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Enhanced Extractable Leachable Compound Identification Using New Database Software with Retention Time and Supplemental Collisional Cross Section

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The identification of unknowns continues to be one of the biggest challenges in extractable leachable (E/L) analysis. In other fields like food and environmental analysis, researchers openly share mass spectral repositories that enable enhanced identification workflows and increased compound coverage.

In contrast, E/L testing labs tend to utilize in-house, confidential libraries and databases (DB), making public information sparse and siloed. Some public information pertinent to E/L analysis does exist in online databases, technical publications, such as the Extractable Leachable Safety Information Exchange (ELSIE) DB, the NIST LC-MS/MS DB, Wiley Mass Spectrometry DB, the EPA Comptox Chemicals dashboard, and commercial E/L-focused DBs and libraries.

Compound data documented in scientific publications (journals, technical presentations, white papers, book chapters, etc.) can also be compiled, reviewed, and downloaded using discovery tools such as CAS SciFinder. But the many different sources and data formats present a bottleneck and require laborious manual curation, merging, and reviewing of data into a single, easy-to-access database.

To address this difficulty, Agilent has developed the new ChemVista Library Manager software, which also includes extensive analytical content. The software enables researchers to generate custom, application-specific databases and libraries for more reliable compound identification. The general overview and structure of the ChemVista software was discussed in Poster MP373.

Herein we present the development of a new user-generated, E/L application-focused database that greatly expands the commercial Agilent E/L database (released in 2018 with 1003 compounds) to well over 5000 compounds. We describe the tools used to integrate and merge information from the various sources described above.

To improve identification of structural isomers, we have begun the integration of method-specific retention time (RT) and collisional cross section (CCS) information into the database.

To encourage information sharing between laboratories, ChemVista accommodates the creation of MassBank txt files (ASMS Poster ThP104) that can be uploaded to the MassBank repository.

Instrumentation

A complex mixture of solvent extracts of commercially available polymer additives, breakdown products, oligomers, surfactants, and parenteral and ophthalmic drug products was analyzed using the Agilent 6560 drift-tube ion mobility LC/Q-TOF system interfaced with reversed-phase LC separation, using the Agilent Jet Stream dual ESI source in both positive and negative polarity (Figure 1).



Figure 1. The 6560 ion mobility LC-Q/TOF system.

IM data was collected using high-resolution multiplexing to enhance the sensitivity, in-spectra dynamic range, and drift-time resolution after demultiplexing. High-resolution MS/MS mass spectra (data-dependent Q-TOF mode) were also acquired for comparison to existing MassBank spectra.

LC separation method

The 1290 Infinity II HPLC binary pump interfaced with a 1290 multiwash autosampler, 1290 temperature-controlled column compartment (TCC), and 1290 diode array detector with 1 μ L DAD cartridge was utilized for the LC separation. An Agilent PS EC-C8 column (2.1 x 150 mm, 2.7 μ m) heated to 45 $^{\circ}$ C was used for the analytical separations, and a PS EC-C18 column (4.6 x 50 mm, 2.7 μ m) was placed after the mixer and before the autosampler to delay the elution of organic impurities from the mobile phase solvents and buffers. The system was operated at a flow rate of 0.350 mL/min with the gradient shown in Table 1.

Mobile phase	A = Water + 2.5 mM ammonium formate + 0.05% formic acid B = Methanol + 2.0 mM ammonium formate + 0.05% formic acid	
Gradient	Time	%B
	0.00	5
	1.00	5
	16.00	100
	25.00	100
	25.10	5
Post Time	3 minutes	

Table 1. General E/L-specific RP separation method.

