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GC/MS Approach for Analysis of Extractables and Leachables (E&L) in Complex Matrices Using Spectral Deconvolution and Retention Indices

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Introduction

Modern drug delivery systems are meant to protect a drug from environmental contamination, but they may also be a source of contamination. It is necessary to identify those compounds that can extract, leach, or migrate from the package or device. Extractables and leachables analysis presents a challenge for GC/MS because of the complexity of the various sample matrices and a diverse range of compounds to be identified. This study demonstrates how unit resolution GC/MS workflow is employed for identifying GC-amenable E&L compounds by leveraging spectral deconvolution in combination with retention index-based time filtering. An addition of the accurate mass high-resolution GC/Q-TOF into the workflow provided extra confidence in compound identification, sensitivity and capability of structural elucidation.

Operating in the OpenLab Electronic Content Management (ECM) XT configuration enabled tools that help facilitate compliance with various national and EU electronic record regulations, including audit trails, and remote data storage.

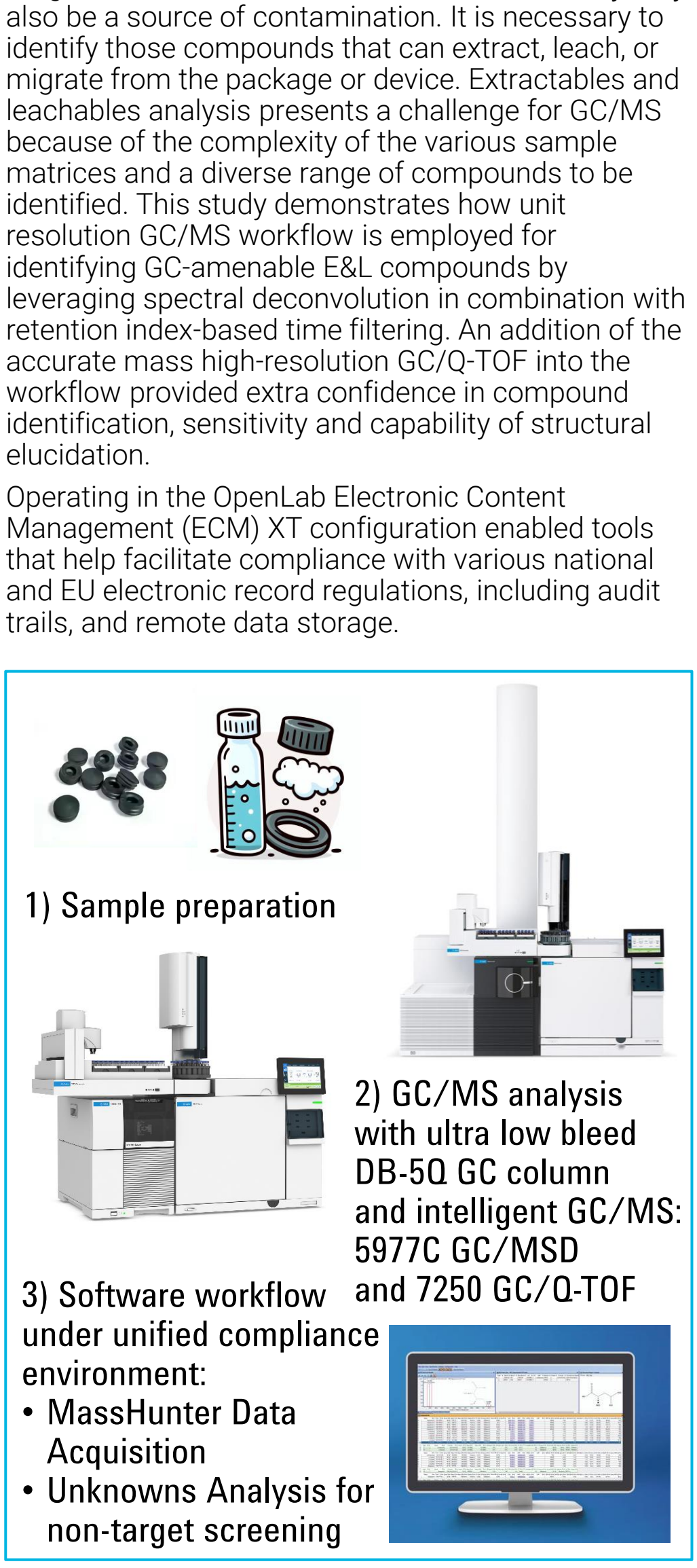

Figure 1. Complete GC/MS workflow for E&L including 5977C GC/MSD and 7250 GC/Q-TOF.

