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Extractables & Leachables Analysis of Pharmaceutical Nasal Spray Samples using the Hi-Resolution Accurate Mass GC/QTOF

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Introduction

Everything we ingest comes in contact with several surfaces during the manufacturing and packaging processes. Some of these compounds could be harmful compounds leaching onto those products. Thus accurate knowledge of compounds that could be coming in contact with our food or medicine needs to be gathered. It is important to accurately detect and identify these known and unknown compounds reliably. This project will demonstrate a reliable and accurate way to identify extractables & leachable compounds using a simple workflow.

The possibility of internal and external influences being introduced onto a manufacturing process, knowledge of all possible unknowns cannot be 100% accounted for, coupled with certain products having complex matrices, the Q-TOF is an important instrument to ensure confident results. Coupled with the Low Energy source functionality, the molecular ion can be enhanced for further confirmation.

The data presented will display the performance of the 7250 GC/Q-TOF and the MassHunter software's ability to streamline the known(targets)/unknown identification process on a pharmaceutical grade nasal spray sample and it's packaging.

Figure 1: Low-Energy capable Agilent 7250 GC/Q-TOF.

Experimental

Sample Preparation:

Samples were obtained from multiple points throughout the manufacturing process in order to perform an extractable study of known compounds which may appear in the final released product. These samples included a control, bottle fixture, gaskets, and a final release sample. Solvent extractions were performed on packaging material using dichloromethane. For spray extractions, ethyl acetate was used. In addition to the compounds which will be identified through extractions, a standard of common plastic, elastomer, ink, and label compounds was prepped, so we can include these known leachable compounds into our workflow.

Low eV & 70eV Data Acquisition:

For GC/QTOF analytical conditions, see table 1. The low energy capable ion source was used at 70eV and low energy of15 eV to analyze samples. Low energy ionization provided additional insight by enhancing abundances of the mol ion or abundances of higher unique m/z ions.

Data Analysis:

A practical workflow was created using the tools in the MassHunter suite to analyze all samples. A typical workflow starts with Masshunter Unknowns Analysis, utilizing the SureMass algorithm to find components, produced a "cleaned" spectrum, then run a library search for annotation. MassHunter Qualitative Analysis was used for further confirmation, using fragment elemental compositions.

Table 1: Agilent 7250 GC/Q-TOF; 7890B GC Parameters.

GC and MS Conditions:		
Column	DB-XLB, 30 m, 0.25 mm ID, 0.25 µm film	
Injection volume and liner	1µL	Single-taper low-pressure drop
Split	100:1 split	
Inlet temperature	275 °C	
Oven temperature program	40 °C for 2 min 5 °C/min to 320 °C; Hold 15mins	
Carrier gas	Helium at 1.0 mL/min constant flow	
Transfer line temperature	280 °C	
Source temperature	250°C	
Quadrupole temperature	150°C	
Spectral range	50 to 750 m/z	
Electron Energy	70 eV and Low Energy 15 eV	

