

Analysis of Extractable and Leachable (E&L) Compounds Using a Low-Energy EI-Capable High-Resolution Accurate Mass GC/Q-TOF

Application Brief

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Introduction

Accurate compound identification is critical to the study of extractables and leachables (E&L) [1]. The complexity of E&L extracts, containing chemicals with a wide range of classes and concentrations, poses challenges for compound identification [2]. The GC-amenable portion of E&L studies is conventionally carried out with a unit mass GC/MS in standard EI full scan mode, with compound identification through NIST GC/MS library searching. Limited knowledge can be obtained from this technique for those compounds detected without a convincing library match score.

This work presents a novel tool to study E&L compounds with enhanced flexibility and confidence using a high-resolution accurate mass GC/Q-TOF equipped with a low-energy EI capable ion source.



Figure 1. Agilent 7250 Series GC/Q-TOF system.



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Experimental

Instrumental analysis

The sample extracts and controls were analyzed by an Agilent 7250 Series GC/Q-TOF system (Figure 1), with operational conditions listed in Table 1. An injection of *n*-alkanes was used to calibrate the retention index (RI) of the acquisition method.

Table 1. Agilent 7250 GC/Q-TOF Operational Conditions

Parameter	Value
Column	Agilent DB-5 MS UI, 15 m × 0.25 mm, 0.25 μm
Inlet	S/SL, 310 °C
Carrier gas	1.5 mL/min Helium
Oven program	50 °C for 5 minutes 10 °C/min to 320 °C, 10 minutes
Transferline	280 °C
Source mode	EI, 70 eV, 10-15eV
Source temperature	200 °C
Quad temperature	150 °C
Spectral range	50 to 1,000 <i>m/z</i>

Data analysis

Compound identification started with Agilent MassHunter Unknowns Analysis B.08.00 using SureMass signal processing [3] and matching against the NIST 14 GC/MS library (Figure 2). The formulas of identified compounds were studied by comparing the standard EI and low energy EI spectra. Agilent MassHunter Qualitative Analysis B.08.00 was used to review MS and MS/MS mass spectra when necessary. The MS/MS spectra-based structure elucidation of the candidates for the unknowns was performed using Agilent MassHunter Molecular Structure Correlator B.08.00. Agilent Mass Profiler Professional (MPP) B.13 was used for differential analysis among sample groups.

