The use of QTOF in forensic toxicology

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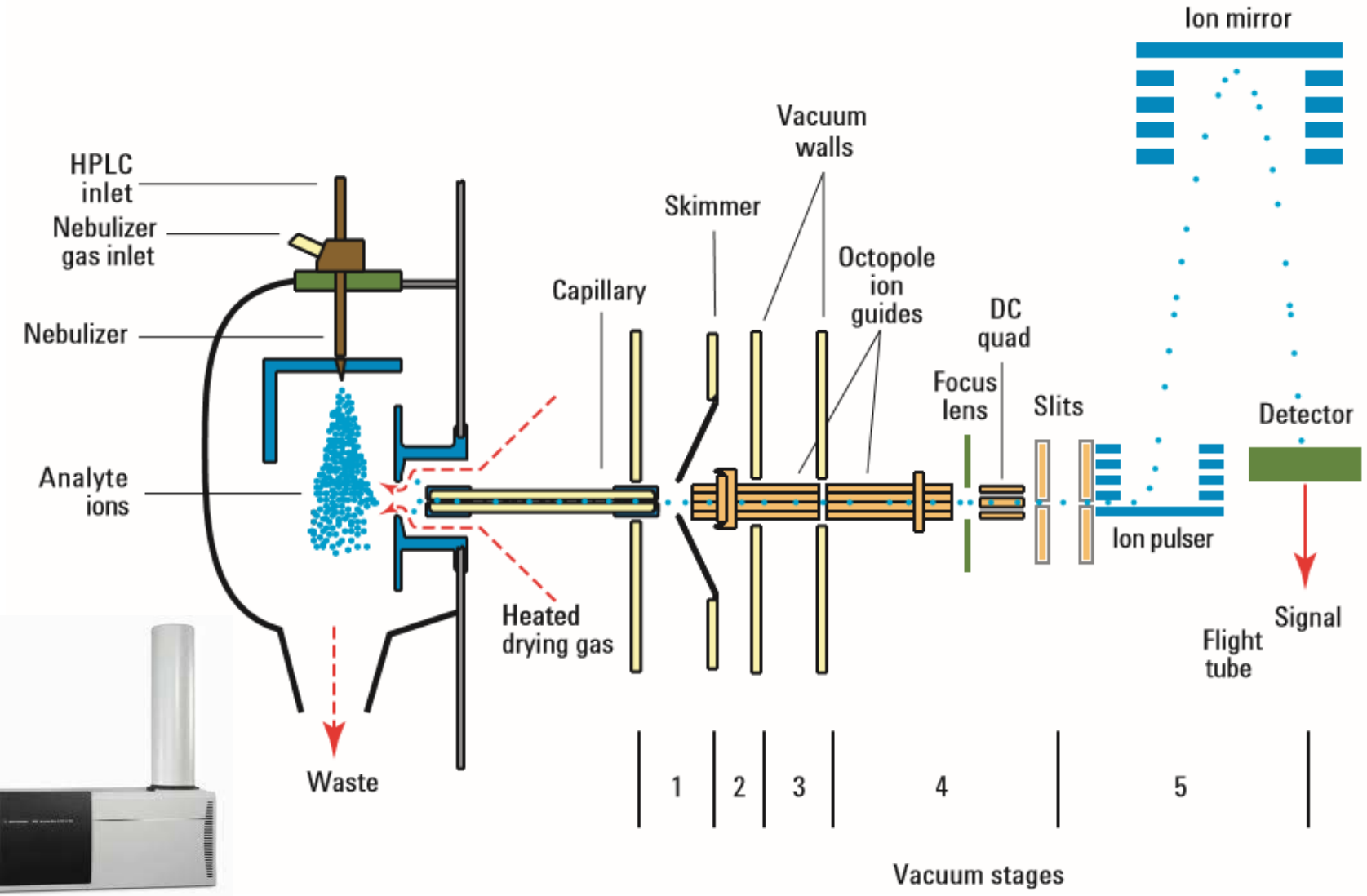
Use of QTOF LC-MS

Accurate Mass LC/MS has been around for a while but its use is now starting to increase rapidly:

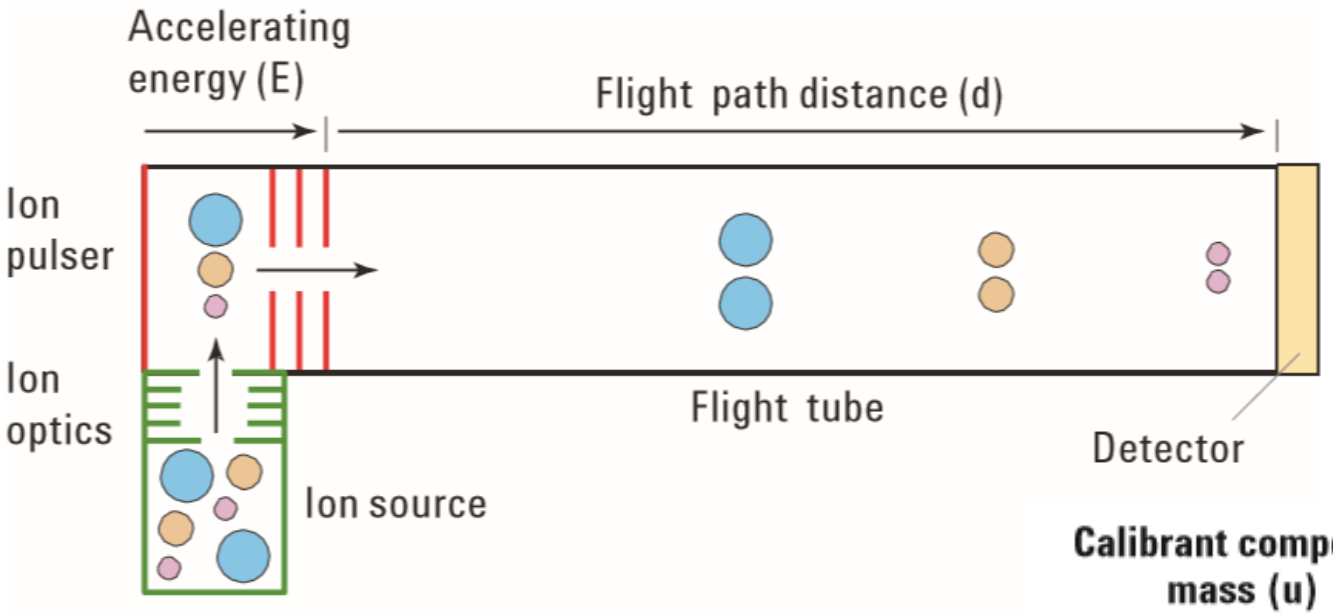
- Can be used for a wide range of applications e.g. multi-drug analysis, proteins, environmental monitoring, etc
- Has particular advantages for analysis of certain compounds
- Provides a complementary method of analysis to existing techniques
- LC-QTOF/MS with CID MS-MS can provide extremely selective and sensitive detection

e.g. Agilent QTOF-MS



Source: Agilent Technologies

e.g. Agilent QTOF-MS



Mass is determined by flight time following a calibration.

The longer the flight path, the *higher* the mass resolution.

Mass accuracy is monitored by introduction of reference masses within run

Calibrant compound mass (u)	Flight time (µsec)
118.0863	20.79841
322.0481	33.53829
622.029	46.12659
922.0098	55.88826
1521.971	71.45158
2121.933	84.14302
2721.895	95.13425

Source: Agilent Technologies

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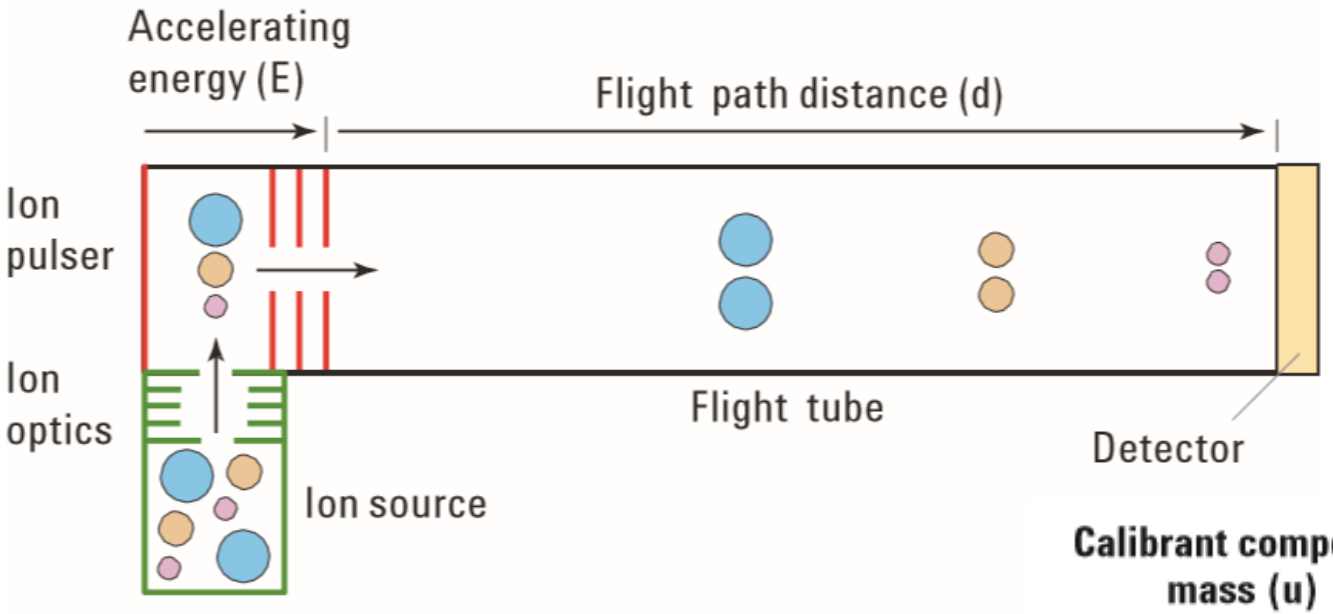

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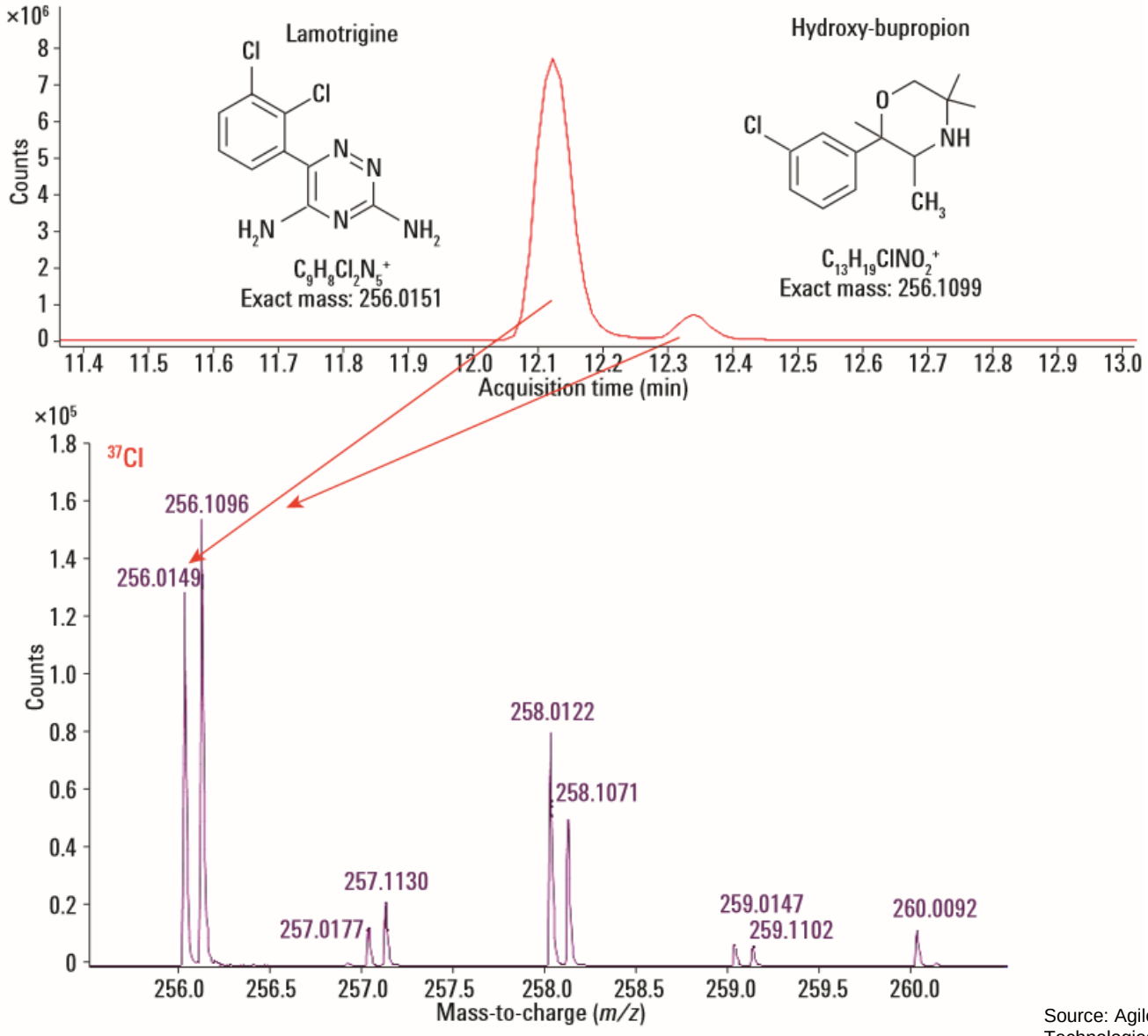
Source: Agilent Technologies

e.g. Agilent QTOF-MS

Low resolution (e.g. LC-MS) would be 256.0 versus 256.1

High resolution MS allows drugs with very similar mass to be distinguished



Source: Agilent Technologies

e.g. Agilent QTOF-MS

Mass accuracy [ppm]	Empirical formulae
100	138
50	67
25	32
10	15
5	7
2	2

Table 1
Mass accuracy vs. number of calculated empirical formulae for reserpine ($C_{33}H_{40}N_2O_9$ M=608.2734; within $C_{1\text{-}100}H_{2\text{-}200}N_{0\text{-}10}O_{0\text{-}10}$).