Experimental

Rubber syringe gaskets were extracted using tetrahydrofuran (THF) solvent at room temperature for six months. An aliquot of the extracts was analyzed using both GC/MSD and GC/Q-TOF systems. The acquisition software operated under a unified compliance environment. The instrumental parameters are shown in Table 1. The chromatographic deconvolution and library search were performed in the MassHunter Unknowns Analysis 12.1. The NIST23 library was used to perform initial compound identification. Structural elucidation was performed using Molecular Structure Correlator (MSC) software 8.2.

Retention time locking was used to achieve the same retention times between multiple GC/MSD and GC/Q-TOF systems allowing for using both retention index and retention time matching.

Parameter	Value
MS	Agilent 5977C GC/MSD and Agilent 7250 GC/Q-TOF
GC	Agilent 8890 GC
Column	Agilent J&W DB-5Q*, 30 m, 0.25 mm, 0.25 µm
Inlet	Multimode inlet, 4 mm Ultra Inert liner, single taper with wool
Injection volume	1 µL
Injection mode	Pulsed splitless (1 min, pulse @ 40psi for 1.1 min)
Inlet temperature program	65 °C for 0.01 min, 300 °C/min to 280 °C
Oven temperature program	45 °C for 2 min; 12 °C/min to 325 °C, 11 min hold
Carrier gas	Helium
Column flow	1 mL/min constant flow
Transfer line temperature	325 °C
Quadrupole temperature	150 °C
Source temperature	200 °C (Q-TOF)/300 °C (MSD)
Electron energy	70 eV (Standard EI); 15 eV, 12 eV and 10 eV (Low Energy EI) (Q-TOF)
Emission current	5 µA (Standard EI); 0.3 µA (Low Energy EI)
Spectral acquisition rate	5 Hz (Q-TOF), 2 Hz (MSD)
Mass range	50 to 1000 *m/z* (Q-TOF), 45 to 450 *m/z* (MSD)

* - available in August 2024

Table 1. GC/MS method parameters

Injection conditions were optimized to maximize the response for both low- and high-boiling compounds as shown with the alkane ladder in Figure 2.

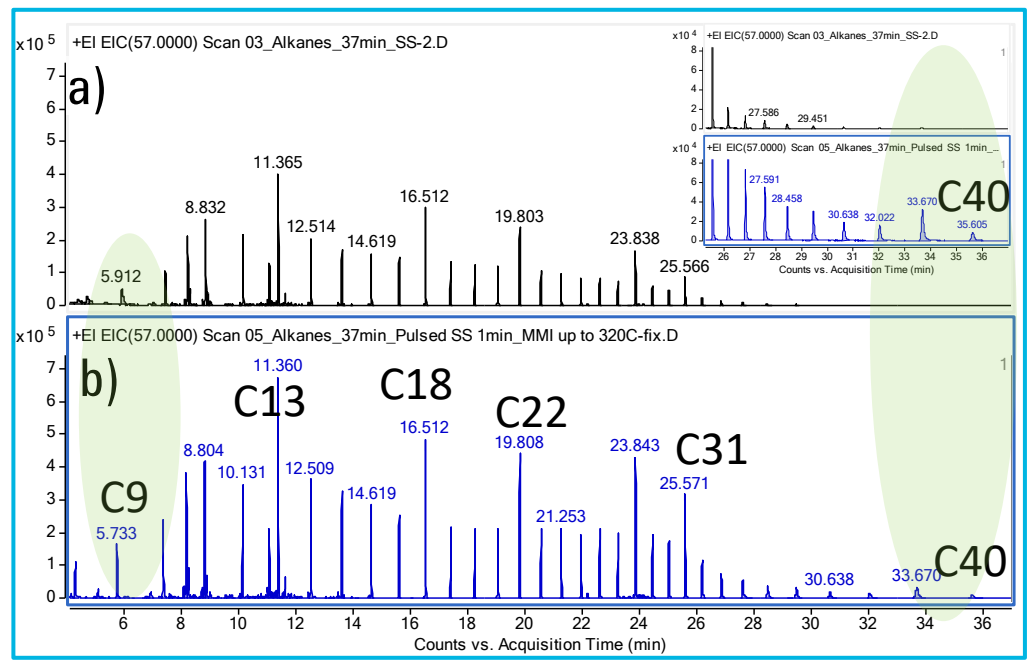

Figure 2. EIC (*m/z* 57) of an n-alkane ladder analyzed under the starting (a) and optimized (b) conditions.

