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S C I E N T I F I C

Improved identification of extractable and leachable substances with Orbitrap Exploris GC 240

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Abstract

Purpose: The goal of this study is to demonstrate the performance of the Thermo Scientific™ Orbitrap™ Exploris™ GC 240 mass spectrometer in combination with Thermo Scientific™ Compound Discoverer™ software to improve detection and identification of extractable and leachable substances.

Methods: Sample preparation involved the extraction of commercially available rubber stoppers for pharmaceutical applications using isopropanol (IPA) and dichloromethane (DCM) at 50 ° C for 72 hours according to the method described in the ISO 10993-12 guideline. Data acquisition was carried out with full scan analysis using EI and PCI with the Orbitrap Exploris GC 240 mass spectrometer.

Results: Using the E&L HRAM mass list within Compound Discoverer software, several compounds matching this molecular weight were identified with mass errors less than 0.01 ppm. A tentative list of 15 additional unknown compounds were detected.

Introduction

In the assessment of potential leachable impurities, controlled extraction studies of polymetric contact materials are performed to provide a worst-case scenario of the potential profile of leachable substances present in the final product. Potential leachable impurities need to be identified, where mass spectrometry is used to assist with structural elucidation. High-resolution accurate-mass (HRAM) GC analysis obtained with Orbitrap technology can provide unsurpassed selectivity (up to 240,000 mass resolution full width at half maximum (FWHM) at m/z 200) and mass accuracy(<1 ppm). This greatly enhances the analysts' capabilities for reliable and accurate identification/structural elucidation in unknown analysis of E&L substances.

This study demonstrates the benefits of incorporating the Orbitrap Exploris GC 240 mass spectrometer for unknown substance identification of sample extracts within an E&L workflow. Statistical differences between samples extracted under different conditions are easily visualized using Compound Discoverer software with simplified identification using both the established NIST and in-house built HRAM libraries. The NeverVent™ technology allows for rapid switching between electron impact (EI) and positive chemical ionization (PCI) for molecular ion confirmation/identification and elemental composition determination with structural elucidation being demonstrated for detected unknown substances.

Materials and methods

Sample Preparation

A screening standard of E&L substances for GC was purchased from Sigma-Aldrich (Germany) to generate HRAM spectra and retention time references for known E&L substances. Sample preparation involved the extraction of commercially available rubber stoppers for pharmaceutical applications using isopropanol (IPA) and dichloromethane (DCM) at 50 ° C for 72 hours according to the method described in the ISO 10993-12 guideline.

Method

Automated liquid injection was performed using a Thermo Scientific™ TriPlus™ RSH SMART autosampler. Data acquisition was carried out with full scan analysis using EI and PCI with the Orbitrap Exploris GC 240 mass spectrometer (table 1).

Data Analysis

Sample acquisition and qualitative processing was performed using Thermo Scientific™ Chromeleon™ version 7.3.2 Chromatography Data System (CDS) software. Unknown analysis and identification performed using Compound Discoverer version 3.3 SP2 software.

Orbitrap Exploris GC-MS parameters	
Transfer line (°C)	290
Thermo Scientific™ ExtractaBrite™ ion source temperature (°C)	320
Electron energy (eV)	70
Acquisition mode and scan range (m/z)	Full scan, 40–650
Resolving power (at 200 m/z)	120,000
Emission current (µA)	50
C-Trap offset (V)	0
Mass accuracy on lock mass	5 ppm
Internal lock mass calibration (column bleed, m/z)	207.02235, 281.05114, 355.06993

Table 1. Orbitrap Exploris GC 240 parameters.

