High resolution mass spectrometry for bioanalysis at Janssen.
Current experiences and future perspectives

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Presentation outline

- Rationale for Quan on HR in non-regulated BAN (only Q-TOF) for *in vivo* samples
- Mass spec workflow considerations
- Three examples
- Conclusions

Rationale for HR MS quantification

PRO

- Sensitivity of HR instruments considerably improved
- Mass spec simplified
  - Optimisation minimal
  - Quan performed on extracted ion chromatogram
- Added value: post-acquisition evaluation of data in Quan/Qual mode
  - Information on metabolites
  - Information on biomarkers

CON

- Expensive equipment
  - Often shared between groups
- Mass spec simplified?
  - Selections to be considered
- Large datasets
- Time consuming data mining and processing

Sensitivity of HR instruments

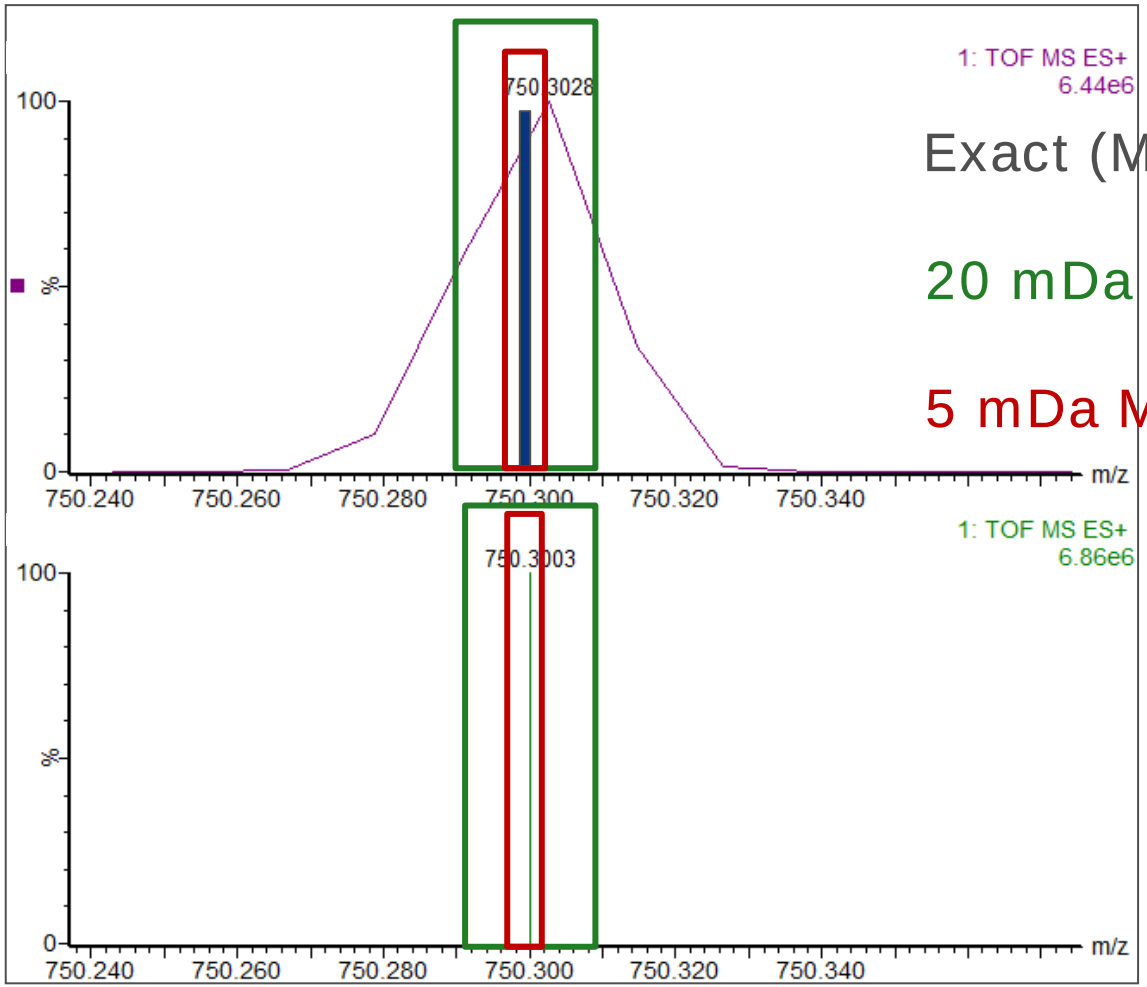
Sensitivity factor relative versus the response on API4000

	Q-TOF1	Q-TOF2	Q-TOF MS/MS
cpd1	0.1	0.5	0.25
acetaminophen	0.4	1	
galantamine	1	2	
loperamide	1	1	5
midazolam	0.1	0.5	0.25
norethindrone	0.4	2	
omeprazole	0.05	0.05	0.5
prednisone	1	1	
risperidone	0.5	1	1
cpd2	0.1	0.4	
cpd3	0.2	0.5	0.5
cpd4	0.1	0.2	
cpd5	2.5	25	5
cpd6	0.4	0.2	2
tolbutamide	0.4	1	
4'-OH mephenytoin	0.1	0.4	

Mass spec workflows can be simplified?