Fast acquisition rate needed to provided enough “accurate” points across a peak.

Reduced ppm reduces number of options – easier to determine identity

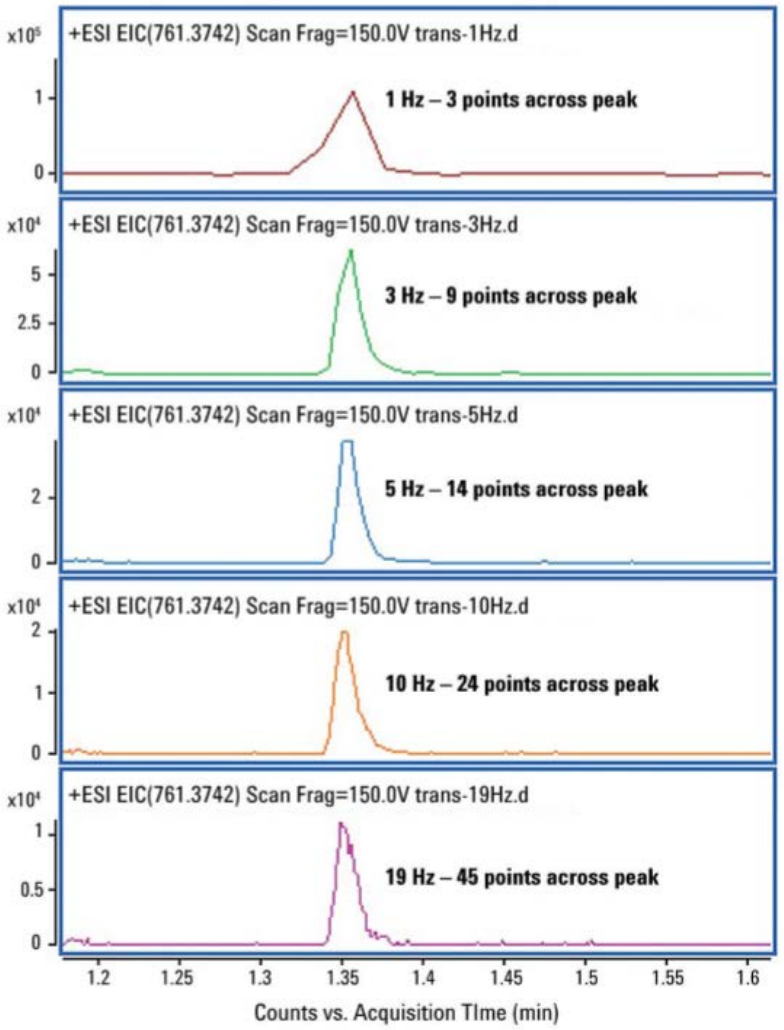
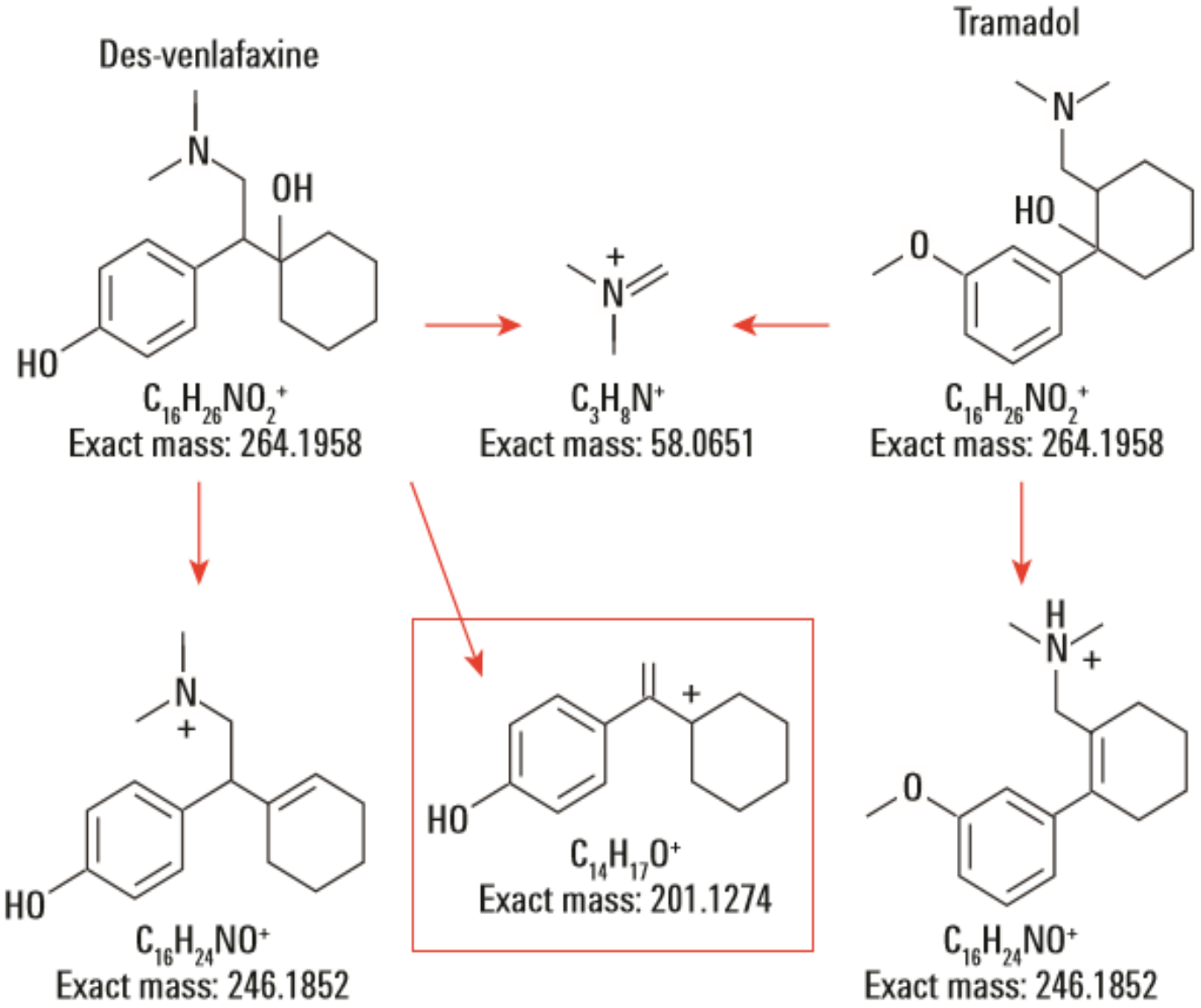


Figure 1. Faster acquisition rates provide better definition for this peptide peak from a UHPLC /MS run. The minimum number of data points that a chromatographer would accept is 10 to 12.

Source: Agilent Technologies

e.g. Agilent QTOF-MS

However, if compounds have same empirical formula, you need LC separation and/or MS/MS fragmentation



Source: Agilent Technologies

e.g. Agilent QTOF-MS

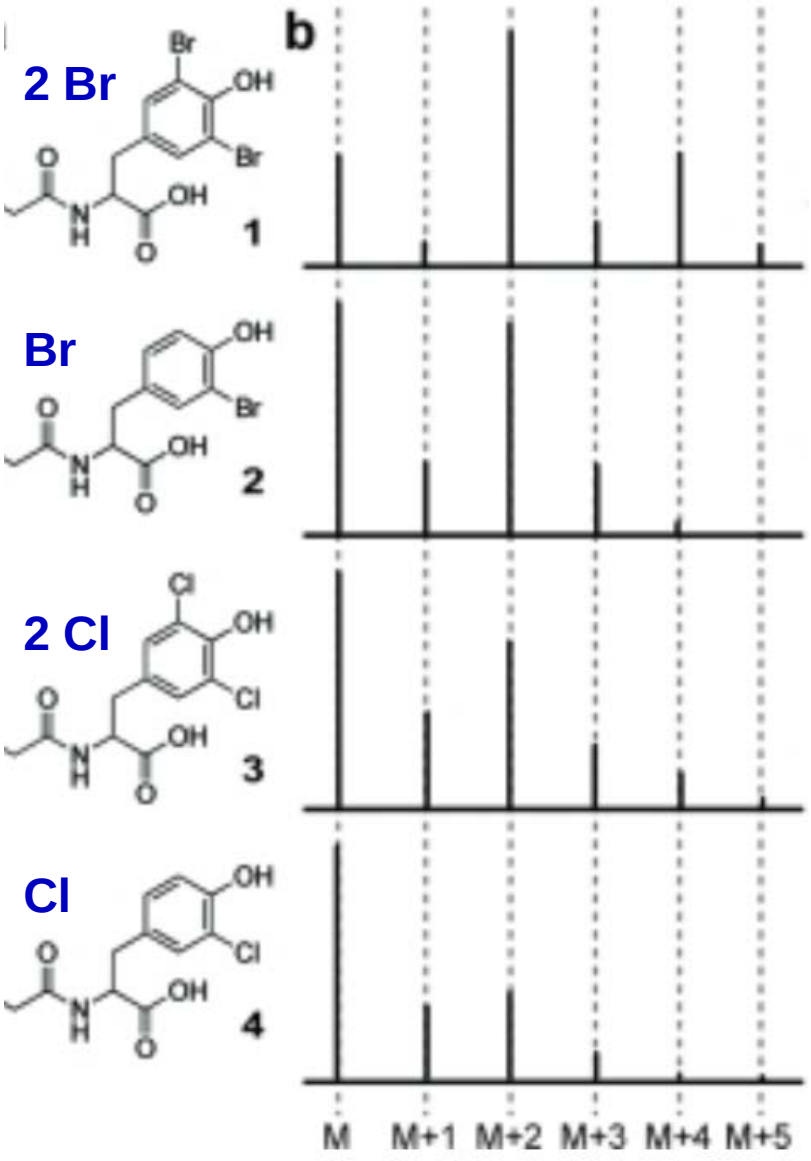
Isotopic ratios can be used for structural elucidation, especially with HRMS due to the mass resolution achieved.

In compounds that contain Bromine or Chlorine there is a distinctive pattern of ions.

If there is no Br or Cl, the pattern exhibits that for C;

High M, lower M+1 and lower even still M+2

As many NPS have Br or Cl, this has proven useful for those compounds (e.g. AH-7921, NBOMes, etc)



Source: Agilent Technologies

Use of QTOF LC-MS

Forensic toxicological applications of LC-QTOF/MS

e.g. Agilent 6540

Source: Agilent Technologies

- **General drug screening**
  - Unknown screening
  - Targeted screening
- **Drug measurement**
  - selective and sensitive

Can be applied to various **specimens**; blood, plasma/serum, urine, oral fluid, hair, post-mortem fluid, tablets/solutions.

Can be applied for a wide range of **requests**; medico-legal, post-mortem, road traffic and criminal toxicology.