Results

In E&L workflows, compound detection and identification with multiple instrumental techniques provides greater confidence to analysts. Such comparison can be easily facilitated using the new dual sequence evaluation feature within the Chromeleon CDS 7.3.2. An example of this shown in Figure 1, where the response and mass spectrum of 2,4-di-tert-buytlphenol at 1 µg·mL-1 is directly compared between sequence data collected using both (A) GC-HRAM and (B) LC-HRAM techniques. Enhanced sensitivity for the molecular ion is clearly demonstrated when using GC-HRAM, with a response that is 127 times greater than that achieved by LC-HRAM. 2,6-di-tert-butyl-4- methylphenol, which was not detected by LC-MS, elutes just after 2,4-di-tert-butylphenol.
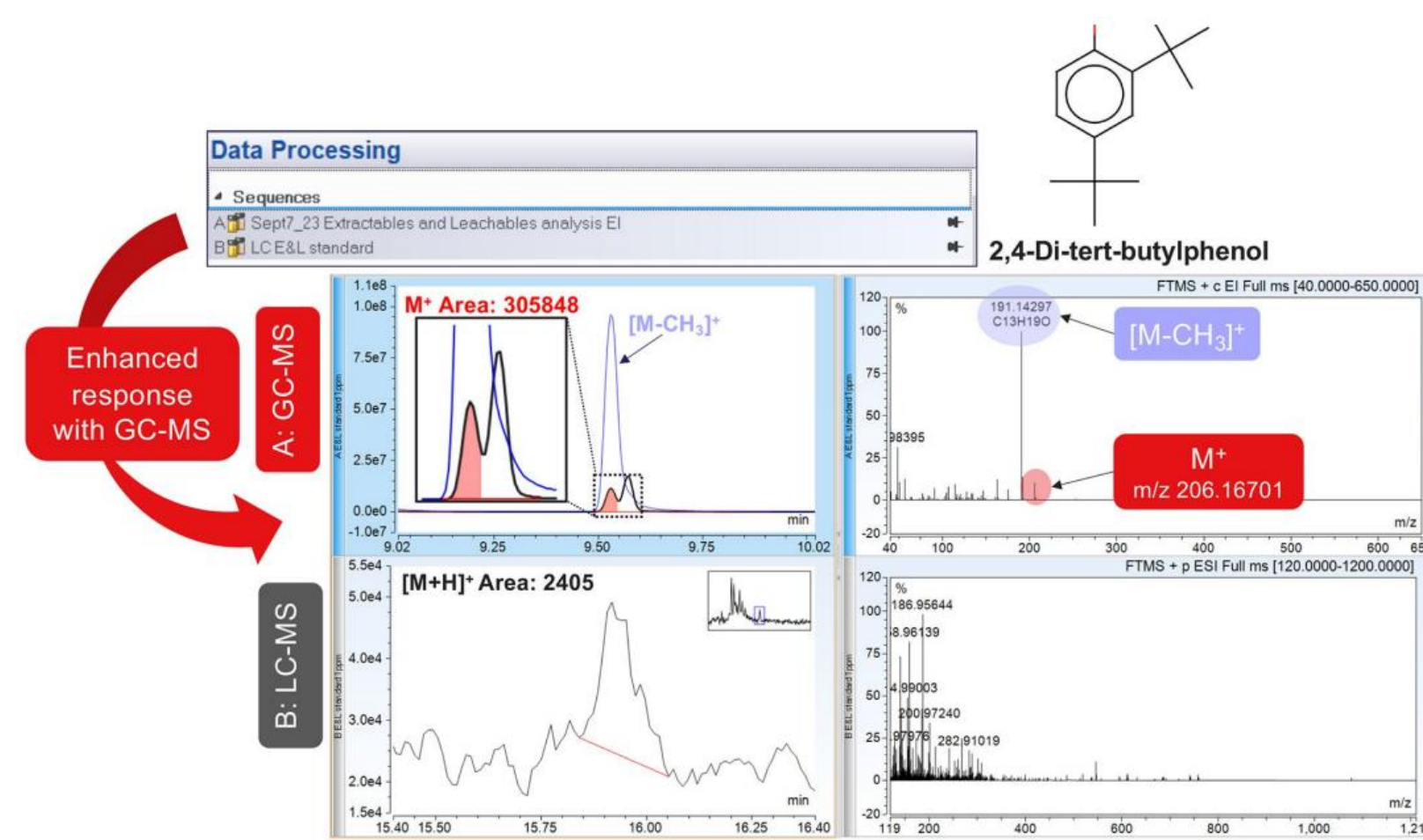

