

level	Serum: bbCID			
[ng/ml]	500	100	50	10
Mix 1	0	0	0	0
Mix 2	1	1	1	1
Mix 3	0	0	0	0
Mix 4	0	0	0	0
Mix 5	0	0	0	0
Mix 6	0	0	0	0

level	Urine: bbCID			
[ng/ml]	500	100	50	10
Mix 1	0	0	0	0
Mix 2	1	1	1	1
Mix 3	0	0	0	0
Mix 4	0	0	0	0
Mix 5	0	0	0	0
Mix 6	0	0	0	0

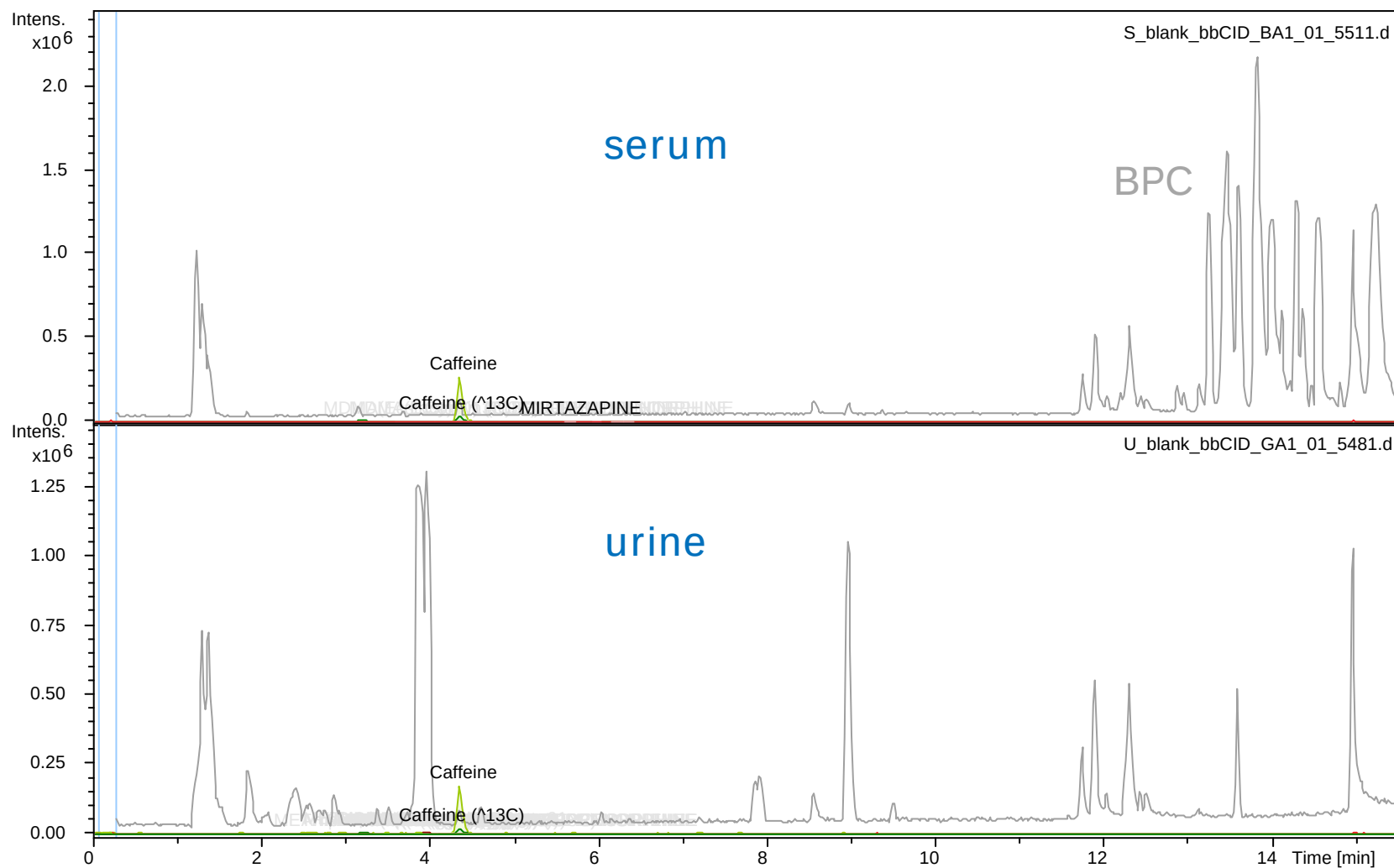
FP statistics for spiked samples
after the application of the “diagnostic ion concept”

NAME	# FP		type
MDMA	345	21.19%	noise
NORBUPRENORPHINE	341	20.95%	noise
MDMA (13C)	154	9.46%	trace peak
Paracetamol	147	9.03%	trace peak
Mirtazepine	108	6.63%	trace peak
METHAMPHETAMINE Fragg 91	70	4.30%	trace peak
Amphetamine Fragg 91	67	4.12%	trace peak
Diphenhydramin	50	3.07%	noise
METHAMPHETAMINE Fragg 119	47	2.89%	noise
MDA Fragg 135	42	2.58%	trace peak
MDMA(Ecstasy) Fragg 135	42	2.58%	trace peak
Amphetamine Fragg 119	40	2.46%	noise
Doxepin / Doxepin	24	1.47%	noise
O-DESMETHYLVENLAFAXINE	16	0.98%	
Amphetamine Fragg 91	16	0.98%	
COTININE	16	0.98%	noise
Nortilidine	15	0.92%	trace peak
Amphetamine Fragg 119	14	0.86%	

NAME	# FP		type
METHAMPHETAMINE Fragg 91 (13C)	9	0.55%	trace peak
AMITRIPTYLINE	8	0.49%	trace peak
TRAMADOL	16	0.98%	
Amphetamine Fragg 91 (13C)	6	0.37%	trace peak
TILIDINE	5	0.31%	noise
NORTRIMIPRAMINE	4	0.25%	trace peak
METHAMPHETAMINE Fragg 119	4	0.25%	
METHAMPHETAMINE Fragg 91	4	0.25%	
TEMAZEPAM	4	0.25%	trace peak
CAFFEINE 13C	3	0.18%	noise
6-MAM	3	0.18%	trace peak
Codeine (13C)	2	0.12%	trace peak
OXYCODONE	2	0.12%	trace peak
OLANZAPINE	1	0.06%	trace peak
METHADONE	1	0.06%	trace peak
PROMETHAZINE	1	0.06%	noise
ECGONINE METHYL ESTER	1	0.06%	noise

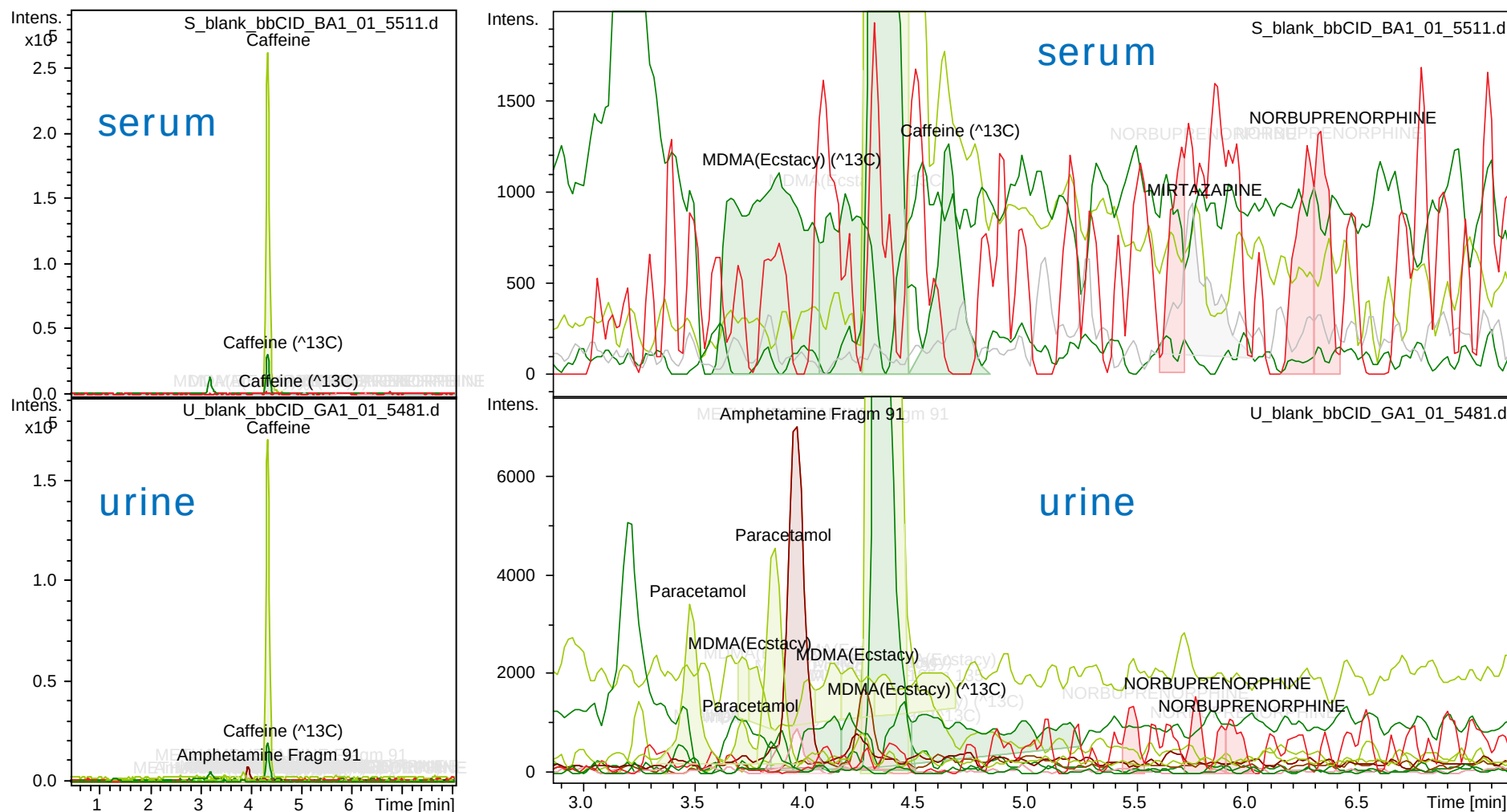
False positives:
compound names, detection type, # of findings

