Targeted and non-targeted analysis of extractables using LC-MS/MS

Introduction

Container-related extractable and leachable (E&L) compounds present increasing challenges for product safety and quality assurance. Extractables migrate from containers under stressed conditions such as exposure to heat and solvents, while leachables migrate out under normal conditions. Enhanced regulatory oversight has heightened the need for robust testing protocols and sophisticated analysis software to detect E&L compounds.

Wiley's KnowItAll[1] provides a comprehensive platform for identifying, analyzing, and managing analytical data across E&L workflows. It supports multiple techniques and vendor formats, including LC-MS with the new LC Expert application for automated untargeted analysis. Users can also create or import a structure database for targeted analysis in KnowItAll's MineIt application.

This application note demonstrates the analysis of a drainage water sample stressed for months with solvent in a high-density polyethylene (HDPE) bottle. The workflow combined both targeted analysis employing a user-created structure database based on the ELSIE E&L compound repository[2] and untargeted screening against a commercial tandem MS library. This integrated approach successfully identified plasticizer compounds extracted into the sample, demonstrating KnowItAll's streamlined LC-MS capabilities and seamless integration of both targeted and non-targeted analytical results.

Experimental

The same sample was analyzed on two different LC-MS systems to demonstrate cross-vendor compatibility:

Sample analysis: The samples were Thermo Q Exactive HF Orbitrap data files acquired in data-dependent acquisition (DDA) mode and converted to the mzML industry standard open format[3] using the ProteoWizard software[4] . The accompanying notes with the data file set indicated that it was acid mine drainage water that had been collected from a water treatment pipeline and stored for approximately eight months in an HDPE bottle with methanol and then filtered.

Software: Data analysis was performed using Wiley's KnowItAll Analytical Edition (Version 2025) with the MineIt and LC Expert applications.

Database: Records from ELSIE, a part of the U.S. Environmental Protection Agency (EPA)'s CompTox Chemical Dashboard, was used for targeted search. NIST MS/MS high-resolution libraries were used for non-targeted compound identification.

Results and Discussion

To investigate potential extractable compounds in the aged water sample, we established a comprehensive analytical workflow combining both targeted and untargeted approaches.

Database preparation and targeted analysis

Records from ELSIE were downloaded as an SDF file and imported into KnowItAll's MineIt application to create a structure database for later use in a targeted analysis (Figure 1). This database would serve as our first line of investigation, allowing us to screen for known extractable compounds that might have migrated from the HDPE container during the eight-month storage period.

When the sample LC-MS datafile was opened in the KnowItAll **LC Expert*** application, the data was automatically deconvoluted and displayed as peaks based on their Extracted Ion Chromatograms (EIC). Focusing on the region from RT 10.2 to 10.7 minutes —where several components of interest appeared— we used the **Include Range Bar** to select this timeframe for detailed analysis (Figure 2).