Figure 1. Dual sequence comparison of analyte response and mass spectrum of 2,4-di-tert-buytlphenol at 1 µg·mL-1 collected by GC-MS (sequence A) and LC-MS (sequence B) in Chromeleon CDS 7.3.2.
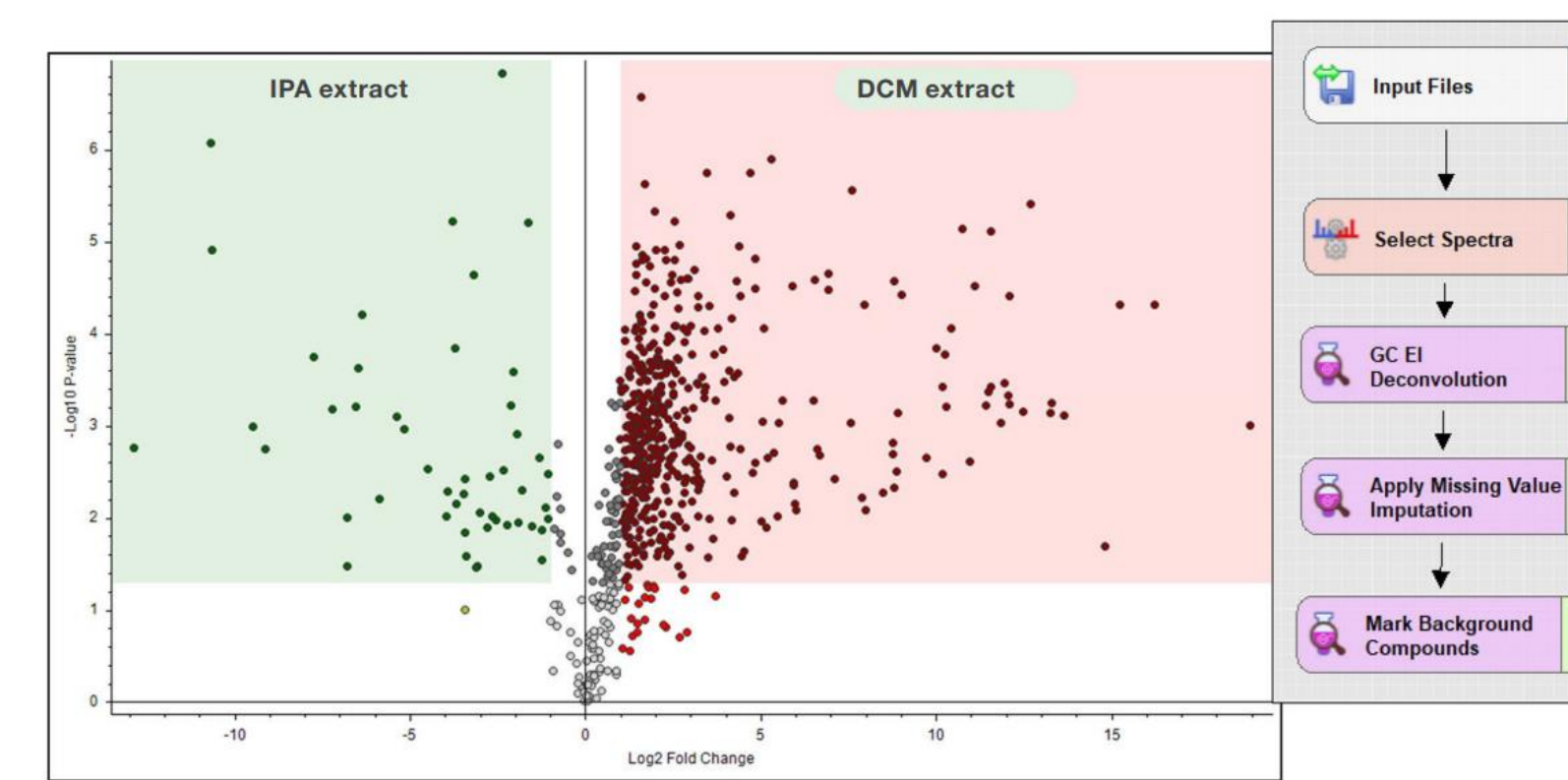

Figure 2 Volcano plot of the unknown compounds (695) identified after spectral deconvolution and background compound removal with the Compound Discoverer software workflow.

Out of a total of 929 compounds detected, 695 unknown E&L substances were identified after background correction from blank analysis using the Mark Background Compounds node within Compound Discoverer software. On visual inspection, greater complexity can be observed in a rubber stopper sample extracted using DCM. Development of HRAM spectra libraries for E&L substances can greatly reduce sample complexity to simplify unknown identification, with new compounds being easily incorporated into the ever-growing E&L HRAM spectral library database.

