



Case Study I: Small Molecule Impurity ID

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Determination of an Unknown E&L Impurity Based on **Accurate Mass Detection, Elemental Composition Matching, Chemicals Database Searching, and MassFrontier Theoretical Fragmentation**

CHALLENGE: Detection of Unknown E&L Impurity

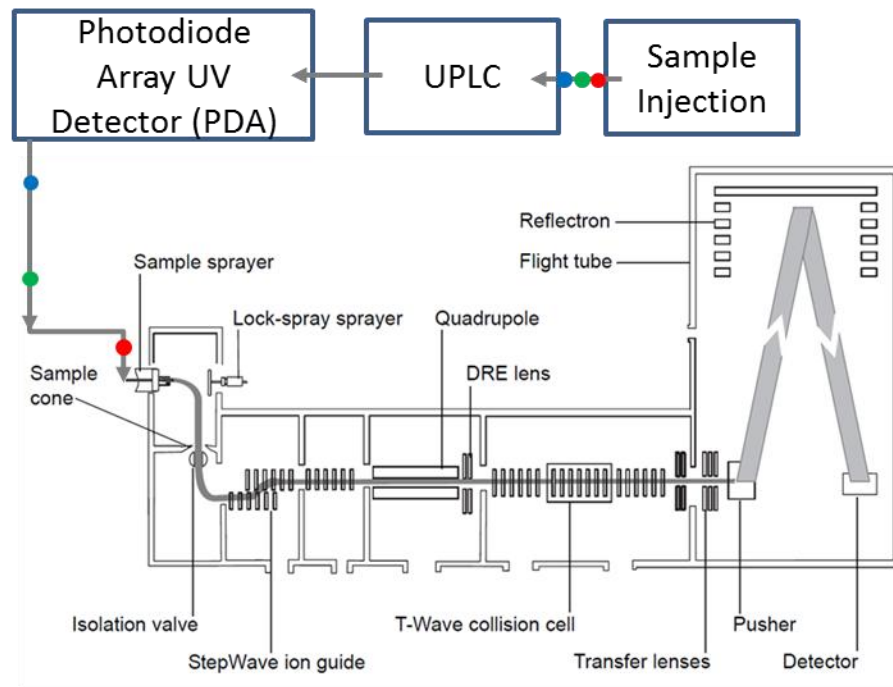
SOLUTION: UPLC – UV – High Resolution Accurate Mass MS Analysis and MassFrontier Theoretical Fragment Library Searching Workflow