Results and Discussion

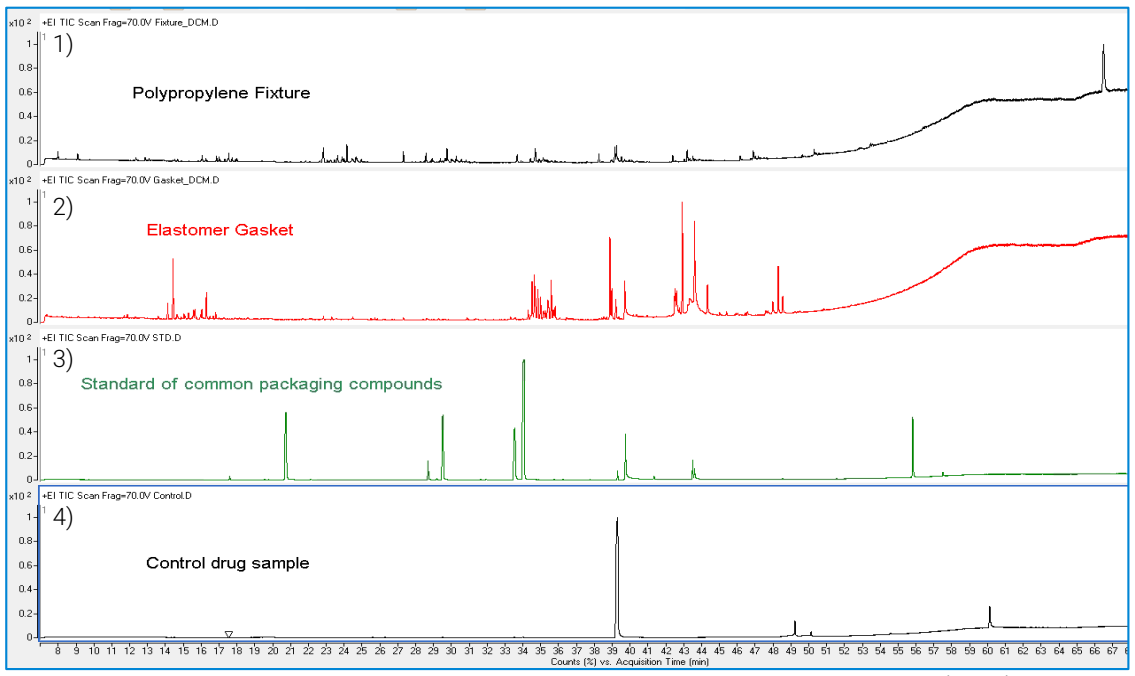

Figure 2: RTICs comparing the extracted samples (1,2) to the standard solutions (3,4).

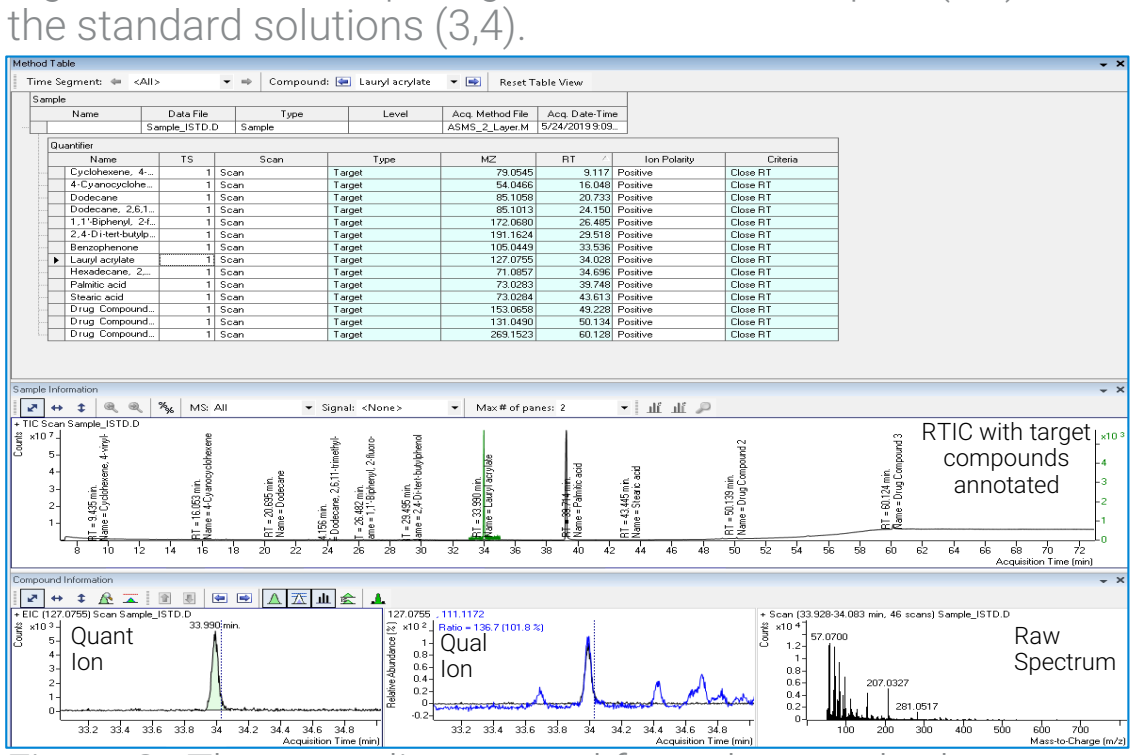

Figure 3: The target list, created from the standard solutions, in MassHunter Quant using method automation to build the compound list.