GC Method Development

For GC/MS analysis both 30 m 0.25 mm x 0.25 µm and 20 m 0.18 mm x 0.18 µm DB-5ms UI columns have been evaluated with respect to the chromatographic separation capability of the complex E&L extracts as well as sensitivity. The GC methods have been optimized for each column. While the 20 m column provided sharper peaks and greater sensitivity for trace-levels compounds, 30 m column offered better separation with higher number of components been reliably identified.

The new Agilent J&W DB-5Q column (available starting August 2024) has demonstrated significant decrease in column bleed at high oven temperatures (Figure 3) and was selected for further experiments.

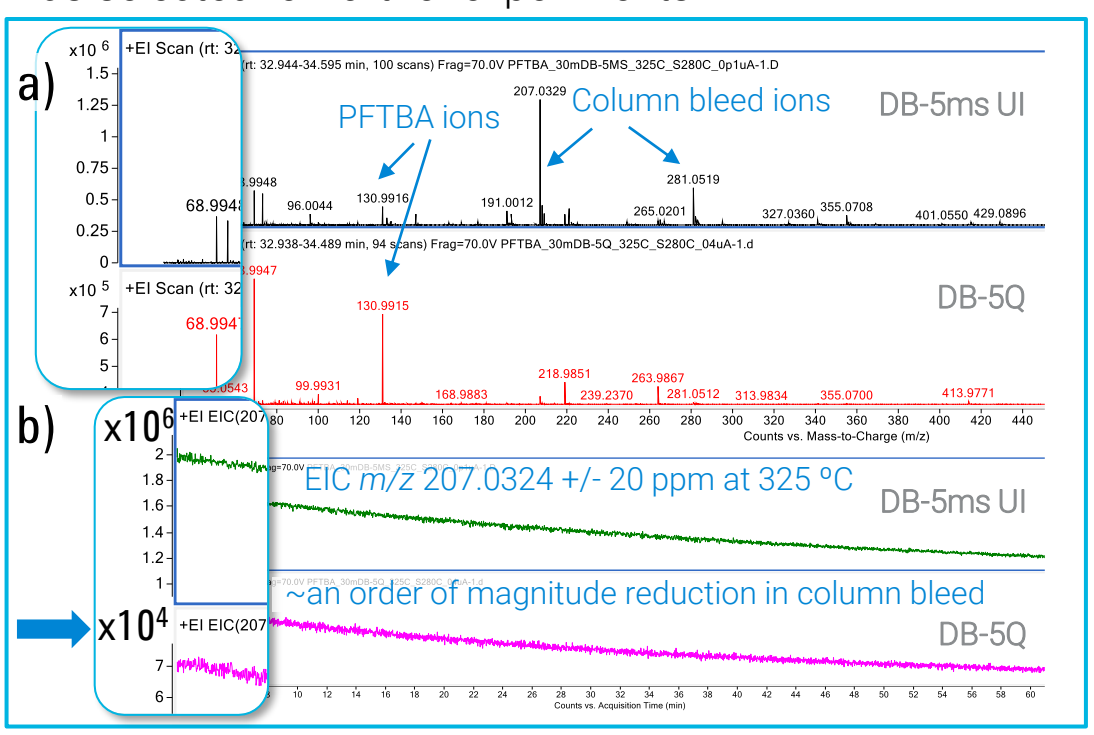

Figure 3. Agilent J&W DB-5ms (top) and DB-5Q (bottom) columns comparison on the GC/Q-TOF. Emission current was selected to yield equal PFTBA abundance, and PFTBA signal was acquired at 325ºC oven temperature. A) PFTBA spectrum. B) EIC of one of the major column bleed ion 207.

Over 100 compounds were initially identified in the sample with GC/MSD by searching deconvoluted spectra against NIST23 and filtering the results based on the retention indices. Figure 4 shows an example of an identified compound, eicosyl acetate, in the presence of coeluting components with a high library match score and excellent RI matching.