IM Q-TOF source and IM AQ conditions

The Agilent 6560 drift-tube ion mobility LC/Q-TOF uses a single compound for temperature correction, and subsequently converts drift-time into CCS by first-principles using the Mason–Schamp equation. The system was operated in MS mode using 4-bit multiplexing with 3800 μ s trap fill time, 150 μ s release time, 60 ms maximum drift time, and 1700 V drift voltage (Figure 2). The system was tuned to ion transmission for stable ions over the 50–1700 mass range.¹

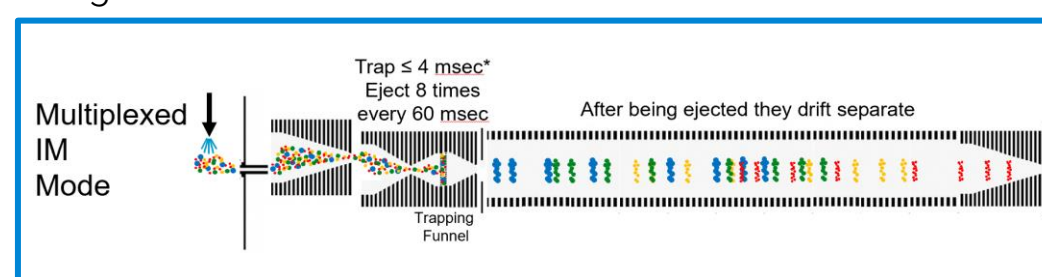


Figure 2. 4-Bit multiplex data acquisition mode.

Results and Discussion

Agilent ChemVista library manager software

Agilent ChemVista software is capable of importing compound and spectral data from *.SDF, *.csv, *.txt (MassBank), and *.cdb (Agilent Personal Compound Database Library, PCDL) formats (Figure 3). The SDF imports support data from both the EPA Chemicals Dashboard and MassBank of North America (MoNA). Data exports of substance information from CAS SciFinder in SDF format can be imported after minor modification of the SDF file. Cheminformatics identifiers guide the imported data from the different sources in a compound-centric manner, eliminating duplicate entries. Efficient sub-setting can be achieved through searching by identifiers (name, CAS, etc.), formulas, and exact mass. Complex queries can be conducted by combining search types to arrive at targeted sets of compounds for specific compound class or E/L method.

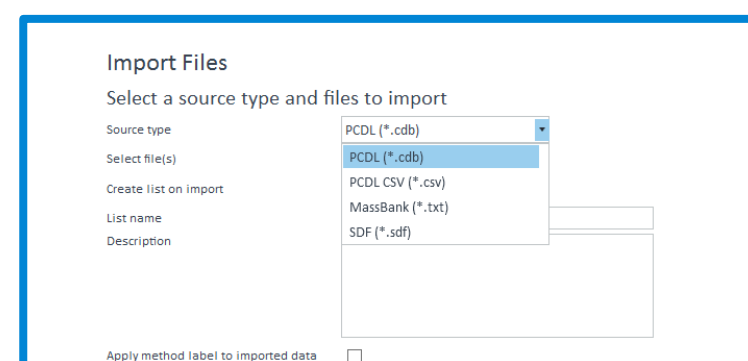


Figure 3. ChemVista file import example.

Importing compounds from EPA Comptox Dashboard

Pre-curated chemical lists exported in *.SDF format from the EPA Comptox Chemicals Dashboard can be directly imported into Agilent ChemVista. Imports can be merged with data imported from elsewhere or used to start a new screening list. Examples of E/L, food contact-, and plastic packaging-related chemical lists downloadable from the dashboard are listed in Table 2.

Database	Number	Content
FOODPLASTICS	81	Chemical Migrants in plastic FC products
WIKIANTIOXIDANTS	271	Antioxidants from public sources
ELSIE	457	Extractable Leachable Safety Exchange Net
CCPDBLISTA	836	Chemicals likely associated with Plastic
CCSEXTRACTS	956	Prediction of CCS values for E/L
E_L_PCLD	1003	Agilent E_L_PCLD
CPPDBLISTB	2861	Chemical possible (List B)
FOODCONTACTSDB	9527	Database created by FPF Version 5
PLASTICMAP	10522	Chemicals related to polymers

Table 2. E/L lists from EPA Comptox Dashboard.