In Figure 3, where the observed sample mass spectra at 12.07 min matched the E&L HRAM library spectra of 3,5-di-tert-butyl-4- hydroxybenzaldehyde. Despite not being visible from the TIC of the sample extract, the mass spectra of this compound could be easily distinguished from the surrounding background using the deconvolution capability of Compound Discoverer software. Extraction of the exact mass of the molecular ion ([M-e]+ : 234.16144 m/z) provides a clean extracted ion chromatogram of 3,5-di-tert-butyl-4- hydroxybenzaldehyde free of background noise/sample matrix interferences, highlighting the high mass resolving power of the Orbitrap Exploris GC 240 mass spectrometer.
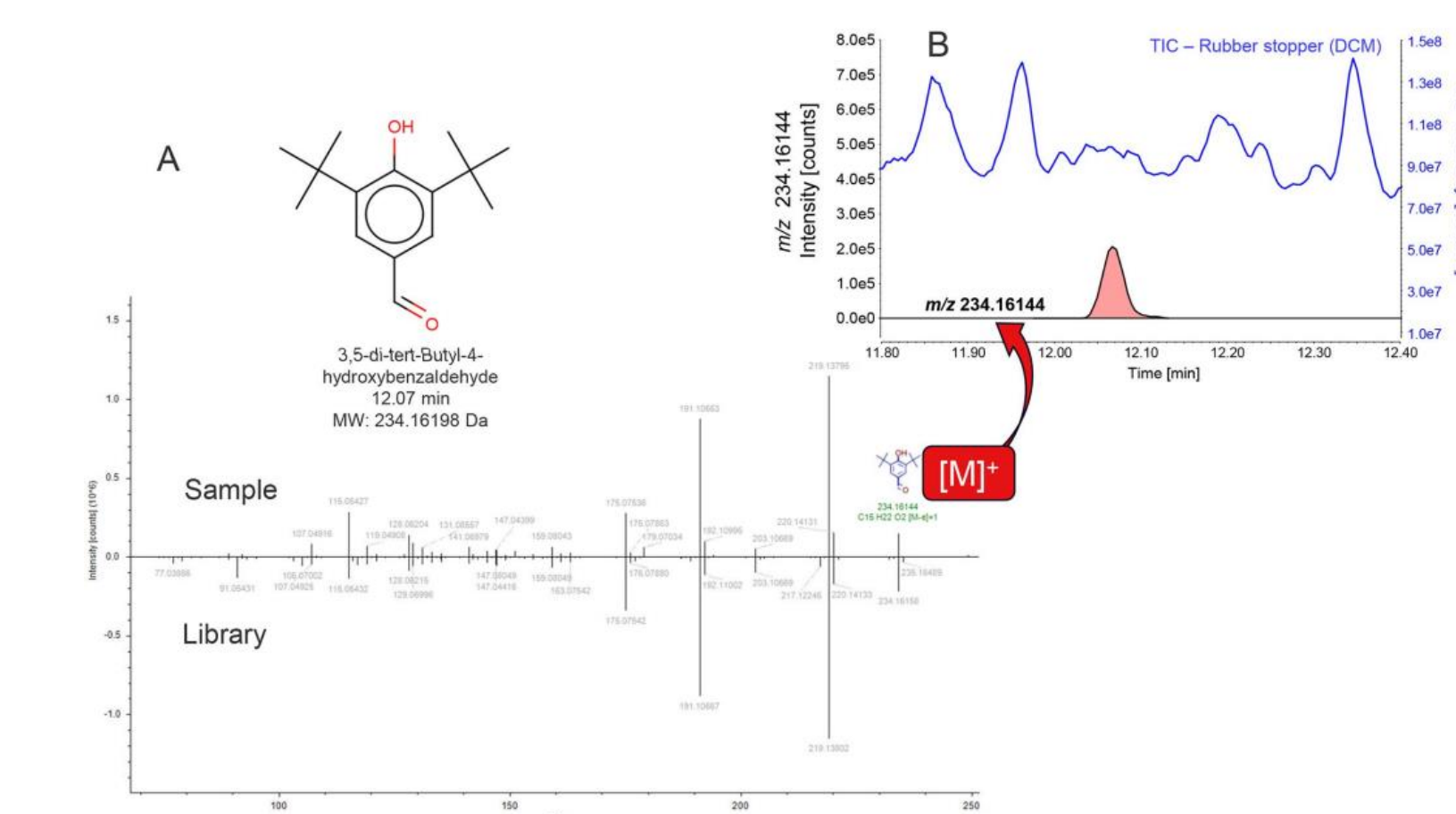