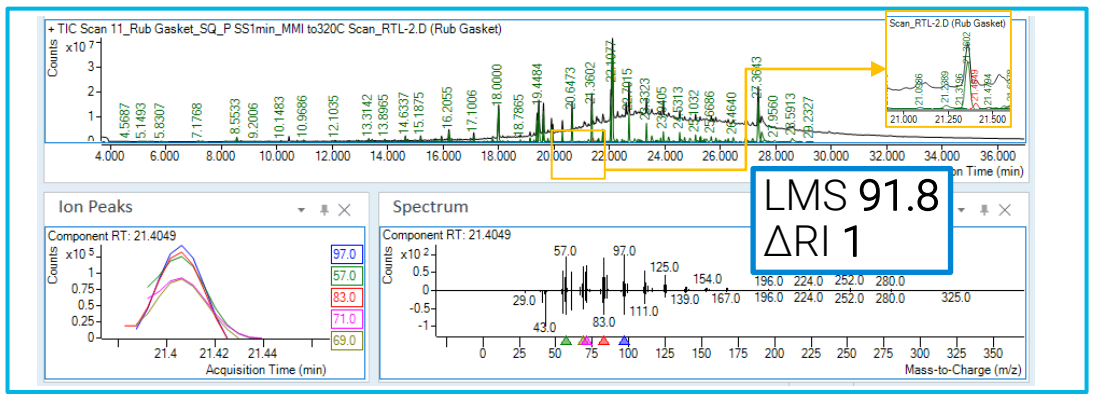

Figure 4. TIC of rubber gasket sample and deconvoluted spectrum for eicosyl acetate.

Compounds Identified in Rubber Gasket Extract by Both GC/MSD and GC/Q-TOF

Over 80 compounds, with selected ones shown in Table 2, were identified by both GC/MSD and GC/Q-TOF.

RT	Compound Name	Formula	CAS#	RT	Compound Name	Formula	CAS#
4.48	Butanoic acid	C4H8O2	107-92-6	15.39	(1-Ethylnonyl)benzene	C17H28	4536-87-2
5.11	Dipropyl acetal	C8H18O2	105-82-8	15.56	n-Hexyl salicylate	C13H18O3	6259-76-3
5.68	N-Ethylacetamide	C4H9NO	625-50-3	15.62	3-Pentadecanone	C15H30O	18787-66-1
5.75	Pentanoic acid	C5H10O2	109-52-4	15.74	4-(1,1-Dimethylheptyl)phenol	C15H24O	30784-30-6
7.13	Hexanoic acid	C6H12O2	142-62-1	15.82	4-(7-Methyloctyl)phenol	C15H24O	24518-48-7
7.15	Glycerin	C3H8O3	56-81-5	15.93	1-Phenyl-1,3,3-trimethylindane	C18H20	3910-35-8
7.22	Phenol	C6H6O	108-95-2	16.20	Tetradecanoic acid	C14H28O2	544-63-8
8.04	2-Acetyl-5-methylfuran	C7H8O2	1193-79-9	16.30	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	C15H22O2	1620-98-0
8.44	Heptanoic acid	C7H14O2	111-14-8	16.67	2,6,10,14-Tetramethylhexadecane (Phytane)	C20H42	638-36-8
8.53	Isovaleraldehyde dipropyl acetal	C11H24O2	1000431-60-3	16.74	3,5-di-tert-Butyl-4-hydroxyacetophenone	C16H24O2	14035-33-7
8.54	Acetophenone	C8H8O	98-86-2	16.81	Isopropyl myristate	C17H34O2	110-27-0
8.55	p-Cresol	C7H8O	106-44-5	16.98	2,4-Diphenyl-4-methyl-2(E)-pentene	C18H20	22768-22-5
8.60	4-Methylbenzaldehyde	C8H8O	104-87-0	17.59	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	82304-66-3
8.79	(1-Methoxypropyl)benzene	C10H14O	59588-12-4	17.60	Farnesyl acetone	C18H30O	1117-52-8
9.23	Triacetonamine	C9H17NO	826-36-8	17.98	Dibutyl phthalate	C16H22O4	84-74-2
9.63	Benzoic acid	C7H6O2	65-85-0	17.99	n-Hexadecanoic acid	C16H32O2	57-10-3
9.72	Octanoic acid	C8H16O2	124-07-2	18.34	18-Norabieta-8,11,13-triene	C19H28	1000197-14-1
10.95	Nonanoic acid	C9H18O2	112-05-0	18.71	N,N-Dimethyltetradecanamide	C16H33NO	3015-65-4
11.69	2,3-Dihydro-1H-pyrrolizin-1-one	C7H7NO	17266-64-7	19.38	Linoleic acid	C18H32O2	60-33-3
12.74	Diphenyl ether	C12H10O	101-84-8	19.60	Octadecanoic acid	C18H36O2	57-11-4
12.85	p-tert-Butylphenetole	C12H18O	17269-94-2	19.80	n-Pentadecylcyclohexane	C21H42	6006-95-7
12.93	Longifolene	C15H24	475-20-7	20.31	N,N-Dimethylpalmitamide	C18H37NO	3886-91-7
13.18	Dimethyl phthalate	C10H10O4	131-11-3	21.40	Eicosyl acetate	C22H44O2	822-24-2
13.41	Ethyl 3-phenylpropenoate	C11H12O2	103-36-6	21.46	Antioxidant 2246	C23H32O2	119-47-1
13.42	1-Dodecanol	C12H26O	112-53-8	21.56	N,N-Dimethyllinoleamide	C20H37NO	2501-33-9
13.76	2,4-Di-tert-butylphenol	C14H22O	96-76-4	21.60	N,N-Dimethyloleamide	C20H39NO	2664-42-8
13.78	Butylated Hydroxytoluene	C15H24O	128-37-0	21.74	Dehydroabietic acid	C20H28O2	1740-19-8
14.38	(3-Decyl)benzene	C16H26	4621-36-7	22.09	Antioxidant 425	C25H36O2	88-24-4
14.54	Pentyl salicylate	C12H16O3	2050-08-0	23.02	Squalane	C30H62	111-01-3
14.63	Diethyl Phthalate	C12H14O4	84-66-2	23.83	13-Docosenamide, (Z)-	C22H43NO	112-84-5
14.79	p-tert-Octylphenol	C14H22O	140-66-9	26.81	Chondrillasterol	C29H48O	481-17-4
15.12	Tributyl phosphate	C12H27O4P	126-73-8	27.37	(24Z)-Ethylidenecholesterol	C29H48O	481-14-1

