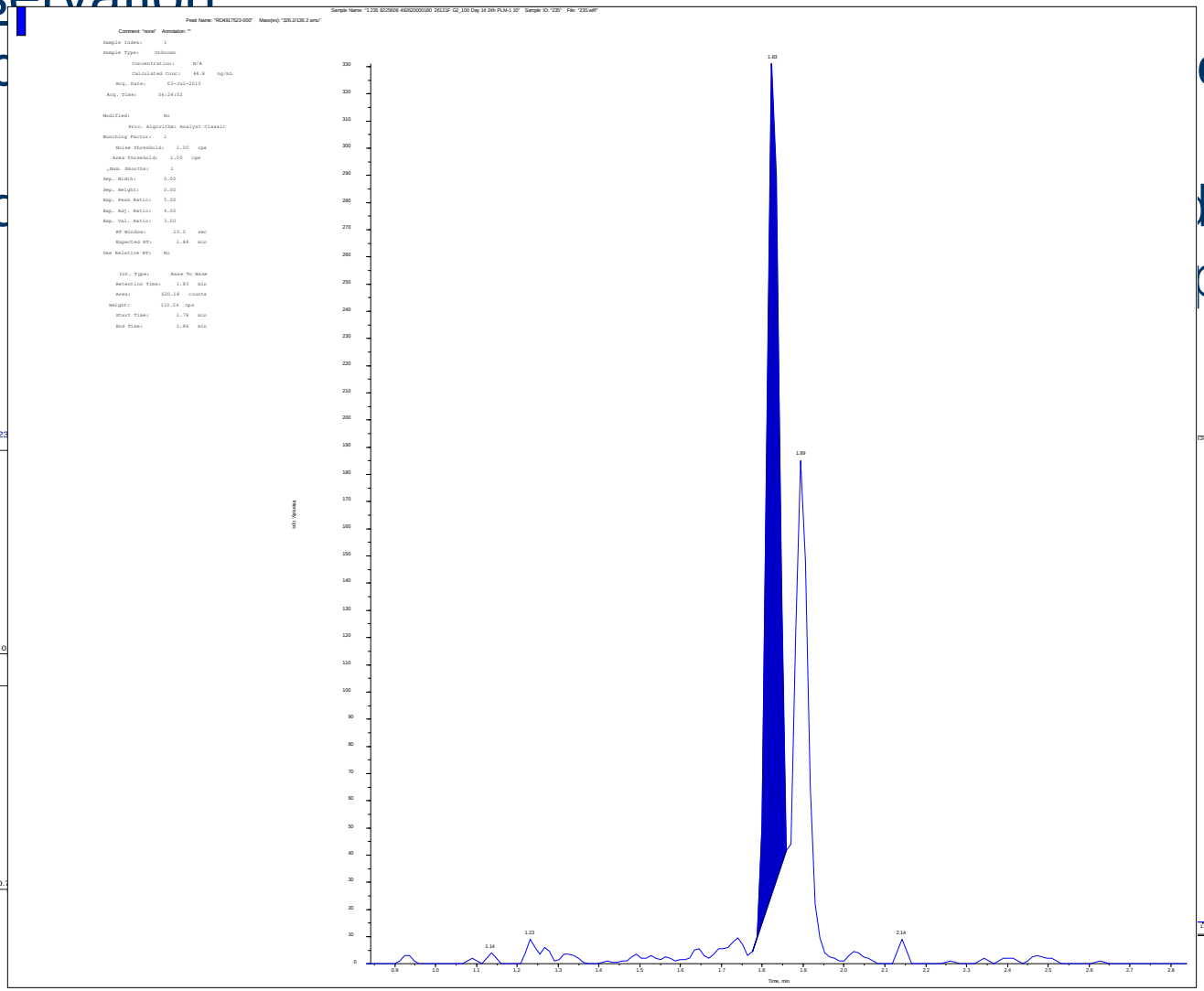
Observation:

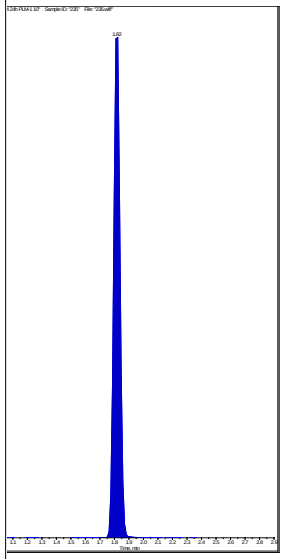
Stud ce in

Stud dy

per





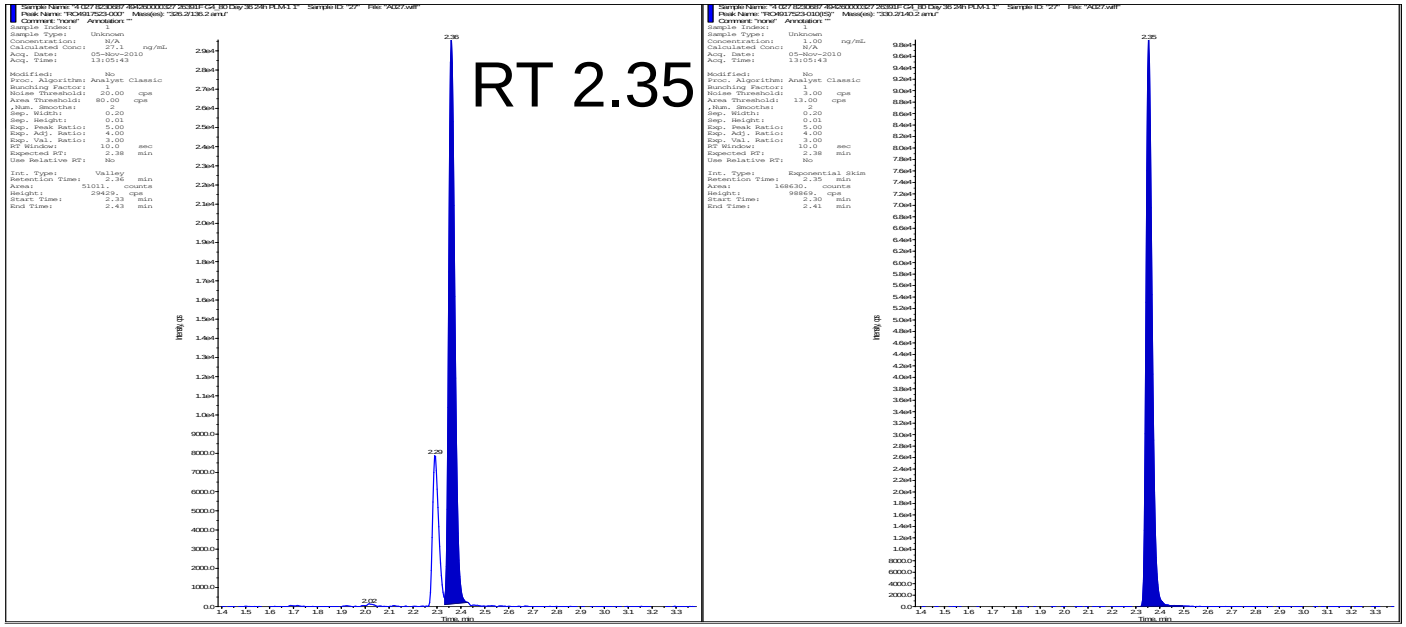


EBF

Anomalous results case studies: #1

Resolution:

- Study conduct was stopped
- SD informed about findings, and MetID involved (O-Gluc)
- Method re-developed and re-validated
- Findings and measures communicated to team and documented in study file



Anomalous results case studies: #2

Basic facts:

- Fully validated ELISA assay for PEG-protein in dog, rat, cyno, and human serum
- Validation was successful with respect to all parameters including dilution linearity
- Sandwich ELISA with anti–PEG mAB for capturing and conjugated binding protein (BP) for detection
- Only intact PEG-protein is detected
- Assay design: serial assay, overnight incubation
  5% plasma
- Con. range: 0.7 – 50 ng/mL (100% plasma); 4PLF
- A&P: 93.8-102.7% & 2.7-10.0%
- dilution linearity $2E^4$-$4E^6$

EBF

Anomalous results case studies: #2

Basic facts:

Assays design

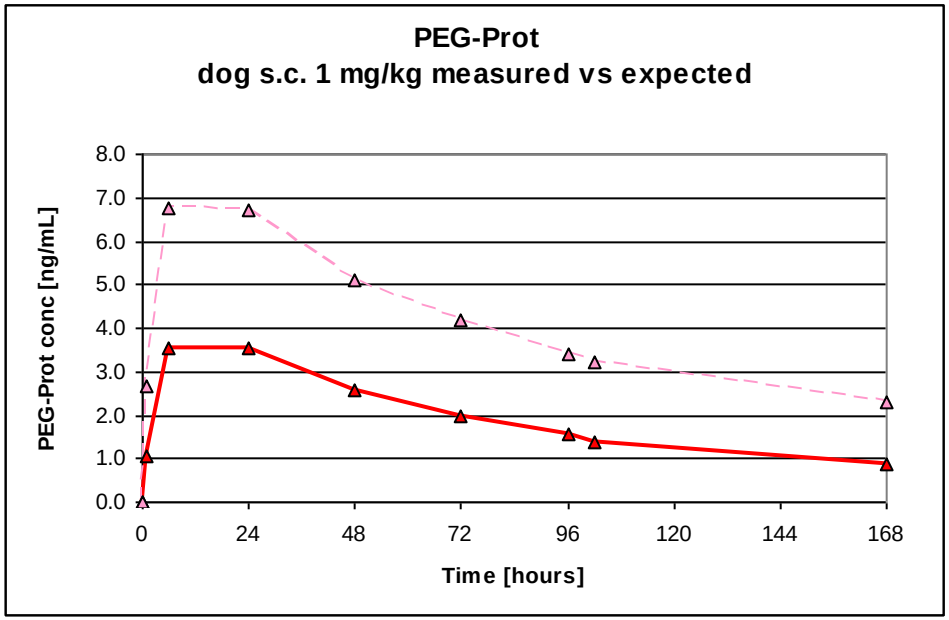
SA-**MTP** mAB**<PEG>***BI* **PEG-Prot** mAb<**Prot**>*DIG* mAb-<**DIG**>POD

(binding protein; IGM)

Anomalous results case studies: #2

Observation:

- Unexpected concentration data (lower as expected from PK properties, biased PK profiles)
- Dilution non-parallelism (concentration dependent effect)
- Time dependence of the effects observed from in-vivo samples



Anomalous results case studies: #2

Resolution:

- Hypothesis for root cause was
  - Non specific binding to container surfaces (PEG)
  - BP (endogenous binding protein)
- PEG - careful selection and washing of sample vials didn't improve the dilution parallelism
- BP - (endogenous proteins) either up-regulated or prolonged half-life by complex formation with PEG-protein
- Resolution: Assay re-developed and validated with samples acidified in order to dissociate the PEG-Prot-BP complexes before application to assay plate
  - Addition of rHProt to saturate free endogenous BP

BA of all studies with unmodified assay repeated
Study reports re-opened and amended

EBF

Anomalous results case studies: #3

Basic facts:

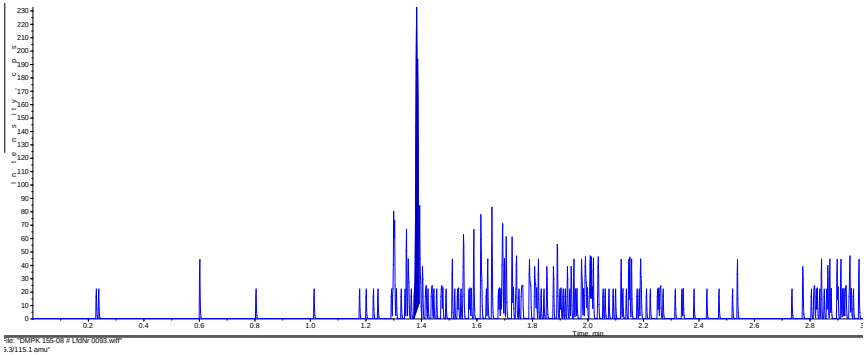
- Qualified LC-MS/MS assay for Wistar rat plasma (2009)
- Drug MW 494, $^{2}H_{3}$-IS used.
- Assay range 0.2 to 100 ng/mL, 20 µL plasma
- Sample prep – LLE using TBME, UPLC-MS/MS
- 0.7 µL plasma equivalents inj. on API4000
- A&P of QCs(93.9-99.4% & 2.45-3.35%)
- No interferences observed