- No optimisation of SRM
- But following considerations
  - Resolution mode
  - Include fragmentation (Ms^E, Ms^{all})
  - Scan time
  - Mass range
  - Centroid or profile data
  - Extraction window

Extraction window, profile vs centroid



Exact $(M+H^+)$ mass: 750.2995

20 mDa MEW (± 10)

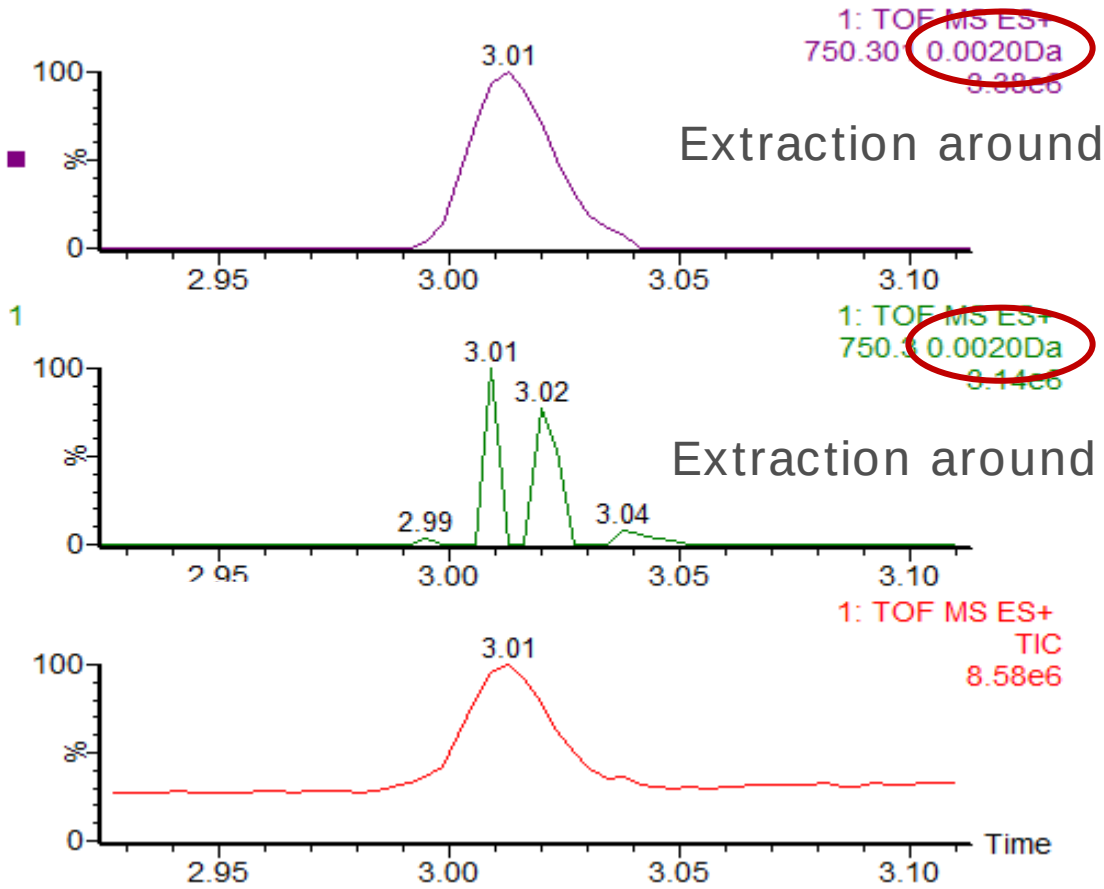
5 mDa MEW (± 2.5)