Use of QTOF LC-MS

General drug screening

Advantages:

- Existing advantages of LC-MS (no derivatisation, sensitive, selective, etc). UHPLC provides rapid analysis. Can also couple to UV.
- Can detect a very wide range of analytes (depending on extraction used)
- Identification based on accurate mass molecular ion (searchable by library or online database)
- Accurate mass MS-MS spectral matching
- Software assists with structural elucidation
- Can return to historic data to revisit possible presence of compounds

Disadvantages:

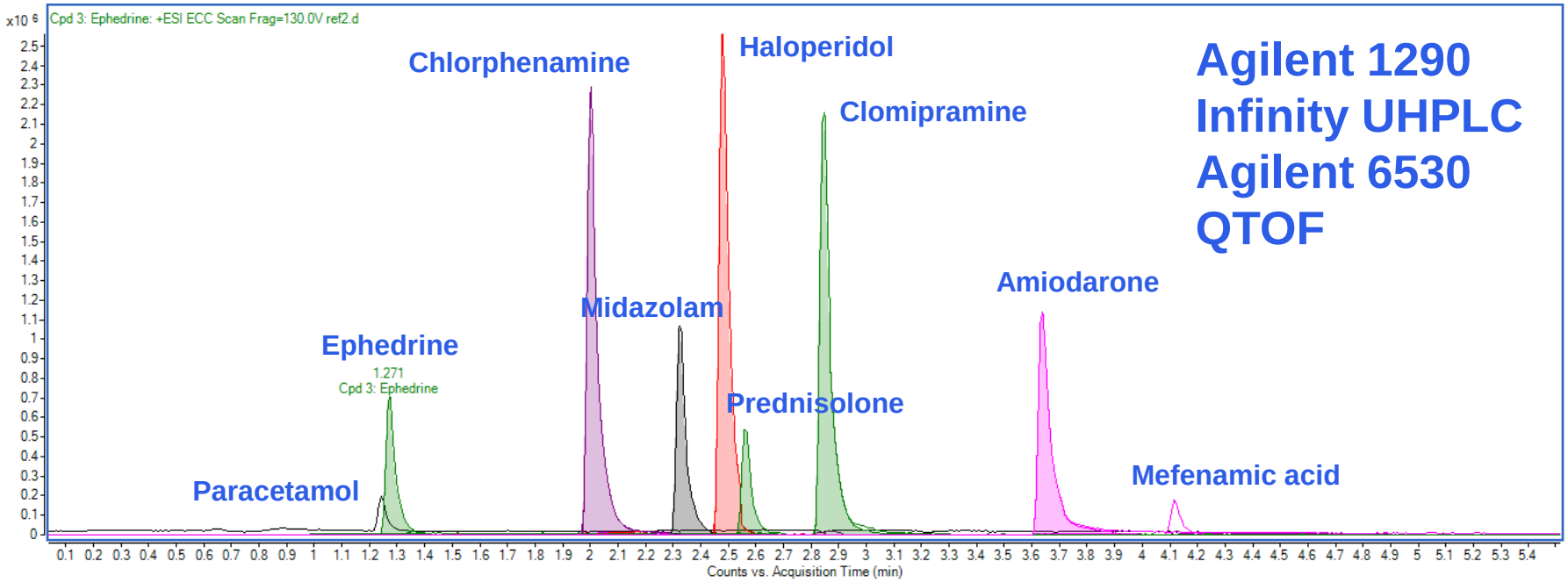
- More compounds are isobaric than you would think, even with accurate mass
- For absolute identification, still need a retention parameter and/or MS-MS data

Application of QTOF LC-MS

General screening

Agilent 2.1 mm x 100 mm Eclipse Plus C18 1.8 micron column

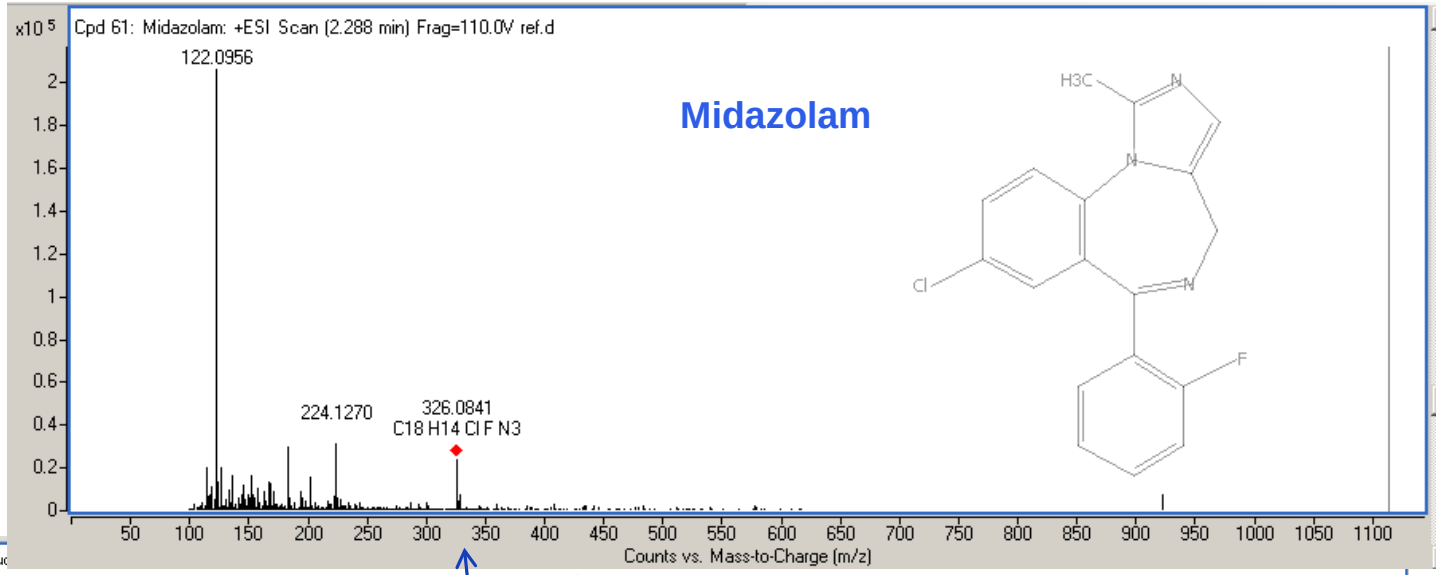
Acetonitrile and 0.1% formic acid mobile phase UHPLC gradient

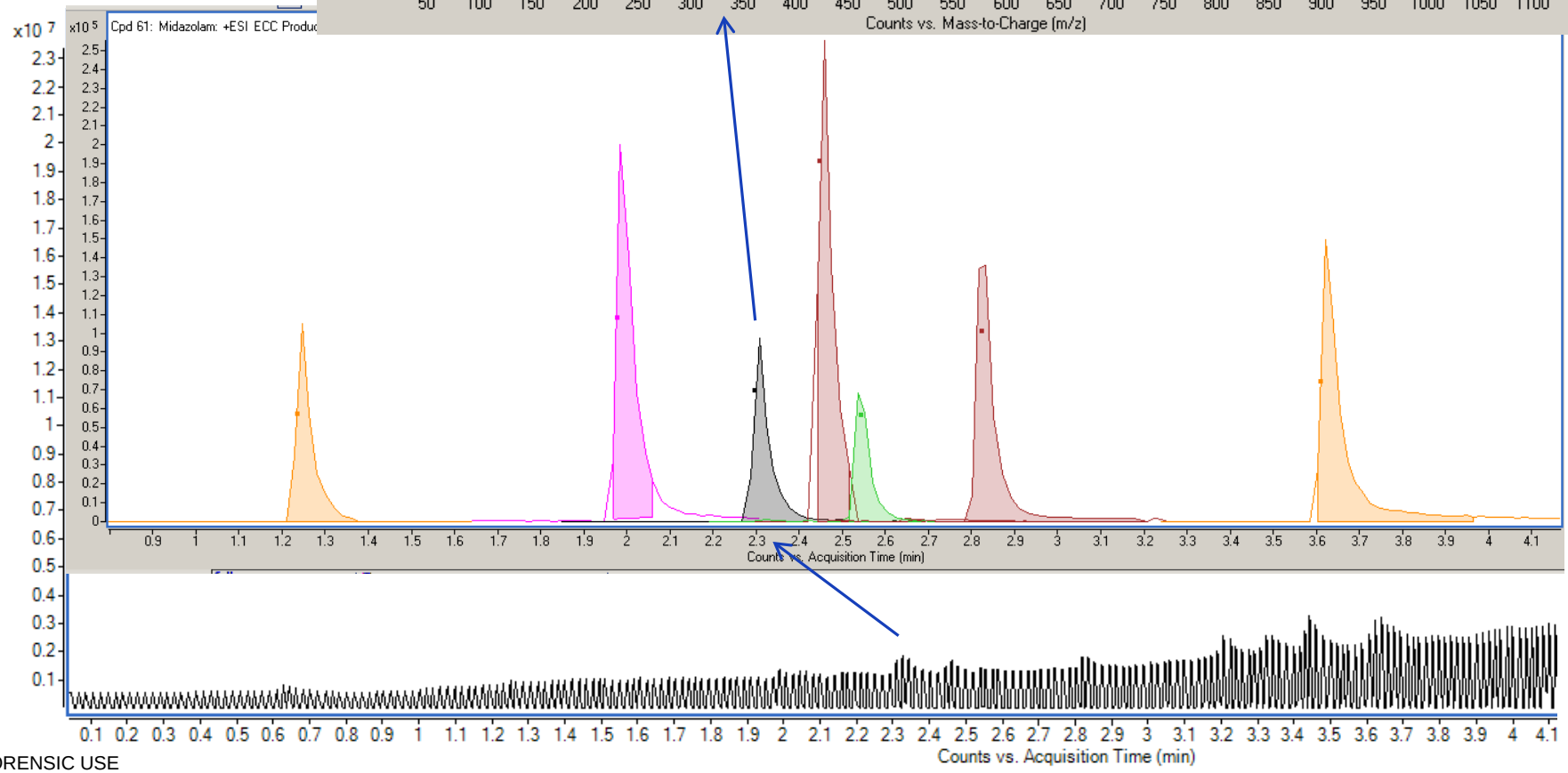


System allows detection by accurate mass and automated MS-MS.

From the TIC, various filters can be used to identify potential peaks of interest, followed by searching against a database/library.

