



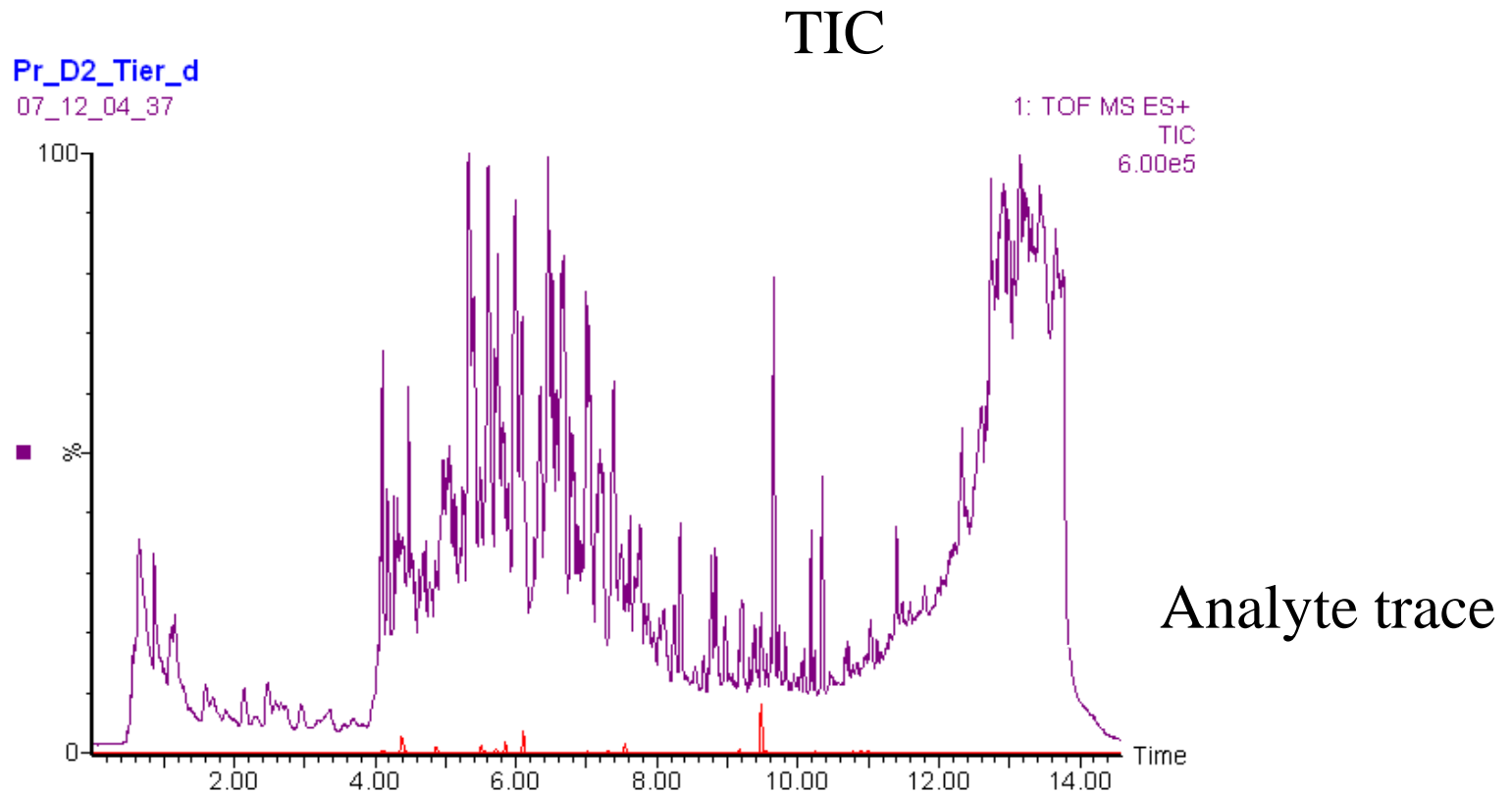
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Optimal MS-based analyte identification techniques (HRMS)

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Mass spectrometer view of the hay stack

