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Ultra High Resolution MS Aids Unknown Extractable Component Identification

Kate Comstock, Seema Sharma, Jesse Canterbury, Vlad Zabrouskov Thermo Fisher Scientific, San Jose, CA, USA

ABSTRACT

Purpose: Demonstrate the value of ultra high resolution MS for unknown extractable ID and structure elucidation.

Methods: Ultra high (1 million) resolution MS

Results: Ultra high resolution MS provided critical information for validating the elemental composition of unknown extractables.

INTRODUCTION

Extractables and leachables (E&L) analysis is an integral part of market approval for a wide range of products from industries such as pharmaceutical, medical devices, and food packaging. The purpose of such analyses is to identify and quantify the E&L compounds for safety evaluation. Many E&L compunds are unknowns and have a broad range of molecular weights and elemental compositions. Ultra high resolution MS generates accurate mass, isotope fine structure, and maintains high fidelity, which provides critical structure information to correctly and confidently determine the elemental composition for identification and unknown structure elucidation.

MATERIALS AND METHODS

Sample Preparation

A mixture of antioxidant standards were dissolved in IPA and diluted to 1 ppm.

The cosmetic product (nail polish) was extracted using ACN/H2O.

Liquid Chromatography

Liquid chromatography separations were carried out on Thermo Scientific™ Vanquish™ LC system consisting of a binary pump, autosampler, column compartment, and diode array detector.

Column: Thermo Scientific™ Hypersil™ C18 column (2.1X100 mm 3 µm).
Column temp: 30ºC
Flow rate (µl/min): 300
Injection volume (µl): 3
Mobile phase: (A) Water/0.1% Formic acid, (B) Acetonitrile/0.1% Formic acid
Gradient:

Time (min)	A%	B%
0	95	5
1	95	5
20.0	5	95
20.1	95	5
25.0	95	5

Mass Spectrometry

The MS analysis was performed on a Thermo Scientific™ Orbitrap Lumos™ Tribrid™ mass spectrometer with 1M option.

Ion source: Thermo Scientific™ EASY-Max™ NG
Ionization mode: ESI positive
Sheath gas flow rate: 45 units N2
Auxiliary gas flow rate: 15 units N2
Spray voltage (KV): +3.5
Ion transfer tube temp (ºC): 350
S-lens RF level: 60.0
Heater temp (ºC): 250

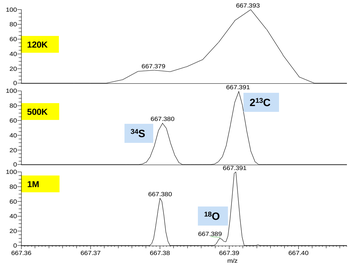
LC-MS analysis was conducted at the 120K resolution setting for precursor ions and the 60K resolution setting for the fragment ions. This was used to generate the list of analytes for subsequent Selected Ion Monitoring (SIM) experiments at the 1,000,000 (1M) resolution setting for obtaining fine isotope structures. Additionally, experiments were also conducted at the 1M setting for full scan.

RESULTS AND DISCUSSION

The power of ultra high resolution MS

At ultra high resolution, mass spectra reveal isotopic fine structure for unknowns. The small (milli-Da) mass differences between isotopes allow determination of the elemental composition with a high degree of confidence. For instance, in the molecular ion range from 600 to 700 amu, the fine isotope structure clearly distinguishes between ^{18}O and ^{13}C isotopes. Furthermore, accurate isotope ratios allow confident assignment of sulfur content. This information could not be obtained at lower resolution. Figure 1 shows the zoom-in spectrum for the A+2 isotope peak family for Irganox 1035 collected at resolution settings of 120K, 500K, and 1M.

Figure 1. Irganox 1035 [M+Na]⁺ A2 Isotope Peak Family at Different Resolutions

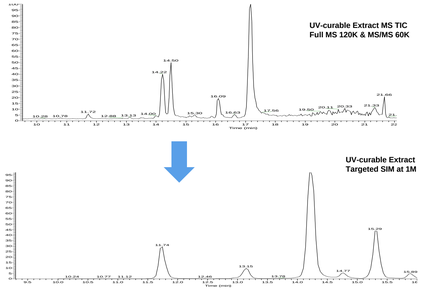


LCMS Analysis of Cosmetic Product Extractables

The cosmetic product, an uncured UV light-curable nail polish, was extracted using acetonitrile/water extract [1]. The extract was directly injected into the LCMS system and the LCMS chromatogram is shown in Figure 2. The sample was run at resolution settings of 120,000, 500,000 and 1,000,000 (FWHM at m/z 200).

To confidently assign the elemental composition, five components in, molecular ion range from 400 to 750 m/z were further analyzed using targeted SIM at 1 M resolution to obtain their fine isotope structure information.