Figure 3 (A) Observed sample mass spectra and matching HRAM library hit of 3,5-di-tert-butyl-4-hydroxybenzaldehyde and (B) total ion chromatogram (TIC) and extracted exact mass of the molecular ion ([M]+: m/z 234.16144) of 3,5-di-tert-butyl-4-hydroxybenzaldehyde
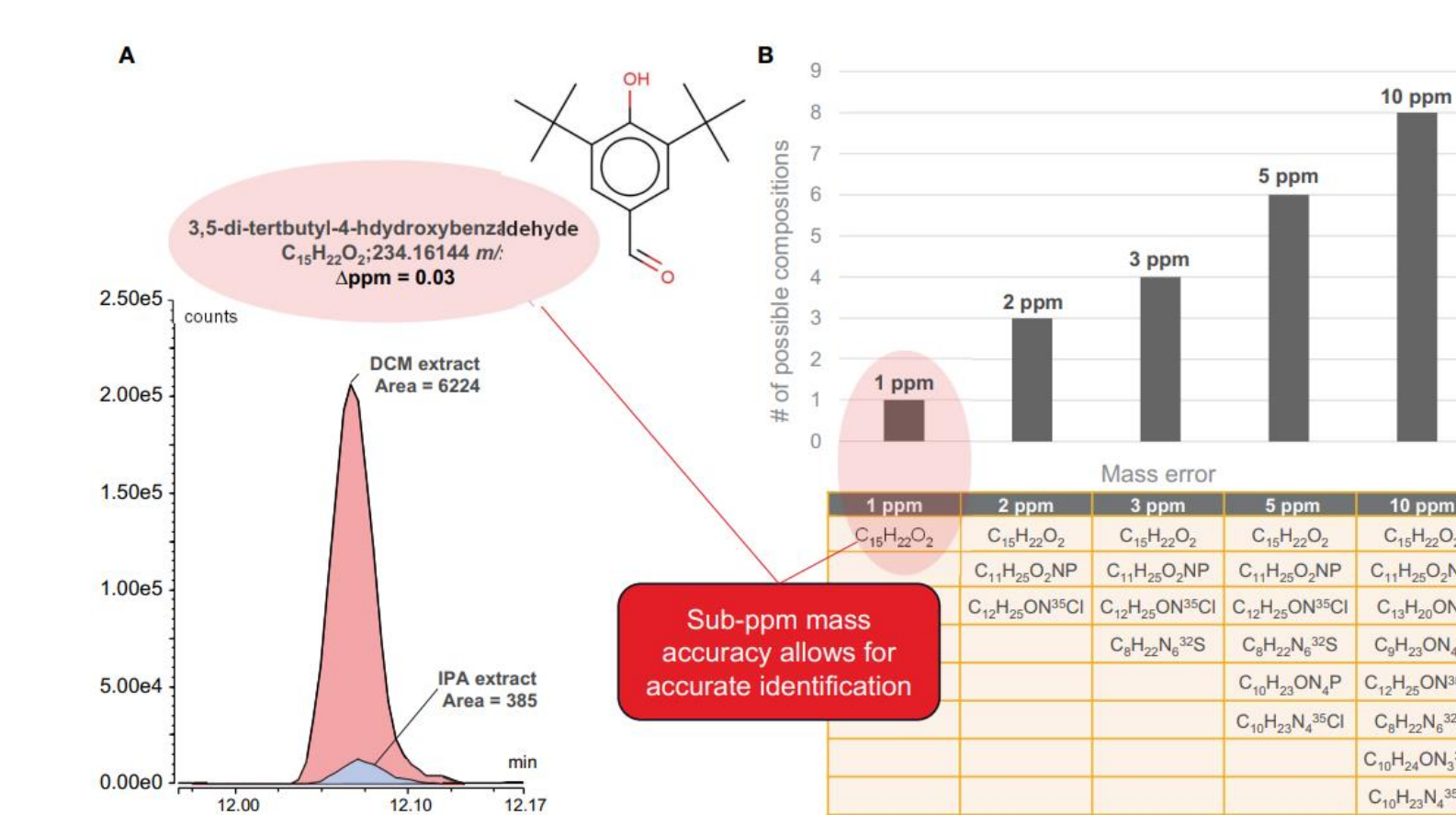

Figure 4 (A) Extracted ion chromatogram and response of molecular ion ([M-e]+: 234.16144 m/z) of 3,5-di-tert-butyl-4-hydroxybenzaldehyde in extractable and leachable sample extracts, (B) Number of possible elemental compositions for 3,5-di-tert-butyl-4-hydroxybenzaldehyde molecular in relation to mass error.