Table 2. Common compounds identified by both GC/MSD and GC/Q-TOF with match factor > 70.

Confirmation of Compound ID Using Accurate Mass

To gain higher confidence in compound identification, accurate mass information was used to either confirm or reject compound ID. Figure 5 shows two such examples, where ExactMass tool of MassHunter Unknowns Analysis software is used to assign fragment ions with formulas based on the accurate mass and the molecular formula of the hit, when possible.

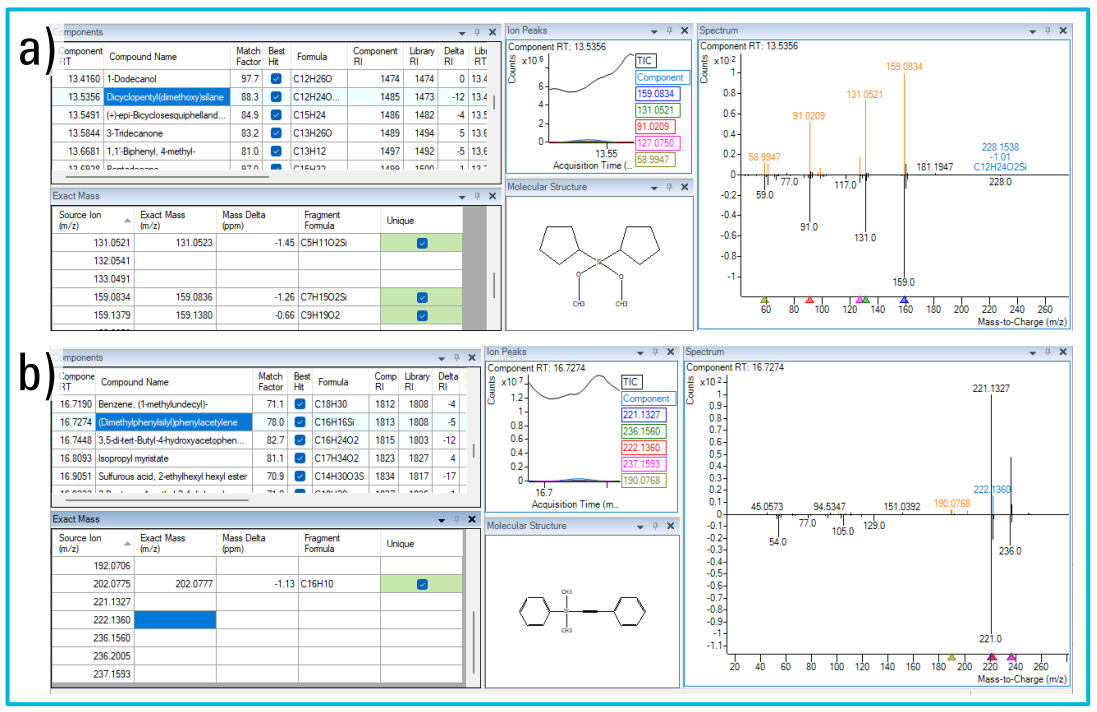

Figure 5. Confirmation of compound ID using accurate mass. Fragment formulas are assigned based on accurate mass and molecular formula of the library hit. Mass error of each prominent fragment ion is then calculated and displayed in the ExactMass table. A) Confirmed compound identified uniquely by GC/Q-TOF. B) Rejected compound by GC/Q-TOF: False-positive with GC/MSD.

Results and Discussion

Additional Compounds Identified by GC/Q-TOF

Table 3 displays compounds identified uniquely by GC/Q-TOF using NIST23 library and confirmed using accurate mass and RI information.

RT	Compound Name	Match Factor	Formula	Delta RI	CAS#
4.17	Methyl Isobutyl Ketone	92.8	C6H12O	-29.7	108-10-1
4.61	Acetylacetone	87.7	C5H8O2	-19.7	123-54-6
4.63	Dimethylformamide	99.1	C3H7NO	-21.2	68-12-2
4.86	Hexanal	96.7	C6H12O	-18.9	66-25-1
5.03	Furfural	80.0	C5H4O2	1.1	98-01-1
5.80	o-Xylene	96.5	C8H10	3.3	95-47-6
5.93	2,6-Lutidine (2,6-Dimethylpyridine)	82.0	C7H9N	-14.1	108-48-5
6.02	2-Heptanone	94.6	C7H14O	-9.3	110-43-0
6.21	Heptanal	94.6	C7H14O	-11.7	111-71-7
6.66	3-Hepten-2-one	79.6	C7H12O	-6.2	1119-44-4