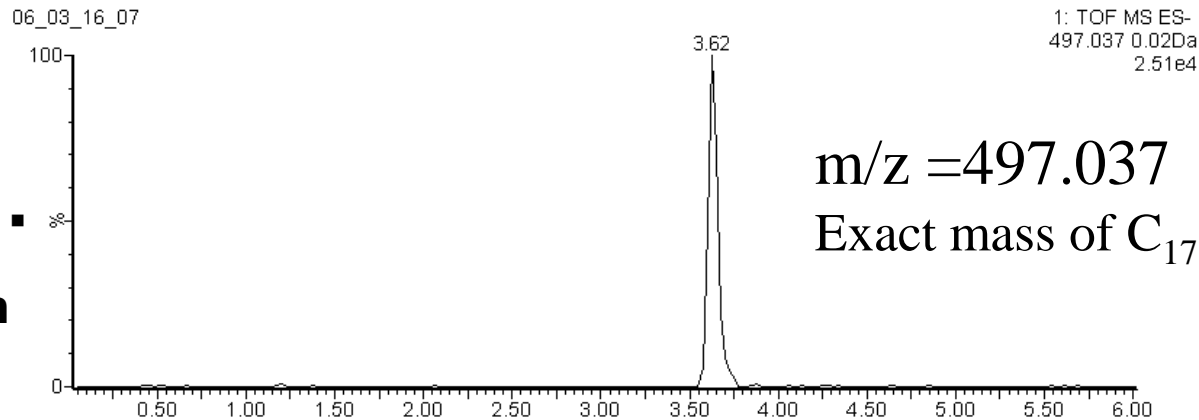
Pork liver extract spiked with 100 vet. drugs



Identification of positive findings

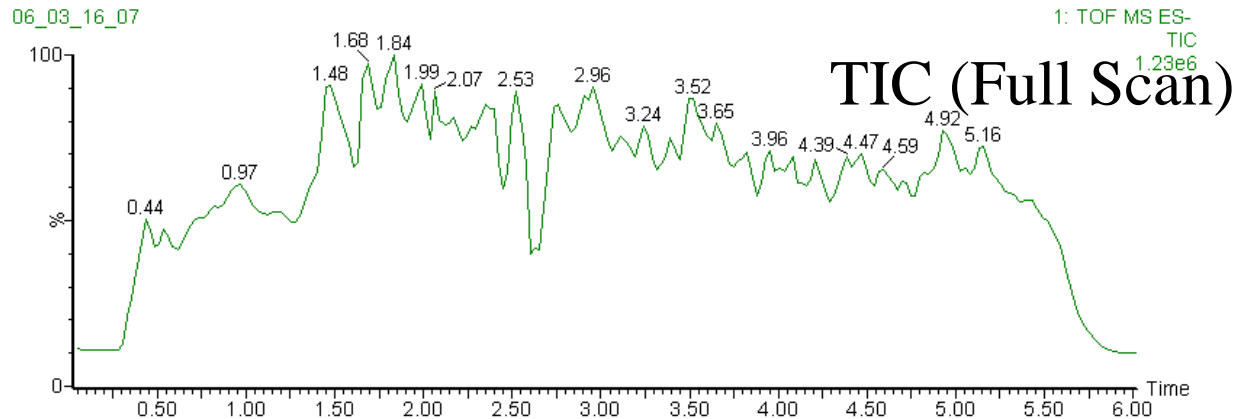
Low $\mu\text{g/l}$ chloramphenicol in urine