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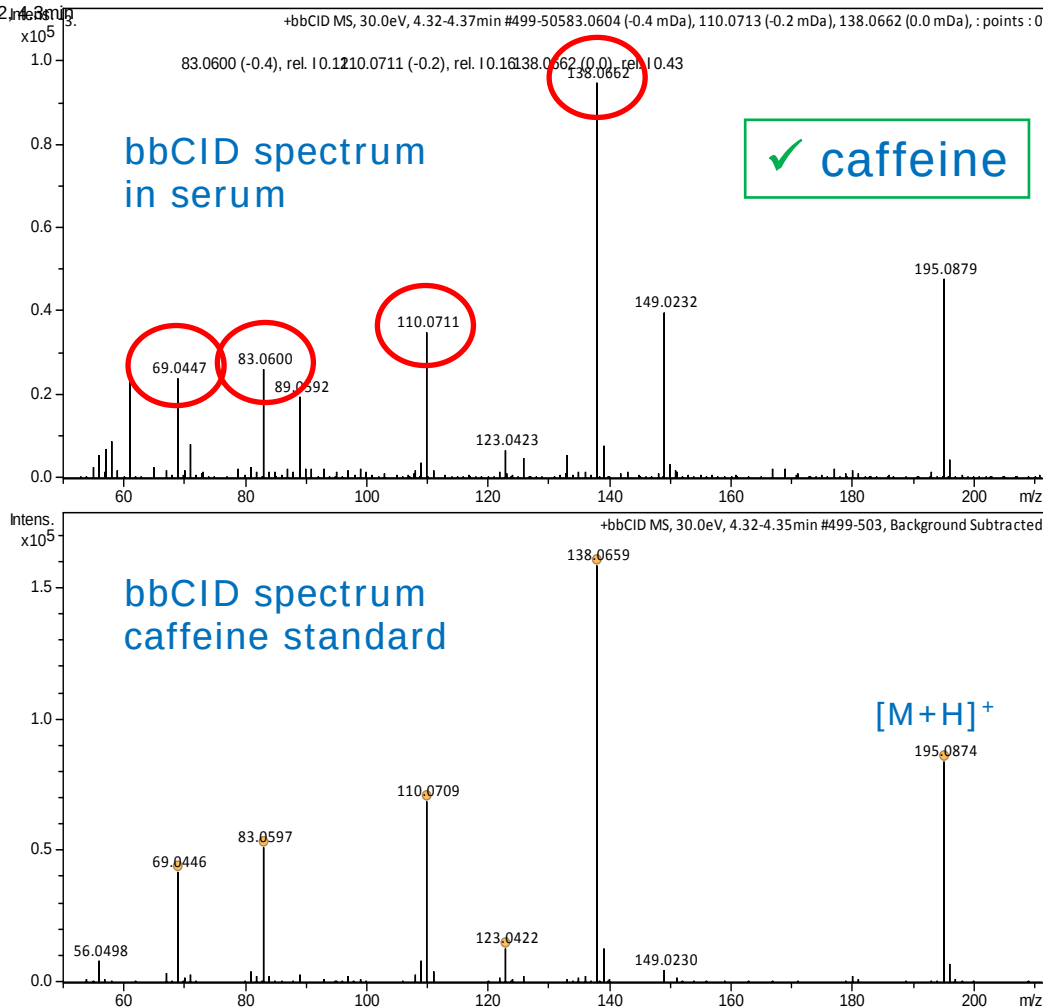
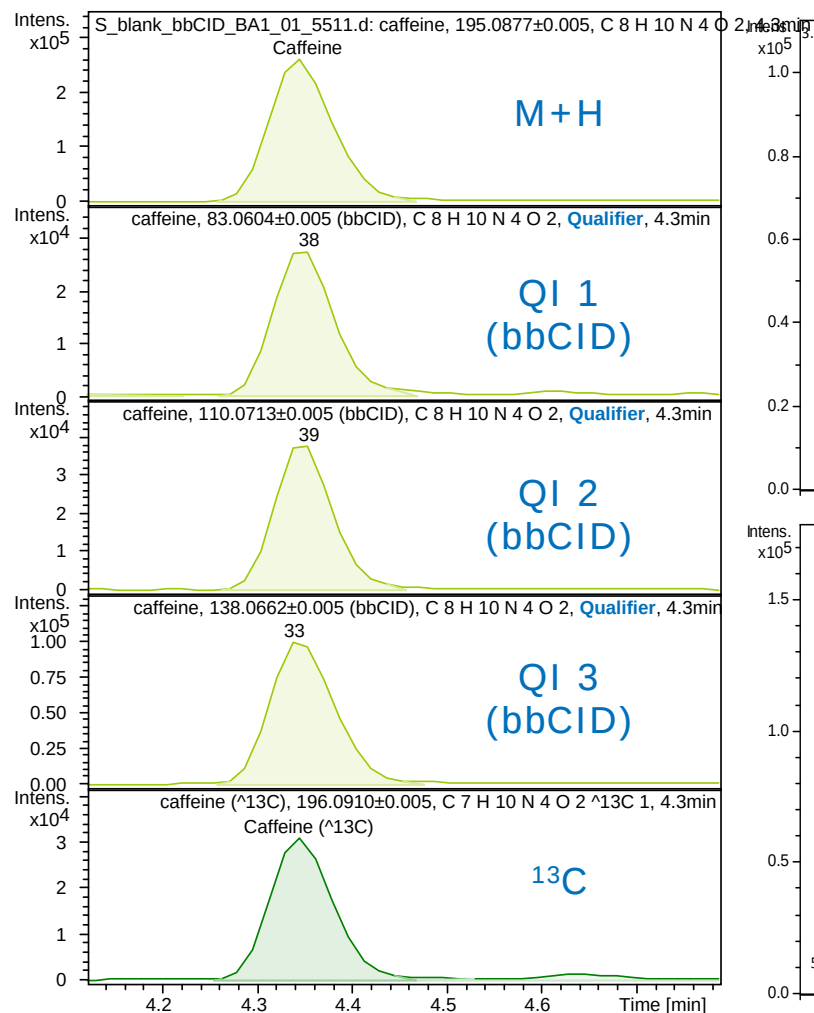


Chromatogram example: Blank serum, blank urine (bbCID data)
a) Complete chromatograms incl. base peak chromatogram
(BPC -> visualization of matrix load)