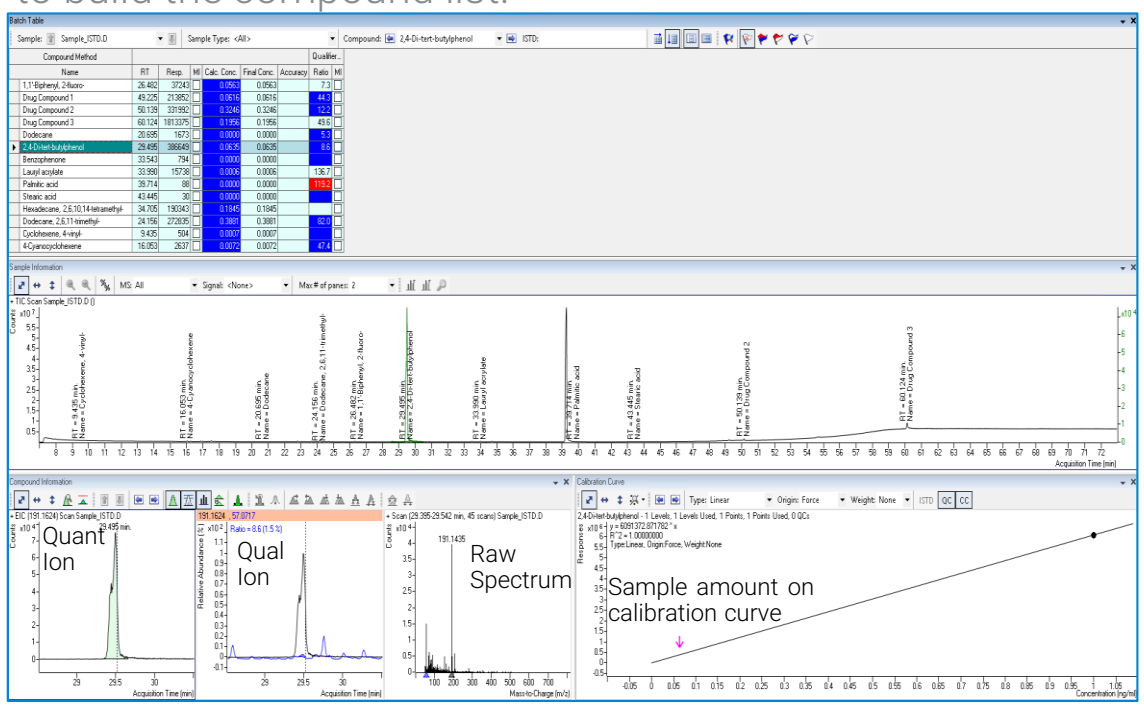

Figure 4: 2,4-Di-tert-butylphenol found in sample using standard RT, quant and qual ion, and mass accuracy for confirmation. A calculated concentration is provided using the 1-point calibration from the standard mix.