6.91	Piperidine, 2,2,6,6-tetramethyl-	91.0	C9H19N	-19.8	768-66-1
7.10	Benzaldehyde	90.9	C7H6O	-10.8	100-52-7
7.36	α-Methylstyrene	95.6	C9H10	-4.2	98-83-9
7.63	Octanal	89.1	C8H16O	-5.5	124-13-0
7.96	2-Ethylhexanol	92.6	C8H18O	-1.7	104-76-7
8.11	N-Methyl-α-pyrrolidone	84.7	C5H9NO	1.4	872-50-4
8.16	2-(2-Hydroxypropoxy)-1-propanol	82.7	C6H14O3	0.1	106-62-7
9.01	Nonanal	96.3	C9H18O	-3.0	124-19-6
10.08	2,4-Dimethylthiophenol	89.1	C8H10S	19.0	13616-82-5
10.29	Benzene, 1,3-dibromo-	91.2	C6H4Br2	14.1	108-36-1
10.70	Benzothiazole	92.2	C7H5NS	-9.3	95-16-9
11.44	m-tert-Butylphenol	72.0	C10H14O	-2.2	585-34-2
12.35	3-Hydroxy-2,2,4-trimethylpentyl 2-methylpropanoate**	73.2	C12H24O3	-3.7	77-68-9
12.57	p-tert-Pentylphenol	74.3	C11H16O	3.2	80-46-6
13.27	BHT-quinol	84.6	C15H24O2	14.2	10396-80-2
13.54	Dicyclopentyl(dimethoxy)silane	88.3	C12H24O2Si	-11.9	126990-35-0
13.58	3-Tridecanone	83.2	C13H26O	4.6	1534-26-5
13.98	Ethyl 4-ethoxybenzoate	82.8	C11H14O3	-5.7	23676-09-7
14.77	(2-Decyl)benzene	88.2	C16H26	10.0	4537-13-7
15.06	(1-Butylheptyl)benzene	83.8	C17H28	-4.1	4537-15-9
15.08	Fenuron	73.1	C9H12N2O	-5.2	101-42-8
15.15	Benzophenone	93.4	C13H10O	-10.0	119-61-9
15.55	2,4-Ditert-butyl-6-nitrophenol	78.7	C14H21NO3	1.7	20039-94-5
15.89	4-(1,1-Dimethylheptyl)phenol	83.2	C15H24O	-25.9	30784-30-6
16.69	Anthracene	86.4	C14H10	-23.5	120-12-7
17.17	Diisobutyl phthalate	88.5	C16H22O4	5.0	84-69-5
17.70	Methyl hexadecanoate	74.6	C17H34O2	1.3	112-39-0
19.01	p-Tolyl disulfide	73.8	C14H14S2	3.4	103-19-5
21.05	Methyl dehydroabietate	79.9	C21H30O2	-17.2	1235-74-1
22.26	Bis(2-ethylhexyl) phthalate (DEHP)	69.6	C24H38O4	0.0	1000377-93-5
25.72	Tinuvin 770	87.1	C28H52N2O4	130.4*	52829-07-9