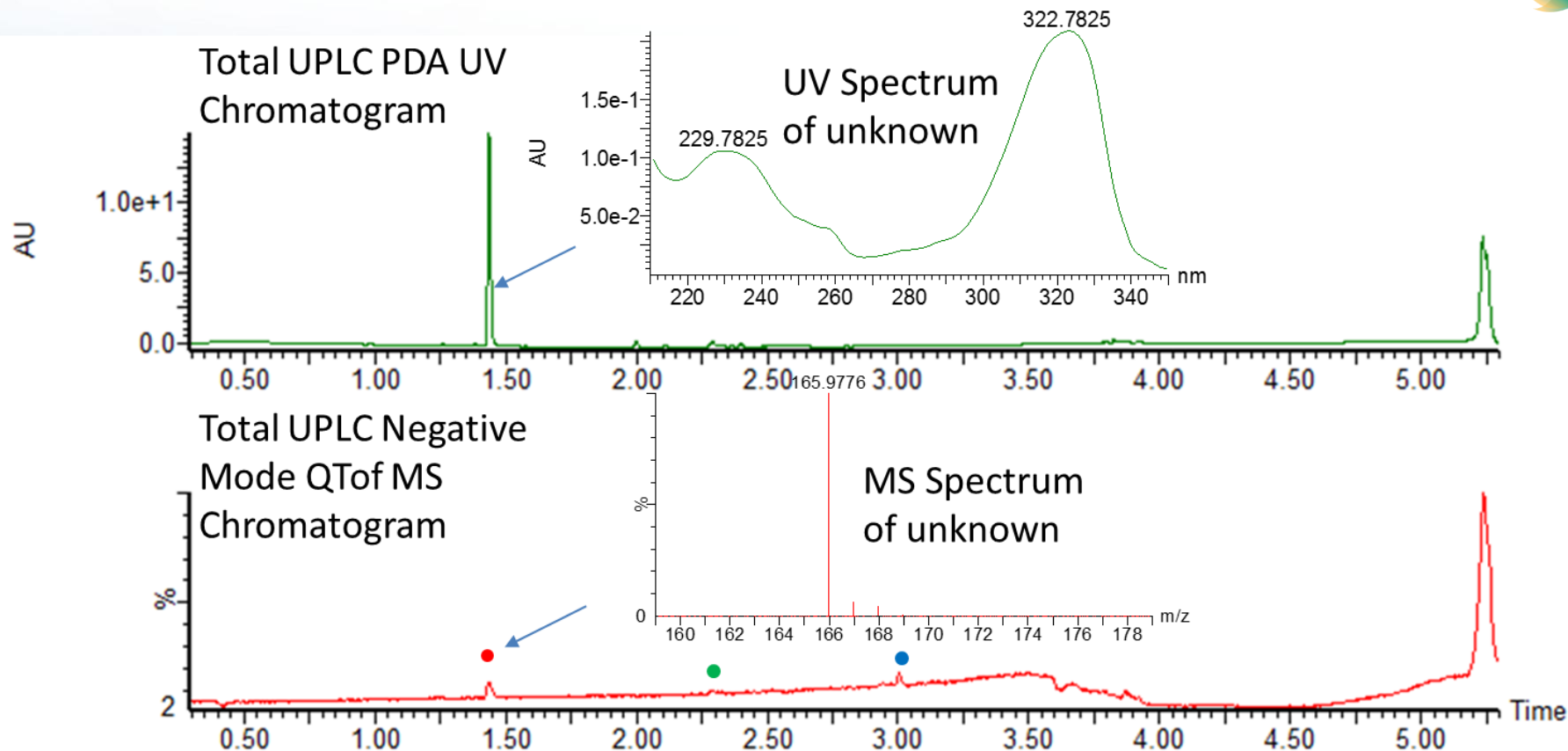
Step 1 - Accurate Mass UPLC-UV-QToF MS and MSMS Analysis