FWHM=M/R

MEW= 2 FWHM

mass accuracy and centroid data

Rs 20000



Impact of resolution on sensitivity

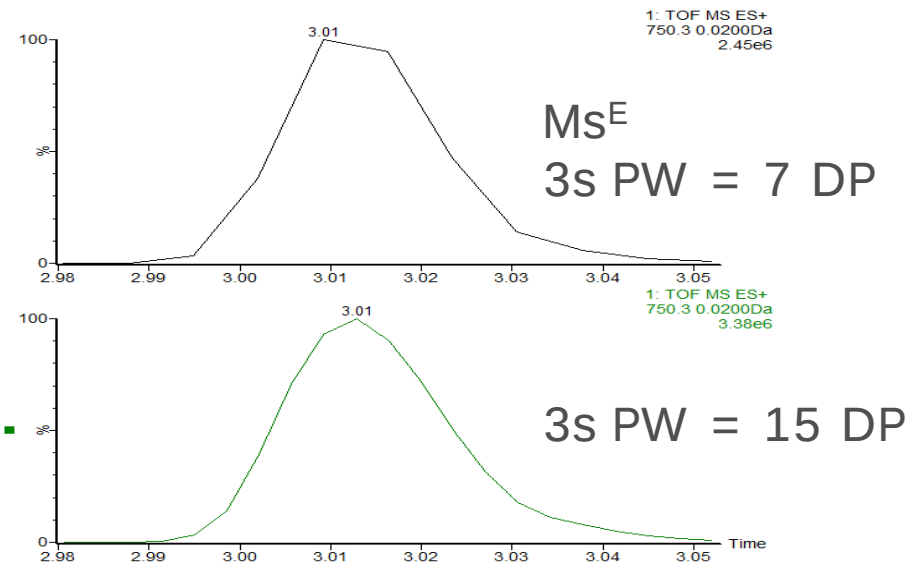
- Chromatography constant
- Scan time constant (200 ms)
- Extraction window 20mDa (+/- 10 mDa)

	ng/mL	Rs 10000 %	Rs 20000 %
cpd 1	20	505	100
	200	380	100
cpd 2	20	315	100
	200	290	100
cpd 3	20	471	100
	200	412	100

	ng/mL	Rs 10000 %	Rs 20000 %
cpd 4	20	416	100
	200	317	100
cpd 5	20	462	100
	200	389	100
cpd 6	20	457	100
	200	348	100

MS^E

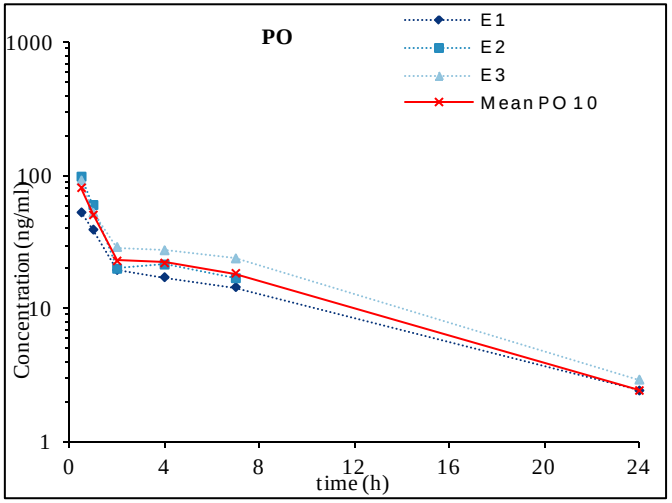
- MS^E for structural info -> Qual
- Do we compromise Quan with MS^E
  - sensitivity
  - # datapoints per peak



	ng/mL	% PA MS^E vs PA Rs20000
cpd 1	20 200	113 119
cpd 2	20 200	57 73
cpd 3	20 200	114 112
cpd 4	20 200	98 119
cpd 5	20 200	108 93
cpd 6	20 200	102 99

Example 1: first study – Q-TOF vs QQQ

- Study design: bile excretion study
- 3 rats dosed PO 10 mg/kg
- Plasma time profile (0.5, 1, 2, 4, 7, 24h)
- Bile and urine collection (0-1h, 1-4h, 4-7h, 7-24h)
- Protein precipitation



Past and Current Approaches

- Predefined separate Quan and Qual approaches
  - Quan -> fast SRM method (< 3 min run) - Bioanalysis
  - Qual -> pool samples per time point
    10 min LC run - Biotransformation

- Integrated Quan-Qual approach
  - Bioanalist analyses on HR-MS – all samples available for Qual – but no compromise in LC run?
  - Data handover to biotransformation for evaluation

Example 1: evaluate Quan/Qual

- Quan with SRM, fast LC
- Quan with HR-MS, fast LC including MS^E
- Comparison Quan performance

- Qual evaluation with fast LC
- Qual evaluation following re-analysis of samples with extended LC run
- Comparison Qual performance

SRM-quantitative results

- **Equipment:** API 4000 Triple Quadrupole LC-MS/MS + UPLC
- **Column:** Acquity UPLC BEH C18 50 x 2.1 mm 1.7 µm
- **Gradient profile**

Time (min)	Flow ml/min	% Formic Acid 0.1 %	% CH3CN
0	0.6	90	10
1	0.6	40	60
1.01	0.6	10	90
1.3	0.6	10	90
1.31	0.6	90	10
1.7	0.6	90	10

	Injection volume (µl)	LLOQ (ng/ml)	linearity (ng/ml)	Batch +QC
Bile	1	**0.5**	0.5-500	ok
Plasma	1	**0.5**	0.5-500	ok
urine	0.1*	**10**	10-5000	ok