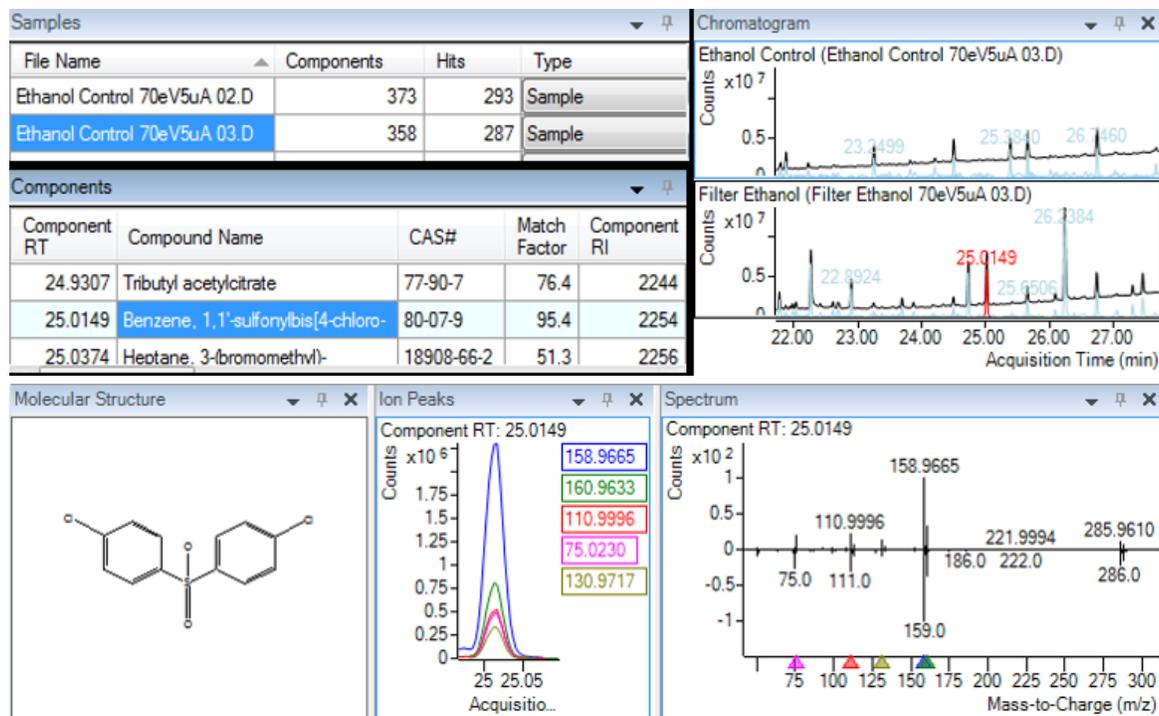


Figure 2. Agilent MassHunter Unknowns Analysis software for SureMass peak detection and library matching.

Sample preparation

A fully assembled single-use bioprocessing system was extracted using flow-through extraction with saline solution at 37 °C for 72 hours. The saline solution was prepared by adding one phosphate buffered saline tablet (Sigma) to each 200 mL of distilled water, resulting in a 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer solution (pH 7.4 at 25 °C). The filter of the device was extracted using ethanol and water/ethanol (1:1) solutions to demonstrate the difference between extraction solvents. Control blanks were prepared for all the extraction experiments. Each extract solution (except ethanol) was extracted with equal volume of dichloromethane, then concentrated 10 times for GC/Q-TOF analysis.

Results and Discussion

Saline extract versus control blank

We used MPP software to perform the differential analysis between sample and control, with saline extract results shown as a representative data set. The results indicate that 113 compounds present in saline extract of the complete device with a fold change ≥ 3 and a p-value ≥ 0.05 compared to the control blank (Figure 3). Table 2 shows the most abundant components.

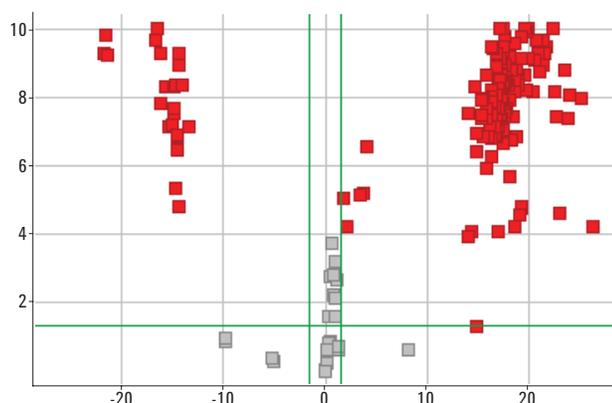


Figure 3. Volcano plot revealing compounds significantly present in the saline extract (upper right).

Table 2. Compound Identification List of Saline Extract (Top List)

Compound	Formula*	RI	Mass diff. (mDa)
Caprolactam	C ₆ H ₁₁ NO	1,268	0.2
Phenol	C ₆ H ₆ O	978	0.0
Tri(1,2-propyleneglycol), monomethyl ether	C ₁₀ H ₂₂ O ₄	1,315	0.0
Dowanol 62b isomer 1	C ₁₀ H ₂₂ O ₄	1,291	-0.2
Dowanol 62b isomer 2	C ₁₀ H ₂₂ O ₄	1,294	-0.2
Dowanol 62b isomer 3	C ₁₀ H ₂₂ O ₄	1,289	0.0
Tentative ID compound	C ₉ H ₁₂ O ₄	1,572	0.5
Dowanol 62b isomer 4	C ₁₀ H ₂₂ O ₄	1,286	-0.1
Benzoic acid, 4-ethoxy-, ethyl ester	C ₁₁ H ₁₄ O ₃	1,527	0.1
Tentative ID compound	C ₁₂ H ₁₅ N ₃ O ₃	1,659	0.2
Vanillin	C ₈ H ₈ O ₃	1,399	-0.1
Hexanamide	C ₆ H ₁₃ NO	1,144	-0.2
Tentative ID compound	C ₈ H ₁₂ O ₃	1,403	0.1
7,9-Di- <i>tert</i> -butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	1,908	-0.2
Tentative ID compound	C ₁₅ H ₂₂ O	1,476	0.4
Ethylparaben	C ₉ H ₁₀ O ₃	1,522	0.2
2-Pyrrolidinone, 1-methyl-	C ₅ H ₉ NO	1,040	0.3
2,4-Di- <i>tert</i> -butylphenol	C ₁₄ H ₂₂ O	1,507	0.0
Tentative ID compound	C ₈ H ₈ O	1,069	-0.2
2-Imidazolidinone, 1,3-dimethyl-	C ₅ H ₁₀ N ₂ O	1,109	0.3
Acetamide, N-cyclohexyl-	C ₈ H ₁₅ NO	1,292	0.2
Butoxyethoxyethanol	C ₈ H ₁₈ O ₃	1,187	-0.2
Di- <i>t</i> -butylhydroquinone	C ₁₄ H ₂₂ O ₂	1,467	0.0
2-Phenylisopropanol	C ₉ H ₁₂ O	1,088	-0.3
Tentative ID compound	C ₅ H ₁₂ O ₂	1,014	0.1
Benzothiazole	C ₇ H ₅ NS	1,232	0.2
Dimethyl phthalate	C ₁₀ H ₁₀ O ₄	1,452	0.1
Tentative ID compound	C ₁₃ H ₂₀ O ₂	1,349	0.5