Acquity UPLC Coupled to G2-S (Waters) Q-ToF High Resolution Mass Spectrometer

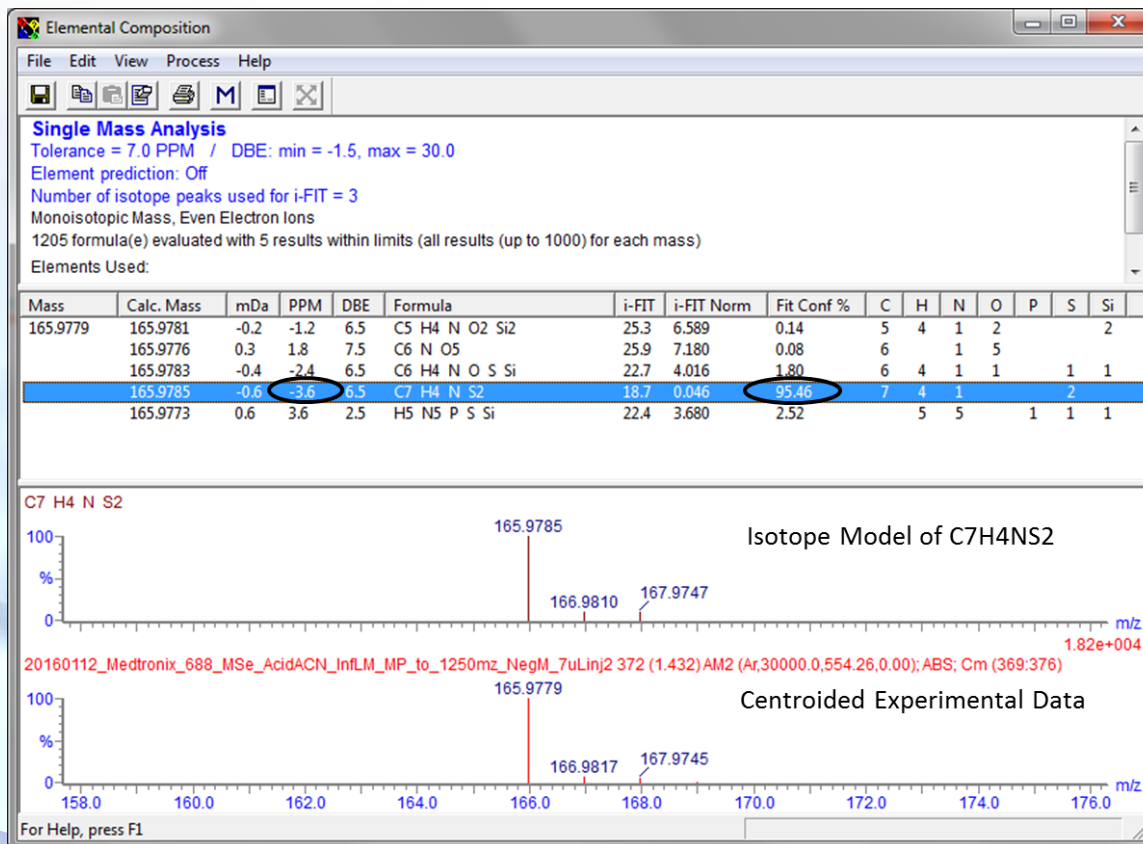
Chemical and Structural Identification of unknown component analytes comprising a complex mixture.



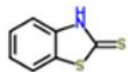
MS and UV Spectra of Unknown Impurity



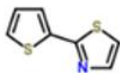
Step 2 – Elemental Composition Determination



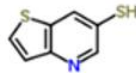
Step 3 – Elemental Composition Search of Online Databases



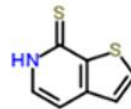
[2-Mercaptobenzothiazole](#)



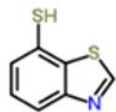
[2-\(2-Thienyl\)thiazole](#)



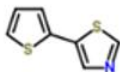
[Thieno\[3,2-b\]pyridine-6-thiol](#)



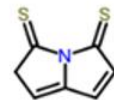
[Thieno\[2,3-c\]pyridine-7\(6H\)-thione](#)



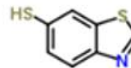
[1,3-Benzothiazole-7-thiol](#)



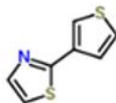
[5-\(2-Thienyl\)-1,3-thiazole](#)



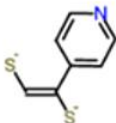
[3H-Pyrrolizine-3,5\(2H\)-dithione](#)



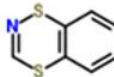
[1,3-Benzothiazole-6-thiol](#)



[2-\(3-Thienyl\)-1,3-thiazole](#)