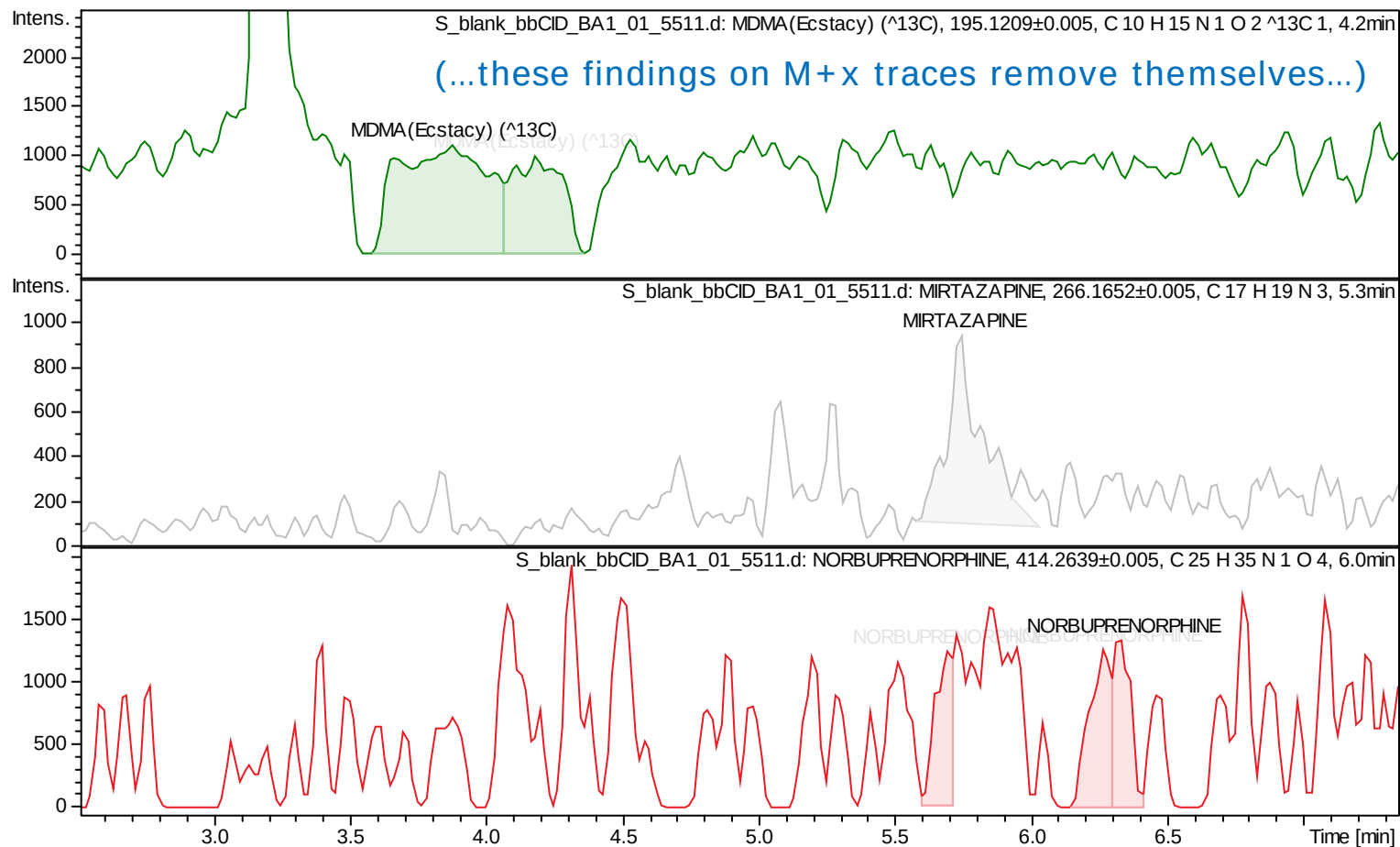
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Blank serum, blank urine (bbCID data):
b) chromatogram details: on some traces with high noise levels
random noise peaks are detected, plus few **low intensity peaks**.

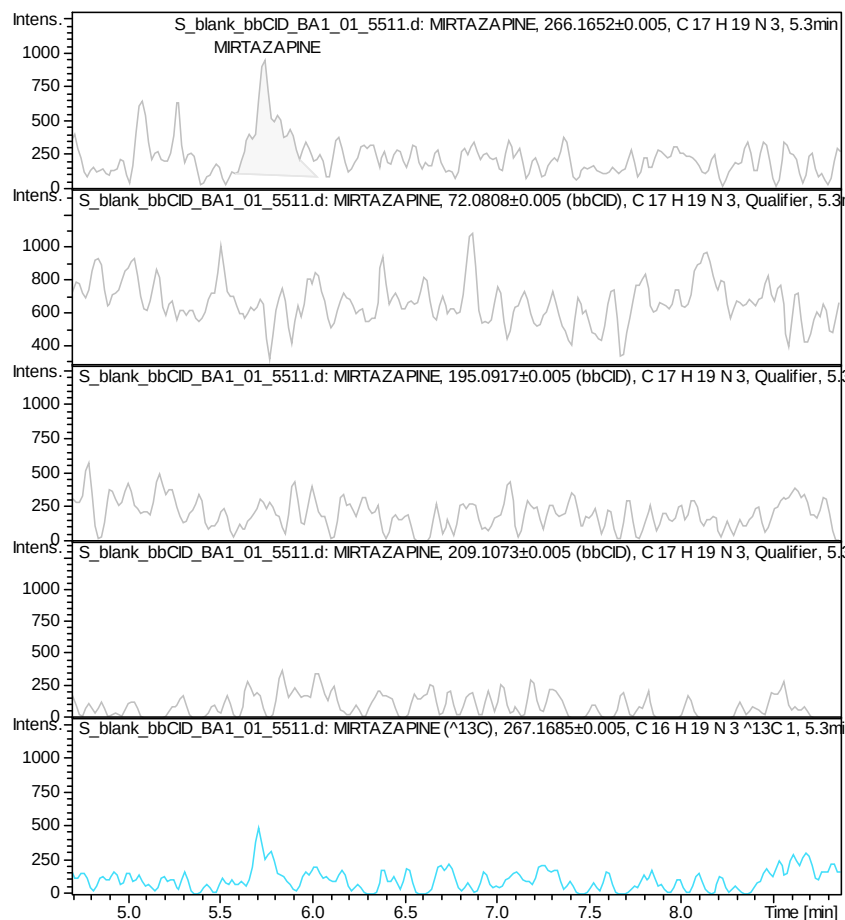


Blank serum (bbCID data):
c) Caffeine is a true positive: fragments (QI) observed with correct intensity ratios; QI peaks correlating with M+H/¹³C peaks

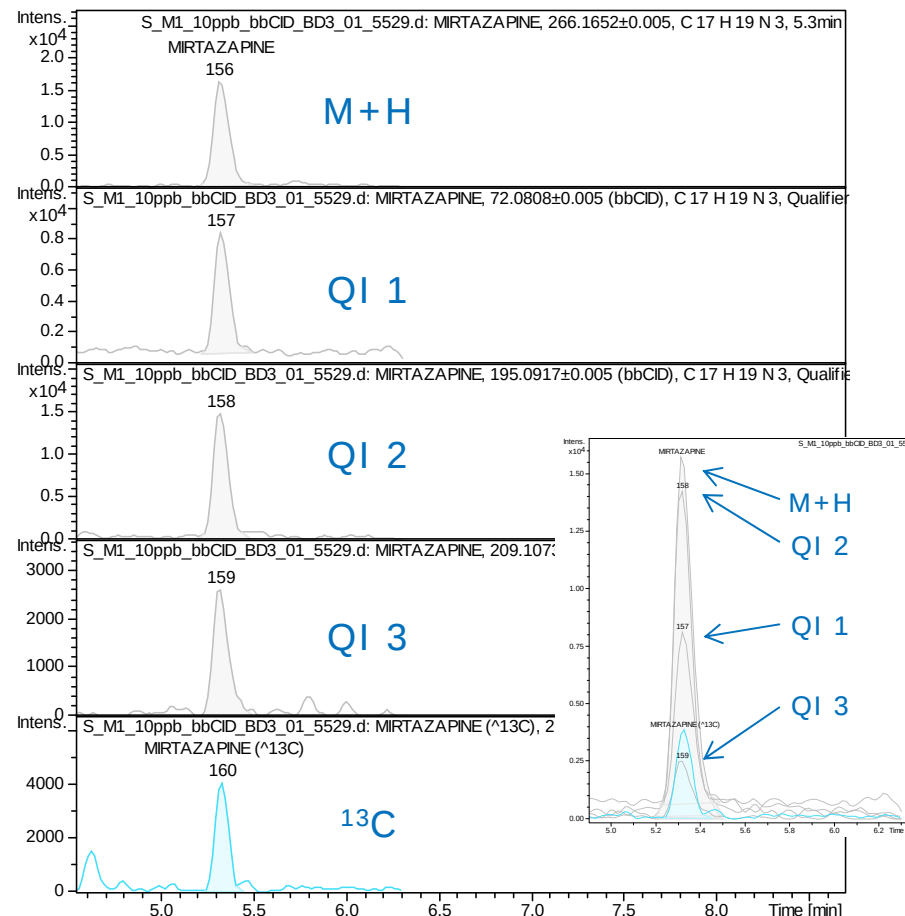


Blank serum (bbCID data): d) Elimination of FP:
no “diagnostic ion” available, no peak on ^{13}C traces
(or on QI traces, see next page) $\rightarrow$ they are gone!

serum blank: clearly different look!



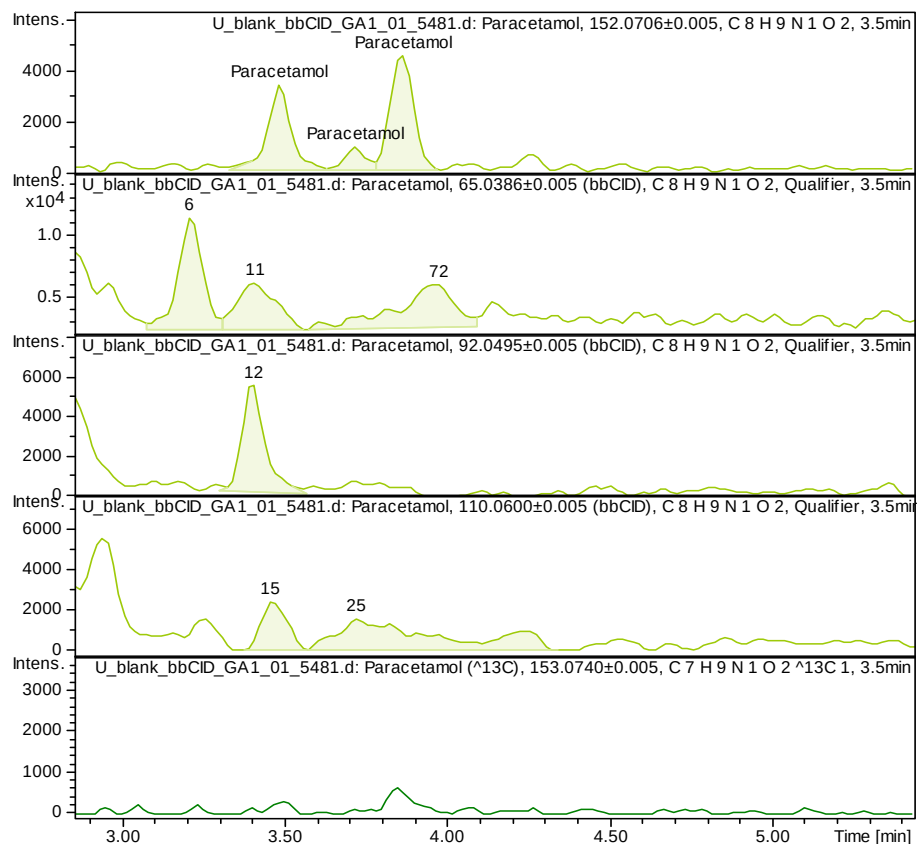
10 ng / ml spiked in serum:



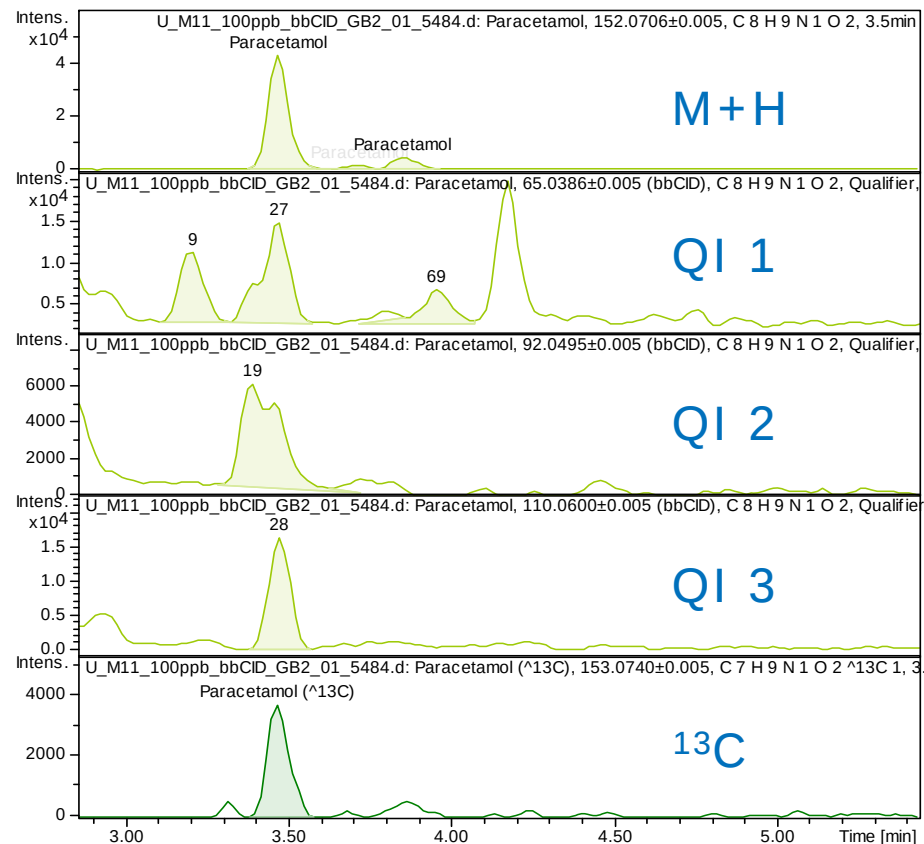
Blank serum (bbCID data): e) Elimination of FP: Mirtazepine
in many cases at least one QI has same or higher intensity than M+H
-> QI very useful for confirmation, here Mirtazepine is gone

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urine blank: <<100 ng/ml (if at all)



100 ng / ml spiked in urine:

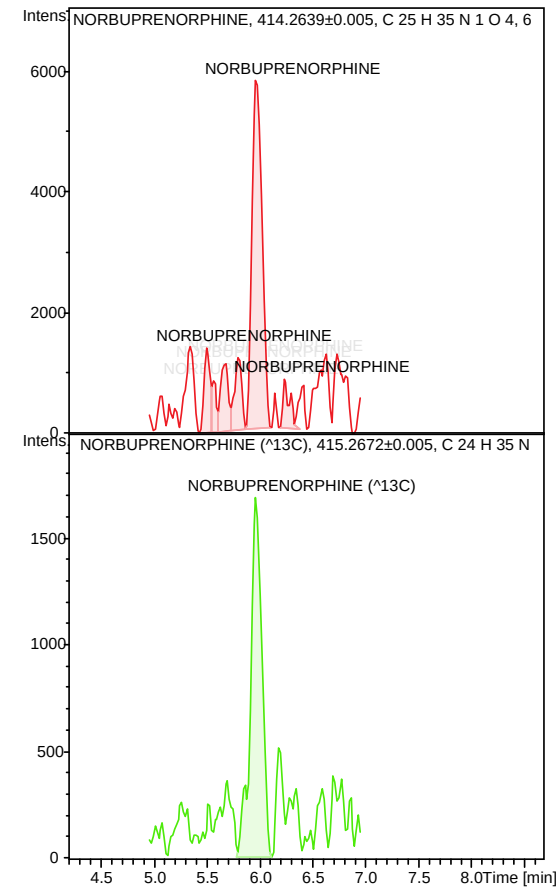
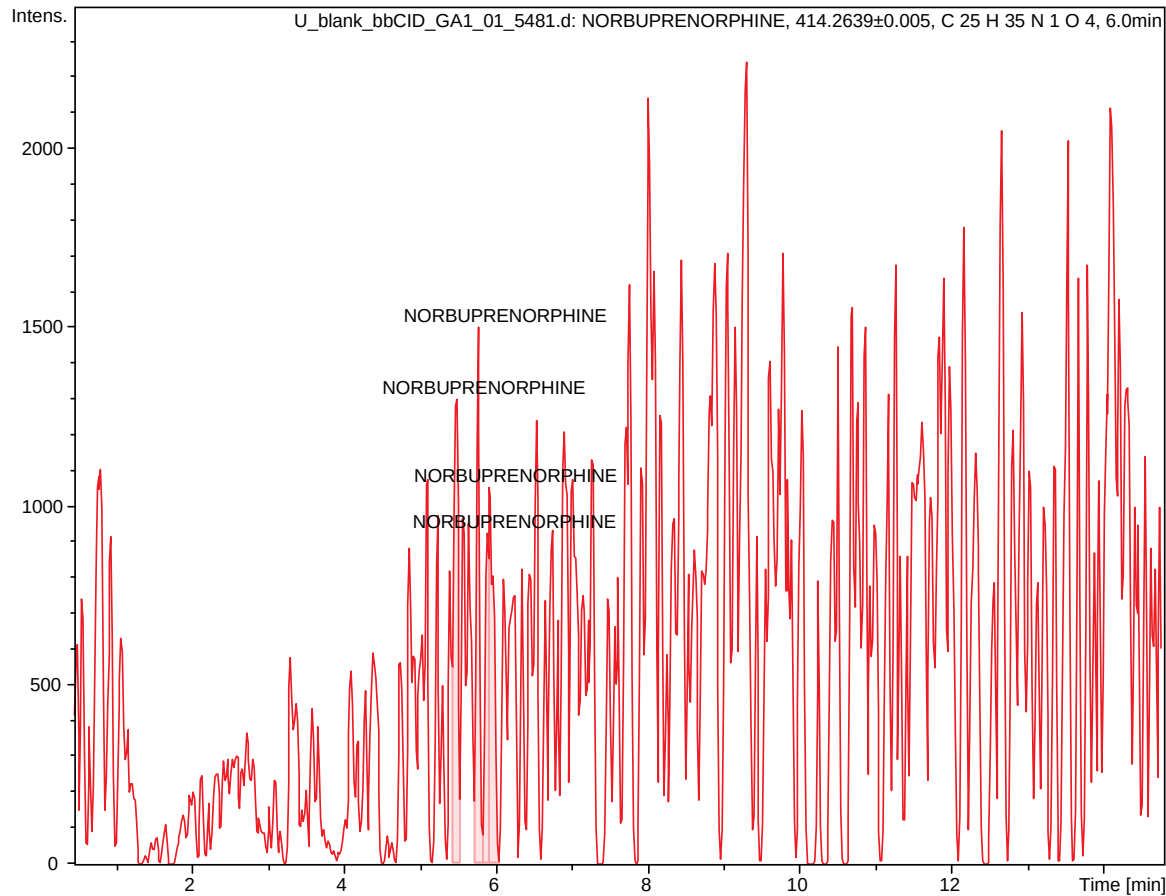


Blank urine (bbCID data): f) Elimination of FP: Paracetamol:

...that's a bit more tricky one... Due to sample history this might be a true trace finding, but low response & interferences on QI traces increase the minimum level for confident confirmation -> rated as FP

serum blank: clearly different look!