* Low IV because of high concentrations

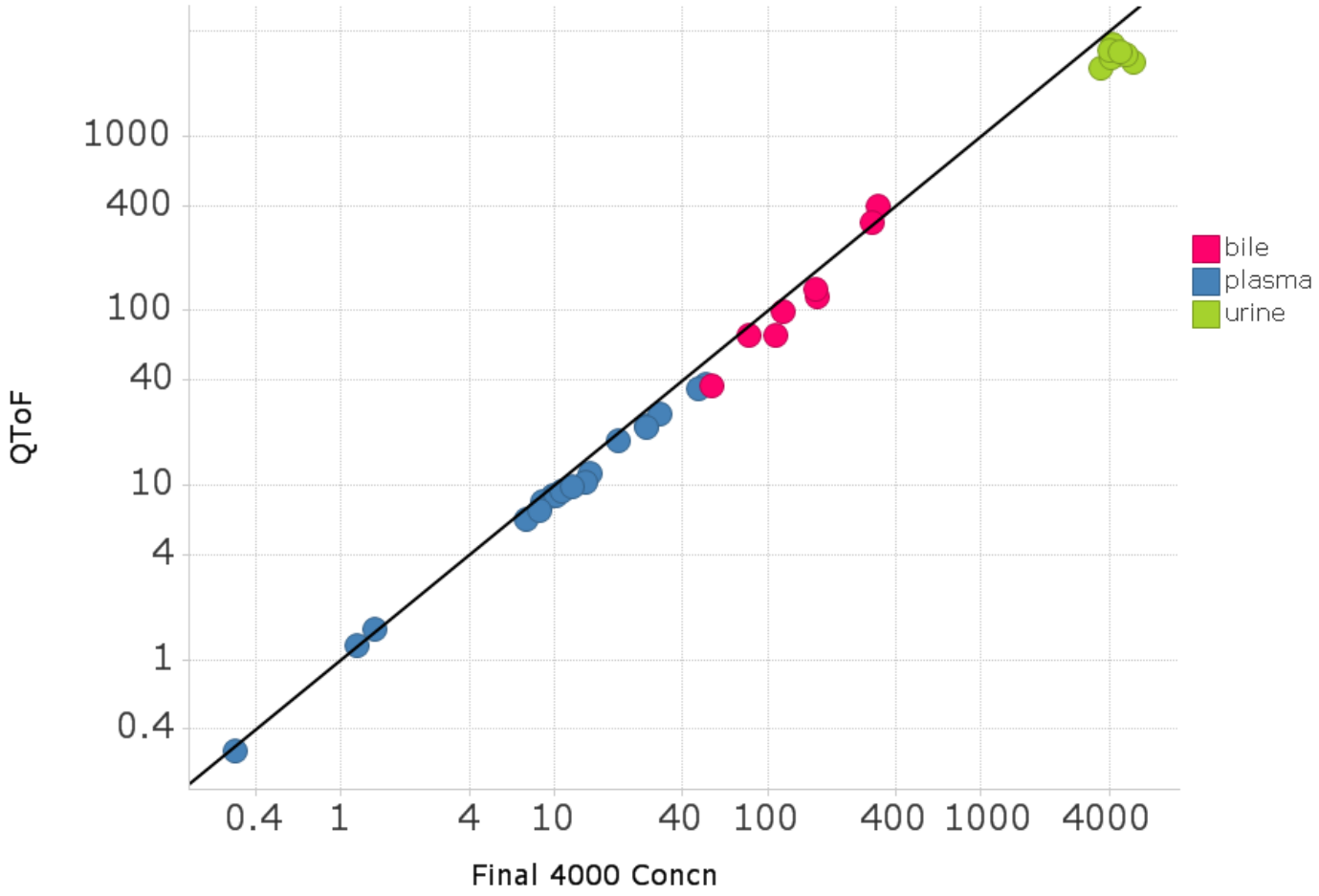
Q-TOF-based quantitative results

- **Equipment :** Synapt G2S + UPLC (identical conditions)
- **Mass range** : 100-1200 (plasma), 50 - 600 (bile, urine)
- **Analyser :** resolution mode
- **Scan time :** 0.1 s
- **QUAN:** 20 mDa MEW

	Injection volume (µl)	LLOQ (ng / ml)	linearity (ng / ml)	Batch +QC's
Bile	1	**2**	2-1000	ok
Plasma	2	**0.5**	0.5-100	Not ok *
urine	0.4	**5**	5-2000	ok

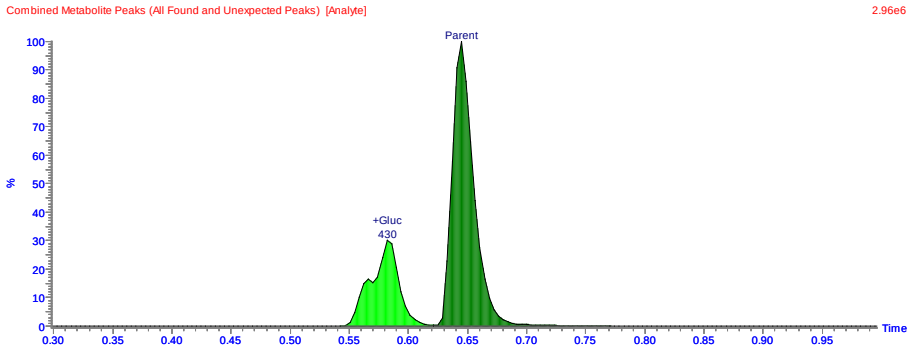
* range of curve too small to cover the range of the 3 QC's