x 50 Amplification

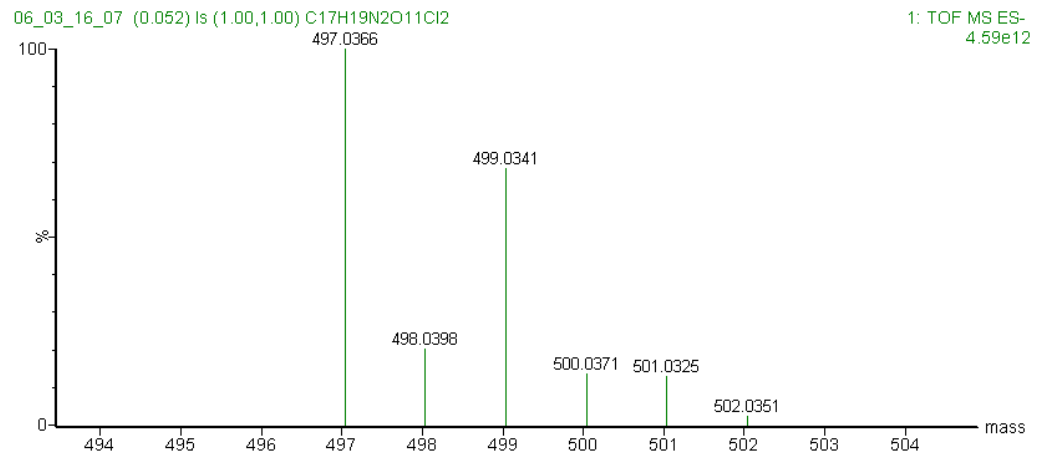
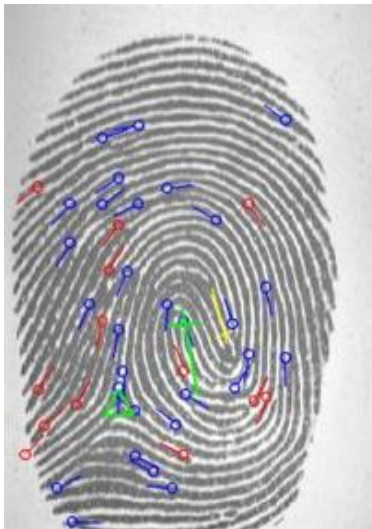
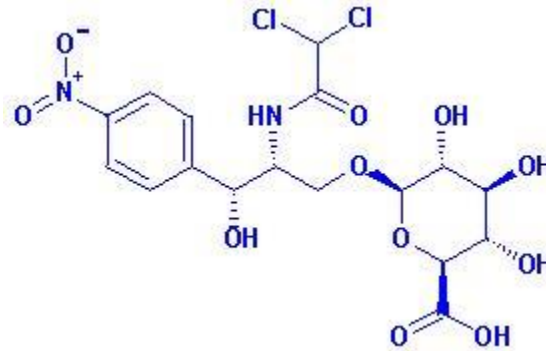


$m/z = 497.037$

Exact mass of $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_{11}\text{Cl}_2$

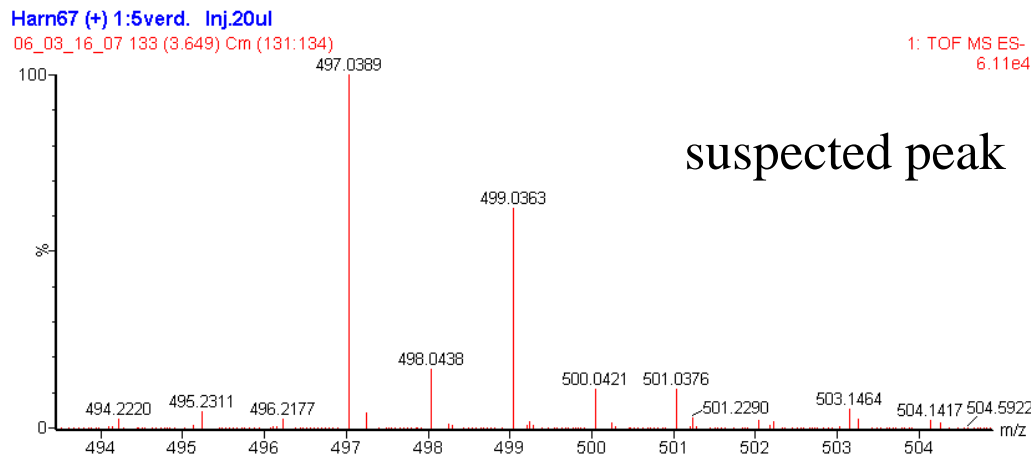
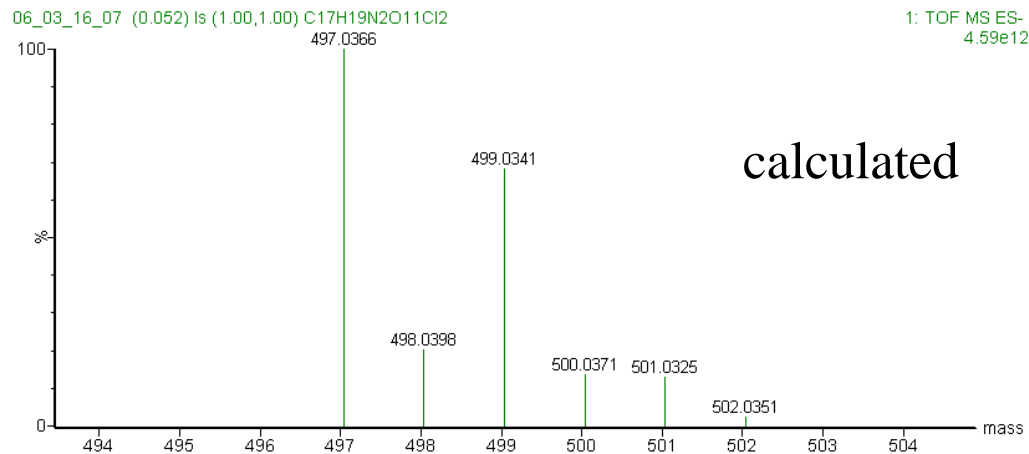