* Formulae of identified compounds were confirmed (or proposed for tentative ID compounds) by comparing the spectra from standard EI and low-energy EI modes.

Impact of extraction solvent

The filter extracts were evaluated to study the impact of using different extraction solvents on the overall extractable profile (Figure 4). The Venn diagram enables the easy visualization of these results, and shows both the unique as well as common extractables detected in each extract.

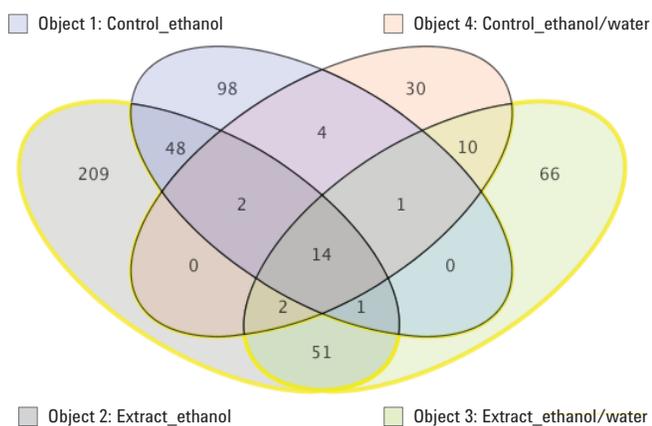


Figure 4. Venn diagram of extractable compounds from the filter of the device extracted by different solvents.

Low-energy EI investigation

Low-energy EI experiments increase the possibility of preserving or confirming the molecular ion (M^+) in the spectrum, as shown in Figure 5. These experiments can offer additional insights into identifying tentative compounds when the library search result is not promising.

Figure 6 illustrates the workflow to study an unknown compound (common between two solvent extraction groups) with low-energy EI and Q-TOF MS/MS. The possible candidate is a benzenemethanol derivative.

Figure 7 shows that the low-energy EI spectra also helped to confidently identify many alkane compounds unique to the ethanol extract.

Conclusions

- Low-energy EI increases the possibility of preserving or confirming M^+ , and accurate mass MS/MS spectra provide valuable insights into structure elucidation of unknown compounds.
- Accurate mass measurements and RI calibration can enhance confidence in compound identification.
- Differential analysis facilitates the comparison of E&L compounds among sample groups.