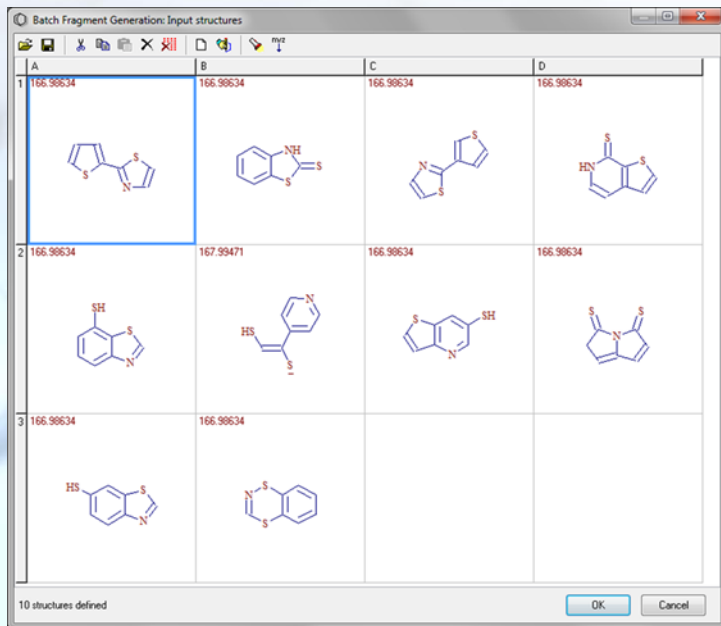


[1-\(pyridine-4-yl\)-ethylene-1,2-dithiolate](#)



[1,4,2-Benzodithiazine](#)

Step 4 – Mass Frontier Theoretical MSMS Spectra Calculation in Batch Fragmentation Mode



Batch Processing Options: Reaction Restriction

Base Ionization & Cleavage H-Rearrangement Resonance Additional Sizes

Ionization Method

☐ $M^{+•}$ Electron Impact (EI)

☐ $[M + H]^+$ Protonation (ESI, APCI ...)

☒ $[M - H]^-$ Deprotonation (ESI, APCI ...)

☐ Cluster Ion Formation: NH_4^+

☐ Alkali Metal Adducts: Na^+

☐ Chemical Ionization: CH_4

Ionization on

☒ Non-bond. el. ($-H^+$)

☒ π bond (π)

☒ Sigma bond (σ)

Cleavage

☒ α (α)

☒ Inductive (I)

☒ Display this window every time Generation of Fragments & Mechanisms is started

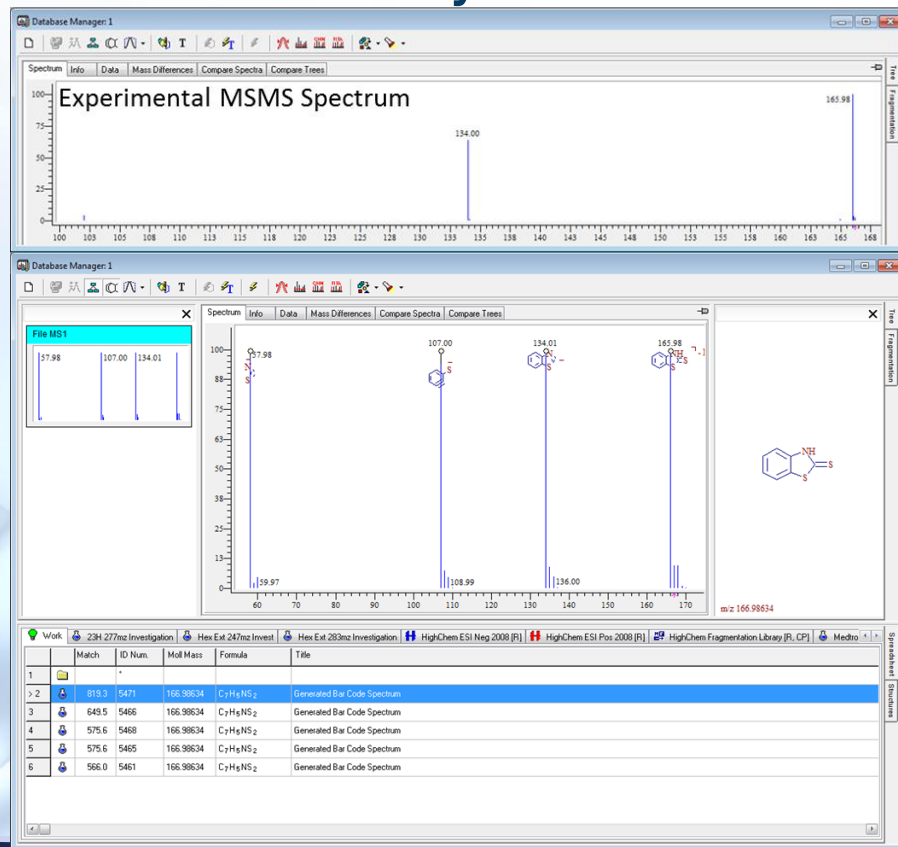
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Step 5 – Search of Experimental MSMS Spectrum Against Calculated MassFrontier MSMS Library