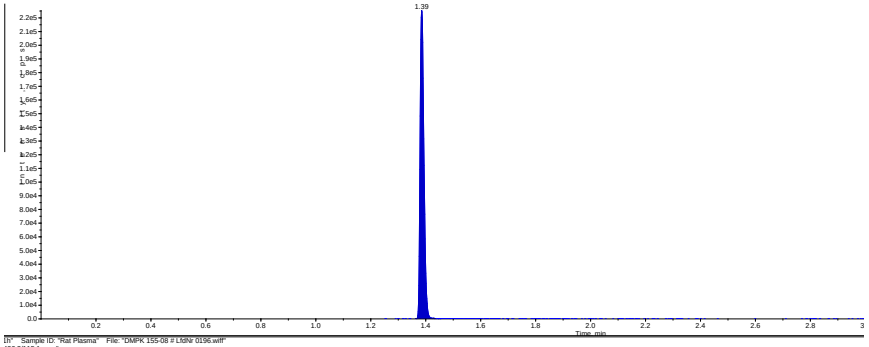
EBF

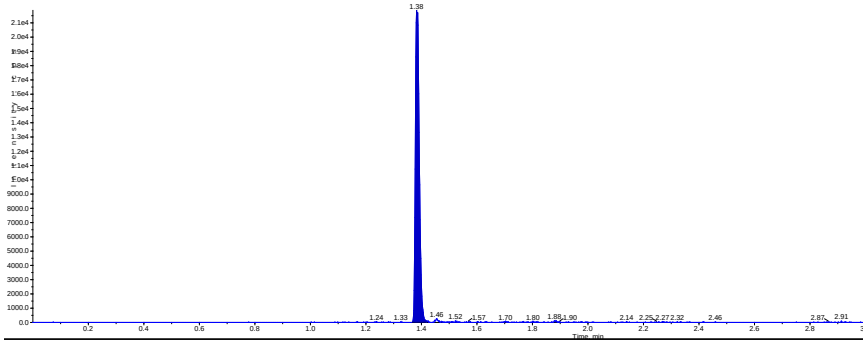
Anomalous results case studies: #3

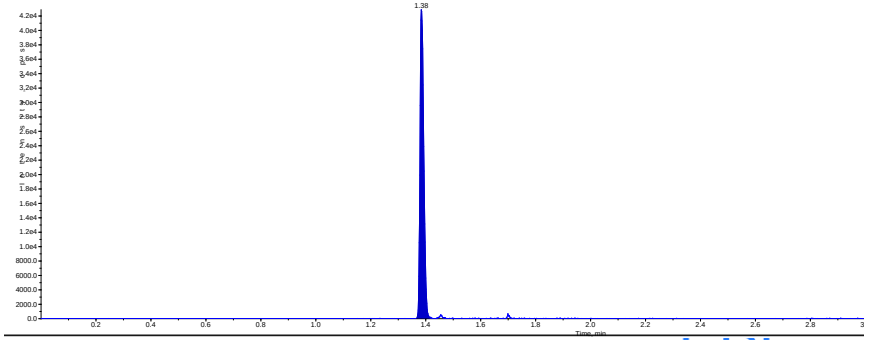
Basic facts:

Zero standard plasma (left) and representative study sample (right) (analyte upper trace, ISTD lower trace)