Importing and merging data from multiple sources

Merging data from various sources using chemical identifiers enables consolidated lists for E/L analysis that were previously isolated. This could include the merging of personal databases with unique metadata, notes, RTs, and MS/MS data with open-source information to supplement workflows. In this work, we supplemented an Agilent internal E/L database containing over 5,700 compounds and a surfactant database containing over 6,800 entries with MS/MS data from MoNA. This yielded over 23,258 Agilent Q-TOF spectra and 47,838 Q-TOF spectra in total (Figure 4).

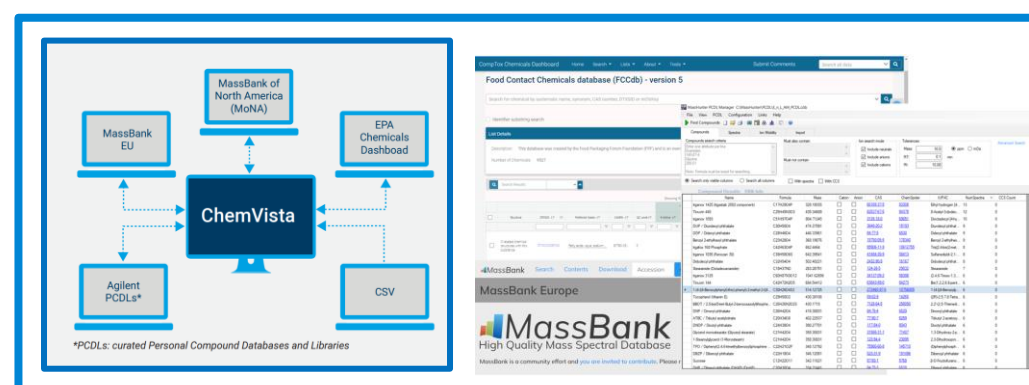


Figure 4. Importing and merging online content.

The addition of collisional cross section (CCS) values from experimental results, prediction programs, or online content^{2,3} provided a means to increase the identification of structural isomers of phthalates and terephthalates and breakdown products of antioxidants. Ion mobility data showed the presence of previously unreported isomers of nonylphenol and alkylsulfonates.

Importing compounds using CAS SciFinder

Searching online technical publications for E/L-related substances using discovery software programs like CAS SciFinder is another method to import previously reported E/L compounds from technical publications into a merged database. Substances can be downloaded from SciFinder (Figure 5) in SDF format and simply modified to meet the specifications of Agilent ChemVista.

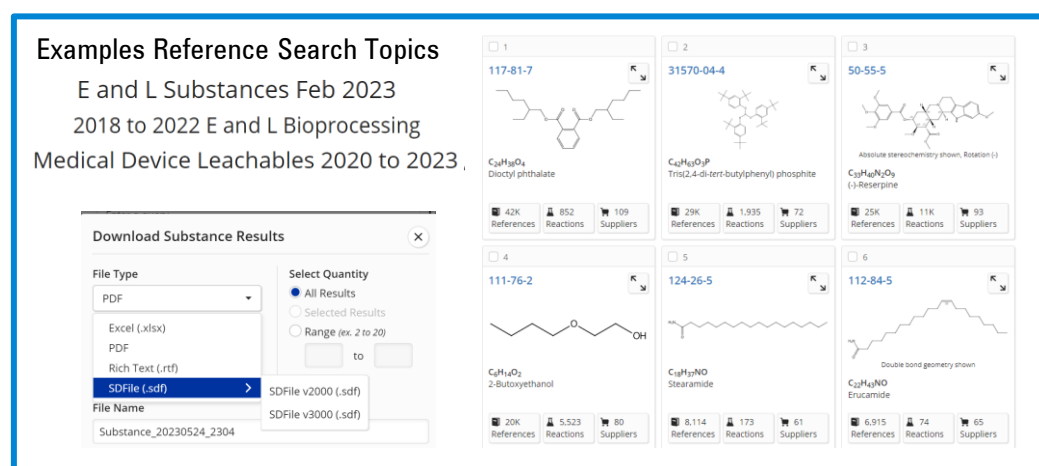


Figure 5. Example of substance export from SciFinder.

