

Nontargeted and Suspect Screening Analysis of Indoor Environmental Chemicals Using the SCIEX X500R QTOF System and SCIEX OS Software



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ABSTRACT

Exposomics and human bio-monitoring (HBM) are rapidly advancing disciplines addressing risk assessment in environmental and occupational health and contributing to the development of policies and programs to protect human health. One of the tools available to the disciplines and utilized with growing popularity is non-targeted and suspect screening using high resolution mass spectrometry (HRMS). Non-targeted screening workflows enable simultaneous detection, characterization, identification (if reference was reported previously) as well as relative quantitation of multiple environmental chemicals, metabolites and other small molecules. Non-targeted screening with HRMS helps overcome challenges of characterization of thousands of compounds present in samples of interest (e.g. environmental exposure samples as well as human samples). The workflow allows scanning extensive mass ranges to determine molecular weight, elemental composition, and molecular structure information. In the study presented here, we report on the analysis of non-targeted and suspect screening of household laundry dryer lint.

INTRODUCTION

Composition of household dust provides valuable insights into the potential health risks associated with the chemical exposure in indoor environment. Previous studies demonstrated presence of a number of compound classes in house dust including plasticizers, pesticides, cosmetics and flame retardants. However, studies directed at non-targeted screening for contaminants in household dust remain limited. Similar studies of dryer lint samples are even more scarce. To our knowledge, the first such study was reported in 2017. [2] Considering physico-chemical properties of the previously reported contaminants, it is essential to note the diversity of the properties and structures of these chemicals. In the study summarized here we applied suspect and non-targeted screening workflows available in SCIEX OS 1.5 software to analyze the data collected for lint samples with the SCIEX X500R QTOF system. Lint matrix was selected as representative of the contaminants present in the indoor environment. In addition to contaminants, it may contain synthetic and cotton fibers, human hair and potentially residues of laundry detergents. Dryer lint is valuable as a proxy for human exposure, as is vacuum cleaner dust. [1]

MATERIALS AND METHODS

Sample Collection and Preparation:
 Three lint dryer samples were collected from one household in Ontario, Canada. The lint was removed from the filter sheet and wrapped into aluminum foil for subsequent sample extraction and cleanup. Two separate portions of the same lint sample were weighted (50 mg each) and placed in 10 mL disposable polypropylene tubes. 5 mL of methanol and 5 mL of acetonitrile respectively were added to the corresponding portions. Each tube was vortexed for 1 min, sonicated for 15 minutes and centrifuged for 5 min at 1500 rpm. Supernatants were removed from both tubes and they were combined and separated into two equal portions. The extracts were loaded on Phenomenex Strata GCB cartridges, pre-conditioned with methanol and acetonitrile respectively (See Figure 1).

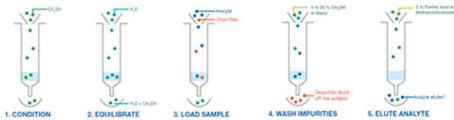


Figure 1. Sample SPE procedure schematic

Elution of the analyte (step 5 in Figure 1) was the only differentiating step as 2% formic acid in 1:1 v/v methanol-to-acetonitrile was used for the subsequent sample analysis in the positive ion mode, while 1:1 v/v methanol-to-acetonitrile was used to elute analytes for the subsequent analysis in the negative ion mode. Before LC/MS analysis, the eluents were reduced to 1 mL volume under nitrogen flow and 1 mL of Milli-Q water was added. The resulting volumes were transferred to LC vials and placed into a cooled (15° C) auto-sampler.

HPLC Conditions:
 A SCIEX ExionLC™ HPLC system was utilized for chromatographic separation. Samples were run twice: with a Phenomenex Luna 3 µm HILIC 200 Å, 100x2mm and with a Phenomenex Kinetex 2.6 µm Phenyl-Hexyl 100 Å, 50x4.6mm in a gradient regime at a flow rate of 500µL/min. Application of two different types of chromatography was due to a diverse nature of the samples which was reported to contain non-polar, polar and very polar compounds. The composition of mobile phases were as follows:
 HILIC separation:
 Eluent A- 90:10 v/v of Acetonitrile/50 mM ammonium formate. Eluent B- 50:40:10 v/v/v of Acetonitrile/Water/50 mM ammonium formate
 Reverse Phase separation:
 Eluent A- 0.1% aqueous formic acid. Eluent B- 0.1% formic acid in acetonitrile.
 The injection volume was set to be 25 µL.

MS Conditions:

A SCIEX X500R QTOF System with Turbo V source and Electrospray Ionization (ESI) probe was used in positive and negative ion mode. SCIEX OS 1.5 software was used for data acquisition, exploration and processing. SWATH® acquisition was used for data collection in 50 to 1000 Da m/z range with 20 Da windows. SWATH acquisition enables the MS/MS analysis of all precursor ions across the desired wide range on LC time scale. It allows detection of virtually every ionizable compound in a sample. Every sample was injected twice in positive and negative polarity.