High mass resolving power, sub-ppm mass accuracy provides identification confidence. For example, in the case of 3,5-di-tert-butyl-4-hydroxybenzaldehyde, the observed molecular ion showed a mass error of 0.03 ppm from the theoretical mass (Figure 5). Based on the number of possible elemental compositions, only C15H22O2 can be associated with this mass at <1 ppm mass error.

After the deconvolution process and library comparison, the compound eluting at 14.02 min was initially identified as hexadecanoic acid. But did not agree with the retention time of the standard. Detection of the molecular ion will greatly assist in compound identification. In positive chemical ionization using methane as a reagent gas, Compound Discoverer software automatically identified the [M+H]+ (m/z 285.27881) and methane adduct ion of the molecular ion [M+C2 H5] + confirming the molecular weight of this unknown to be 284.27153 Da, (Figure 6).
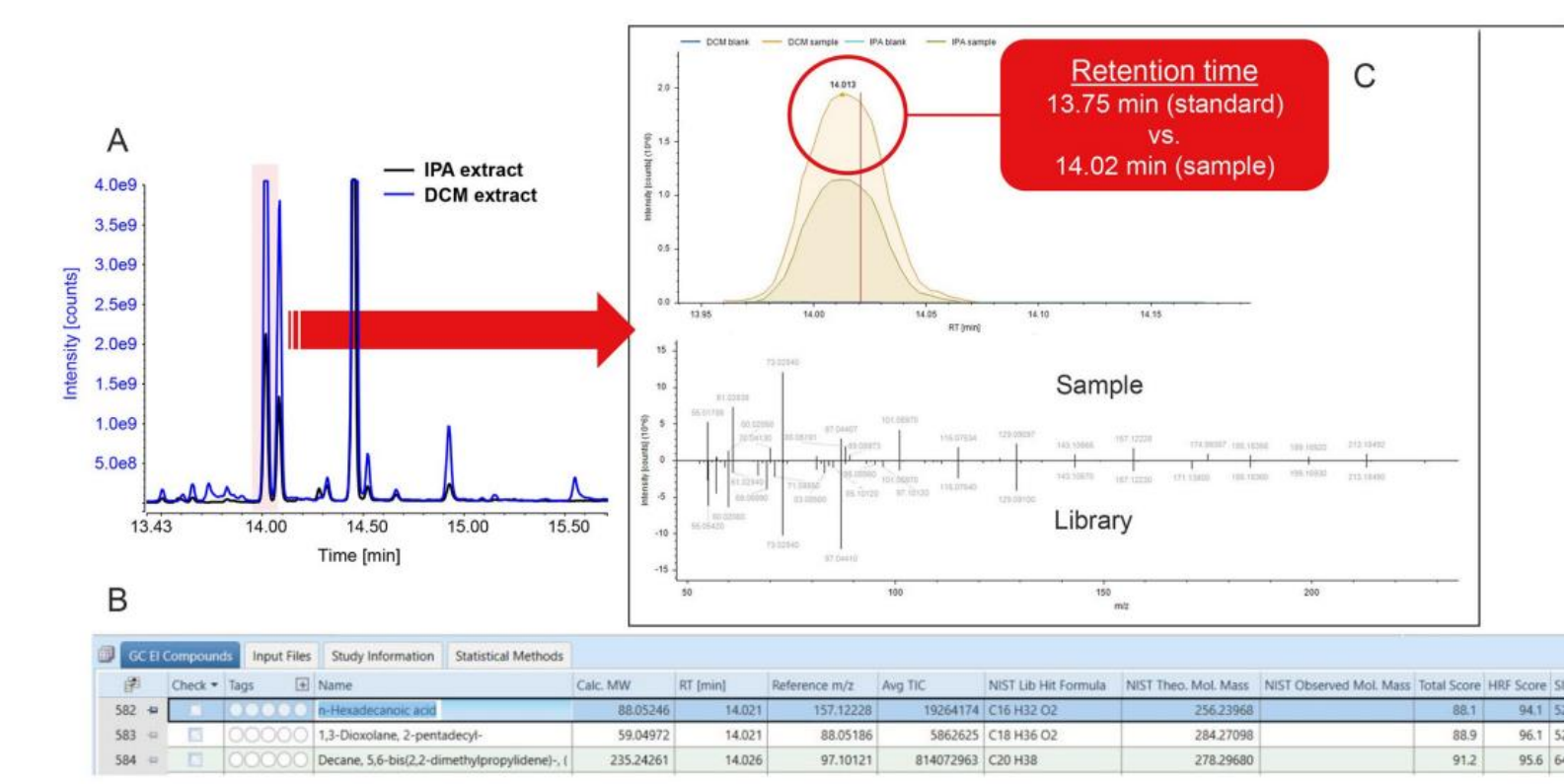