The fingerprint: The Isotope Profile



The fingerprint: The Isotope Profile

Isotopic pattern of chloramphenicol glucuronide



This was a «textbook» example

Compounds containing halogens are easy to identify

“CHO” compounds are much more challenging

Extracting a narrow mass window out of a HRMS scan may still be insufficiently selective

The lower the analyte concentration, the less unique the analyte, the more complex the matrix, the higher the need for orthogonal identification & confirmation

Example SARMs (Selective Androgen Receptor Modulator)



Example SARM's

The European Reference Laboratory for hormones in meat suspected the use of SARMs for animal growth promoting purposes

A MS/MS method covering 3 SARMs was developed and sent to the National Reference Laboratories for testing and finally including these new analytes into the monitoring program.

The sample processing and clean-up technique was rather similar with our in-house HRMS based multiresidue hormone monitoring method

Example SARMS

We reprocessed datafiles of some 2000 samples measured over the last years. All were negative.

In addition we tested for the presence of other SARMS for which we had no reference substances.

Some suspected detects were observed. MS based identification at the low $\mu\text{g/l}$ level was difficult.

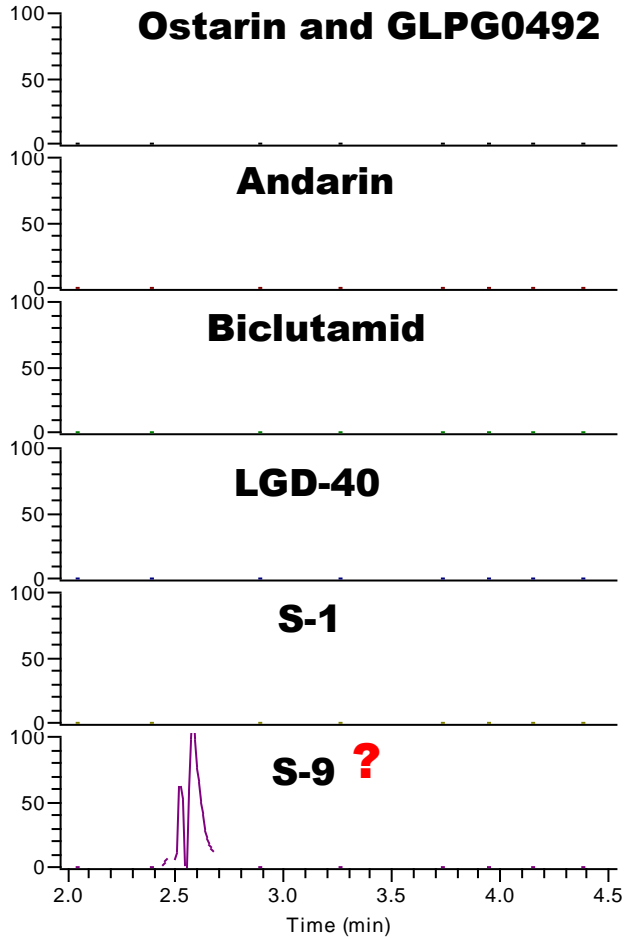
These findings could not anymore be confirmed after having access to reference substances (analyte retention time is still a very important identification & Confirmation criteria)

