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RESEARCH ARTICLE

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Optimized workflow for unknown screening using gas chromatography high-resolution mass spectrometry expands identification of contaminants in silicone personal passive samplers

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Rationale: Silicone wristbands have emerged as valuable passive samplers for monitoring of personal exposure to environmental contaminants in the rapidly developing field of *exposomics*. Once deployed, silicone wristbands collect and hold a wealth of chemical information that can be interrogated using high-resolution mass spectrometry (HRMS) to provide a broad coverage of chemical mixtures.

Methods: Gas chromatography coupled to Orbitrap™ mass spectrometry (GC/Orbitrap™ MS) was used to simultaneously perform suspect screening (using in-house database) and unknown screening (using vendor databases) of extracts from wristbands worn by volunteers. The goal of this study was to optimize a workflow that allows detection of low levels of priority pollutants, with high reliability. In this regard, a data processing workflow for GC/Orbitrap™ MS was developed using a mixture of 123 environmentally relevant standards consisting of pesticides, flame retardants, organophosphate esters, and polycyclic aromatic hydrocarbons as test compounds.

Results: The optimized unknown screening workflow using a search index threshold of 750 resulted in positive identification of 70 analytes in validation samples, and a reduction in the number of false positives by over 50%. An average of 26 compounds with high confidence identification, 7 level 1 compounds and 19 level 2 compounds, were observed in worn wristbands. The data were further analyzed via suspect screening and retrospective suspect screening to identify an additional 36 compounds.

Conclusions: This study provides three important findings: (1) a clear evidence of the importance of sample cleanup in addressing complex sample matrices for unknown analysis, (2) a valuable workflow for the identification of unknown contaminants in silicone wristband samplers using electron ionization HRMS data, and (3) a novel application of GC/Orbitrap™ MS for the unknown analysis of organic contaminants that can be used in exposomics studies.

1 | INTRODUCTION

Silicone wristbands have become a popular noninvasive technique for assessing personal exposure to environmental organic contaminants in children[1–6] and adults.[7–28] The wristband samplers are generally worn for a 7-day period to capture a wide range of chemicals that represent typical environmental exposures. Despite their promise, there remain unresolved challenges surrounding sample preparation and chemical analysis to extract and analyze potentially thousands of chemicals from the wristband in a single analytical method.[12] Current methods analyzing silicone wristbands primarily use targeted analysis[1–4] which limits the number of compounds detected in a single run to those that are previously selected for analysis. Gas chromatography (GC) coupled to high-resolution mass spectrometry (HRMS) is well suited to address these limitations due to the high mass resolving power that allows for non-target analysis to detect a greater number of compounds in a single run. In addition, retrospective analysis can be performed as chemical databases continue to be developed; this means that data can be reanalyzed against an expanded database without the need to run the samples on the instrument again.

One main advantage of HRMS for the analysis of complex sets of analytes, over single and triple quadrupole mass spectromerty (MS) instruments, is that it allows for simultaneous quantitative analysis of targeted compounds and qualitative analysis (identification) of unknowns. In addition, the high-accuracy masses of fragment ions obtained from tandem MS (MS/MS) fragmentation allow for the structural elucidation of compounds that can facilitate the tentative identification of unknown compounds without a library. For example, the Orbitrap™ HRMS instrument can overcome many of the limitations of other MS instruments because it can use the synchronous full-scan MS and MS/MS acquiring capabilities that are advantageous for both confirmation of the structure and quantification of the analyte. The fast data acquisition rate afforded by Orbitrap™ MS also provides low detection limits and higher sensitivities, making it more suitable for applications in *exposomics* research.[29] Specifically, GC/Orbitrap™ MS can provide a full scan over a wide mass range of each sample with a sub-ppm mass error and can reach a resolution of up to 120 000 at full width at half-maximum at m/z 200.[30–32] Data deconvolution then generates specific values of m/z to be matched with data available in both high- and low-resolution MS libraries to perform unknown screenings without the use of costly standards.[30–32]

Several workflows for suspect and unknown screening analysis using HRMS have been developed and evaluated for environmental contaminants in various matrices using liquid chromatography with HRMS (LC/HRMS).[33–38] However, applications of GC/HRMS in unknown analysis are still very limited and relatively undeveloped. Current workflows for GC/HRMS included targeted pesticide analysis in fruit matrices,[39] fatty acid methyl ester analysis,[40] honey bee extracts,[41] and wastewater samples.[42] Therefore, there is an immediate need for optimized methods that can lead to a higher confidence in identification as over half of tentative detections remain unconfirmed.[43] Confidence levels have been proposed and developed for unknown analysis using electrospray LC/HRMS (see Schymanski et al[44] and Xue et al[45]); however, a similar classification of "confidence levels" in unknown identification using data from GC/HRMS electron ionization (EI) data is limited. The extensive fragmentation resulting from EI (hard ionization) often produce a mass spectra without the molecular ion, which is a criterion necessary for level 1 identification as defined by Schymanski et al[44] in LC/HRMS, which involves soft ionization.