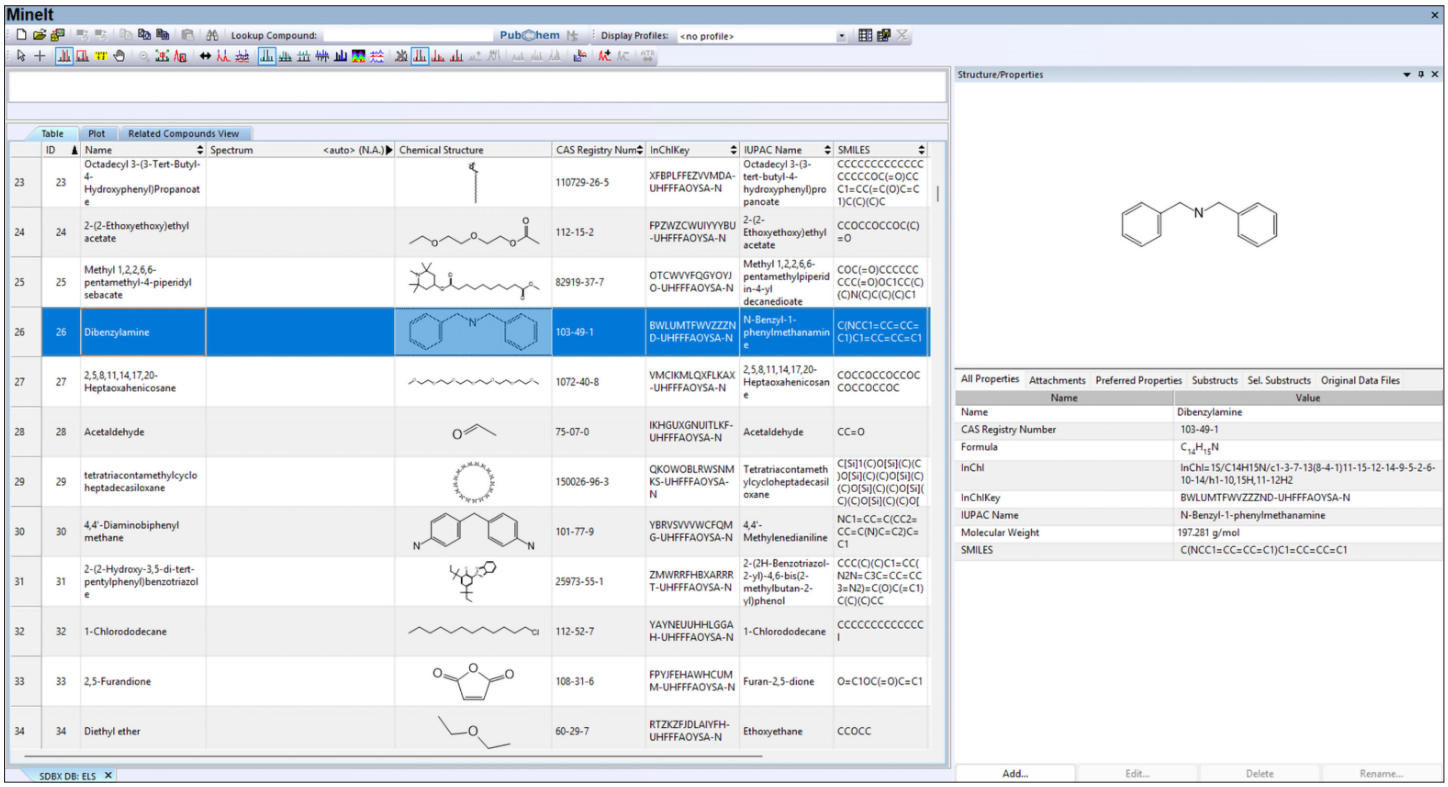

Figure 1. *A new structure database of extractables was created in MineIt by importing an SDF file from the ELSIE compound repository.*

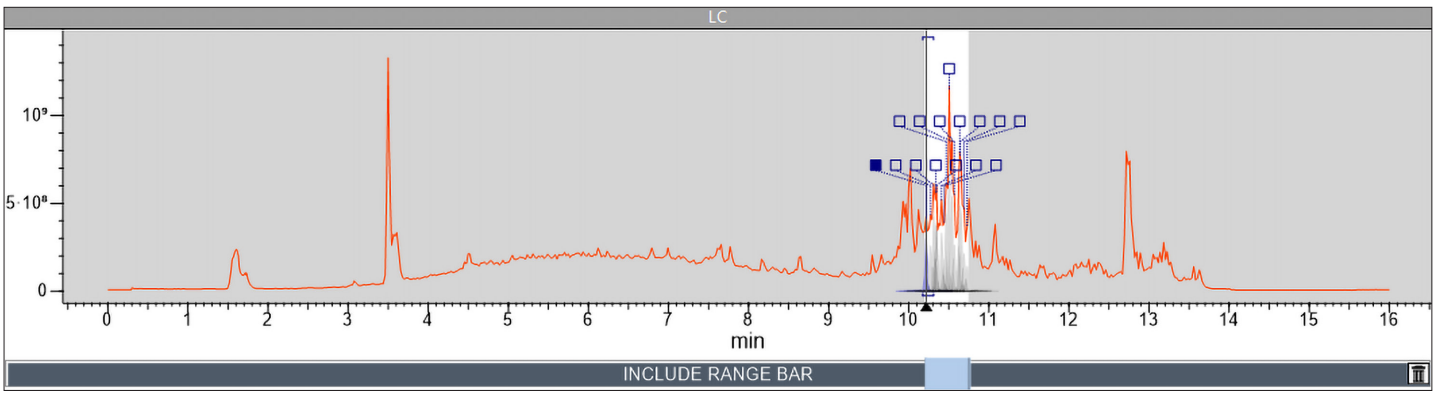

Figure 2. *The LC-MS chromatogram range was selected using the* ***Include Range Bar****.*