*- Only predicted RI is available

** - Component of texanol

Table 3. Compounds identified uniquely by GC/Q-TOF.

Identification of Unknown Compounds in Rubber Gasket

Low electron energy was used to help identify molecular ions of the unknown compounds (Figure 6).

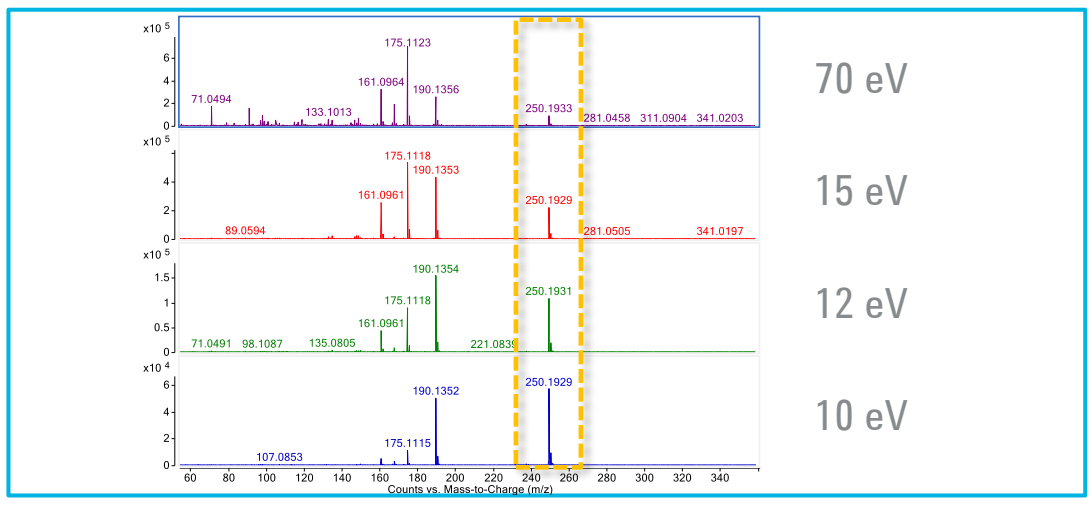

Figure 6. Low energy EI. Gradual increase of the relative abundance of the tentative molecular ion at lower electron energies is observed.

Tentative molecular ions Identified using low energy EI were selected as precursors in MS/MS experiments (Figure 7) to further perform structure elucidation carried out in MSC software (Figure 8).