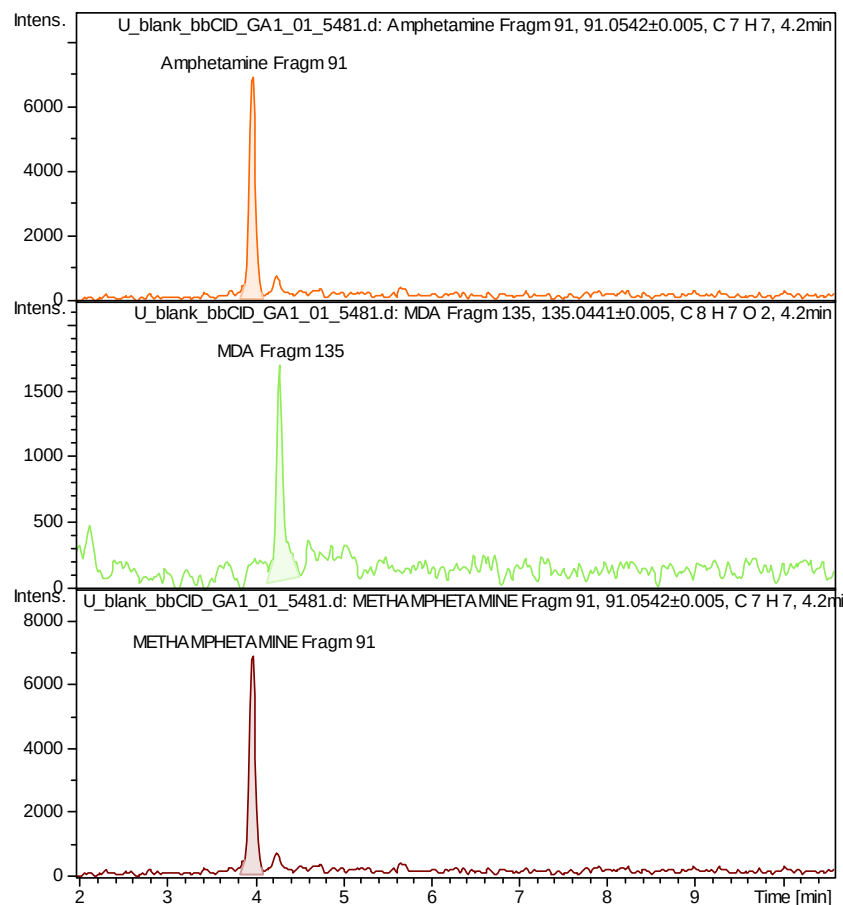
10 ng / ml spiked in serum:



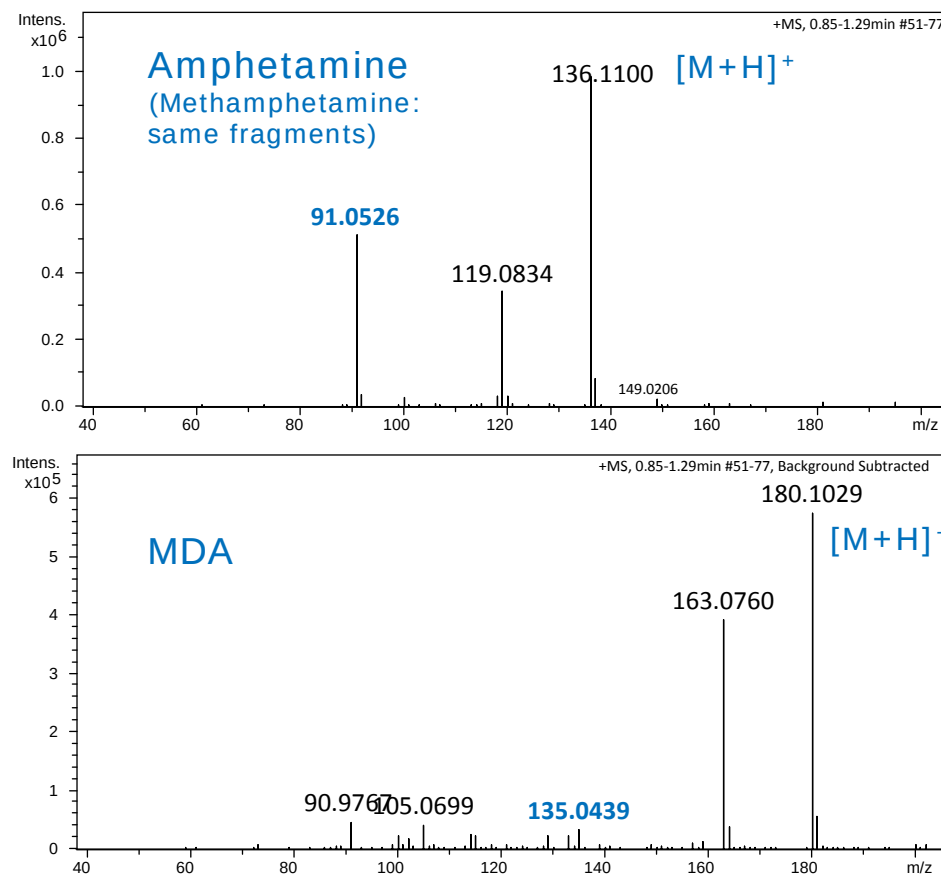
Blank urine (bbCID data): g) Elimination of FP: Norbuprenorphine:
an easy one: just noise, no peak on ^{13}C traces → gone!
(for comparison: Norbuprenorphine/ ^{13}C are detectable at 10 ng/ml level)

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urine blank:



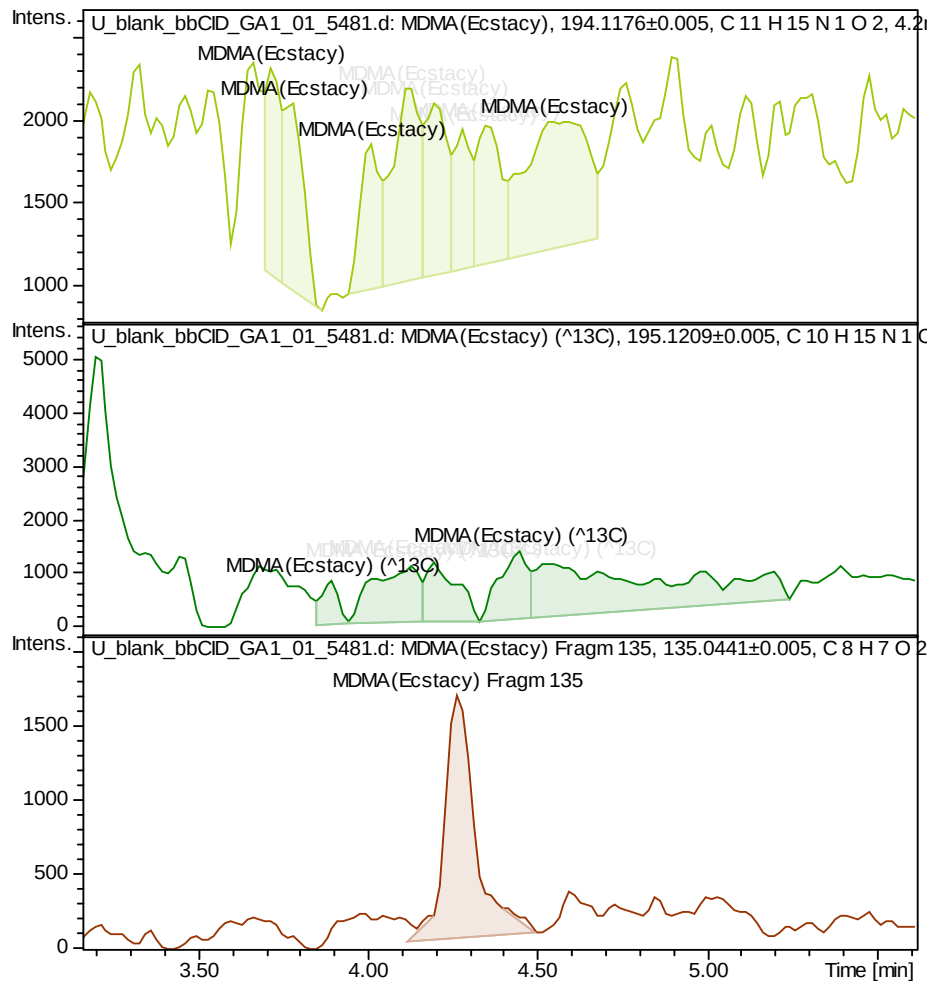
MS spectrum of standards:



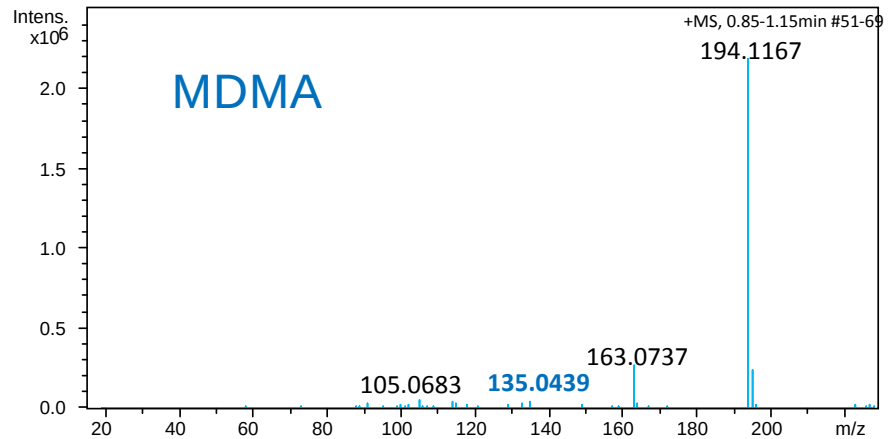
Blank urine (bbCID data): h) Elimination of FP:
(Meth)Amphetamine, MDA: easy ones, the fragments never come alone... (missing finding for M+H ; retention times are quite off) -> gone