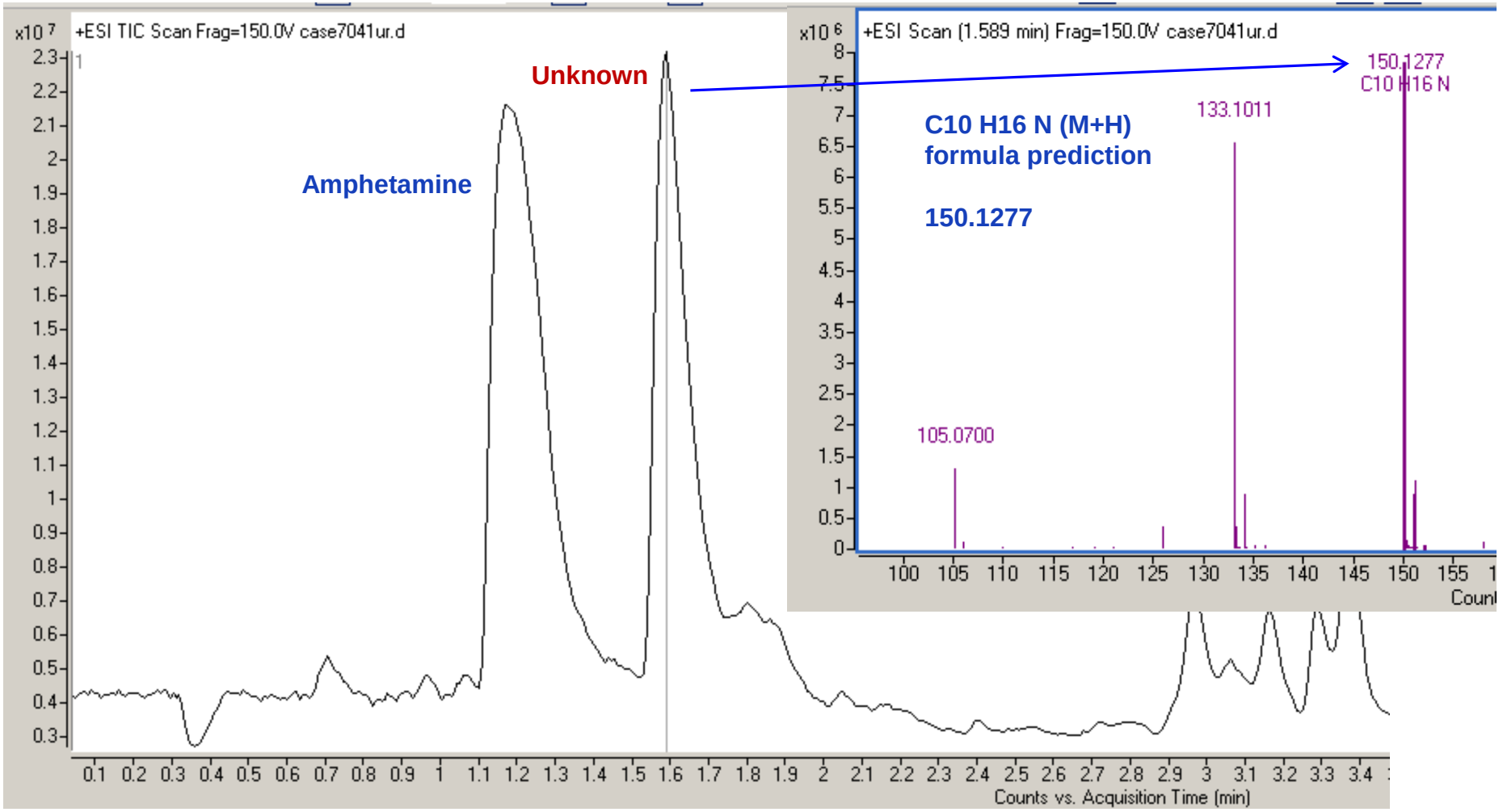
Auto MS-MS detection & identification





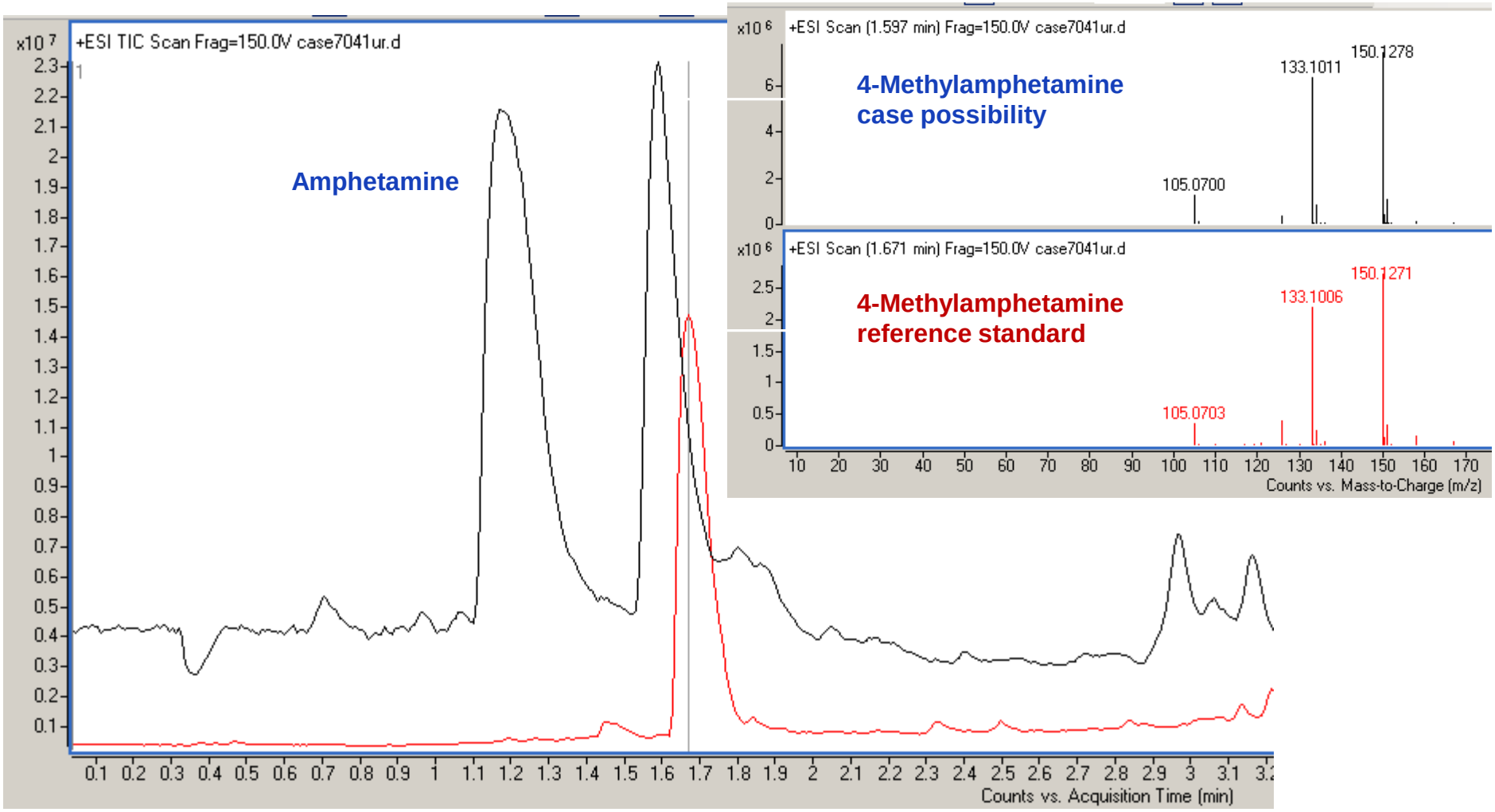
Case Examples

New Psychoactive Substances (NPS)



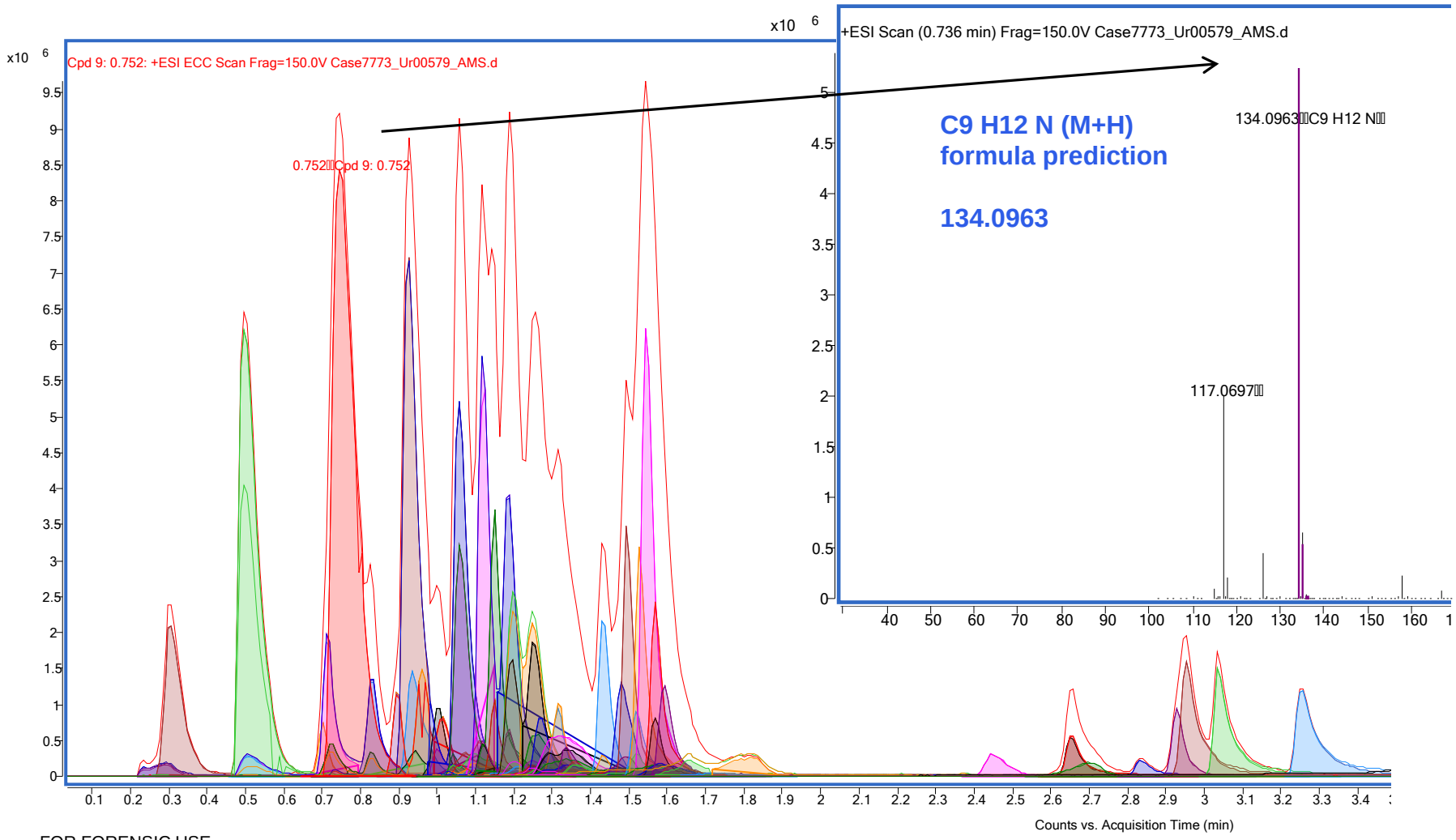
Case Examples

New Psychoactive Substances: 4-methylamphetamine (4-MA)



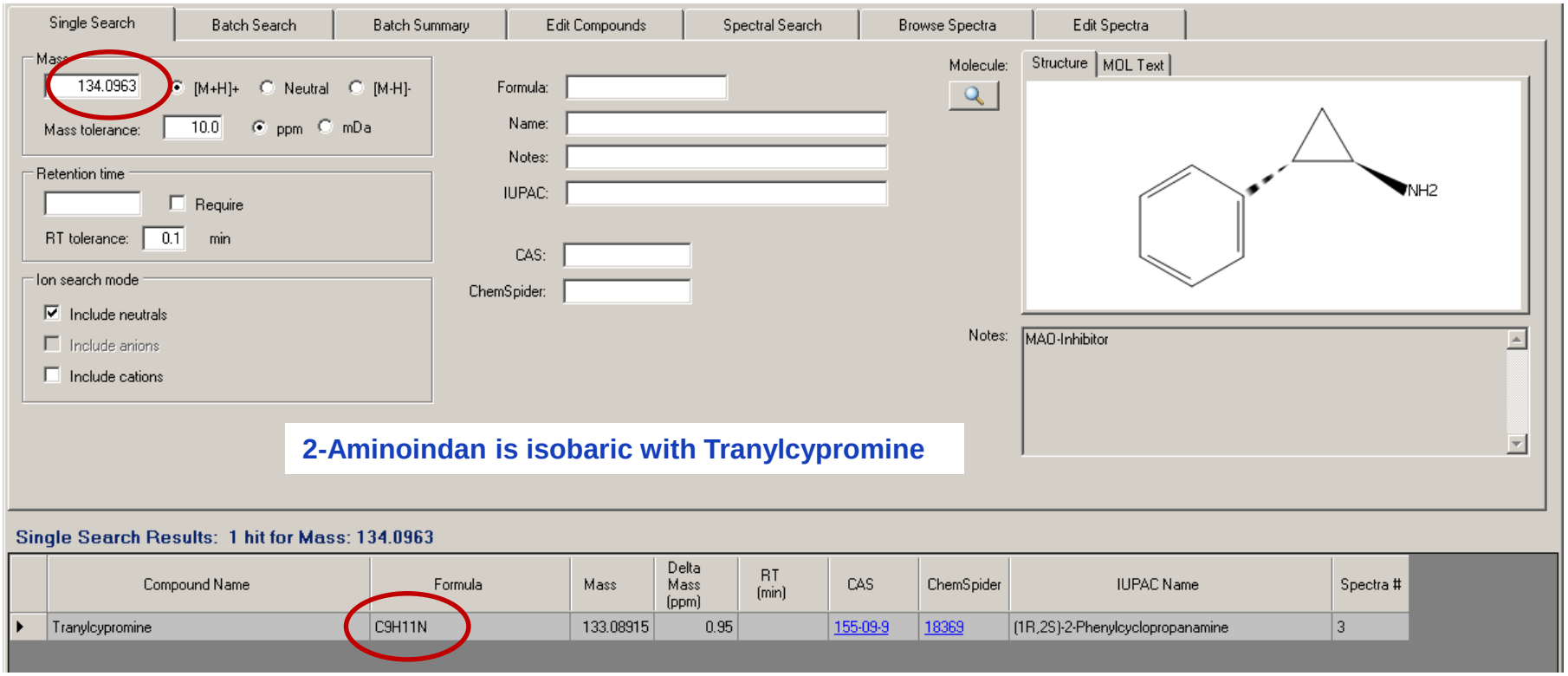
Case Examples

New Psychoactive Substances: 2-aminoindan



Case Examples

New Psychoactive Substances: 2-aminoindan



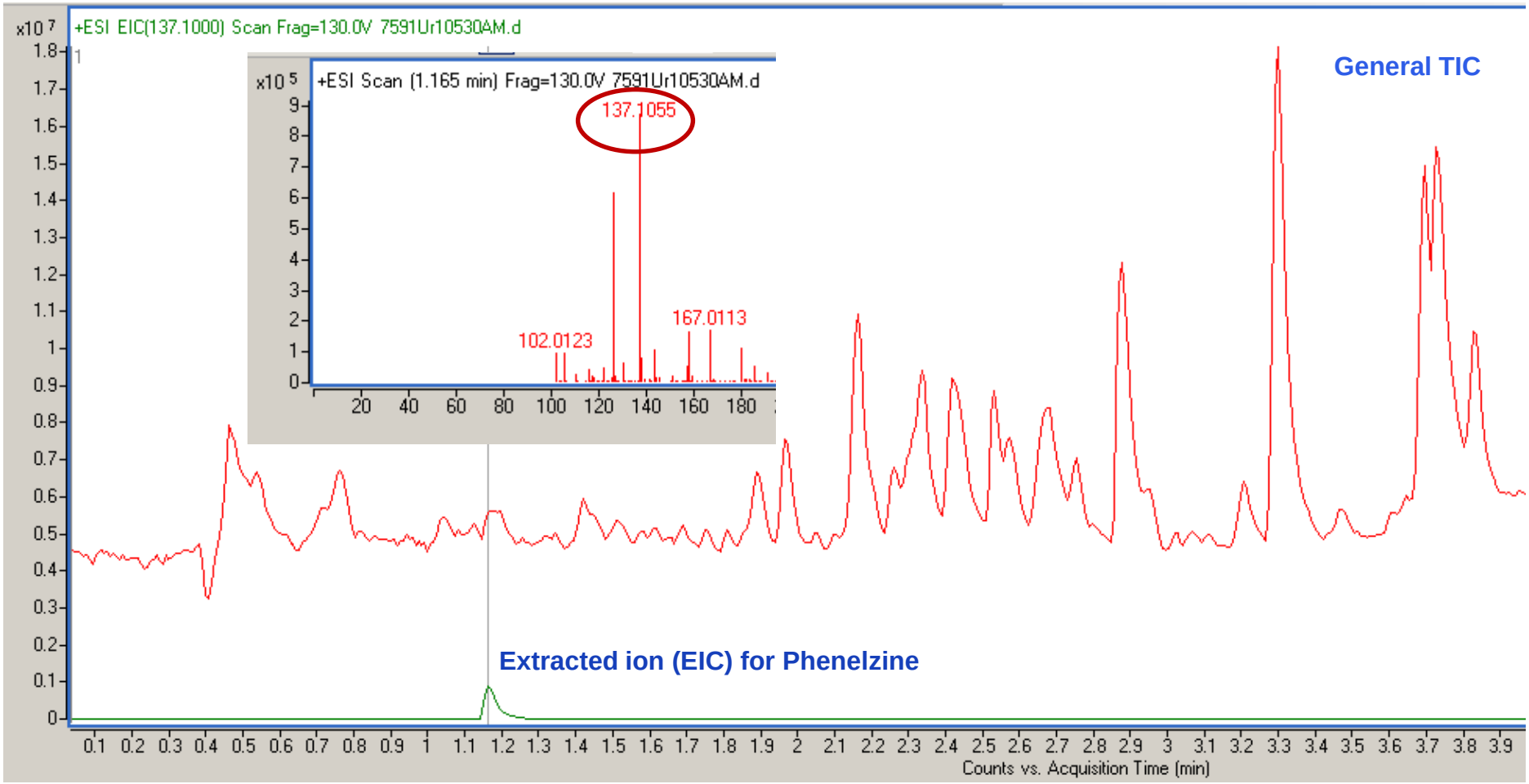
68787	NH_2 $C_9H_{11}N$	133.1903	54	81	26	7	