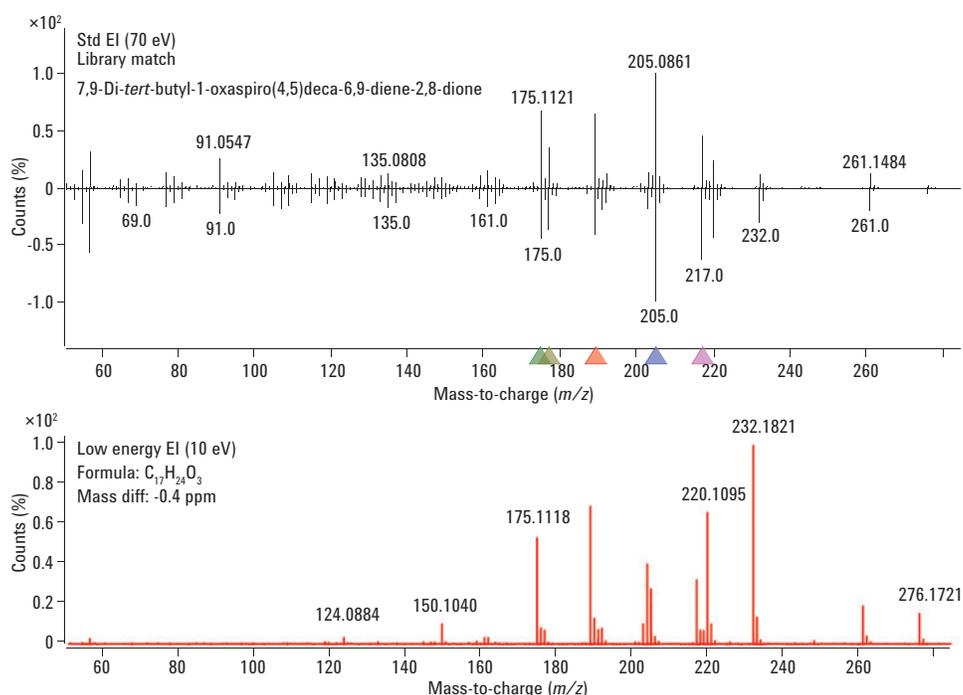
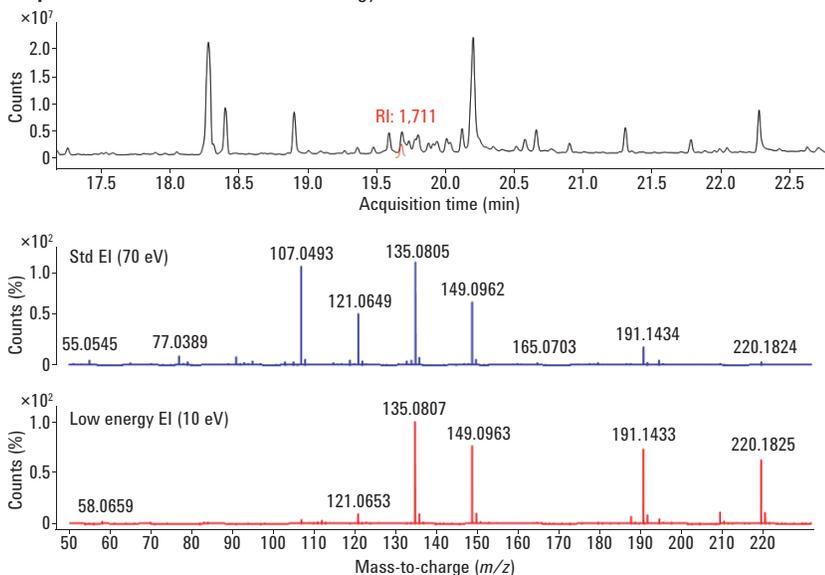
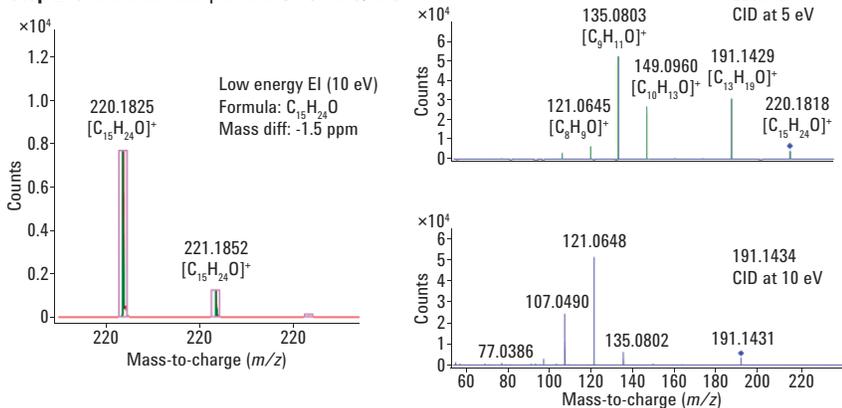


Figure 5. Low-energy EI increases the relative abundance of M^+ in the spectrum of a compound confidently identified with match score of 92.6 (RI: 1908).