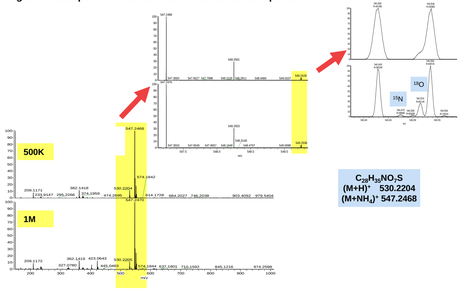
Figure 2. LC-MS Chromatogram of UV-curable Extract



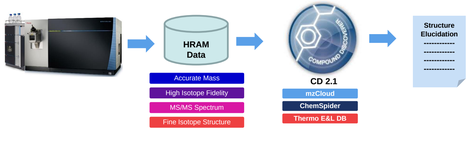
Results and Discussion

The HRAM data of LCMS analysis was processed using Thermo Scientific™ Compound Discoverer™ 2.1software (CD 2.1). The sample was analyzed at 500,000 and 1 million resolution. For m/z 547.2468, the 1M resolution spectra show the ^{15}N and ^{18}O isotopes while these two peaks weren't observed in the 500K resolution data. This information is critical for assigning the correct elemental compositions.

Figure 3. Isotope Fine Structure of the Identified Component



Workflow of Ultra High Resolution MS for E&L Analysis



Workflow for E&L analysis using ultra high resolution MS and Compound Discoverer 2.1

The ultra high resolution mass spectrometer provides accurate mass, fine isotope structure, high isotope fidelity, and MS/MS spectra. The high quality HRAM data are processed by the CD 2.1 advanced small molecule structure analysis software. Based on the HRAM data, CD 2.1 extracts components, predicts composition, groups the adducts, and carries out multiple database and spectral library searching automatically to identify known compounds. The "structure proposals" facilitate unknown component ID utilizing the predicted compositions and FISh scoring (Fragment Ion Search) function.

Ultra high resolution MS greatly facilitates analysis of high mass unknowns. Even with sub-ppm mass accuracy, without the fine isotope structure information, it is difficult to determine the elemental composition with confidence.

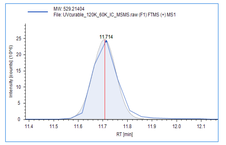
Data Processing using Compound Discoverer 2.1

Determining the Correct Composition

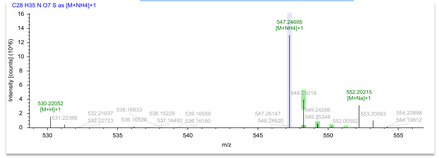
CD 2.1 processing of the results generated predicted compositions with good mass accuracy. To determine the correct elemental compositions, fine isotope structure was used to distinguish the correct elemental composition from other candidates. This is especially true for higher mass (> 500 amu). Figure 5-a shows the extracted ion chromatogram of MW.529.21404. The mass spectrum shows the color coded ions (M+H) and (M+NH4)+, see Figure 5-b. Based on the accurate mass measurement, CD 2.1 predicted 10 formula within the identification threshold, see Figure 5-c and Figure 5-d which show that only the correct predicted composition fine isotope structures match the experimental result.

Figure 5. CD Result

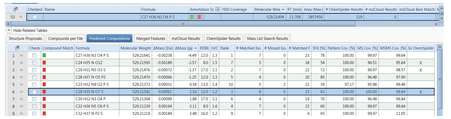
5-a. XIC of MW.529.21404



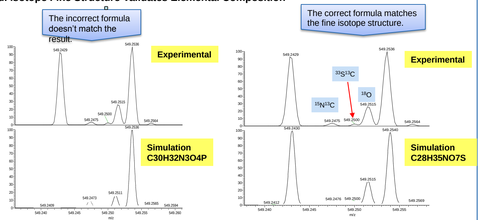
5-b. Mass Spectrum



5-c. Predicted Compositions



5-d. Isotope Fine Structure Validates Elemental Composition

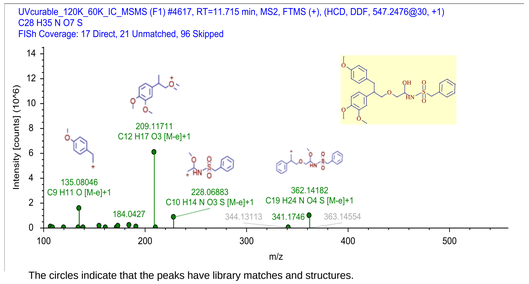


The above results demonstrate that the fine isotope structure made available ultra high resolution mass spectrometer greatly enhances the ability to confidently determine the correct unknown elemental composition.

Structure Proposals and FISh Scoring for Unknown ID

The known components were identified through multiple database searching. Based on the proposed composition and the MS/MS fragments, the unknown structures were proposed using "Structure Proposals" function. The FISh scoring (Fragment Ion Search) feature search's embedded " "Fragments and Mechanisms" library auto-annotates the matching fragments matching with library.

Figure 6. Auto Annotation for Proposed Structure



The circles indicate that the peaks have library matches and structures.

CONCLUSIONS

This study demonstrates that ultra high resolution MS is valuable for extractable and leachable analysis. The isotopic fine structure, combined with the high mass accuracy, enables confident elemental composition assignment. Coupled with CD 2.1 data processing software and the mzCloud spectral database, this workflow greatly facilitate E&L compound analysis and small molecule impurity ID, especially for unknown structure analysis.

ACKNOWLEDGEMENTS

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