Source: www.chemspider.com

Case Examples

Re-examination of findings – phenelzine

(following new evidence of use)



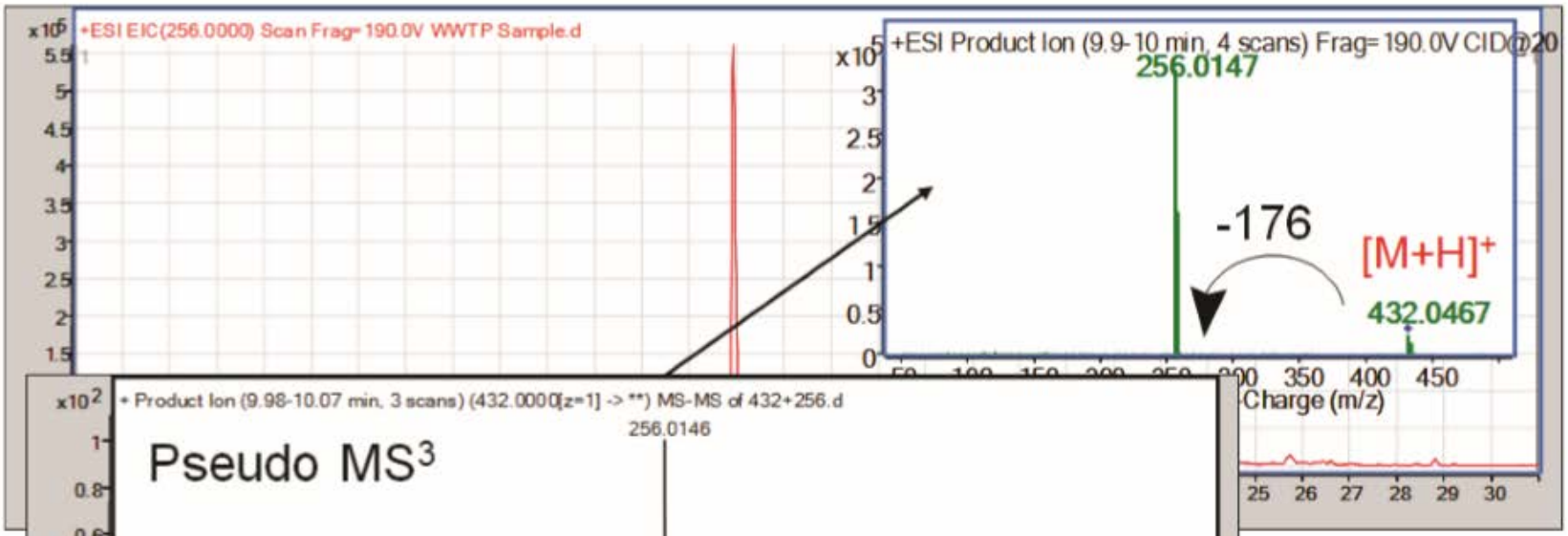
Application of QTOF LC-MS

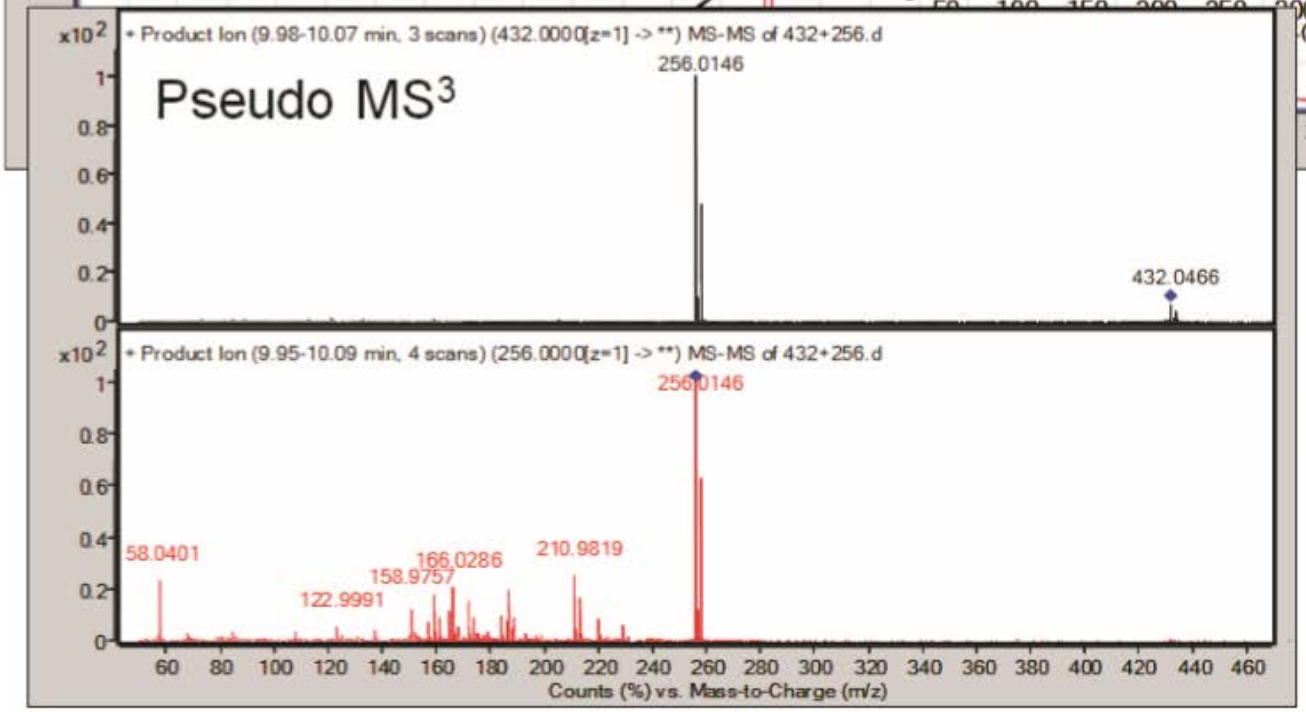
Analysis of drug glucuronides

- Glucuronic acid is a carboxylic acid which is heavily involved in the secondary (Phase II) metabolism of many drugs. Invariably occurs with drugs that have a “OH” or a “NH” group
- Generally results in a more polar compound which is easier to eliminate from the body. Reported to remain detectable longer than parent or Phase 1 metabolites in the urine
- Urine analysed by dilute-n-shoot and/or by solid phase extraction
- Accurate mass “neutral loss” data interrogation (m/z 176.03209) was used post run to determine any potential glucuronide compounds in the extract
- Glucuronide metabolites can be identified by database matching of the accurate mass of the parent compound (after loss of 176.03209) and then by the library match of the MS-MS fragments
- Detection provides useful forensic evidence (especially in cases where long time elapsed between incident and sampling e.g. DFSA cases)
- Typically glucuronides are hydrolysed for metabolite detection but this can remove important forensic timeline information (e.g. ratio of Phase I:II)

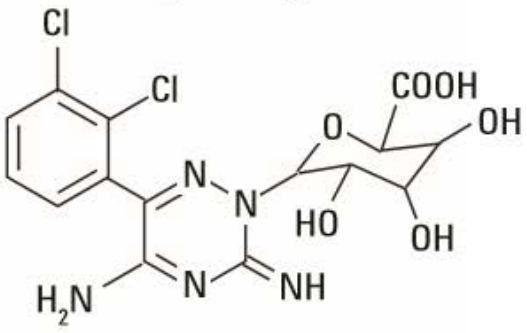
Case Examples

Glucuronide metabolites (e.g. prescription compliance)





Lamotrigine N2 glucuronide



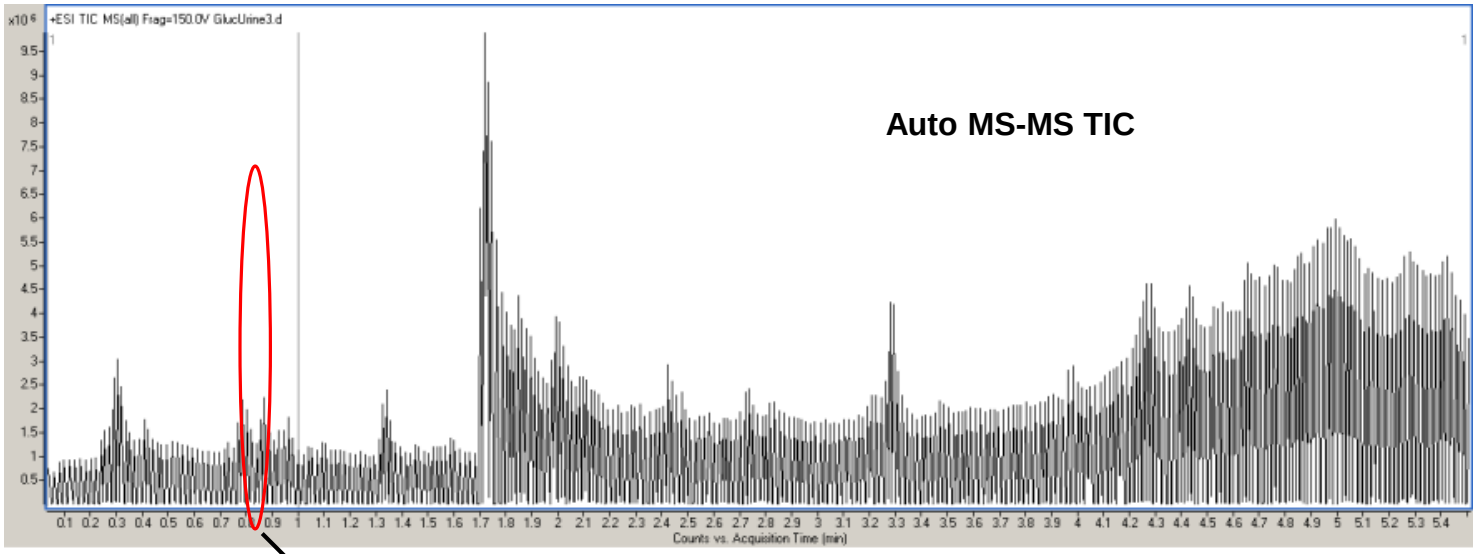
$C_{15}H_{16}Cl_2N_5O_6^+$

Exact mass: 432.0472

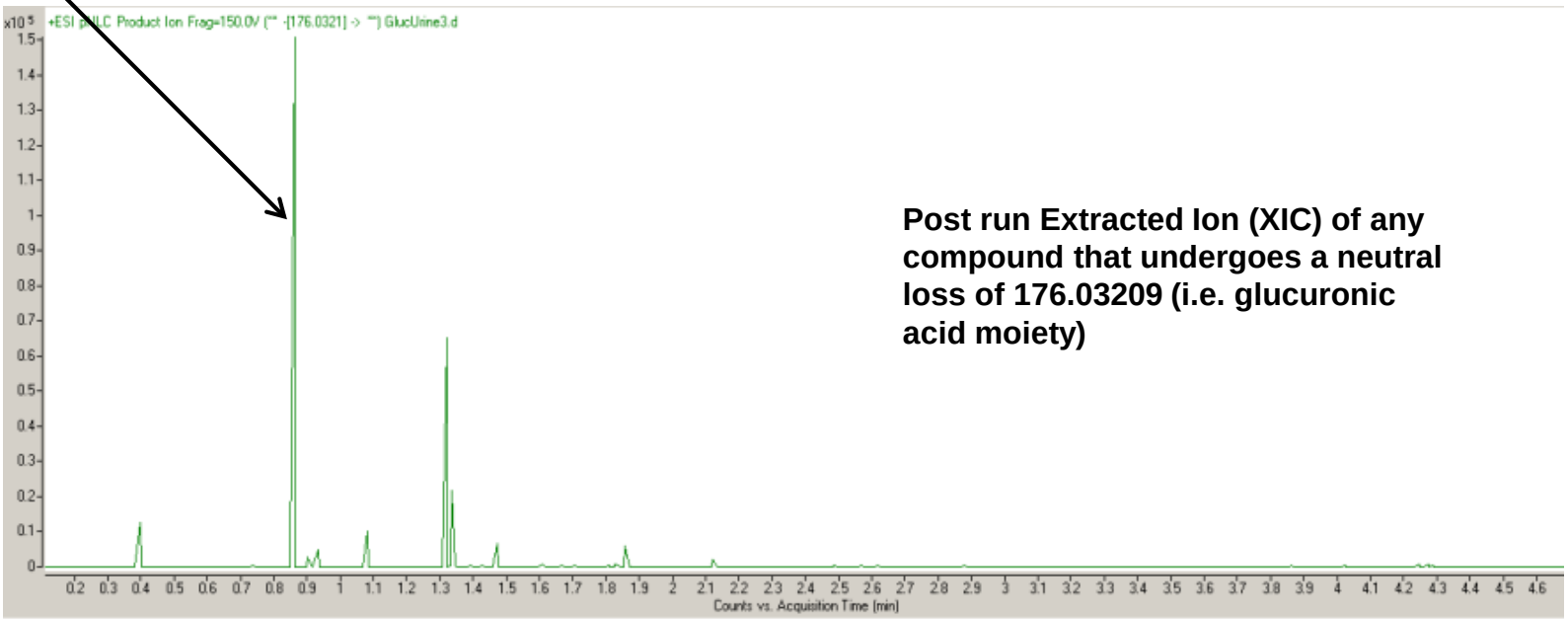
Source: Agilent Technologies

Case Examples

Glucuronide metabolites (e.g. metabolite identification)