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urine blank:



MS spectrum standard:

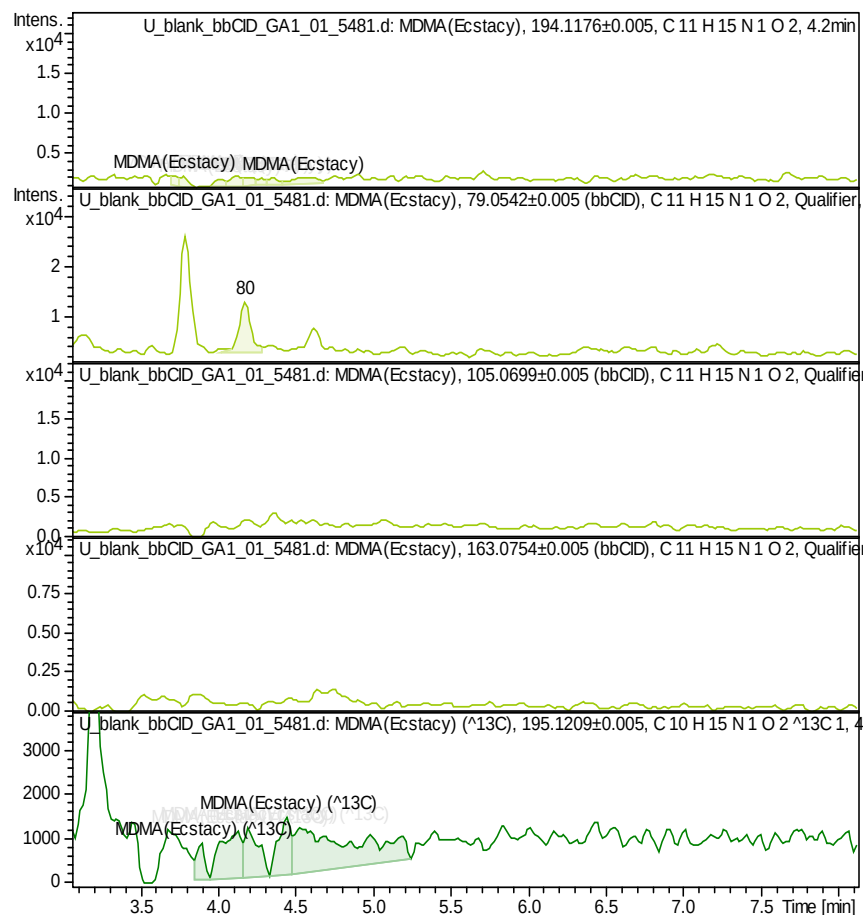


“the fragments never come alone...”
If this would be real, there should be a huge peak for $M+H$! -> this one is gone

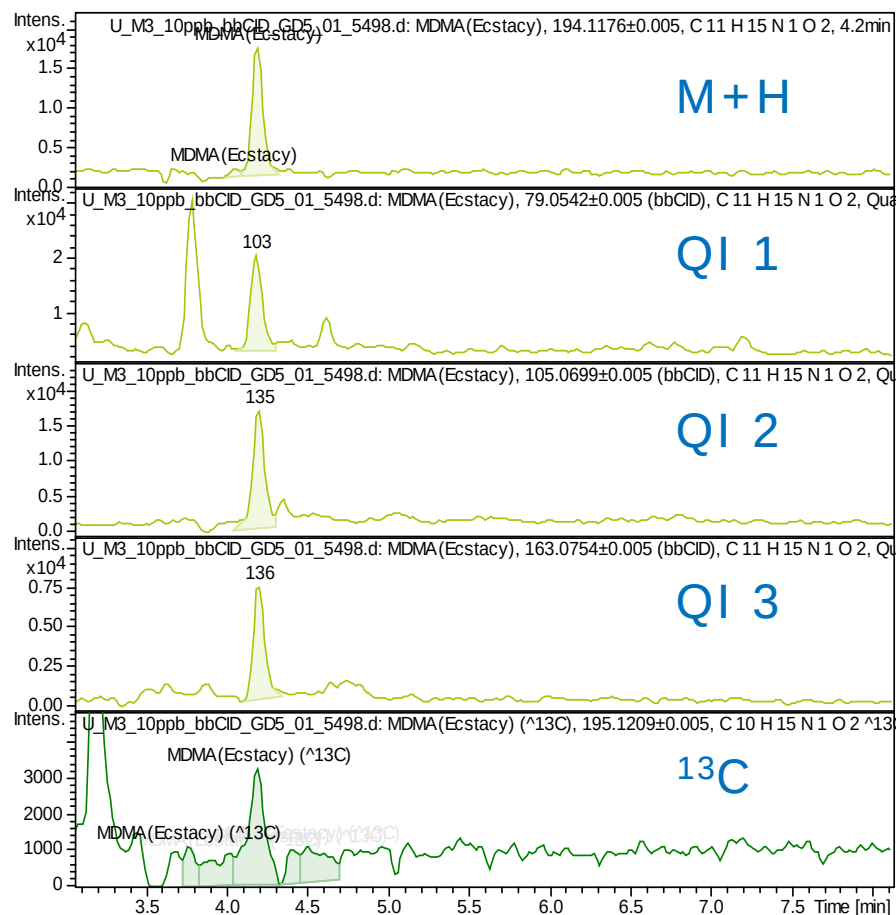
Blank urine (bbCID data): i) **Elimination of FP: MDMA**: another tricky one: just noise traces, but peaks on ^{13}C trace seem to confirm M+H findings -> add-on criteria needed for removal (see next page)

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urine blank: clearly different look!



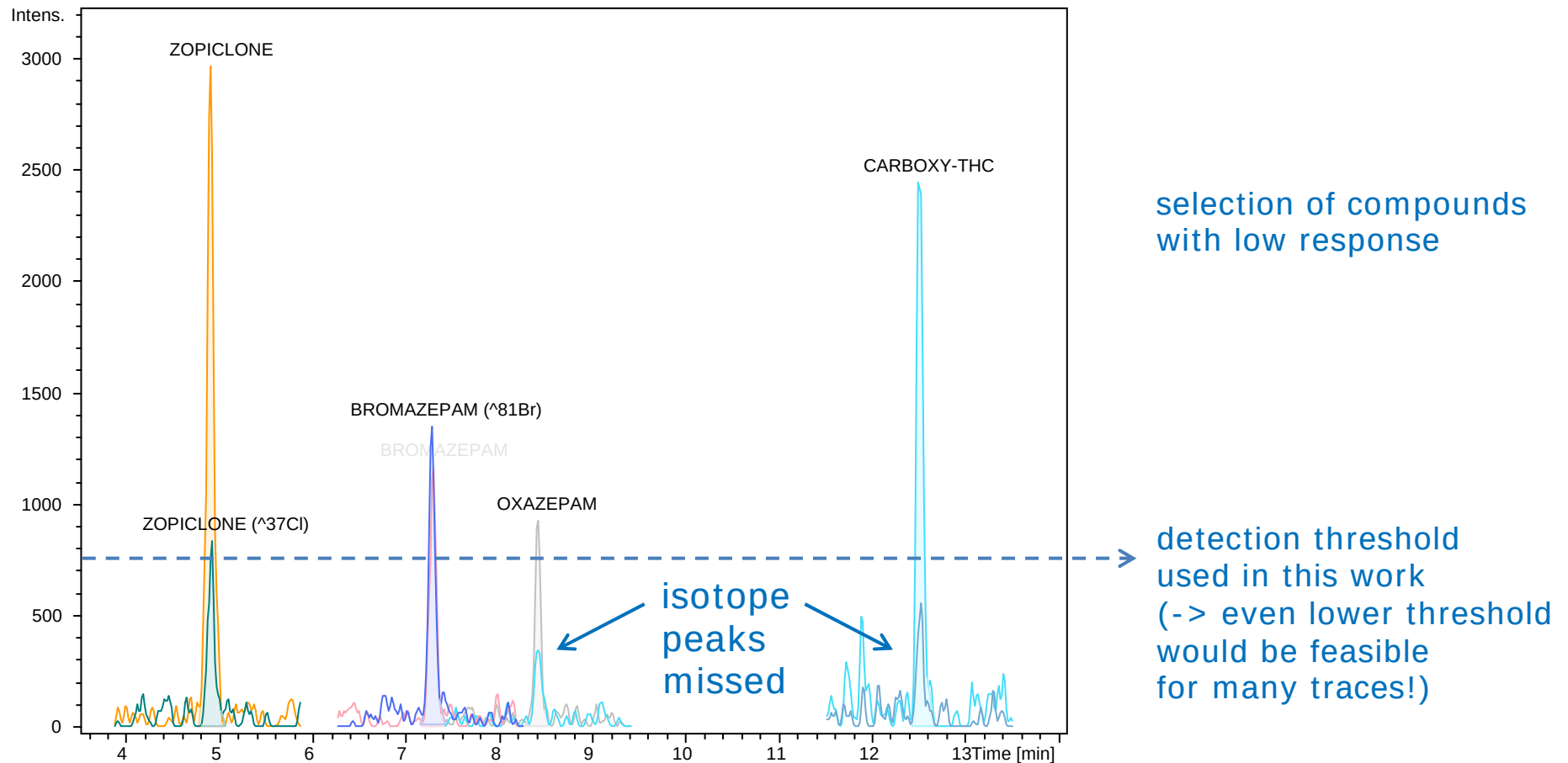
10 ng / ml spiked in urine:



Blank urine (bbCID data): j) Elimination of FP: MDMA:
¹³C traces useless in this case
 -> use requirement of detecting QI 2/3 instead

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Example compound traces, 10 ng / ml in urine

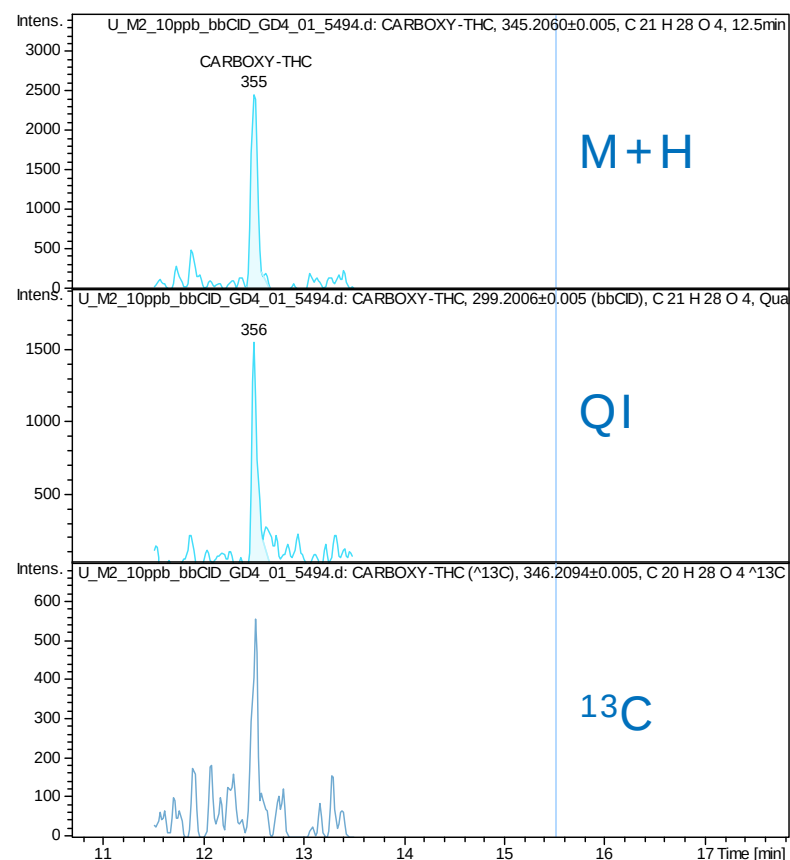
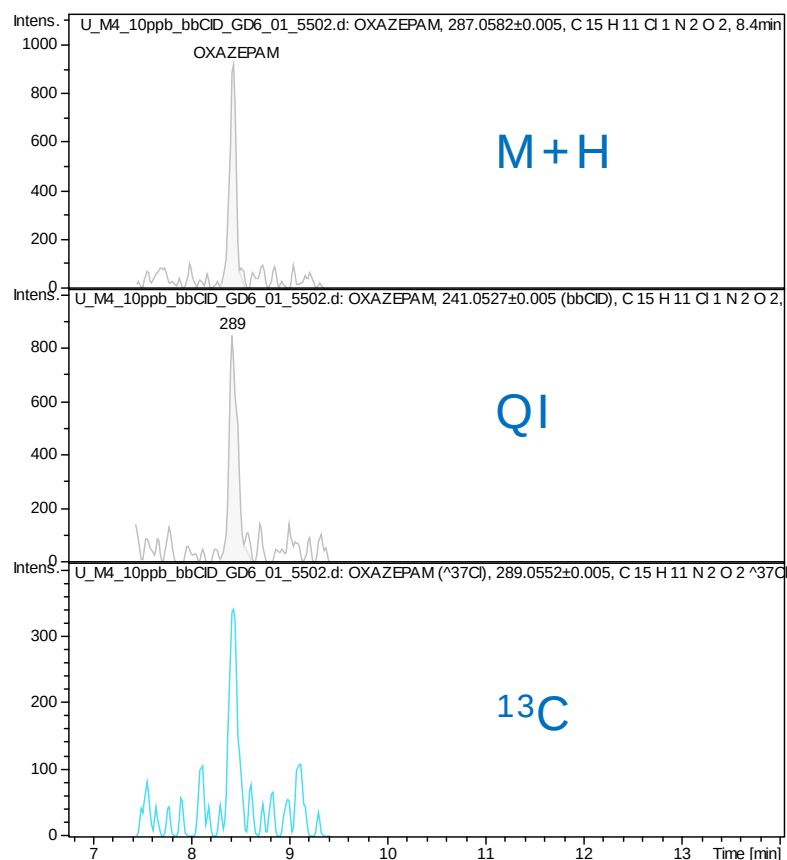


Do I really need to work with such a low threshold at all ?

Yes, if you need to detect **compounds with low response factors** at these low levels.

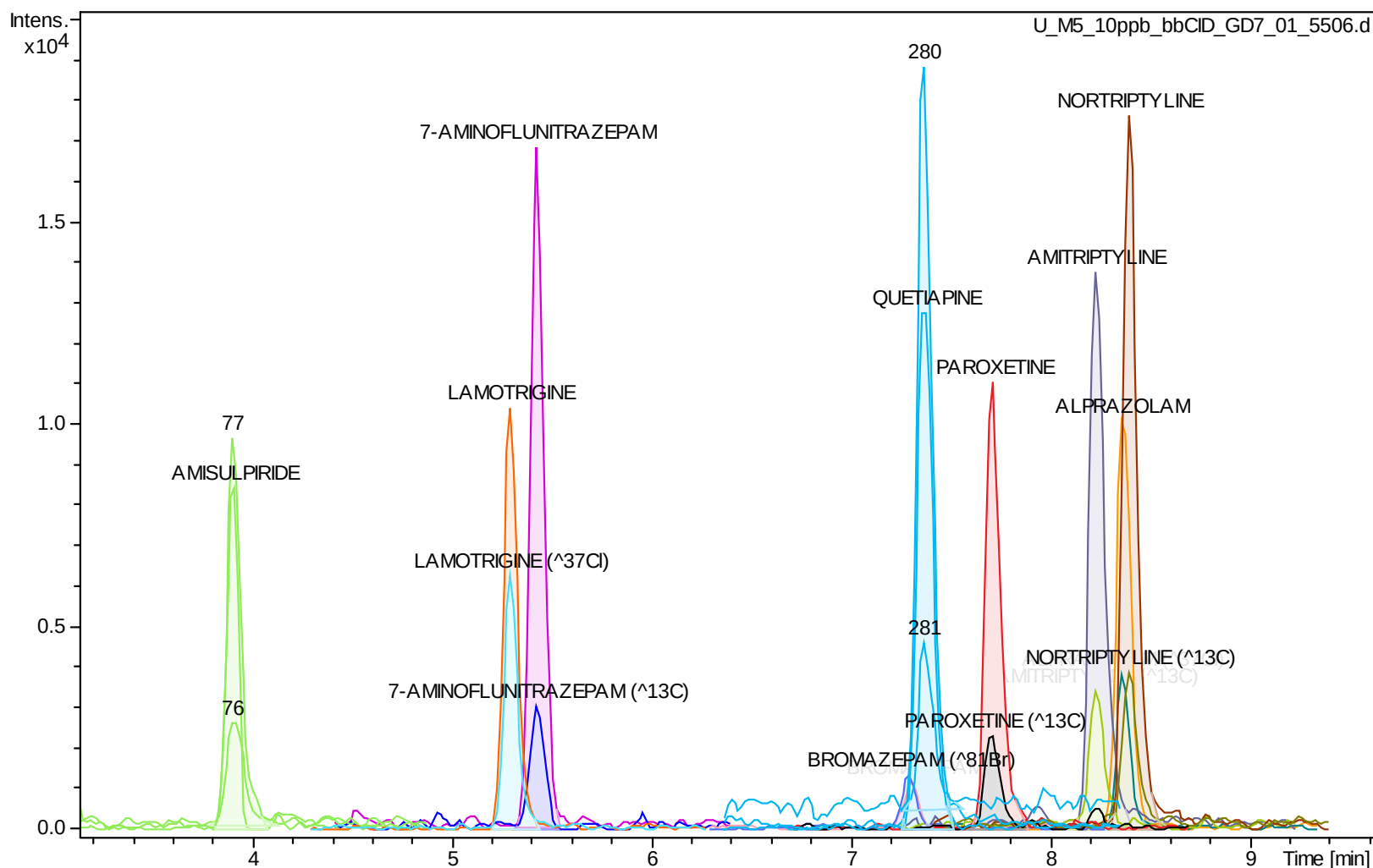
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Oxazepam, Carboxy-THC , 10 ng / ml in urine



Does the QI confirmation work for low level compounds?
Yes! Even for low-response compounds at low level QI detection can be possible and a good alternative to the ^{13}C trace.