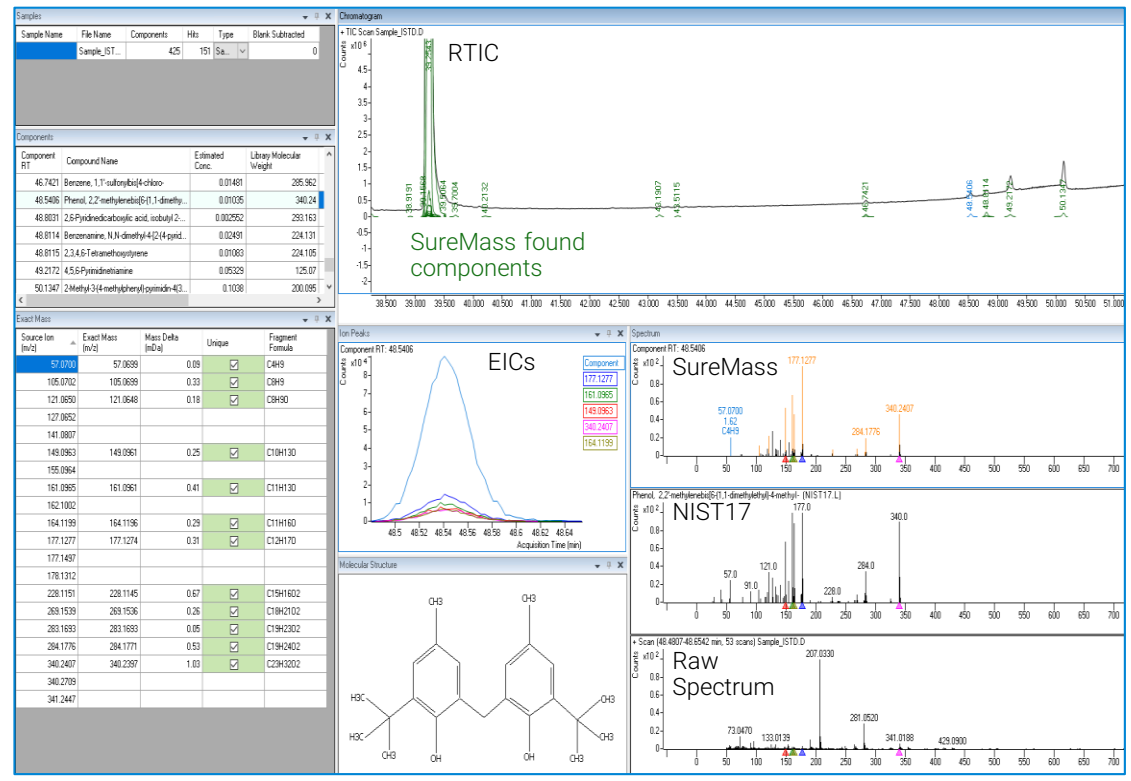

Figure 5: Using SureMass signal processing, the sample is quickly analyzed for unknown compounds and estimated concentrations are calculated, from the Quant method in figure 4. The "cleaned" SureMass spectrum created a better NIST17 library match. The formula from the library hit was used to confirm the molecular ion using the accurate mass. The mass table calculates the fragment ions using the NIST hit formula as the elemental limits.