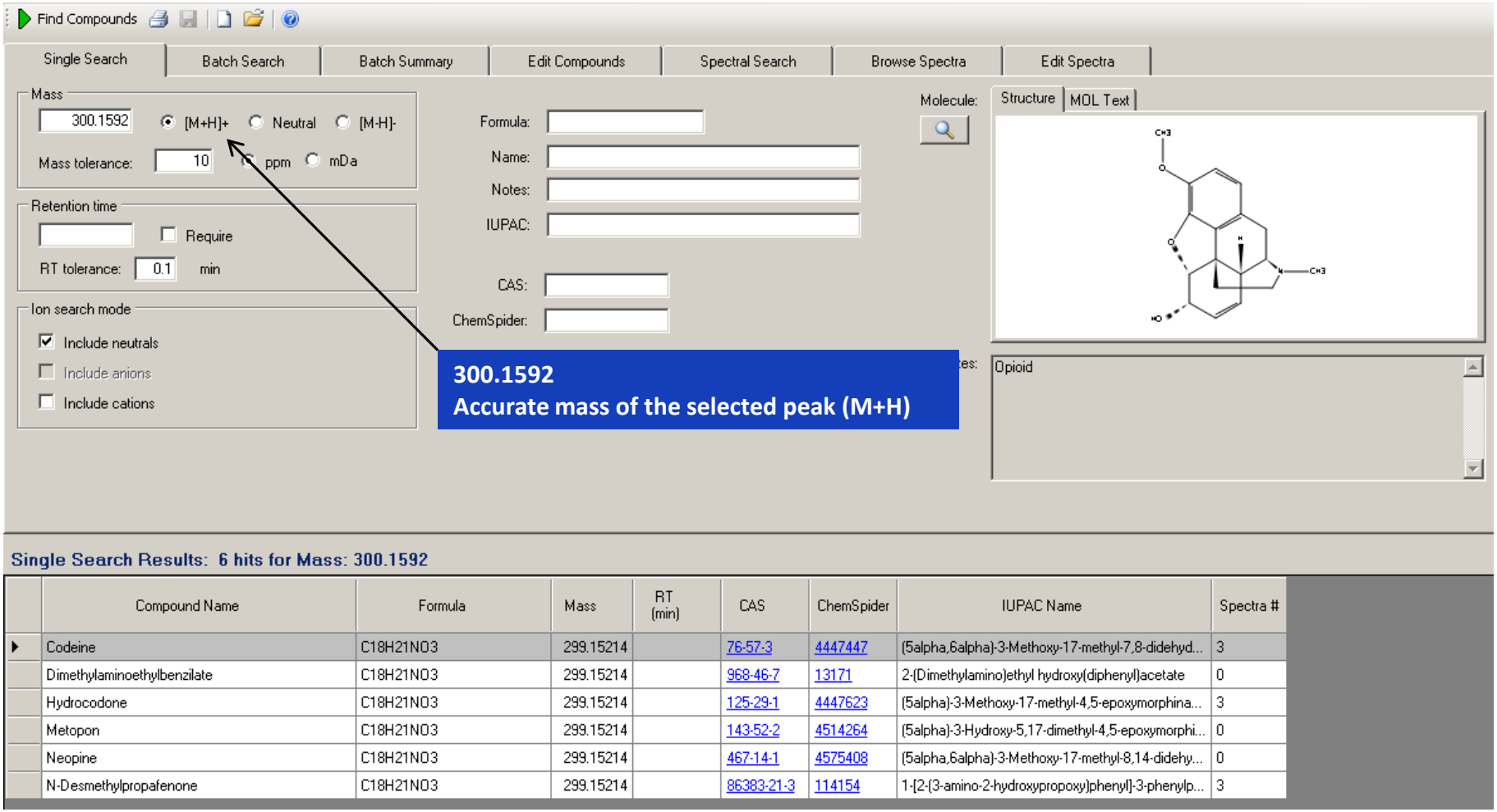
Auto MS-MS TIC



Post run Extracted Ion (XIC) of any compound that undergoes a neutral loss of 176.03209 (i.e. glucuronic acid moiety)

Case Examples

Glucuronide metabolites (e.g. metabolite identification)

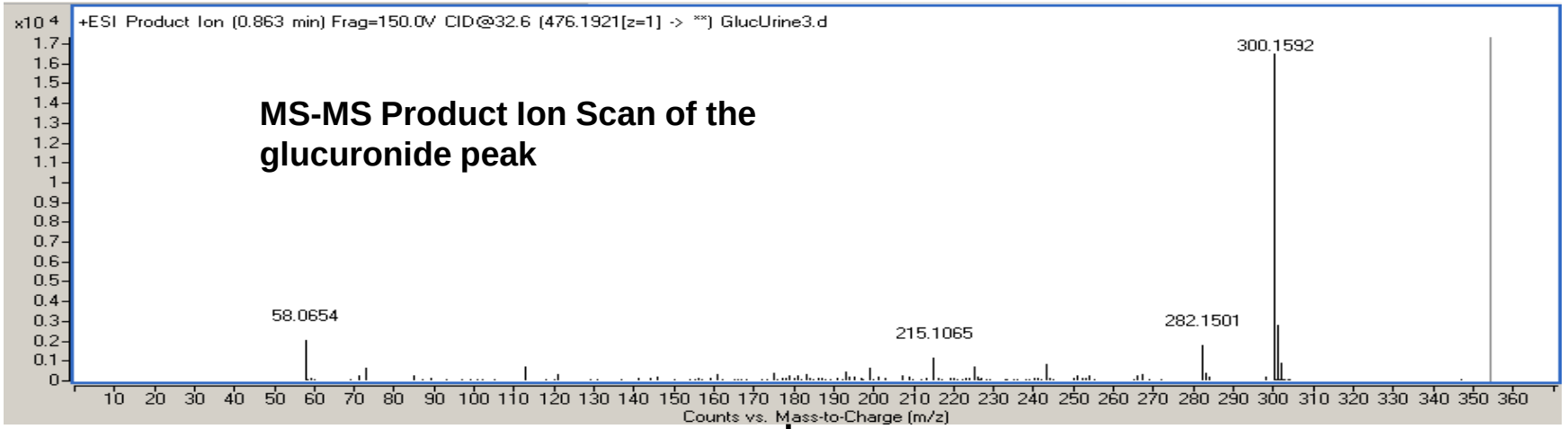


Compound Name	Formula	Mass	RT (min)	CAS	ChemSpider	IUPAC Name	Spectra #
Codeine	C18H21NO3	299.15214		76-57-3	4447447	(5alpha,6alpha)-3-Methoxy-17-methyl-7,8-didehyd...	3
Dimethylaminoethylbenzilate	C18H21NO3	299.15214		968-46-7	13171	2-(Dimethylamino)ethyl hydroxy(diphenyl)acetate	0
Hydrocodone	C18H21NO3	299.15214		125-29-1	4447623	(5alpha)-3-Methoxy-17-methyl-4,5-epoxymorphina...	3
Metopon	C18H21NO3	299.15214		143-52-2	4514264	(5alpha)-3-Hydroxy-5,17-dimethyl-4,5-epoxymorphi...	0
Neopine	C18H21NO3	299.15214		467-14-1	4575408	(5alpha,6alpha)-3-Methoxy-17-methyl-8,14-didehy...	0
N-Desmethylpropafenone	C18H21NO3	299.15214		86383-21-3	114154	1-[2-(3-amino-2-hydroxypropoxy)phenyl]-3-phenylp...	3

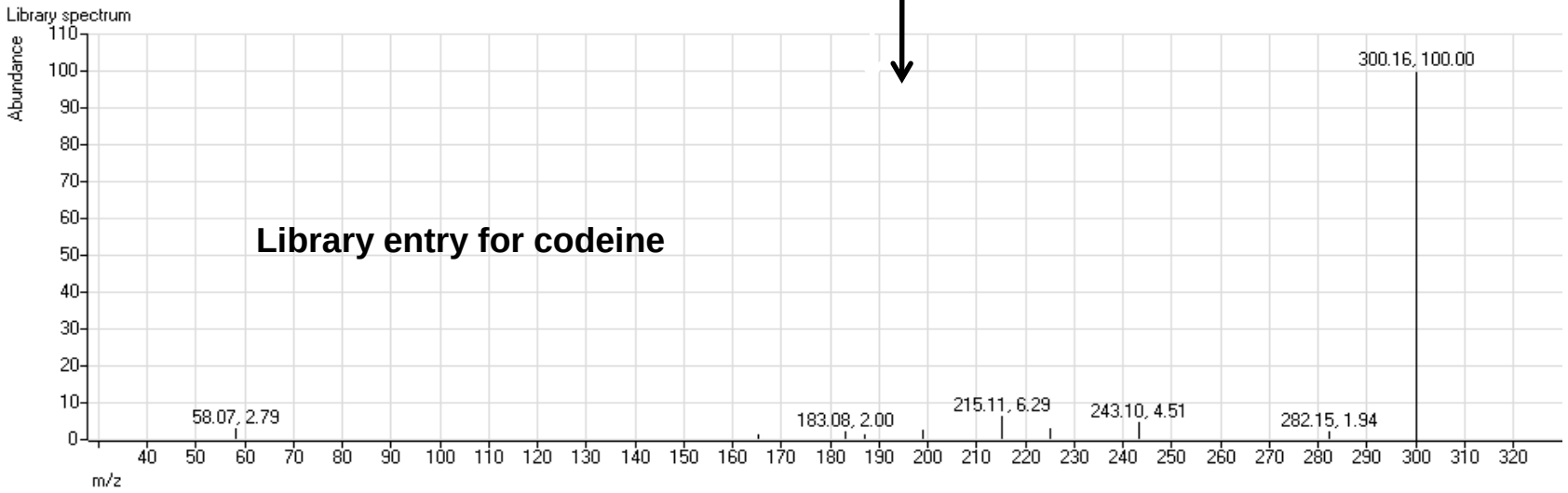
Accurate mass of the resulting product of the neutral loss of 176.1321 gives six potential isobaric matches for this peak HOWEVER.....

Case Examples

Glucuronide metabolites (e.g. metabolite identification)



MATCH!