Bovine blood

SARMs standard 2 µg/l

RT: 1.96 - 4.54 SM: 3B

RT: 2.00 - 4.56



m/z = 388.0915

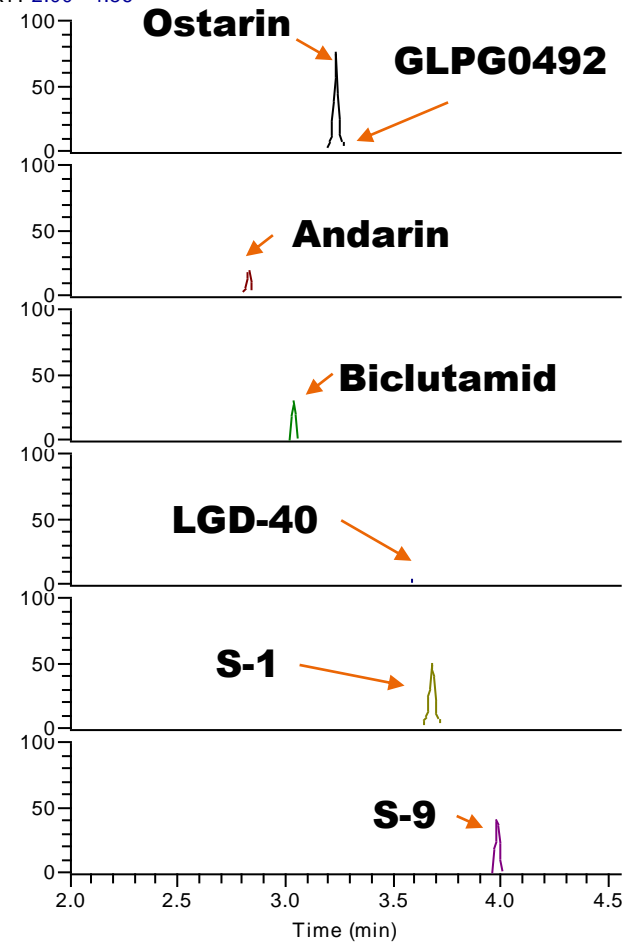
m/z = 440.1075

m/z = 429.0538

m/z = 337.0781

m/z = 401.0766

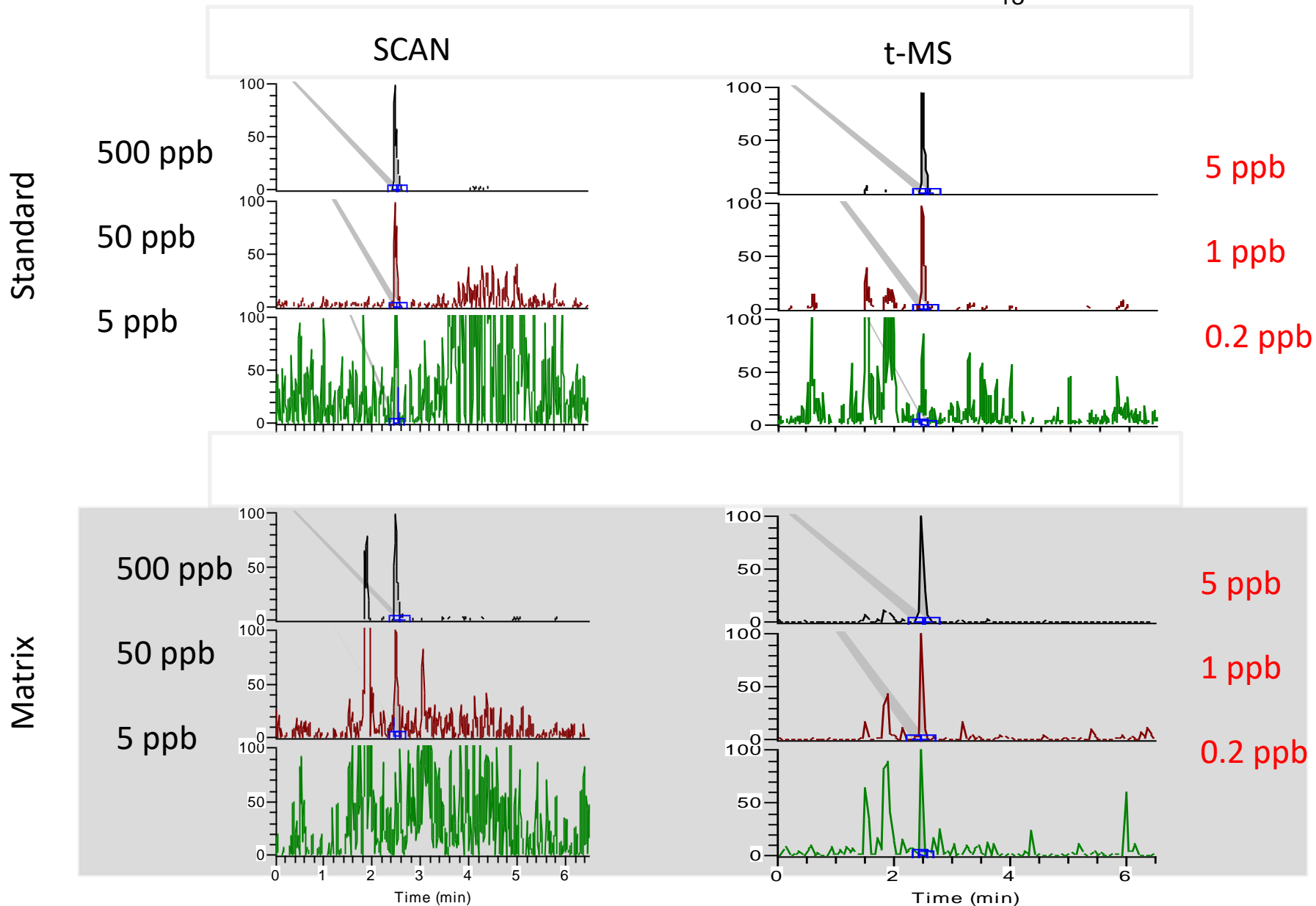
m/z = 417.0471



Even for HRMS, MS/HRMS may still be needed

Quadrupole based precursor selection, followed by fragmentation and HRMS based product ion detection is highly selective

HRMS versus MS/HRMS (Ciprofloxacin)



Precursors can be calculated, fragments can be proposed