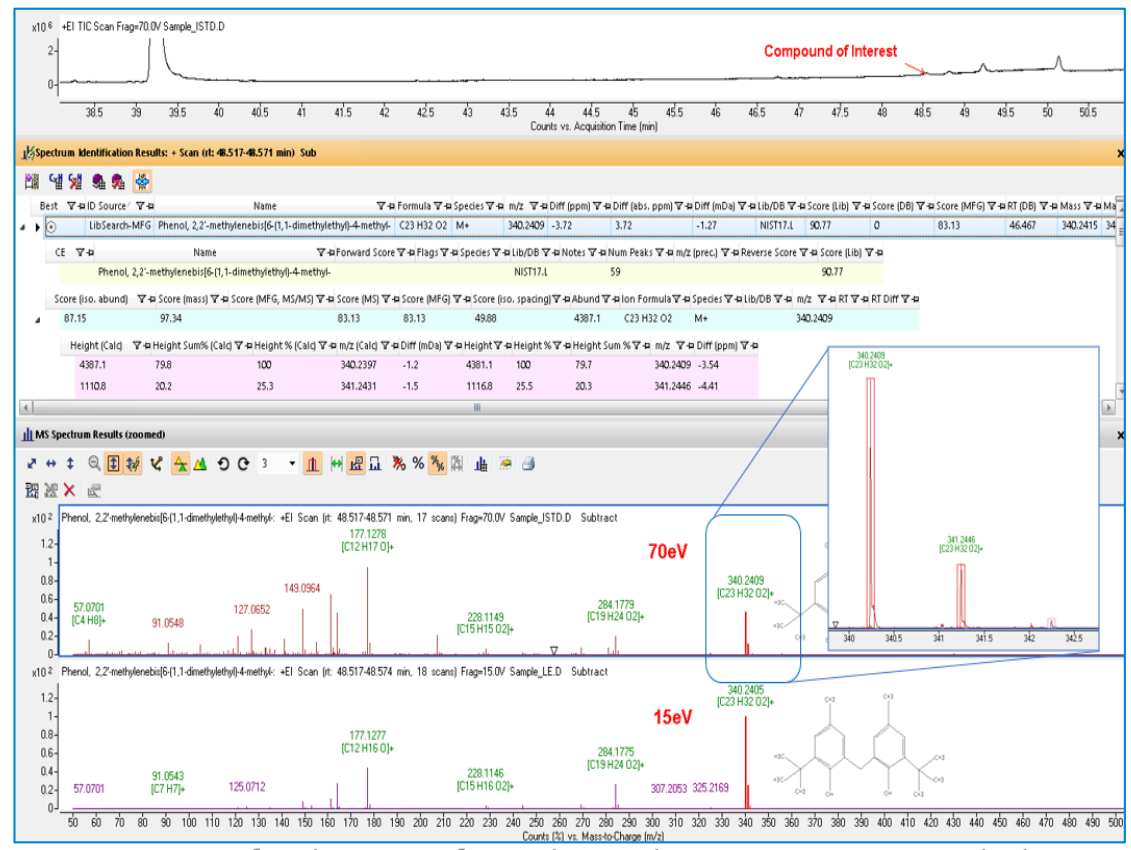

Figure 6: To further confirm the unknown compound, the sample was run using 15eV to enhance the molecular ion and use isotopic fidelity to analyze it's elemental composition. The green annotations provide confirmation of the molecular ion's elemental composition, because the fragments could be produced from the $C_{23}H_{32}O_2$ formula.

Results and Discussion

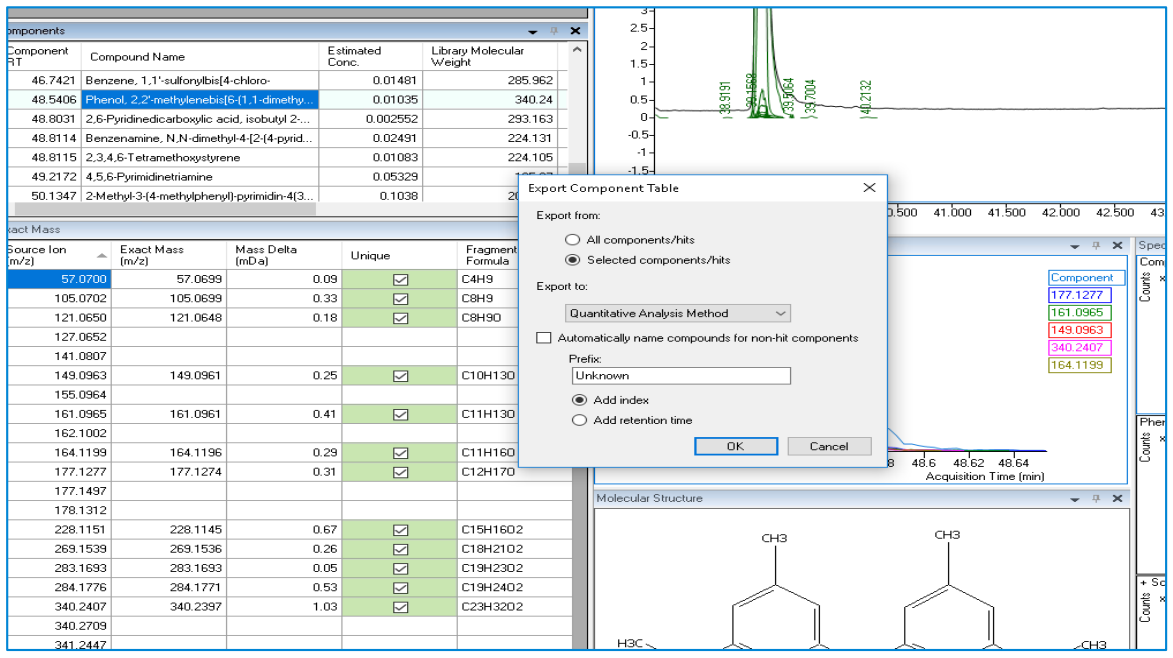

Figure 7: Utilizing the information found through SureMass, library search results, molecular ion confirmation, and fragment ions; this compound can be quickly added to the MassHunter Quant method as a new target compound.