Comparison Q-ToF vs API-4000 data

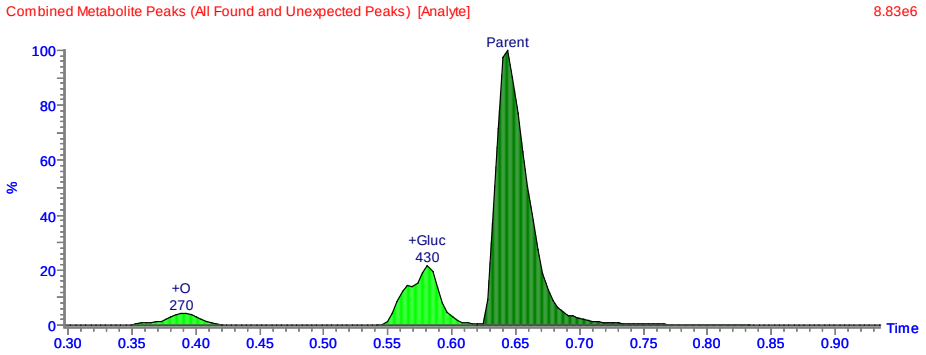


Qualitative outcomes from Q-ToF data

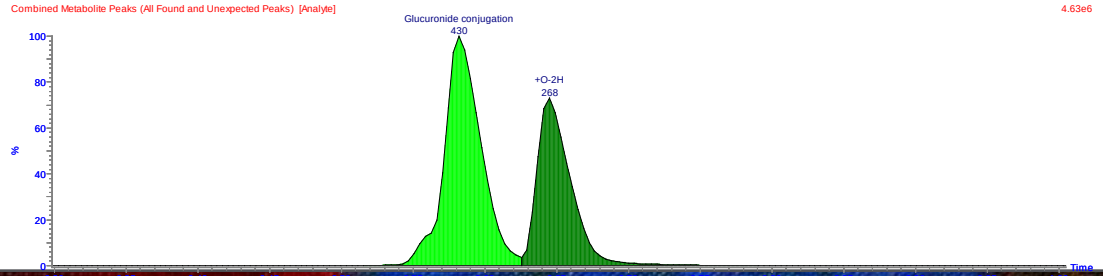
Plasma



Urine



Bile



Are QUAL results inferior due to LC compromise?

- Samples re-analysed with 10 min LC gradient
  - Acquisition kept identical (except for scan time)
  - Treshold settings identical for met ID

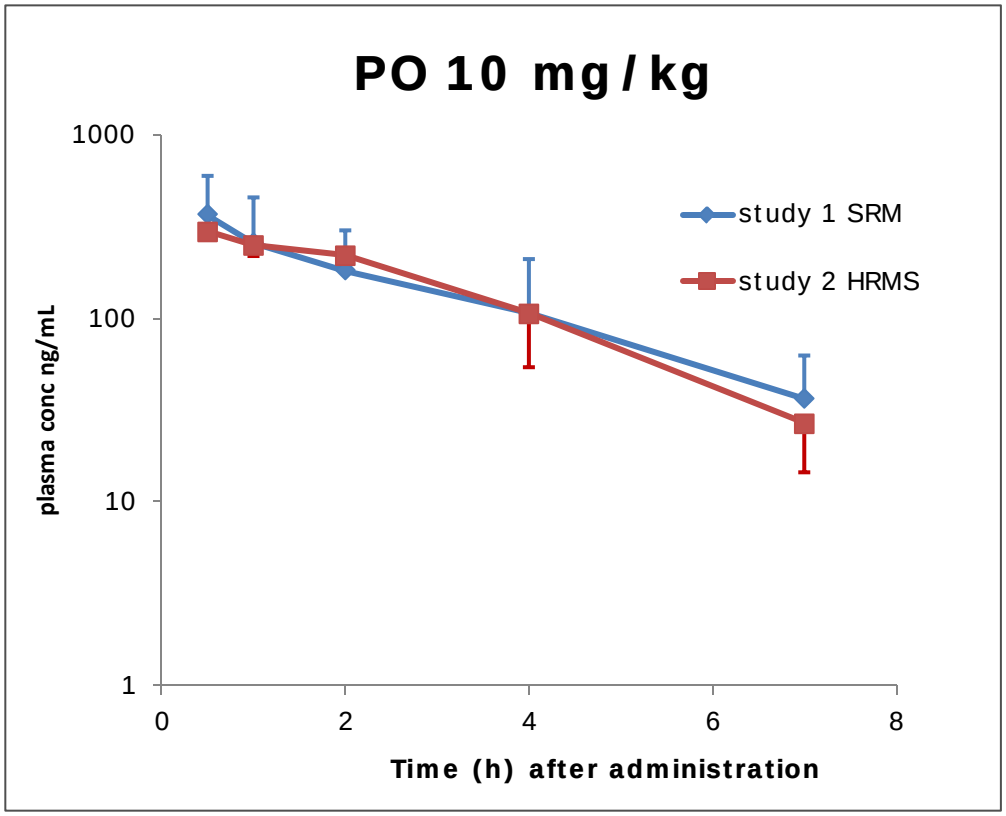
- More metabolites measured in Qual mode (different scan time)
  - But XIC in Quan results also revealed metabolite
  - Differences in response btw Quan and Qual (matrix impact)