	RT [min]	Peak Area	Peak Area [%]	Peak Height	FWHM [	Base Ion [m/z	Annotated Name	Molecular Formula	Match Score	Mass Accuracy [ppm	Calculate
9	10.4092	7531245	4.73	330086938.7	0.0304	195.1227	Tetraethylene glycol	$C_8H_{18}O_5$	98.73	0.06	195.1227
10	10.4411	5159560	3.24	181744468.0	0.0337	282.2791	(9Z)-Octadec-9-enamide	$C_{18}H_{35}NO$	84.06	-0.31	282.2791
11	10.4411	5159560	3.24	181744468.0	0.0337	371.1012	Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	59.36	-0.18	371.1012
12	10.4677	6893517	4.33	442123948.7	0.0332						
13	10.5048	29395052	18.48	925952004.8	0.0222	403.3051	Octanoic acid, 1,2-ethanediylbis(oxy-2,1-ethanediyl) ester	$C_{22}H_{42}O_6$	96.52	-0.75	403.3054
14	10.5048	29395052	18.48	925952004.8	0.0222	403.3051	Triethylene glycol bis(2-ethylhexanoate)	$C_{22}H_{42}O_6$	96.52	-0.75	403.3054
15	10.5386	19211605	12.08	539410580.9	0.0310	282.2791	(9Z)-Octadec-9-enamide	$C_{18}H_{35}NO$	99.40	-0.31	282.2791
16	10.5680	6738406	4.24	260997371.1	0.0312	409.3118	3,3',5,5'-Tetra-tert-butyl-4,4'-dibenzoquinone	$C_{28}H_{40}O_2$	91.20	4.14	409.3101
17	10.5680	6738406	4.24	260997371.1	0.0312	319.2995	Phenol, dioctyl-	$C_{22}H_{38}O$	97.52	-0.23	319.2995

Figure 3. *The* ***Peaks*** *table displays the results of an accurate mass search against the structure database. It lists each component with its characteristic information such as retention time (RT), peak area, and peak height.*

The targeted search against our ELSIE-derived database immediately yielded results: 12 compounds were found within the selected retention time window (Figure 3). This confirmed our hypothesis that extractable compounds were indeed present in the sample.

Untargeted Screening for Unknown Compounds

We proceeded with an untargeted search against the NIST MS/MS high-resolution libraries*[5]. The **Database Search Bar** menu item displays the search progress and spectral matches with their scores (Figure 4). Individual matches were then evaluated directly through the **Database Match** panel, which overlays experimental spectra with library references, along with structural information if available (Figure 5).

Database Search

RT [min]	#	Match	Score	HQI	R.HQI	Notes
10.2194	1	1-(2-Ethyl-2-methyltetrahydro-2H-pyran-4...	84.91	54.16	88.33	
10.2734	1	2S-Amino-4E-pentadecene-1,3R-diol	97.24	84.81	98.62	
10.3127	1	Acetyl tributyl citrate	97.54	94.43	97.89	
10.3383	1	Benzyldimethyldodecylammonium cation	93.21	39.90	99.13	
10.3472	1	2-Amino-1-phenylbutane	96.75	91.88	97.29	
10.4092		No match found				
10.4411		No match found				
10.4677	1	N-Lauryldiethanolamine	97.43	86.65	98.63	
10.5048	1	Triethylene glycol bis(2-ethylhexanoate)	72.29	70.71	72.46	
10.5386		No match found				
10.5680	1	Palmitoleic acid ethyl ester	90.70	85.16	91.31	
10.6352	1	Palmitoleic acid ethyl ester	90.70	85.16	91.31	
10.6395	1	Palmitoleic acid ethyl ester	90.70	85.16	91.31	
10.6893	1	2,2'-(Tridecylazanediyl)bis(ethan-1-ol)	83.26	80.87	83.52	
10.7499	1	Stearamide	92.81	90.57	93.05	

Figure 4. *The* ***Database Search*** *panel displays non-targeted matches with their scores. Scores that are closer to the maximum of 100 are the best matches.*