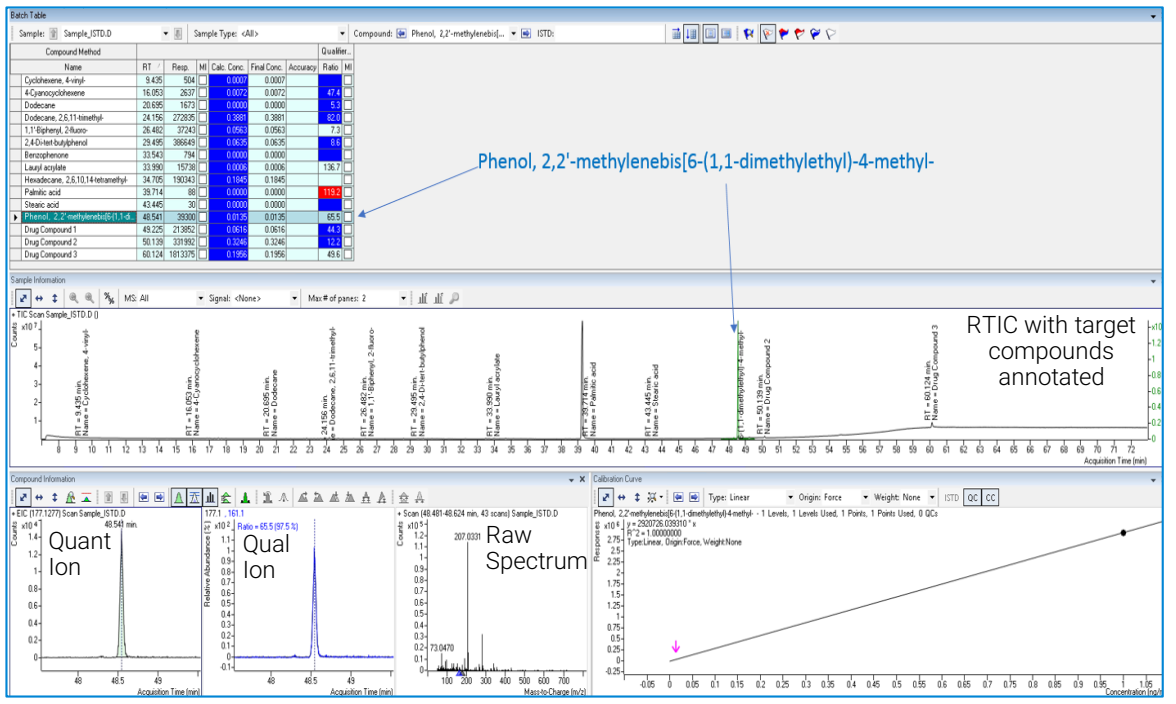

Figure 8: Added compound from figure 7 now provides semi-quant results, with a calculated concentration of 14 pg μL^{-1}.