Conclusions example 1

- Analytical performance comparable between QQQ and Q-TOF
- PK Profiles comparable
- Chromatography very compressed for MetID
  - Metabolites largely co-eluting
  - Difficult to make good use of MS^E data (IMS?)
  - Some optimization of retention time for the parent feasible
- Qualitative outcomes still valuable
  - Identified major excretion pathways and circulating metabolite
- Increased LC peak capacity will improve data quality

Example 2: Bile excretion study

- Decision upfront for longer LC run (Rt UD 3.5 min)
- Quan for parent compound



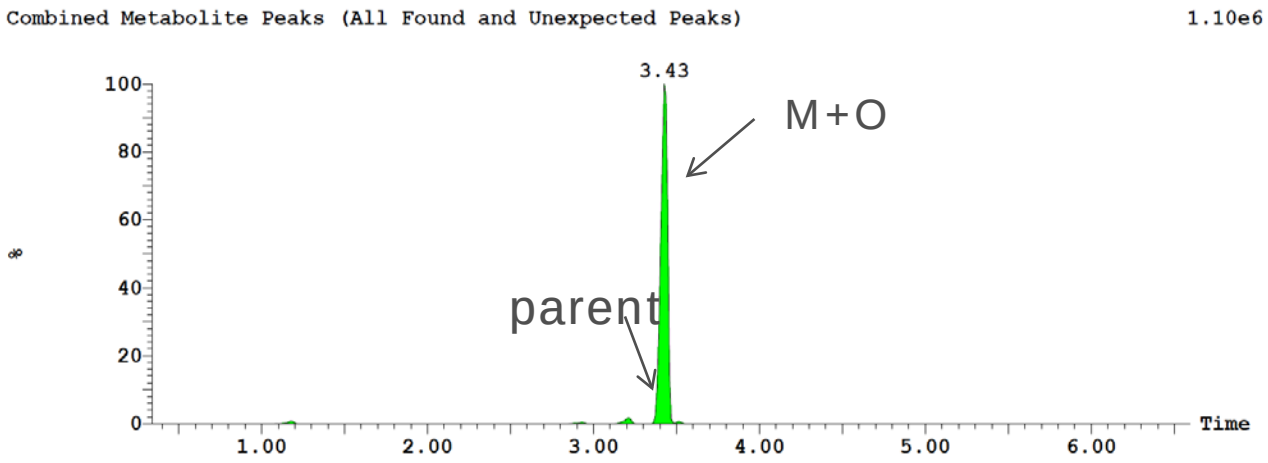
Time (h)	study 1 SRM ng/mL	study 2 HR MS ng/mL
0.5	369 ± 227	227 ± 27
1	258 ± 197	197 ± 31
2	183 ± 118	118 ± 26
4	106 ± 104	104 ± 51
7	36.6 ± 26.1	26.1 ± 12.1
24	BQL	BQL
	LLOQ 0.500	LLOQ 2.00

	Rat 1		Rat 2	
Bile	ng/ml	% of dose	ng/ml	% of dose
0-7h	2410	0.453	851	0.161
7-24h	870	0.345	464	0.149
Total	3280	**0.798**	1315	**0.311**

	Rat 1		Rat 2	
Urine	ng/ml	% of dose	ng/ml	% of dose
0-7h	25.5	0.002	13.3	0.002
7-24h	9.43	0.002	21.4	0.008
Total	35	**0.003**	35	**0.010**