Data Processing:

The data was processed in Suspect and Nontargeted screening workflows with SCIEX OS 1.5 software.

In non-targeted screening workflow a comparison (blank matrix) sample was acquired separately to correctly identify features present only (or in significantly larger amounts) in the lint samples. A group of ions including adducts and isotopes of the same chemical that form a chromatographic peak at a specific retention time was considered to comprise the same feature. For each datapoint (within a feature) non-targeted screening analysis workflow returns a number of ion characteristics such as retention time, molecular mass, fragment spectra and signal intensities as well as elemental composition and elemental formula derived from the accurately detected mass.

In suspect screening workflow which is particularly suited for identification of analytes present in spectral database but without actual reference standards, a list of suspect compounds is exported from the available database (Figure 2). SCIEX OS software enables importing Compound ID, chemical formula and up to 3 fragment ions of the highest intensity from the MS/MS spectrum is the fragment ions are to be used for additional confirmation or ion ratio calculations. [3]

Figure 2. Library Database Importer

RESULTS AND DISCUSSION

Results of Suspect Screening

The suspect screening analysis was conducted for samples acquired in both positive and negative ion mode. The specific content of the lint compared to overall household dust may be related to considerable exposure to human body, chemicals in textile debris, dyes, laundry detergent, surfactant and flame retardants. [2] The library list of suspects included phthalates, pesticides, surfactants and parabens as well as certain pesticide classes. The compound database contained 6844 compounds with elemental formulas, MS1 and MS/MS spectral information.

Suspect screening workflow with the reference compounds from the library database led to tentative identification of the compounds presented in Table 1. The three samples were from the same household but from different laundry loads, so similar results were expected. As no standard references were available for tentatively identified compounds, a cross-sample comparison was conducted to gain a larger level of identification confidence. The recurring features were compared to establish consistency in the retention time values, precursor mass accuracy, isotopic pattern and MS/MS library score. We have also ensured consistency in the content of the feature. E.g. in the group of ions which included a target precursor ion, its solvent adducts or metal ion clusters eluting at close RT values. One good example of a positively identified compound across all analyzed samples is a flame retardant and plasticizer *Triphenyl phosphate*.

Figure 3A illustrates the difference between the content of one of the lint sample and a comparison sample. Figure 3B demonstrates extracted ion chromatograms, MS1 and MS2 information for the tentative analyte in three different samples.

Table 1: Suspect screening results of the laundry dryer lint samples

Tentative Compound	CAS	Molecular Formula	Ion form	Theoretical m/z	Experimental m/z	Source/Uses	Comment
Diethyl phthalate	84-72-2	C16H22O4	[M-H] ⁻	279.159	279.1654	Plasticizer	Detected in all analyzed samples
Triphenyl phosphate	115-86-6	C18H15O4P	[M-H] ⁻	327.078	327.0782	Flame retardant	Detected in all analyzed samples
17-Alpha-Methyltestosterone	58-18-4	C20H28O2	[M-H] ⁺	303.180	303.1785	Steroid	Detected in one out of 3 analyzed samples
Diphosphoramide	122-39-4	C12H11N	[M-H] ⁺	170.096	170.0975	Fungicide	Detected in one out of 3 analyzed samples
Simetryn	1014-70-6	C8H15N3S	[M-H] ⁺	214.110	214.1092	Pesticide	Detected in one out of 3 analyzed samples
4-Hydroxybenzoic acid propyl ester	94133	C10H12O3	[M-H] ⁻	179.071	179.0716	Paraben (Cosmetic products)	Detected in all analyzed samples
Methyl 3-Hydroxybenzoate	19438100	C8H8O3	[M-H] ⁻	151.046	151.040	Paraben (Cosmetic products)	Detected in all analyzed samples
Decylbenzenesulfonic acid	13222-98-1	C16H22O3S	[M-H] ⁻	297.167	297.1513	Laundry detergent	Detected in all analyzed samples
Benzenesulfonic acid 4-methyl	39156495	C7H8O3S	[M-H] ⁻	311.170	311.1684	Laundry detergent	Detected when HILIC separation was performed
Ethylene glycol diethyl ether sulfate	48073445	C14H26O6S	[M-H] ⁻	309.170	309.1576	Laundry detergents, hair sprays, and hair care compositions	Detected when HILIC separation was performed
Tris(2-chlorobenzenesulfonic acid)	25496019	C19H9O6S3	[M-H] ⁻	339.203	339.2021	Surfactant, cleaning products	Detected when HILIC separation was performed