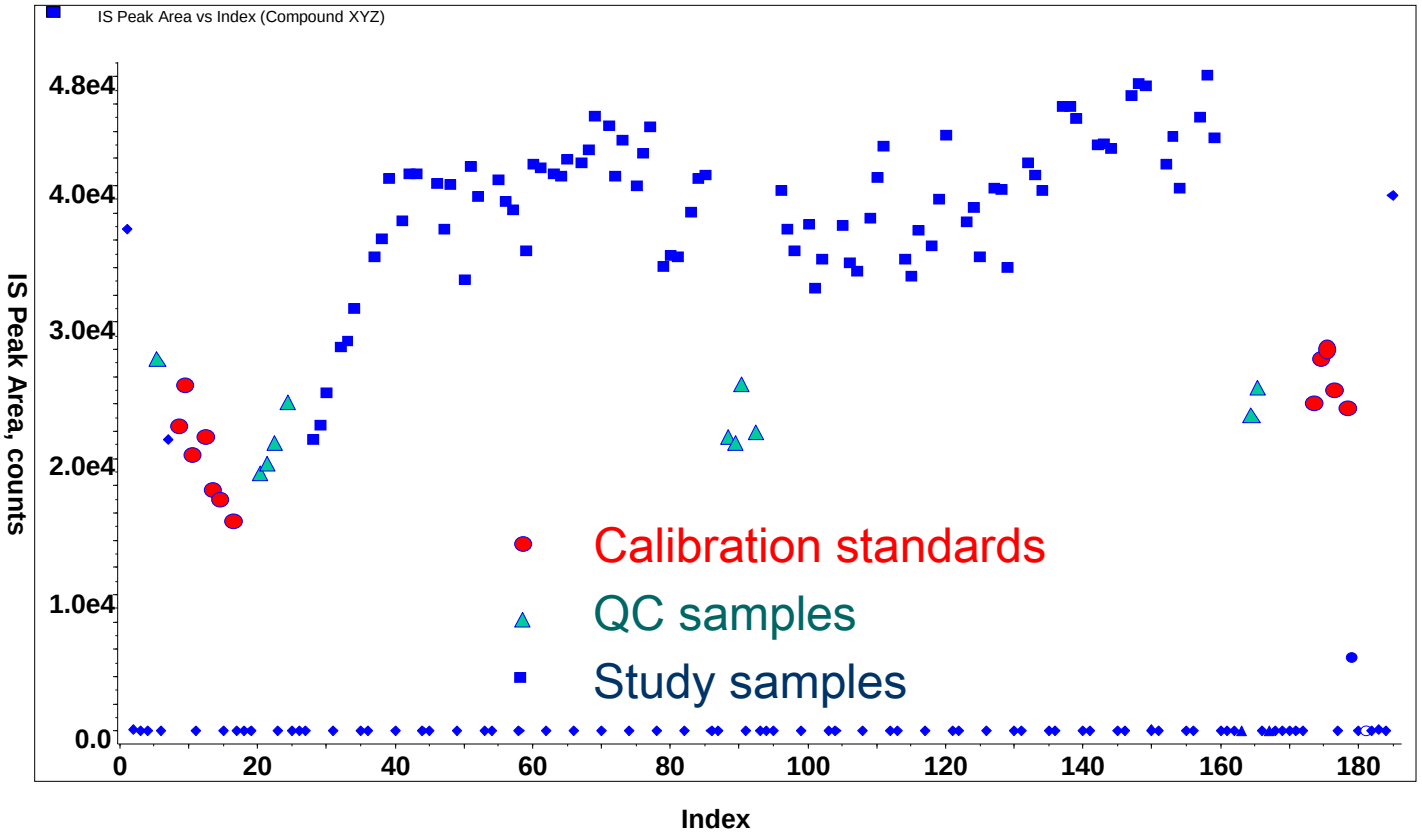




Anomalous results case studies: #3

Observations:

Bias in IS response between Cals/QCs (spiked into commercial plasma) and study samples



EBF

Anomalous results case studies: #3

Resolution:

- Additional QCs prepared in plasma from reserve animals showed no systematic bias vs. calibration samples in commercial plasma (determined by accuracy of QC samples).
- Differences considered to be due to Wistar rat plasma from different breeders:

  study animals = Charles River

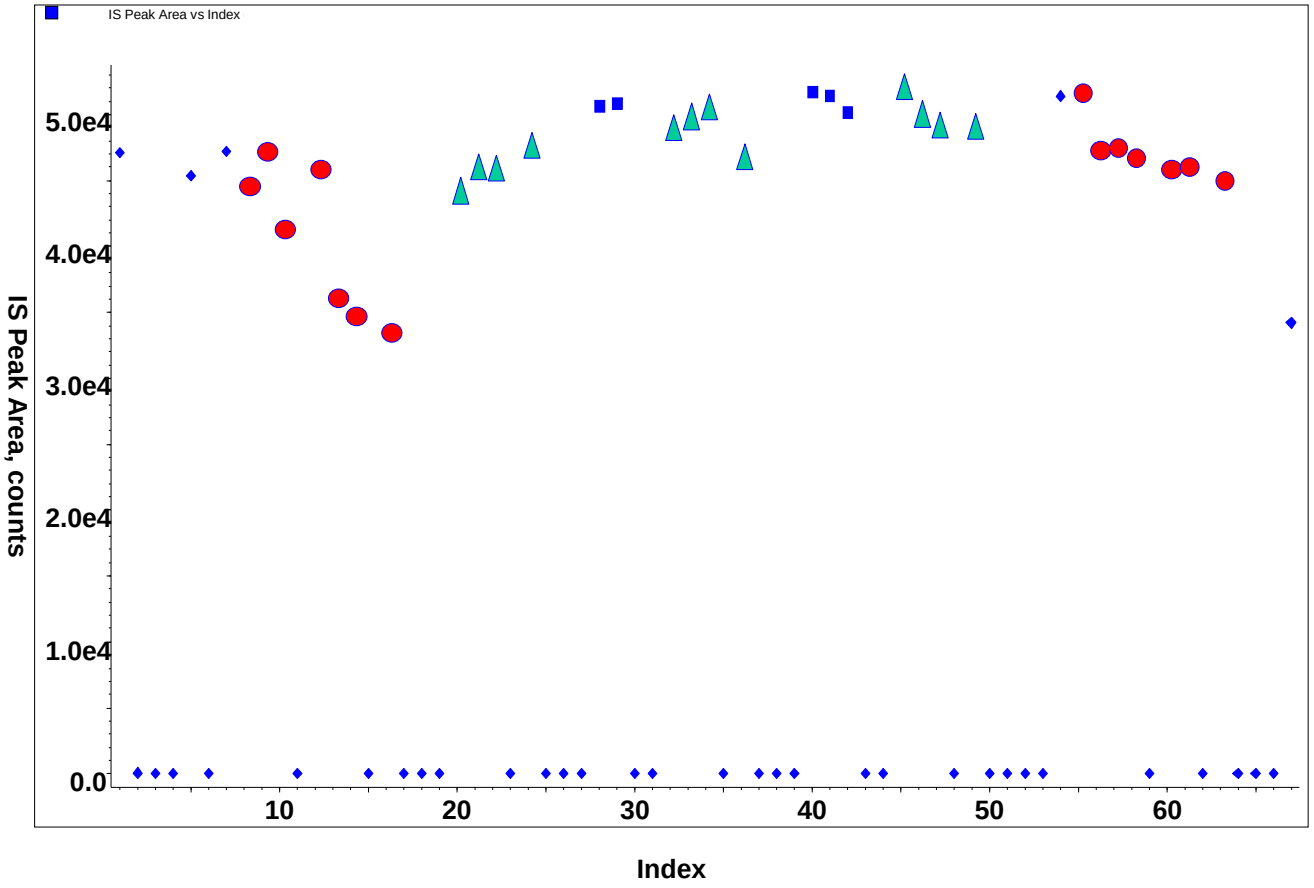
  calibration samples = Harlan

Results confirmed that initial measured values were valid and IS was working as expected!

EBF

Anomalous results case studies: #3

Resolution:



Anomalous results case studies: #4

Basic facts:

- Fully validated assay successfully applied to several studies
- Batch length: 96 or 192 samples
- Analysis runs often over night – samples stored in cooled autosampler
- 52-week toxicity study to be analysed
- Repeat analysis for day 1, week 4 and week 13 samples:
  - All batches valid

	QC low	QC mid	QC high
N	44	43	43
Dev (%)	-1.2	-2.7	-0.8
CV (%)	6.6	3.9	4.1

EBF

Anomalous results case studies: #4

Observations:

- New set of calibration standards and quality control samples prepared for analysis of week 26 samples
- Blank plasma checked for interference
- Per SOP spiking protocol QCed prior to use of samples in a study
- System suitability samples (SST) → o.k.
- Calibration samples → o.k.

Invalid study batches obtained because batch acceptance QCs failed:

- no systematic pattern

- deviation of QC independent of position in batch

EBF

Anomalous results case studies: #4

Resolution:

- MS + pipetting robot used for sample preparation checked
  → no finding, SST ok
- Second set of calibration standards and quality control samples prepared – QC check performed
- Samples analysed in pre-study batch → valid
- Study sample analysis continued - invalid study batches obtained

- Batch length reduced: 1 valid batch followed by invalid batches again
- Third set of calibration and quality control samples spiked with different source of blank plasma
- Meanwhile same problem occurred for other well established assay too

Mismatch between air conditioning "performance" in lab and "comfort temperature level" of MS on hot summer days

EBF

Anomalous results case studies summary

Case #	Method Validation(V) Study Conduct(C)	Observation and resolution covered by SOP (S) or by scientific awareness (A)
1	C	A – interfering peak
2	C	S – failed dilution parallelism
3	C	A – systematic IS response diff.
4	C	S – failing batches

Summary:

Anomalous results mainly observed during assay conduct

Anomalous results case studies

Acknowledgement:
All EBF members who provided the case studies
Sirpa Lakso, Silke Lüdtke, Peter van Amsterdam, Magnus Knutsson, Berthold Lausecker for the preparation of this session