Step 1: Measure unknowns with low energy EI source



Step 2: Confirm M⁺ and perform Q-TOF MS/MS



Step 3: Structure elucidation on possible candidate

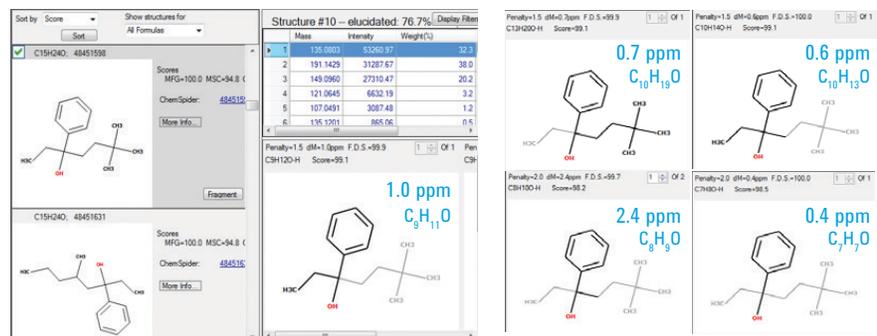


Figure 6. Study of an unknown compound with low-energy EI and structure elucidation on a possible candidate using Agilent MassHunter Molecular Structure Correlator.

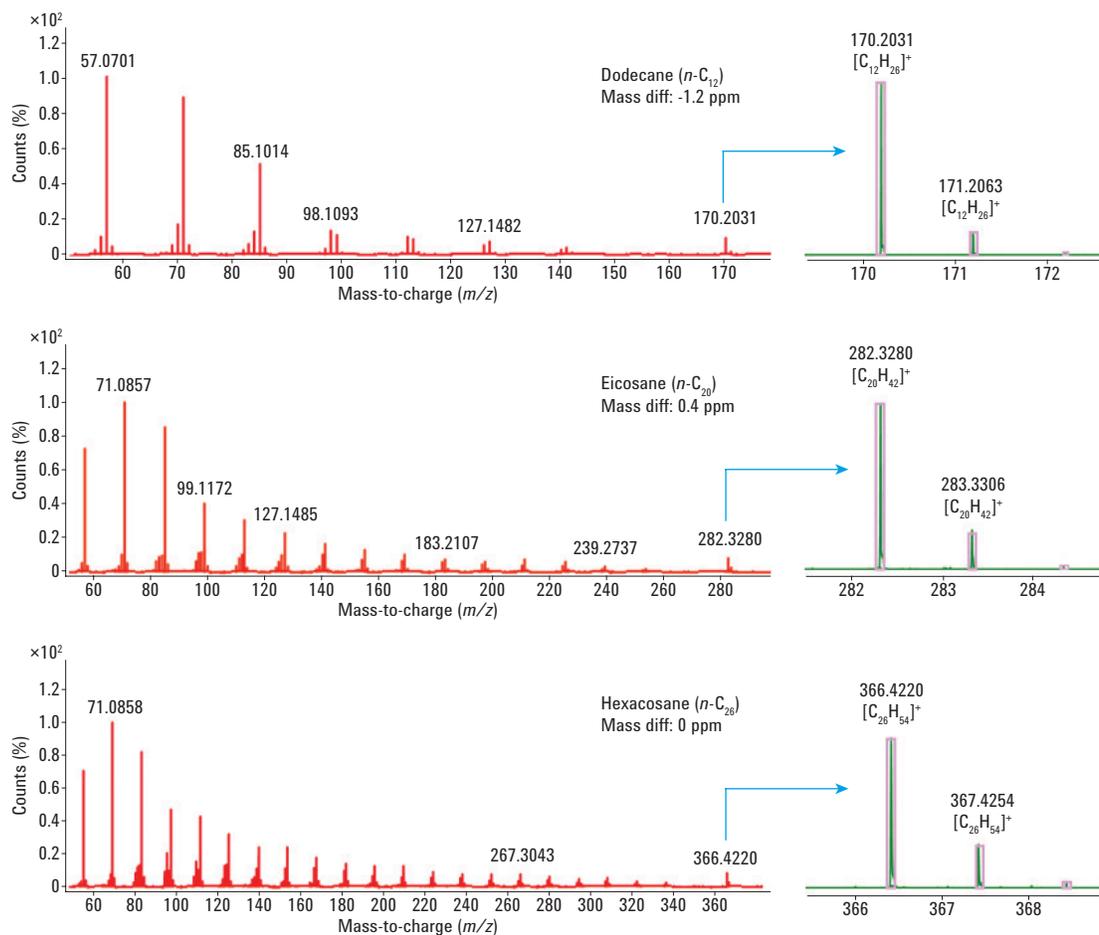


Figure 7. Low-energy EI (12 eV) spectra of *n*-alkanes. M^+ clusters show good mass accuracy and isotopic fidelity.

References

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