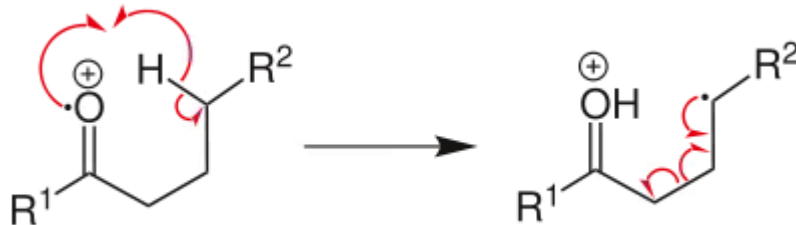
In QqQ we do a tuning of pure, concentrated reference substances to obtain collision energies and fragment masses

In non-targeted HRMS we would like to detect and identify analytes without having access to reference substances

Brain against muscles

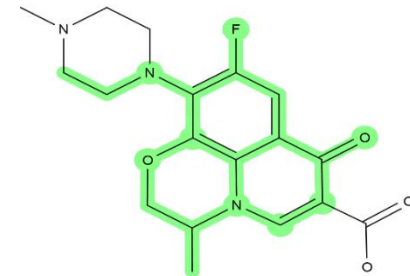
Predicting
(e.g. Mass Frontier)

**Based on expert knowledge
and fragmentation library**

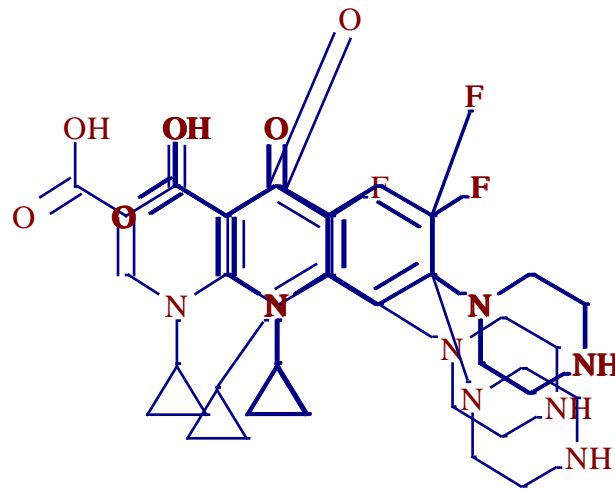


Explaining
In Silico «chopping» algorithm
(e.g. MassFragment)