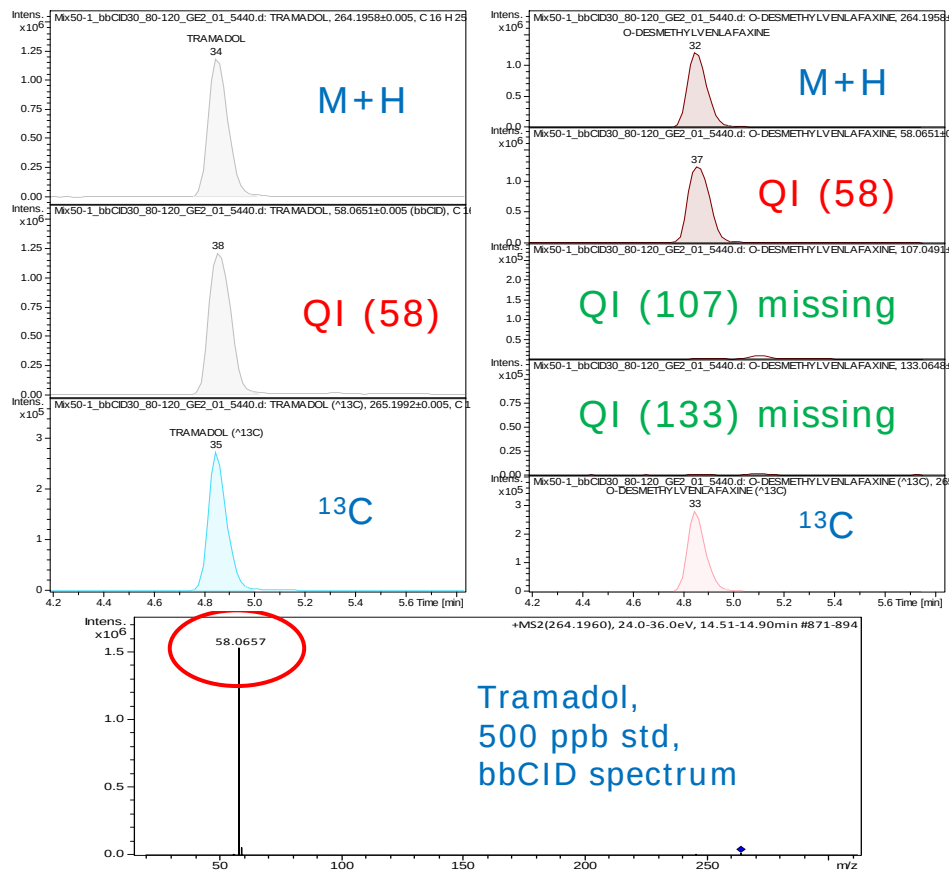
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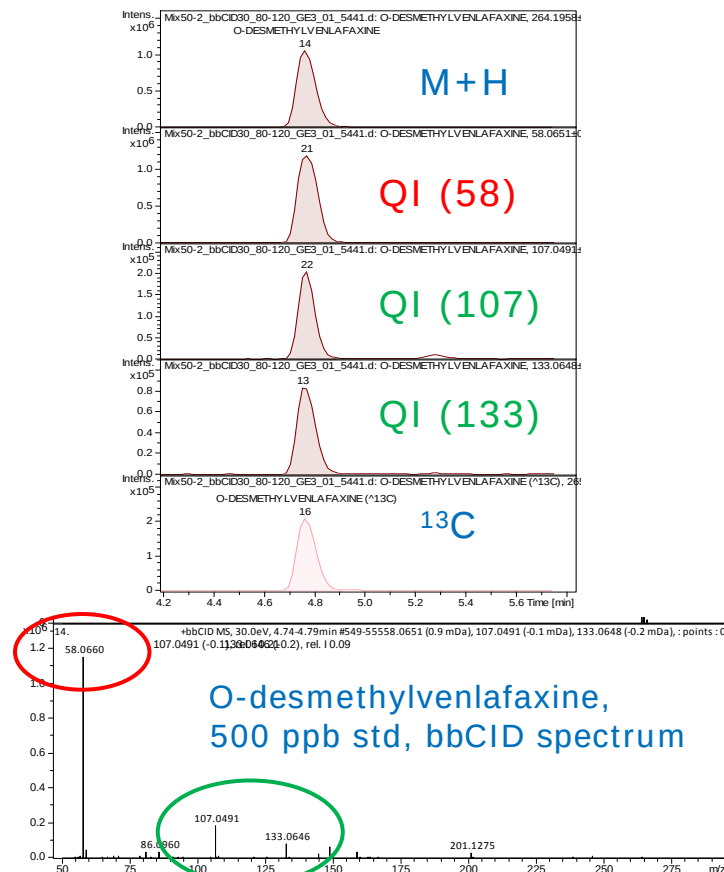
Example chromatogram, 10 ng/ml level (bbCID data): all nine spiked compounds detected and confirmed, no false positives. For most compounds much lower detection/confirmation levels will be achievable!

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Tramadol standard: O-desmethylvenlafaxine will show up as finding as well, but can be removed due to missing QI m/z 107 & 133.



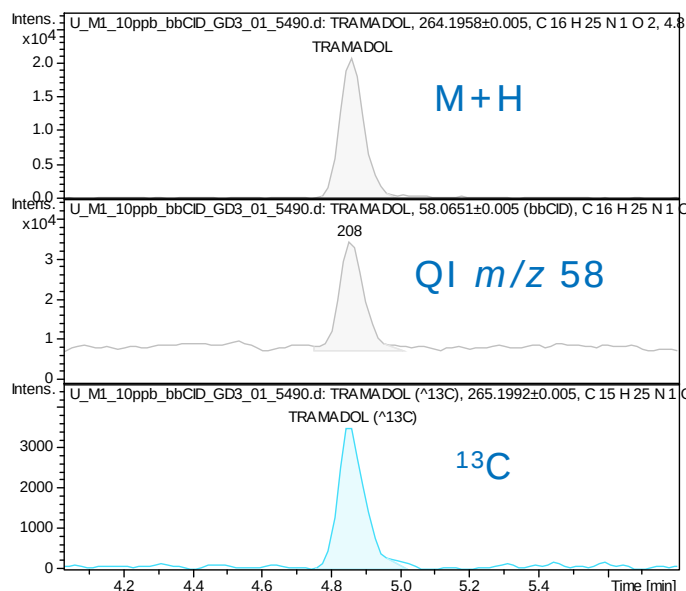
O-Desmethylvenlafaxine standard: tramadol will show up as finding as well, and can not be removed due to common QI m/z 58.



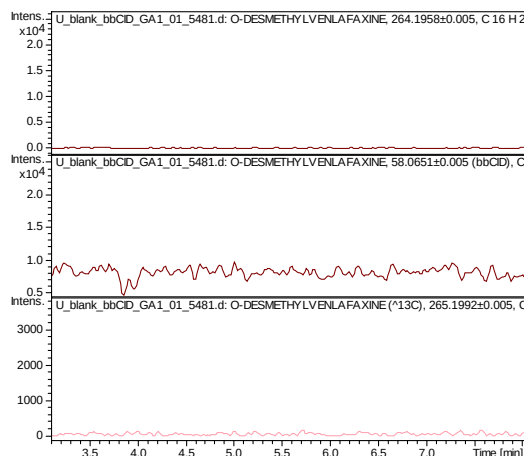
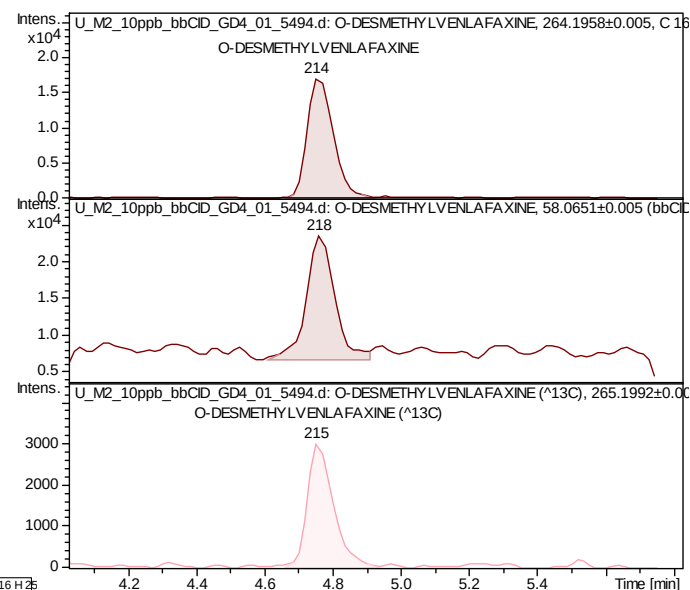
Tramadol: has same sum formula & RT as O-desmethylvenlafaxine: the only false positive finding that is unremovable in cases, when O-desmethylvenlafaxine is present due to the common QI m/z 58

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Tramadol:



note the high
noise level
on this QI trace!



same traces
in urine blank

Can an unspecific QI like m/z 58 (C_3H_8N) work at 10 ng/ml level in matrix? Obviously yes, even low level gives a clear peak.

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