Example 2: Bile excretion study

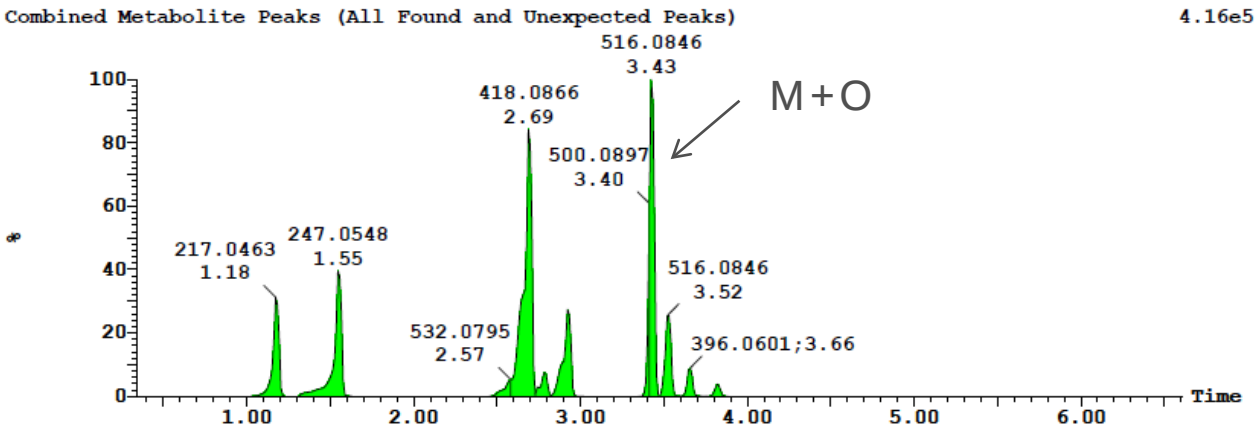
Plasma 0.5h: combined metabolites



Met Id	Rt (min)	PPM	Area Abs
Met	1.19	1.9	382.7
Met	2.92	-1.9	202.1
M+O+glucuronide	3.21	-1.8	858.5
parent	3.41	0.9	12242
M+O	3.43	1.2	**40759**
M+O	3.51	1.8	236.5

Example 2: Bile excretion study

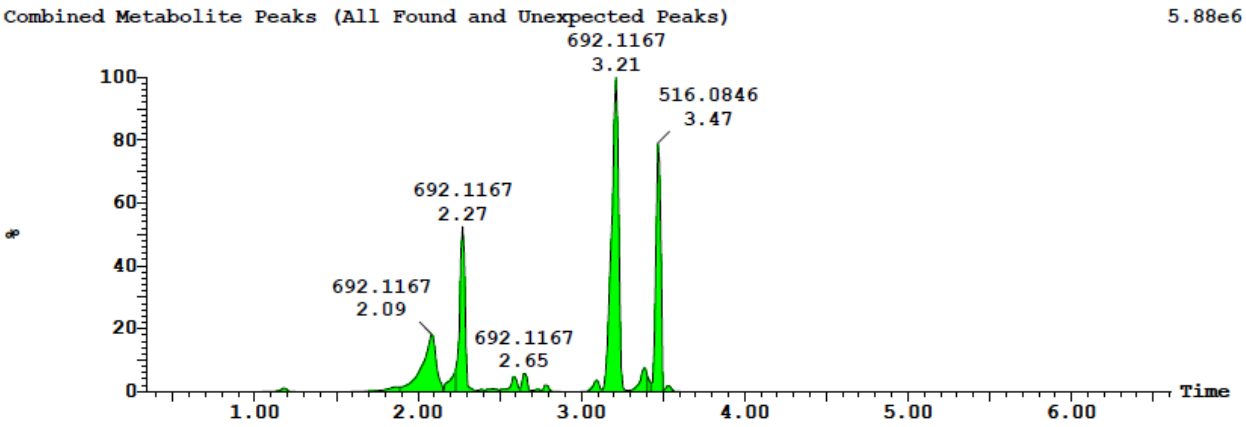
Urine 0-7h: combined metabolites



Met Id	Rt (min)	PPM	Area Abs
Met	1.18	1.9	5362
Unassigned	1.55	-1.9	8384
Met	2.69	0.6	19383
parent	3.40	0.9	**556**
M+O	3.43	1.2	**14095**
M+O	3.52	1.8	4347

Example 2: Bile excretion study

Bile 7-24h: combined metabolites



Met Id	Rt (min)	PPM	Area Abs
gluc	2.09	1.8	108175
gluc	2.27	0.6	133686
M+O+gluc	3.21	0.6	295018
Parent	3.41	-0.3	**6325**
M+O	3.47	-0.7	**167524**