Top search match yielded a candidate for the unknown in the extractables and leachables study, namely

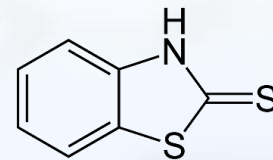
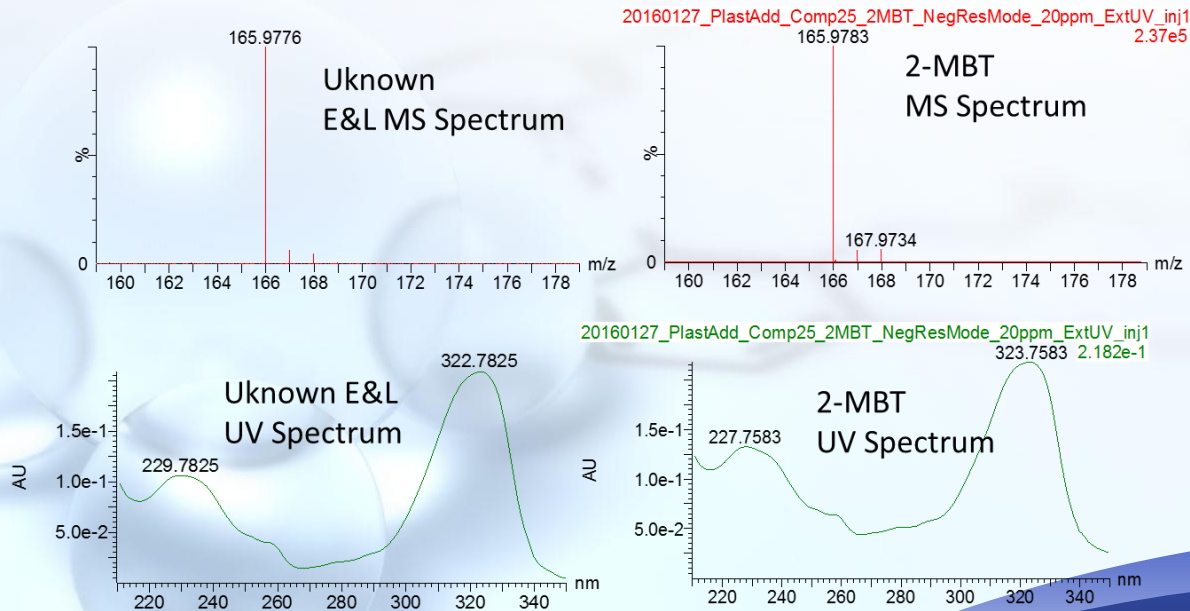
2-Mercaptobenzothiazole



Step 6 – Confirmation of 2-mercaptobenzothiazole (2-MBT) as the correct identification of the extractables/leachables unknown



Experimental standard data confirmed the MassFrontier identification.



2-MBT



SUMMARY:

Mass Spec Lab's UPLC-UV-high resolution accurate mass workflow successfully identified a completely unknown E&L analyte present at trace levels (≈ 20 ppm) with no information other than that provided by the work flow.

We gratefully acknowledge technical assistance from HighChem (Bratislava, Slovakia, EU), the maker of MassFrontier, in assistance with the theoretical fragmentation spectral matching algorithms.

Questions? More Information?



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