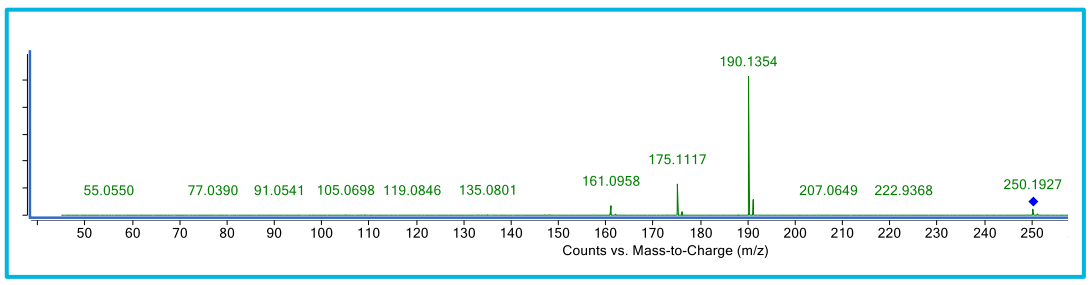

Figure 7. MS/MS using tentative molecular ion as precursor.

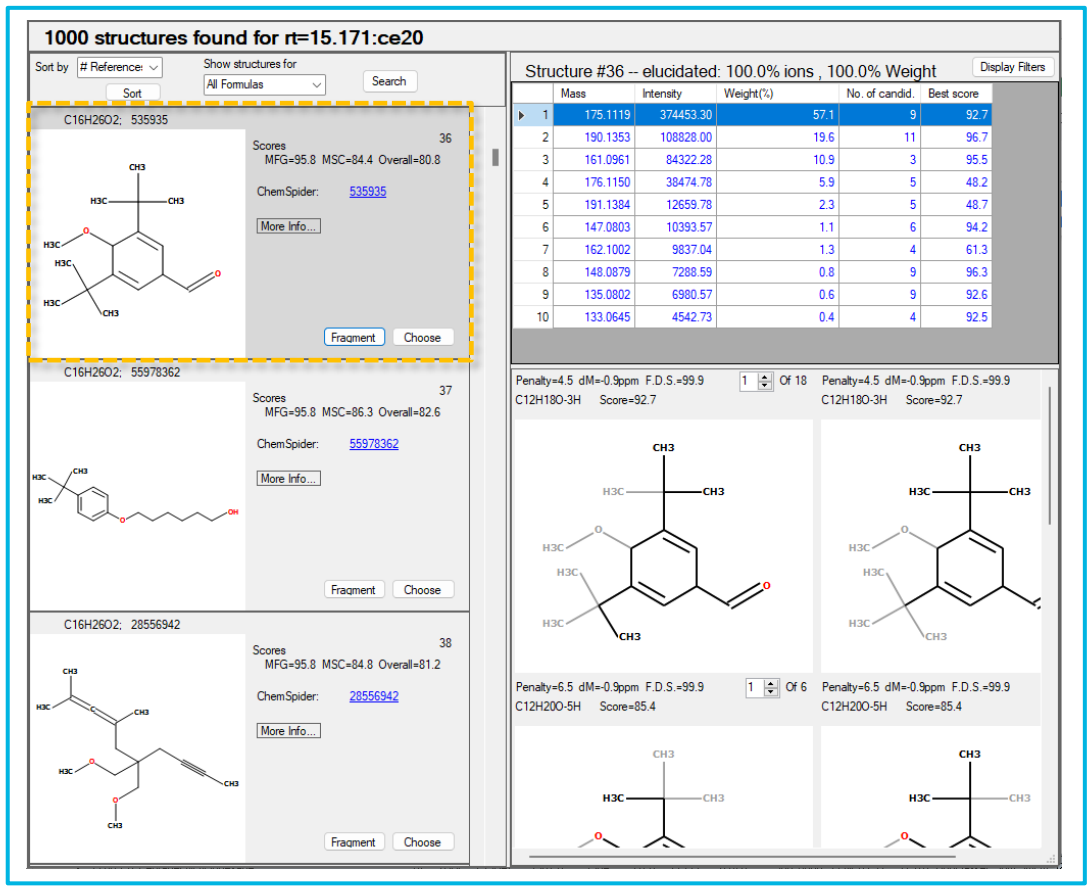

Figure 8. Proposed structure for one of the unknown compounds in rubber gasket extract using MSC.

Conclusions

- The optimized GC/MSD and GC/Q-TOF approach for analysis of extractables and leachables allowed for identifying over 150 compounds in a complex E&L extract
- High-resolution GC/MS enabled identification of over 60 additional components with increased confidence and structure elucidation of the unknowns
- The novel ultra low bleed Agilent J&W DB-5Q GC column resulted in significant decrease in background, that could potentially yield higher number of identifications of late eluting compounds.

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