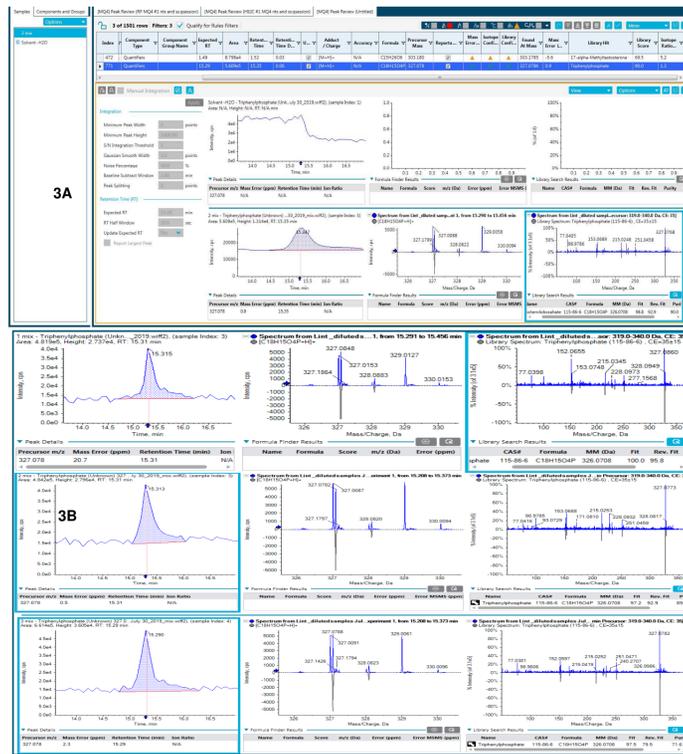


Figure 3. Extracted ion chromatogram, MS1 and MS2 spectra of Triphenyl phosphate tentatively identified in Sample "2mix", in the first replicate 1- 3A. Extracted ion chromatograms, MS1 and MS/MS spectra in 3 different dryer lint samples.

It is essential to note that the compound identification results were somewhat different between reverse phase separation and that with HILIC. Overall, the number of features tentatively identified was larger with RP in the positive ion mode while HILIC allowed exclusive identification of two compounds in the negative ion mode (see Table 1). These results demonstrate the advantages of the suspect screening workflow in SCIEX OS 1.5 software that utilizes curated spectral libraries and automated software searching and matching algorithms for identification of dozen of compounds without the need to purchase reference standard chemicals to optimize targeted screening.

Results of Nontarget Screening

We have used a comparison sample e.g. "blank" to identify the features present only in the lint sample. In order to streamline relevant feature extraction, we first set "Area Ratio Threshold (Unknown/Control)" parameter to 100000, 10000 and 1000. The difference between 100000 and 1000 threshold was found not to exceed 5 features for the three analyzed samples. A total number of features detected in all replicates of sample 1 mix, for example, was 127 in the positive ion mode with reverse phase separation and 145 – with HILIC. In the negative ion mode the features detected were 90 and 77 respectively.

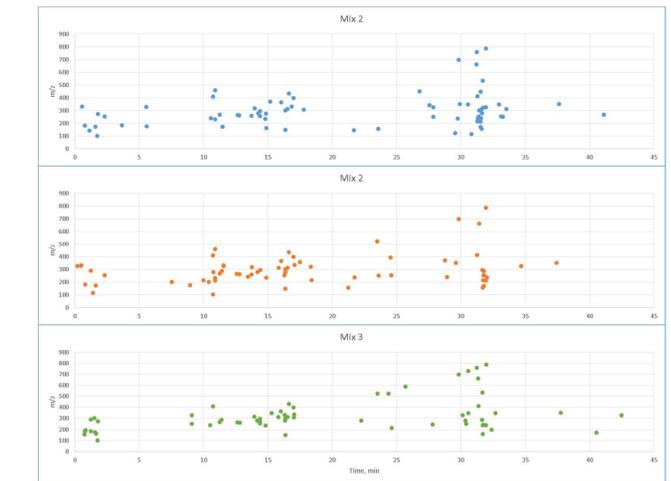


Figure 4. Non-targeted feature distribution detected in three separate lint samples with reverse phase chromatographic separation. Figure 4 illustrates distribution of the features observed in the positive ion mode in three lint samples analyzed with reverse phase chromatographic separation. It is essential to note similarities in the observed patterns, the fact that suggests characteristic indoor contaminants composition for this specific household. Complete chemical identification of such large number of compounds remains challenging. Prioritization approach is required to select recurring features with high formula finding score to subsequently investigate MS/MS pattern with a software enabling *in silico* fragmentation prediction, such as ChemSpider.

CONCLUSIONS

This study demonstrated advantages of suspect and non-targeted screening analysis of dryer lint sample using SWATH acquisition with X500R QTOF system and corresponding processing workflows available in SCIEX OS 1.5 software. The detection of pesticides, surfactants, flame retardants and plasticizers in the analyzed samples was found to be consistent with previous findings. [1,2] Similarities in feature distribution across different lint samples suggests a possibility of creating specific fingerprints of indoor dust for subsequent connections between chemical composition of the indoor environment and health impacts.

REFERENCES

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