Case Examples

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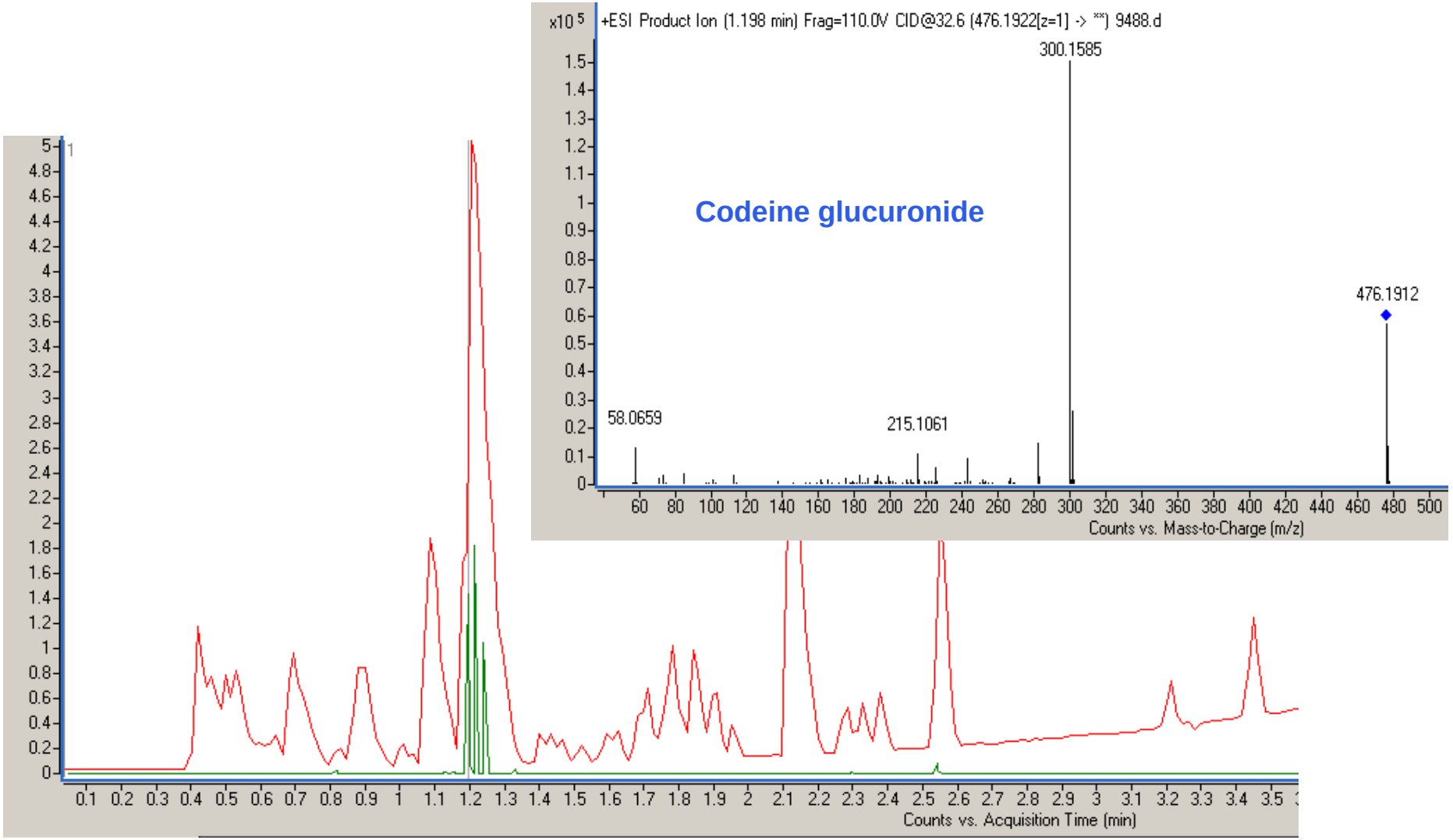
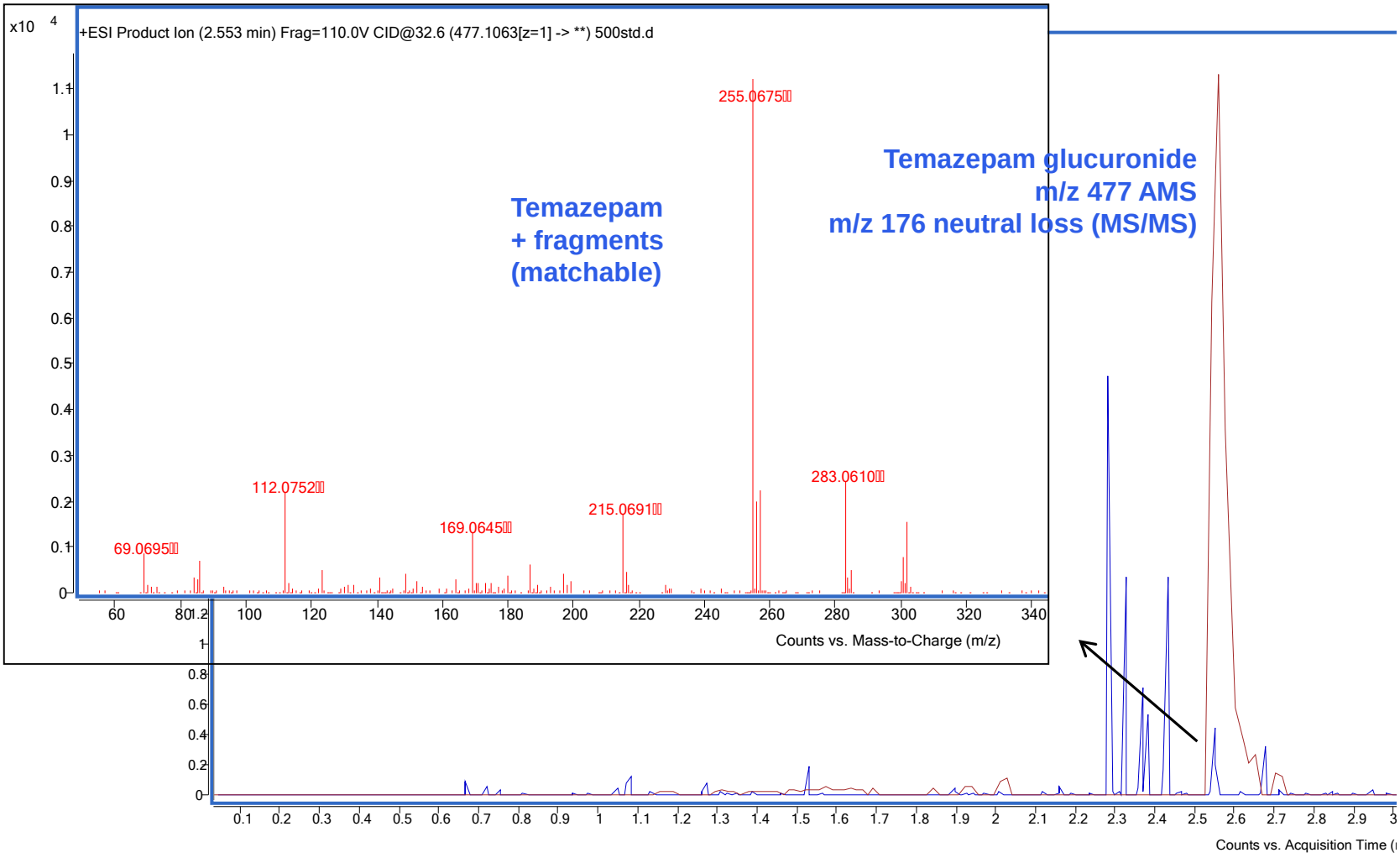


Case Examples

Glucuronide metabolites (e.g. extended window of detection)



Case Examples

Glucuronide metabolites detected in casework

PARENT/PHASE 1 METABOLITE ALSO FOUND IN CASE	GLUCURONIDE METABOLITE ONLY FOUND IN CASE	NOVEL GLUCURONIDES (nothing published)
Propranolol-glucuronide Mirtazapine-glucuronide Amitriptyline-glucuronide Hydroxynortriptyline-glucuronide Hydroxyamitriptyline-glucuronide Oxymorphone-glucuronide Norcodeine-glucuronide Lorazepam-glucuronide Citalopram-glucuronide Quinine-glucuronide Clozapine-glucuronide Dosulepin-glucuronide Venlafaxine-glucuronide ODV-glucuronide	Paracetamol-glucuronide Dihydrocodeine-glucuronide Dihydromorphine-glucuronide Lamotrigine-glucuronide Morphine-3-glucuronide Morphine-6-glucuronide Oxazepam glucuronide Temazepam glucuronide Desmethylpapaverine glucuronide	Omeprazole-glucuronide Hydroxymethadone-glucuronide Desmethylnoscapine-glucuronide α-Hydroxyalprazolam-glucuronide Hydroxydesmethylmirtazapine-glucuronide

Source: Kerry Taylor, ROAR Forensics (now Alere Forensics, part of Abbott)

Application of QTOF LC-MS

Synthetic cannabinoids and metabolites

Challenges:

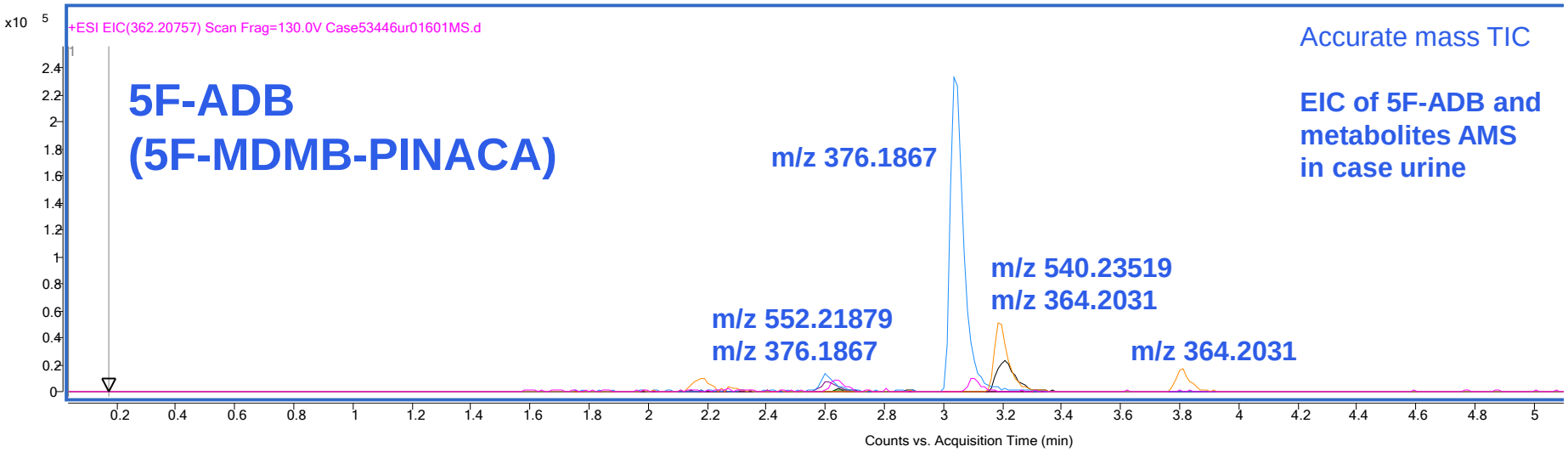
- Very low concentrations of parent drugs and metabolites
- Parent drugs predominantly in blood, metabolites in urine
- Reference material not available for all drugs or metabolites

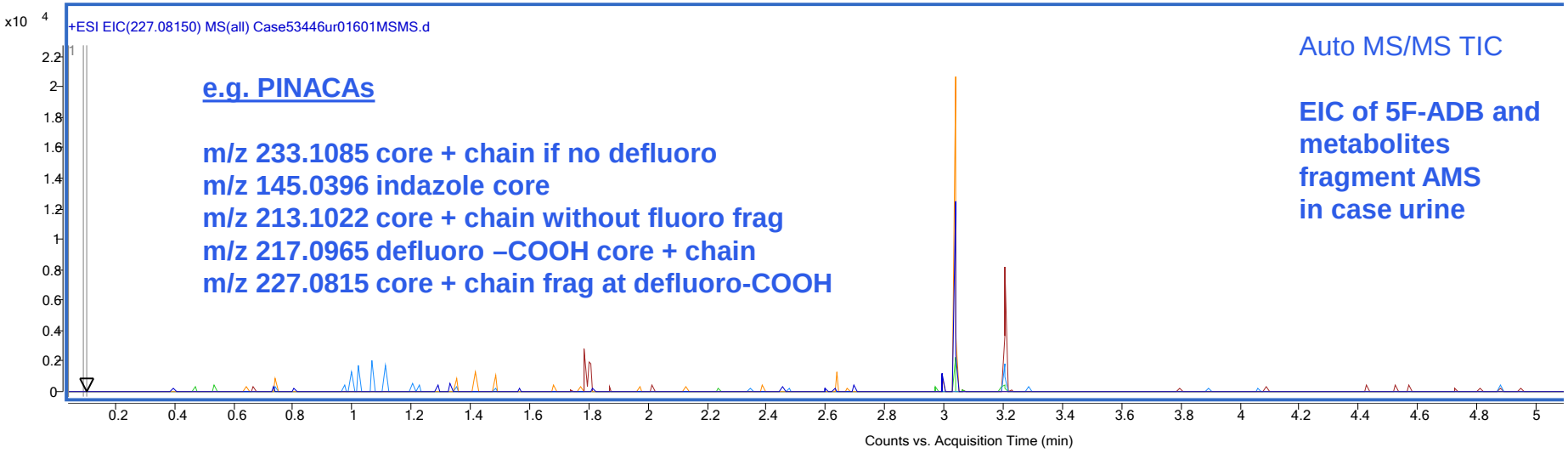
What can you do?:

- Use targeted QTOF-MS/MS for accurate mass of parent drug or metabolites
- Extract accurate mass ions in accurate mass TIC of parent drug or metabolites
- Perform Auto MS/MS and extract ions in MS/MS TIC of common fragments for common classes of synthetic cannabinoids (e.g. PINACA, PICA, CHMINACA, FUBINACA, CUMYL, etc)
- Overlay EICs and look for responses in both chromatograms
- Check with literature and/or reference material (especially for RT)