Conclusion : example 2

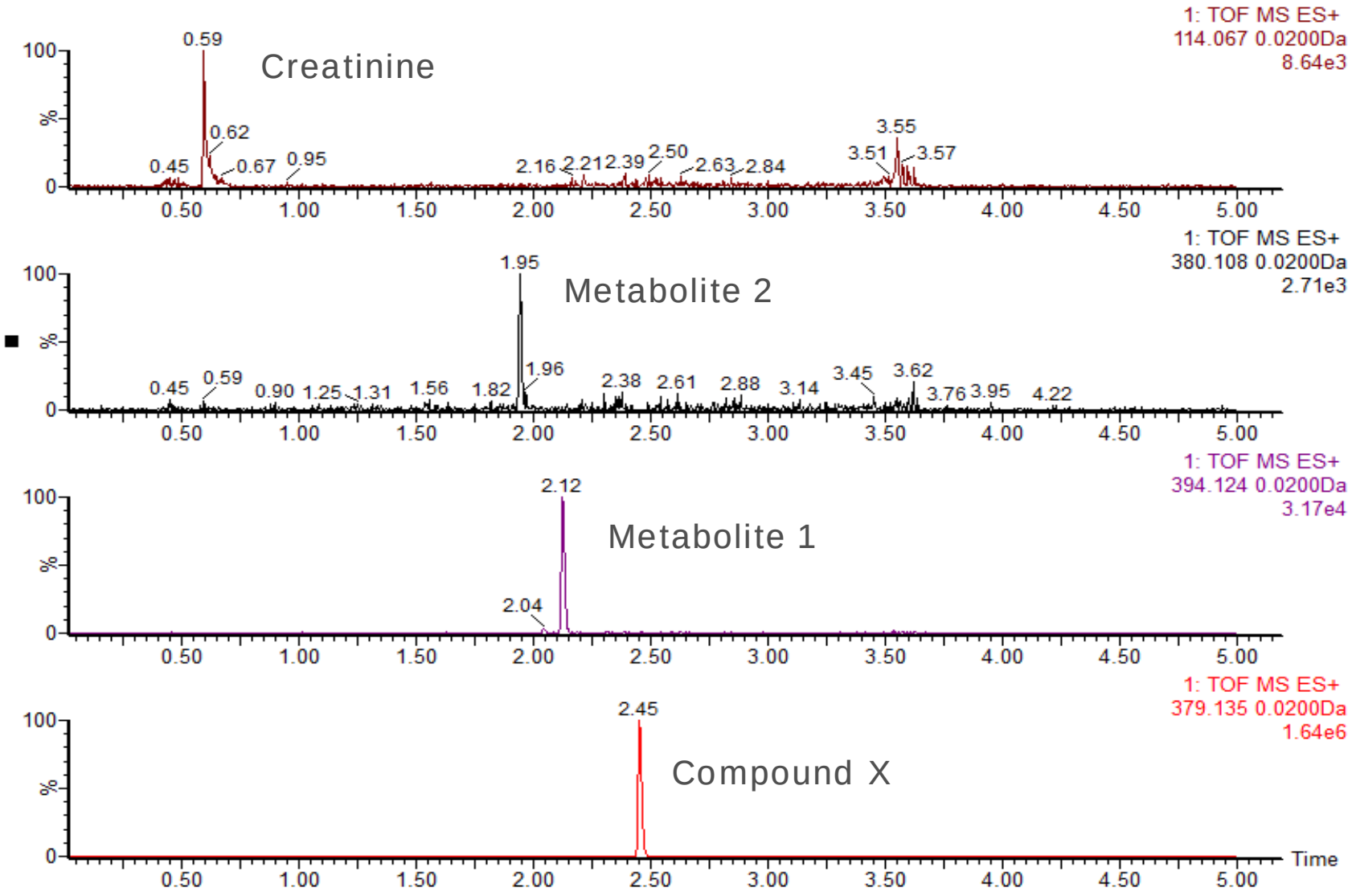
- Major drug related material is not UD
- Bioanalytical method can be optimized
  - Separation UD and M+O
  - Consider stab testing of N-oxide early on in project
- PK/PD correlation difficult
  - Evaluate activity of metabolite
  - Monitor metabolite in PD experiment

Example 3: metabolite + biomarker evaluation

- Lead project: nephrotox due to metabolite
- Bu project: evaluate *in vivo* metabolites early on
- Include biomarker
- Evaluate creatinine in plasma
- Chromatography adapted for creatinine analysis

- Acquity UPLC HSS T3 2.1x100mm 1.8µ
- Solvent A: Ammonium Acetate 0.01M
- Solvent B: Acetonitrile
- LC gradient @ 0.5 mL/min

Time (min)	% A	% B
0	99	1
2.5	40	60
3	5	95
3.1	99	1
5	99	1


1: TOF MS ES+
114.067 0.0200Da
8.64e3
Creatinine
0.59
0.62
0.45
0.67
0.95
2.16
2.21
2.39
2.50
2.63
2.84
3.55
3.51
3.57
1: TOF MS ES+
380.108 0.0200Da
2.71e3
Metabolite 2
1.95
1.96
0.45
0.59
0.90
1.25
1.31
1.56
1.82
2.38
2.61
2.88
3.14
3.45
3.62
3.76
3.95
4.22
1: TOF MS ES+
394.124 0.0200Da
3.17e4
Metabolite 1
2.12
2.04
1: TOF MS ES+
379.135 0.0200Da
1.64e6
Compound X
2.45
100
%
0
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Time

Conclusions

- Select studies for Quan/Qual
- Workflows not as simple as promised
- Always compromise between Quan efficiency and Qual data quality
- Untargeted Quan/Qual for endogenous markers, sample prep and LC matters
- Q needed? -> LC-TOF or LC-exactive

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