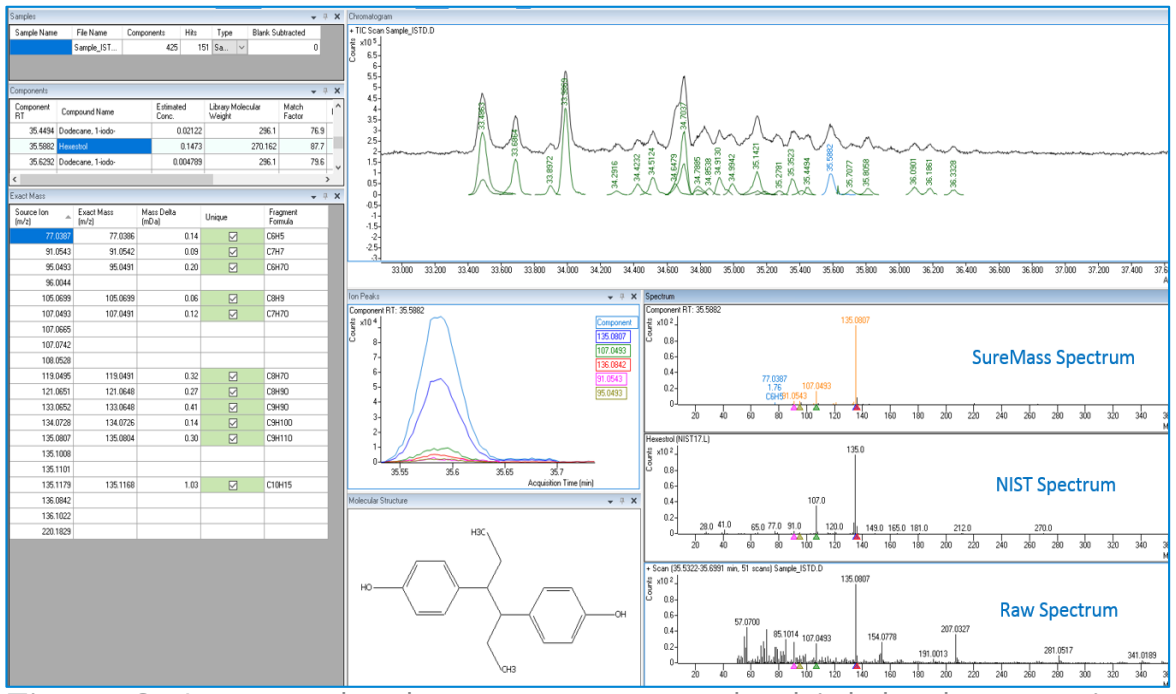

Figure 9: A second unknown compound, which had excessive background interference due to neighboring components and matrix. SureMass provided a library searchable spectrum for only the ions representing the compound of interest.

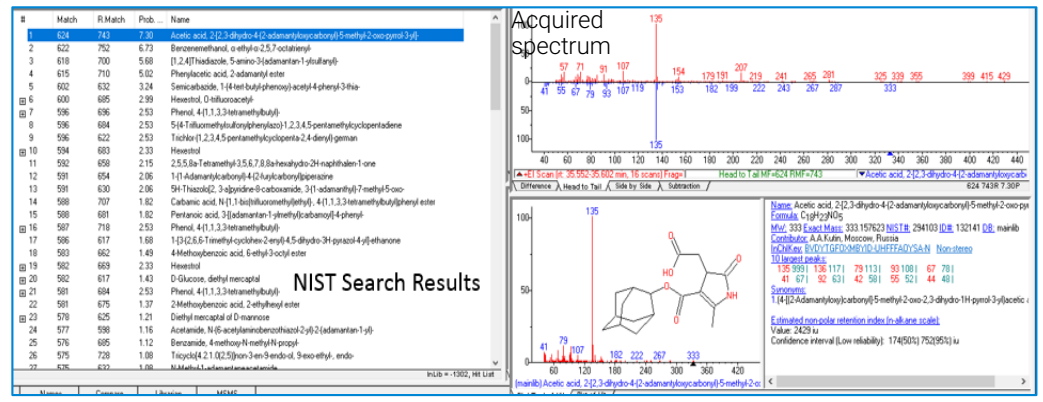

Figure 10: The raw spectrum from figure 9 was imported into the NIST MS Search for a library search identification. The top hit, with a match score of 624, cannot be the compound because the accurate mass does not support this formula. The spectral matching in combination with accurate mass provides a higher confidence in the compound identifications.

Conclusions

- The 7250 QTOF accurate mass data provided crucial information needed to confidently identify unknowns.
- MassHunter allowed for a simplified workflow to help quickly and efficiently identify targets and unknowns.
- SureMass signal processing significantly assisted in confidently finding compounds and ensuring compounds did not go undiscovered.
- Low eV provided additional information related to the molecular ion and confirmation of the elemental composition of the ion representing the intact molecule.
- Quick tools to help append newly discovered compounds of interest into a targets list, enables for workflow adaptability and quick sample screening.

Future Work:

- Collect additional data to create a Personal Compound Database and Library(PCDL) for screening.
- Further optimize and shorten acquisition method for quicker sample analysis and further enhancing results.
- Utilize MassHunter Molecular Structure Correlator (MSC) for structural elucidation.

References

Degenhard Marx, Gerallt Williams and Matthias Birkhoff (June 3rd 2015). Intranasal Drug Administration — An Attractive Delivery Route for Some Drugs, Drug Discovery and Development

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