Case Examples

New Psychoactive Substances: synthetic cannabinoids





Case Examples

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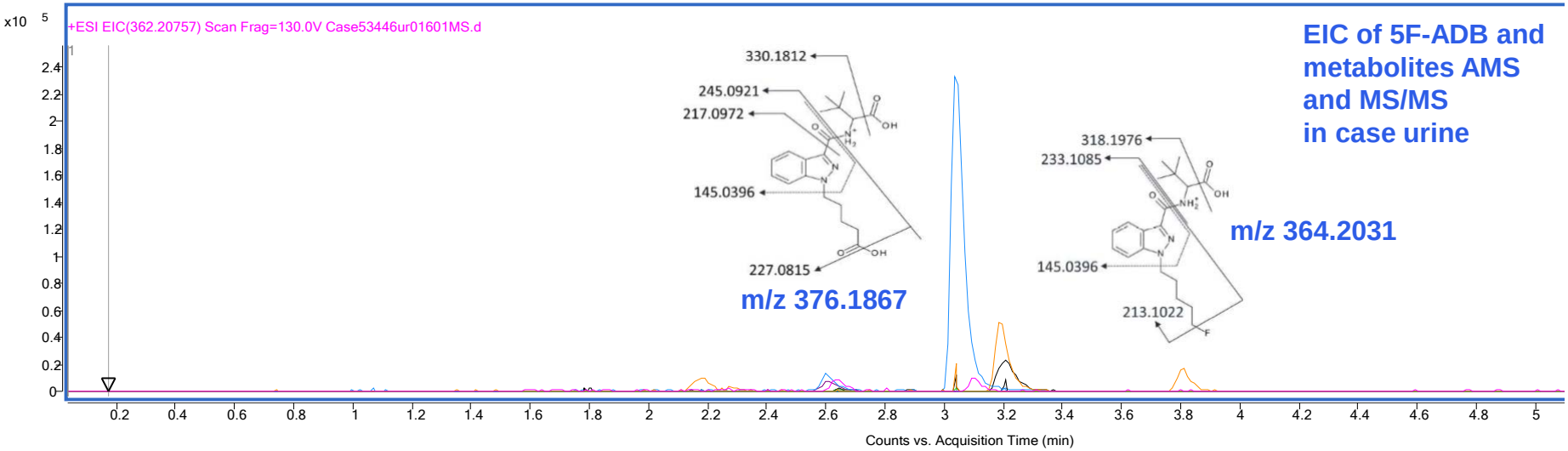
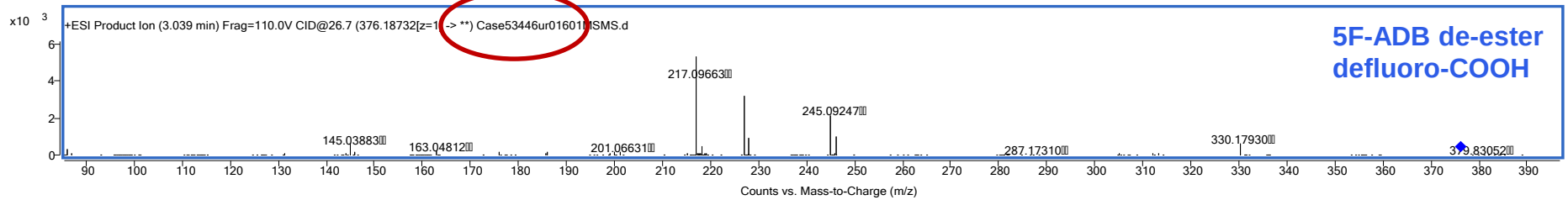
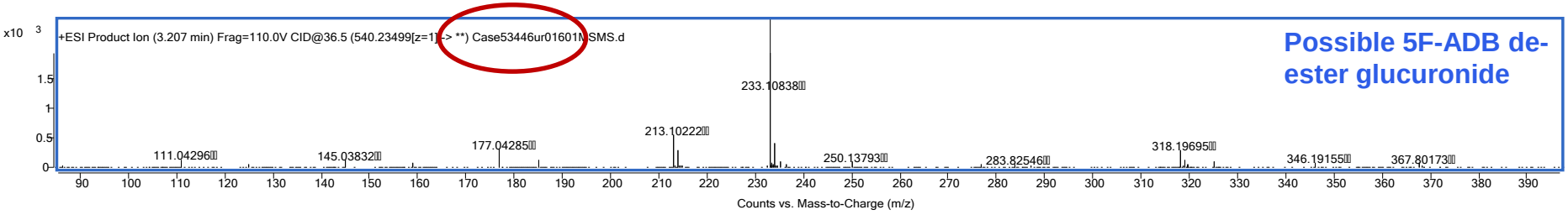
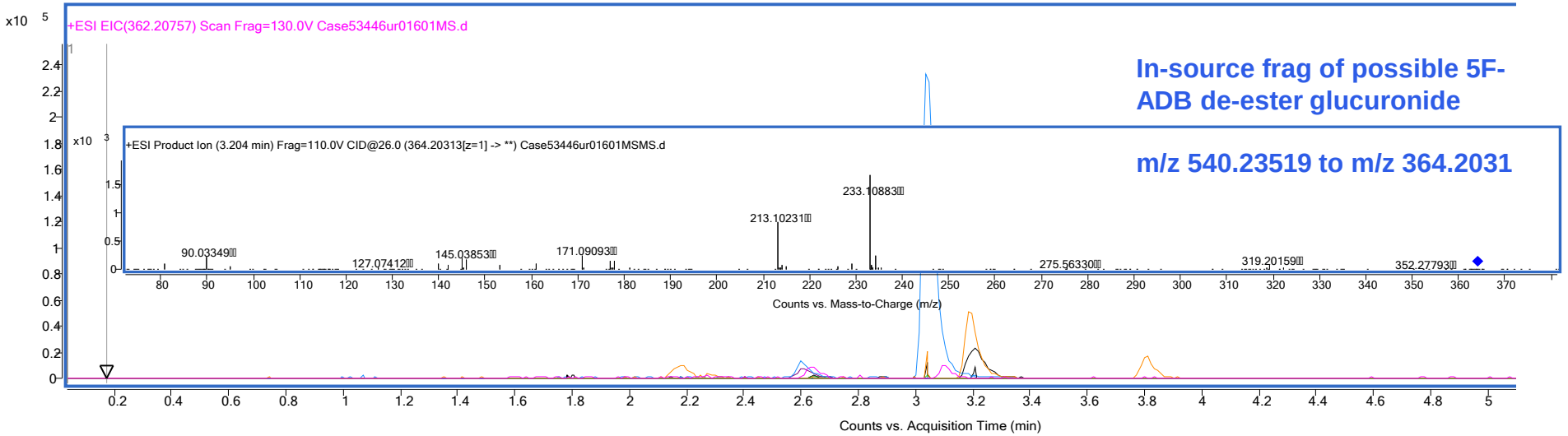


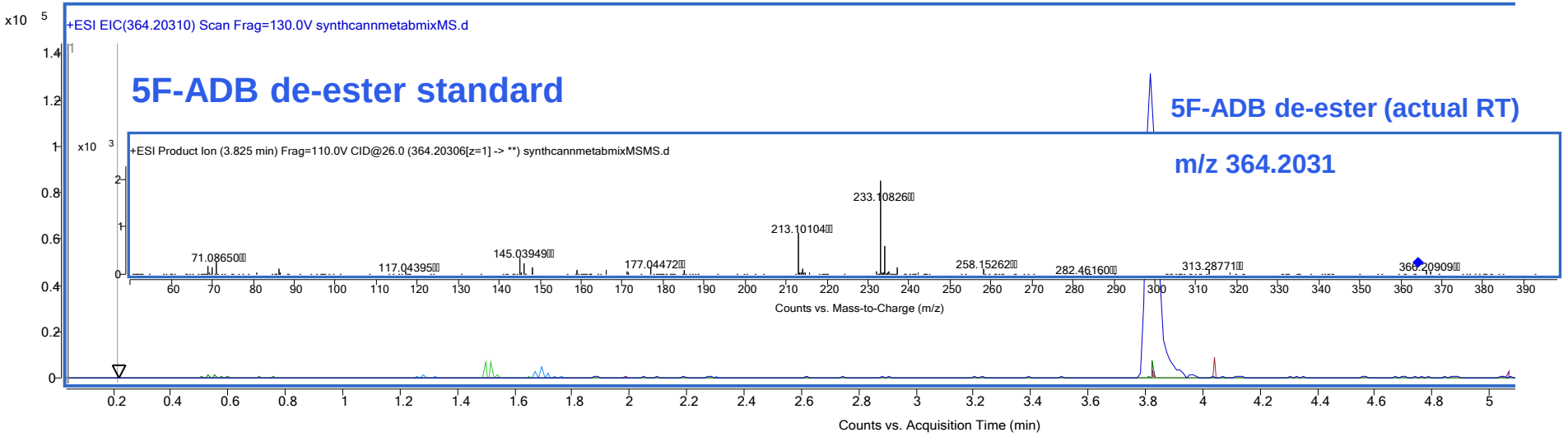




Case Examples

New Psychoactive Substances: synthetic cannabinoids





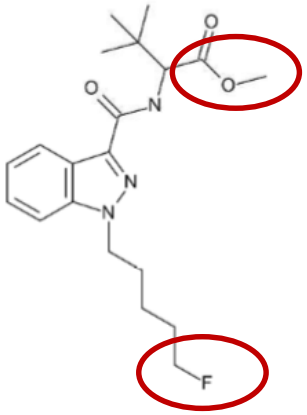
Case Examples

New Psychoactive Substances: synthetic cannabinoids

Further reading and MS/MS of 5F-ADB & metabolites:

Kusano M et al. “Fatal intoxication by 5F-ADB and diphenidine: Detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS”, Drug Test Anal. 10(2):284-293 (2018)

5F-ADB

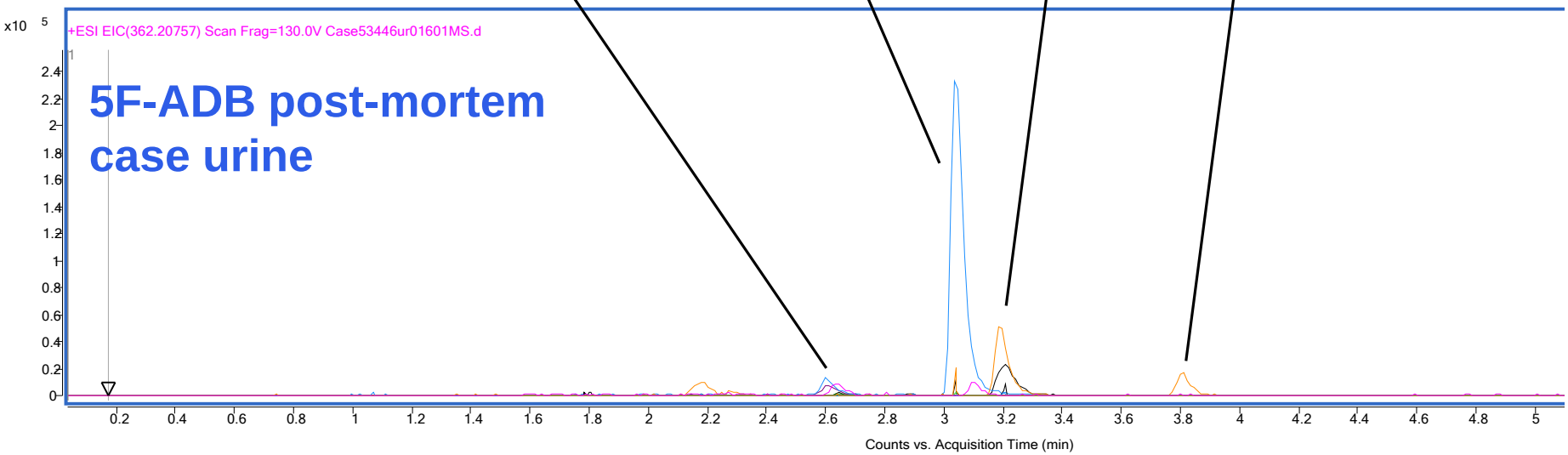


Possible 5F-ADB de-ester defluoro – COOH glucuronide with in-source fragmentation
m/z 552.21879
m/z 376.1867

5F-ADB de-ester defluoro -COOH
m/z 376.1867

Possible 5F-ADB de-ester glucuronide with in-source fragmentation
m/z 540.23519
m/z 364.2031

5F-ADB de-ester (valine)
m/z 364.2031



5F-ADB post-mortem case urine

Use of QTOF LC-MS

- **Complements existing systems**
  - Immunoassay
  - GC-MS
  - HPLC-DAD
  - LC-MS/MS

- **Replaces existing systems**
  - Immunoassay
  - GC-MS
  - HPLC-DAD
  - LC-MS/MS

Use of QTOF LC-MS

Complementary implementation

- Ensure you are aware of analytical coverage overlap or differences
- Possible to use same extraction procedures or extracts
- Provides additional confidence in results (particularly screening)
- Method can be validated and evaluated alongside existing systems

Replacement implementation

- Ensure it performs as well as if not better than the method it is replacing (especially if replacing LC-MS-MS)!
- Implementation should be carefully planned to fit in with the existing workflow
- The new method should be fully validated and evaluated
- The workforce should be trained appropriately for effective use

Use of QTOF LC-MS

PROBLEMS AND PITFALLS

If using sensitive QTOF MS for the first time, you will find even more things than traditional LC/MS-MS! This can alter detection window comments, reporting cut-offs and other interpretative aspects of forensic toxicology

Be aware of in-source fragmentation this can affect identification of molecular ion

Experiment with positive and negative mode – some drugs can do both

Using deuterated internal standards or matrix dilution can minimise any ion suppression/enhancement effects, especially in quantification

Don’t forget about U/HPLC – the better the chromatography, the better the MS data obtained

Use of QTOF LC-MS

SUMMARY

QTOF LC-MS provides a complementary method of analysis or can replace existing techniques (including LC-MS/MS)

Can be applied to a very wide range of analytes so has particular advantages for general screening (inc. ability to re-interrogate historic data) as well as drug and metabolite detection & identification

Need to be aware of interpretative and analytical issues

Careful implementation and training can enhance workflow and significantly improve the laboratory service

simon.elliott@alere.com & simontox@yahoo.co.uk