Figure 5 Total ion chromatogram overlay of IPA and DCM sample extracts between 13.4 and 15.7 min (A), proposed identification based on library match search (B), and deconvoluted spectrum with head to tail mass spectrum comparison between sample and library match for peak at 14.02 min (C)
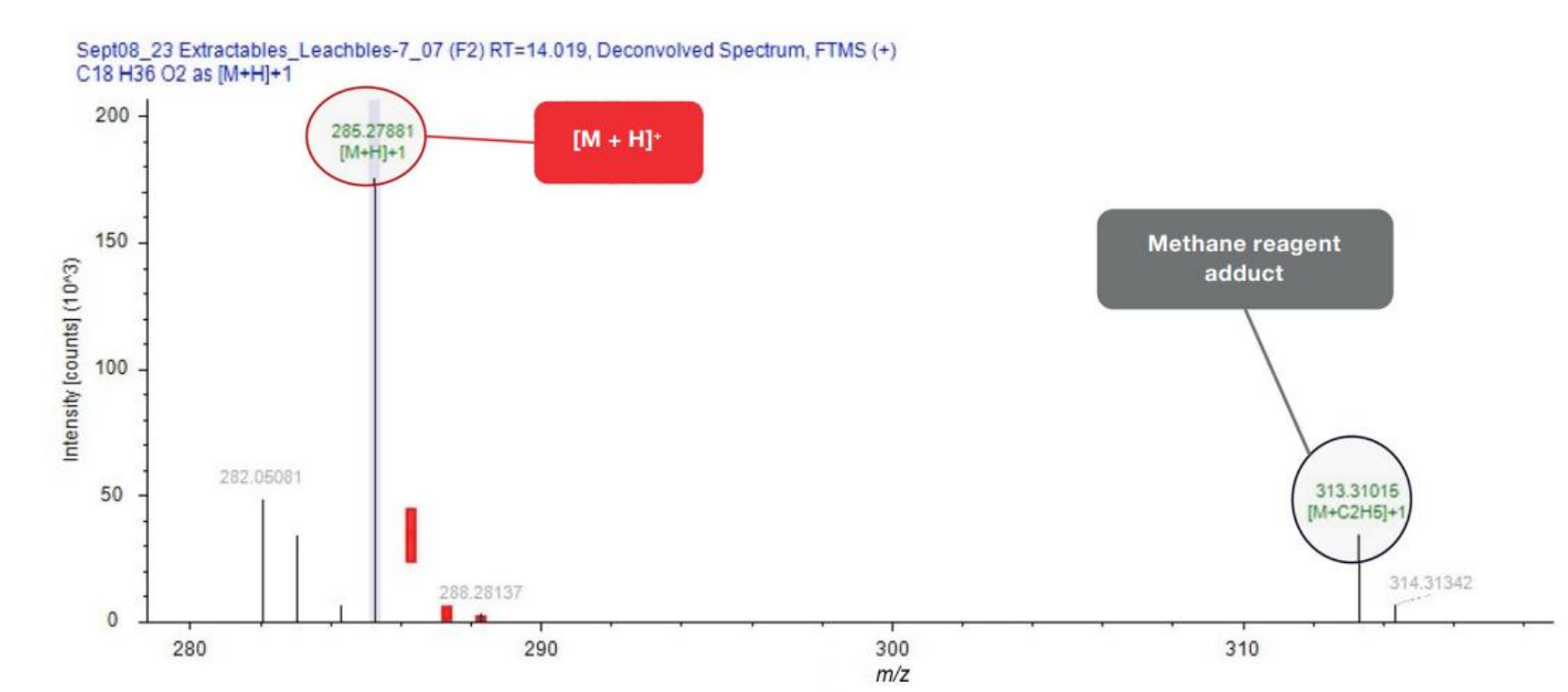

Figure 6. PCI mass spectra of compound eluting at 14.02 min with molecular ion ([M+H]+) and methane reagent adduct ([M+C2H5] +) detected for molecular weight confirmation.