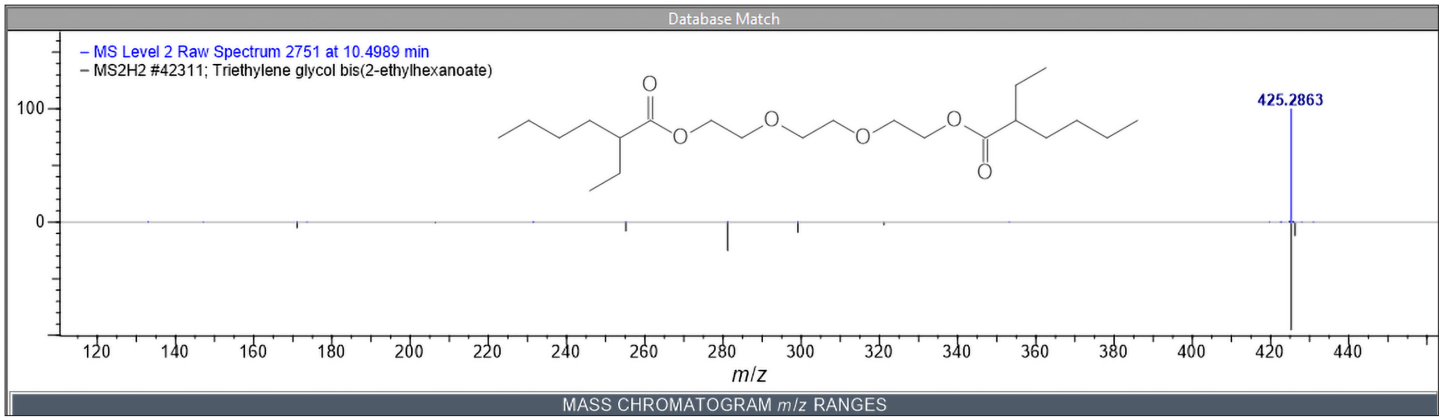

Figure 5. *The* ***Database Match*** *panel displays the matched spectra and a structure for the selected hit.*

Confirming the Discovery

Because a targeted search was performed earlier, we were able to see a corresponding accurate mass hit for Triethylene glycol bis(2-ethylhexanoate) is at the same retention times in the Peaks table (see Figure 3) as the Database Search table at 10.5048 min. This compound, used in the manufacture of plastics, was likely extracted from the container or filter into the water sample during methanol exposure.

To validate this hypothesis, we analyzed the blank sample (Blank_1.mzML) using the identical protocol. The absence of Triethylene glycol bis(2-ethylhexanoate) in both targeted and untargeted searches of the blank confirmed that this compound was likely extracted during the storage and filtering process, as it was not present in the original sample.

Conclusion

This study successfully demonstrated how KnowItAll Analytical Edition can tackle real-world challenges for extractables and leachables through an integrated analytical approach combining both targeted and non-targeted analyses. The workflow began by quickly downloading and creating a large structure
repository from a public source into a KnowItAll structure database for use in targeted analysis. LC Expert then seamlessly processed industry-standard mzML files, performing automated searches against both the user-created database and comprehensive NIST MS/MS libraries.

The dual approach proved its value when Triethylene glycol bis(2-ethylhexanoate) was identified through untargeted screening and confirmed by targeted analysis at the same retention time. This plasticizer's presence in the aged sample but absence in the blank sample provided evidence of container-related extraction during methanol storage, validating our contamination hypothesis.

The entire analytical workflow—from database preparation through compound identification and source confirmation—demonstrates KnowItAll's capability to rapidly generate, test, and validate hypotheses in E&L investigations. This automated, integrated approach provides analysts with a powerful solution for addressing complex extractables and leachables challenges in pharmaceutical and environmental applications.

References:

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3. Martens, L.; Chambers M.; Sturm M.; Kessner D.; Levander F.; Shofstahl J.; Tang WH.; Römpp A.; Neumann S.; Pizarro A.D.; et al. (2001) mzML—a *Community Standard for Mass Spectrometry Data*. Mol. Cell. Proteomics, 10 (1), 1-7. DOI: 10.1074/mcp.R110.000133
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5. National Institute of Standards and Technology (NIST). *NIST / EPA / NIH Mass Spectral Library 2023*: John Wiley and Sons, Inc.: Hoboken, NJ, 2023; https://sciencesolutions.wiley.com/solutions/technique/gc-ms/nist-epa-nih-mass-spectral-library/ (accessed 2025-03-27).

∗Subscription required to the NIST MS/MS Mass Spectral Libraries and LC Expert application.