Based on brute force



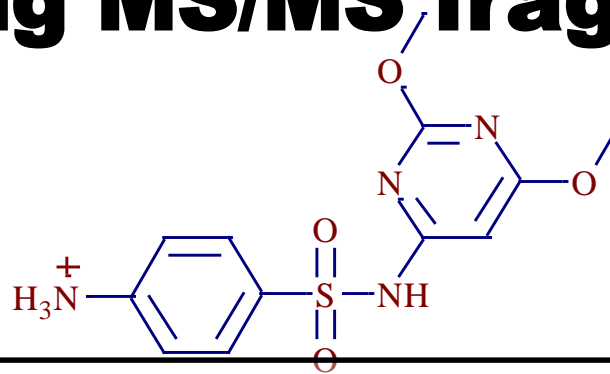
Explaining MS/MS fragmentation (In silico)



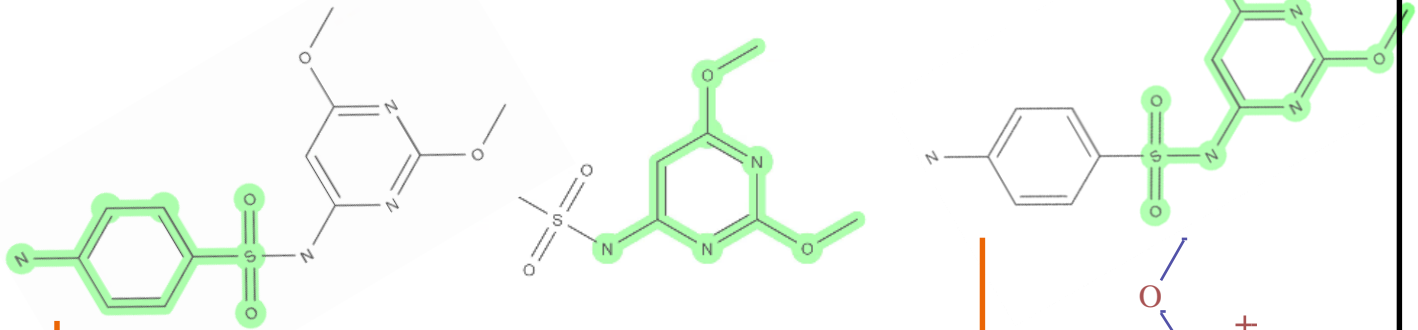
Bonds are systematically chopped

Resulting fragments are ranked according to the stability of the broken chemical bonds