Importing/exporting compound with method

ChemVista software allows to associate a compound with multiple RT (chromatographic separation conditions) and spectra (MS analyzer details). The choice of mobile phase, gradient, and column all impact RT values. The lack of a standardized LC methods necessitates that each lab methodically determine their own RT information based on standards. Being able to export compounds linked to specific LC/MS methods (e.g., methanol vs. acetonitrile organic mobile phase) will simplify the use of RT to identify E/L compounds and structural isomers.

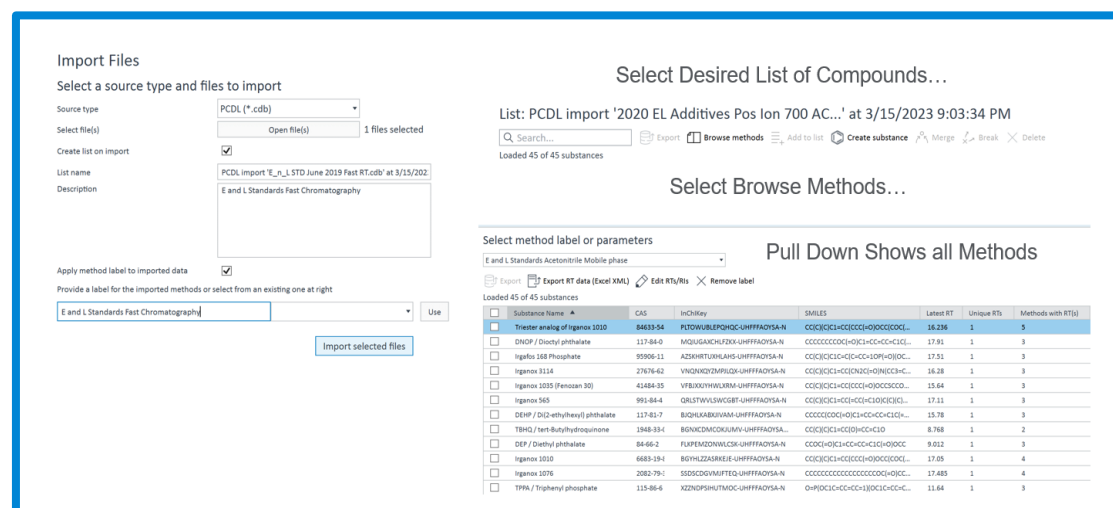


Figure 6. Exported PCDLs linked to specific LC/MS method to include retention time (RT) and ionization method parameters.

Compound identification using expanded E/L database

Ion mobility mass spectral data was acquired from organic solvent (IPA/THF) extractables for several ophthalmic drug product containers, rubber gaskets in disposable syringes, disposable pipette tips, PET plastic bottles, and silicone rubber caps. CCS values were added to the PCDL exported from ChemVista to further enhance the identification workflow.

In general, compound identifications using MS/MS spectra increased 3-fold with incorporation of the imported spectra compared to previously released Agilent database. The addition of RT information linked to LC separation methods (methanol vs. acetonitrile mobile phase) provided means to accurately identify previously unresolved structural isomers (phthalates, terephthalates). The addition of experimental, predictive and literature CCS values led to enhanced identification of compounds.

Work is in progress to utilize TAGs, metadata, mass defect filtering, and MS/MS fragmentation patterns to enhance the identification of E/L products that can potentially occur from sterilization of medical devices, container closure system, or single-use systems.

Conclusions

- Identification of unknowns in E/L analysis remains a challenge.
- Agilent ChemVista can enhance E/L analysis by consolidating compound and spectral data from many sources into a consistent, compound-centric structure.
- Exporting newly compiled data from ChemVista and supplementing with RT and CCS enhances Agilent identification workflows.

References

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2. Song, X.-C. et al. Prediction of Collision Cross-Section Values for Extractables and Leachables from Plastic Products. *Environmental Science & Technology*. 2022, 56 (13), 9463–9473.
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<https://www.agilent.com/en/promotions/asms>

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