Using the E&L HRAM mass list within Compound Discoverer software, several compounds matching this molecular weight were identified with mass errors less than 0.01 ppm (Figure 7A).several ions that correspond to specific fragments of ethyl palmitate (Figure 7B), providing additional confirmation on the identify of this compound.
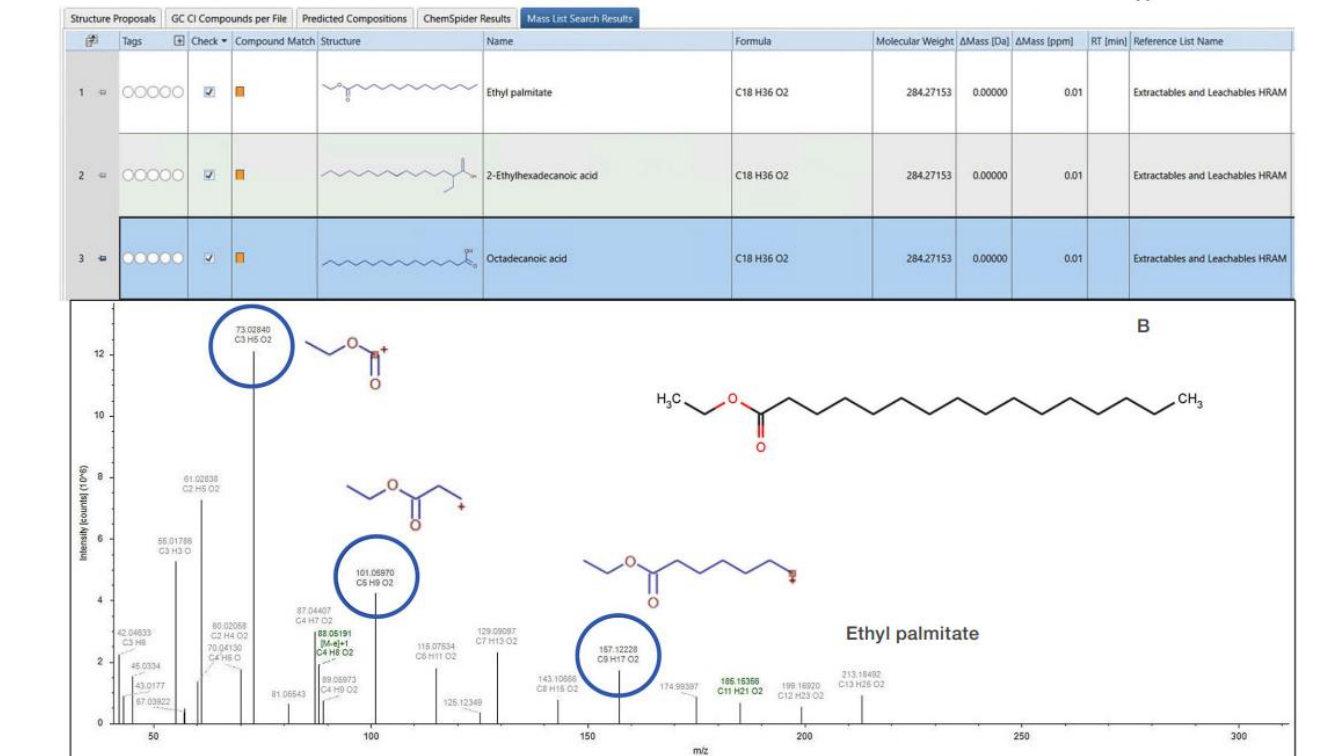

Figure 7. Compound Discoverer software proposed candidates from E&L HRAM mass list (A) and EI mass spectrum of unknown compound eluting at 14.02 min (B)

Conclusions

- GC combined with HRAM provides enhanced sensitivity for detection of E&L substances with poor response using LCHRAM techniques

- Spectral deconvolution with Compound Discoverer software combined with dedicated E&L HRAM spectra libraries simplifies identification, providing confidence in unknown identification.

- High selectivity from mass resolving power helps facilitate compound detection within complex sample matrix with sub-ppm mass accuracy, providing confident compound identification.

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