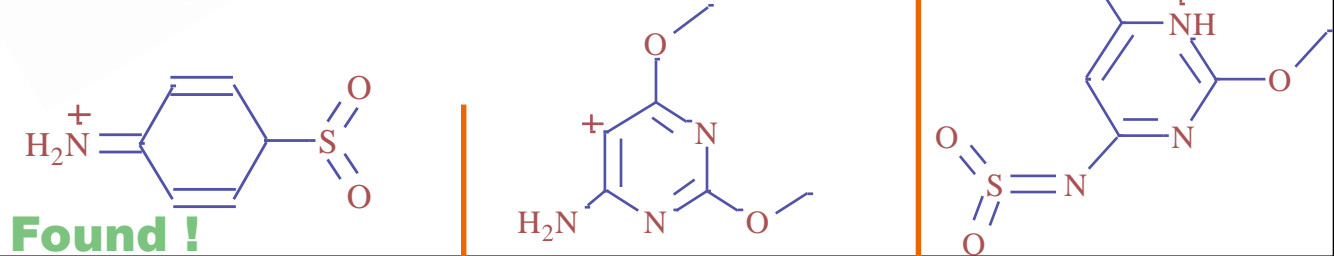
Explaining MS/MS fragmentation



156.0119



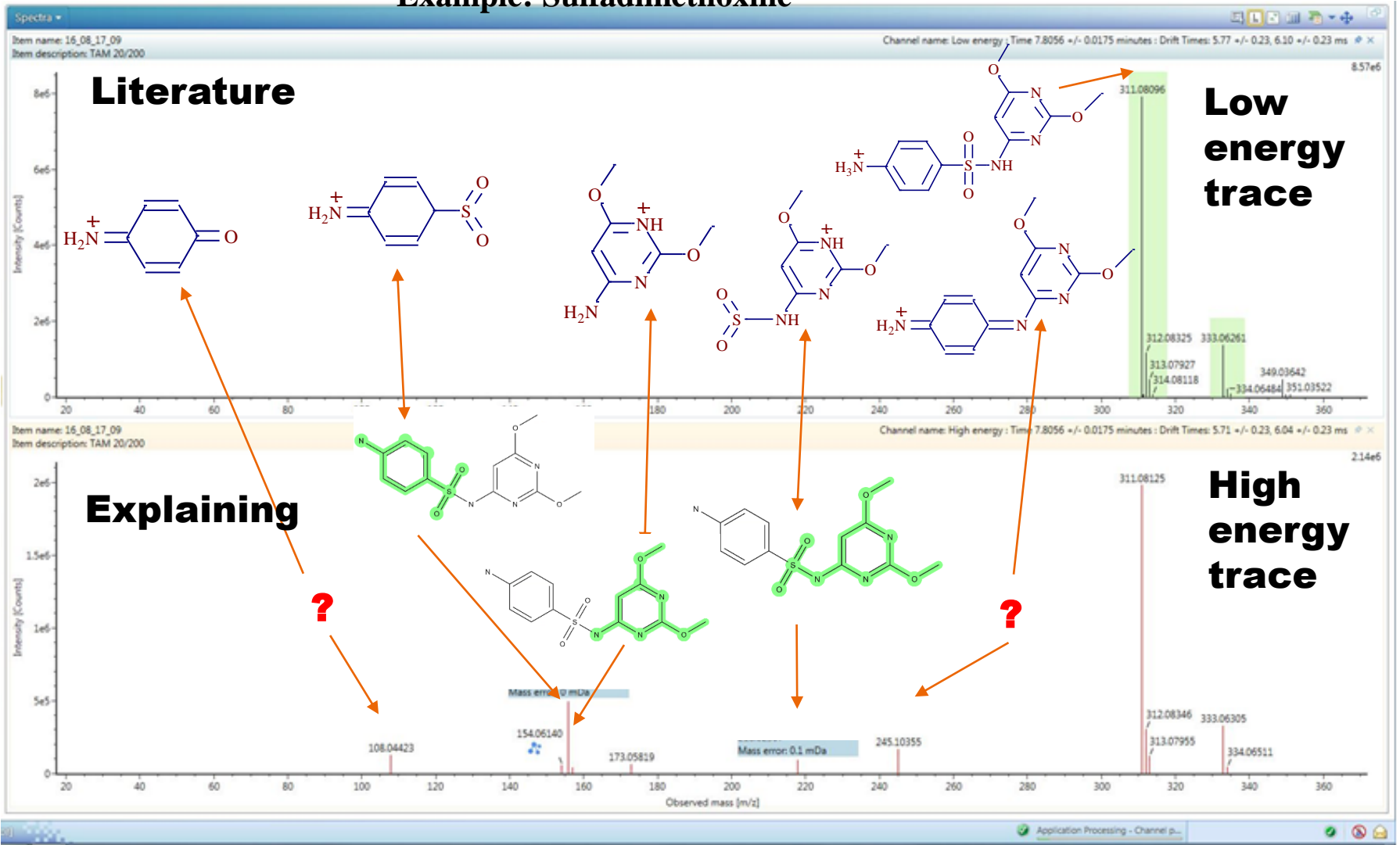
156.0112



Does a «chopping» correspond to the accurate mass of an experimentally observed fragment ?

Not everything can be explained

Example: Sulfadimethoxine



Isobaric

Current HMRS based identification

Conventional collision induced fragmentation (CID):

The weakest bonds are fragmented

There is no generic fragmentation energy suitable for all compounds

Stable compounds produce no fragments, labile compounds produce small ions with little diagnostic properties

Future of HMRS based identification

Electron activation dissociation (EAD):

Virtually every bond undergoes fragmentation

The technique permits structural elucidation (similar to NMR), including positional isomers

EAD should greatly enhance in silico fragmentation based techniques

Future of HMRS based identification

Electron activation dissociation (EAD):

Producing many fragments will spread the ion abundance to many ions and reduces sensitivity

EAD energy can be set to suit the need for small molecule analysis, yet it is slower technique than CID (an issue for UHPLC?)

Future of HMRS based identification?

Ion Mobility (IMS):

IMS is not really orthogonal to m/z

Higher IMS resolving power will certainly help

Collision cross sections (CCS) cannot yet be well calculated and show some platform dependency

Conclusion

Precise retention time (chromatography) is still very important

MS based identification without physical reference substances at trace level in complex matrix is in many cases not possible

Emerging technology like IMS or EAD may bring us closer to this aim