The objectives of the study reported here were to: (1) optimize a workflow for more efficient data processing to identify unknowns in silicone wristbands, (2) provide confidence levels to features observed in EI-HRMS data, and (3) evaluate the effect of different sample preparation steps on the detection limits of Orbitrap™ MS. Analytical interferences are a major challenge in the analysis of wristbands for trace organic contaminants.[2,17,27] Therefore, sample preparations with and without cleanup were examined to determine if analytical performance could be improved with minimal preparation, without adding extreme bias to the analytes present in the final extract. Data processing parameters were optimized to increase confidence in unknown identification (reducing false positives) and to improve analytical efficiency (reducing data analysis time). Lastly, as proof-of-concept towards the application of the optimized sample preparation and data processing workflow for exposure assessment, three wristbands worn by volunteers were analyzed to identify the environmental contaminants to which the individuals had been exposed.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Analytical standards for pesticides, flame retardants, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and other compounds were obtained from Wellington Labs Inc. (Guelph, ON, Canada), Cambridge Isotope Laboratories Inc. (Tewksbury, MA, USA), and AccuStandard Inc. (New Haven, CT, USA). LC/MS and pesticide grade solvents including acetonitrile (ACN), ethyl acetate, hexanes, isooctane, and methanol were purchased from Fisher Scientific (Pittsburg, PA, USA). Sep-Pak™ C18 solid-phase extraction (SPE) cartridges (500 mg, 3 cm^3) were obtained from Waters Inc. (Milford, MA, USA).

2.2 | Wristband analysis

Three volunteers wore wristbands for a period of 7 days in March and April of 2019. Two participants (WB1 and WB3) remained local to western New York while the other participant (WB2) traveled overseas to Southeast Asia. Wristbands were pre-cleaned prior to deployment and prepared according to a method previously described by Travis et al,[1] with slight modifications to sample weight and final volume. Briefly, using solvent-rinsed surgical scissors, wristbands were cut into

eight equal pieces. Four pieces (2 g) were transferred to 50 mL acid-washed glass centrifuge tubes. Extraction was performed twice, each using 25 mL of ethyl acetate on an orbital shaker at 60 rpm for 2 h. Ethyl acetate extracts were combined and concentrated to 300 µL, then ACN (3 mL) was added to samples prior to SPE cleanup. The SPE cartridges were rinsed with 6 mL of ACN. Each of the extracts was passed through an SPE cartridge, collecting the eluent in a 10 mL acid-washed glass centrifuge tube and further eluted with 6 mL of ACN into the same collection vessel. Sample eluents were then evaporated to dryness and reconstituted in 200 µL of $^{13}C_{12}$-PCB-138 (50 ng/g) in isooctane and transferred to 2 mL amber vials. An aliquot (50 µL) of each sample was transferred to an insert and diluted 1:2 with isooctane for analysis via GC/Orbitrap™ MS.

2.3 | GC/HRMS method

A Thermo Scientific TRACE™ 1310 gas chromatograph coupled to a Q-Exactive Orbitrap™ mass spectrometer (Thermo Scientific, San Jose, CA, USA) was used for sample analysis. A splitless inlet was utilized for injection and was set at a constant temperature of 200°C, with a split ratio of 1:100 and a splitless time of 1.00 min. A 30 -m Thermo Scientific TG-5SILMS column with a 0.25 mm internal diameter and a 0.25 µm film thickness was used for separation. The initial oven temperature was set at 70°C and held for 2 min. The temperature was then ramped to 330°C at 20°C/min, and held at 330°C for 5 min. Helium (99.999% purity) was used as the carrier gas and the flow rate was set at 1.0 mL/min. The transfer line and source temperature were both maintained at 250°C. Full-scan acquisition was used in profile mode using EI mode at 70 eV with a mass range of *m/z* 50–650. The resolution and automatic gain control settings were set at 60 000 and 3E6, respectively, and the maximum ion injection time was set to "AUTO."

2.4 | Data processing: suspect screening

An in-house compound database was developed for 123 compounds including pesticides, flame retardants, PCBs, organophosphate esters (OPEs), and PAHs. Standards were run at 1 µg/mL, and the most abundant *m/z* value corresponding to the compound's spectra in the National Institute of Standards and Technology 17 library (NIST, Gaithersburg, MD, USA) and the compound's structure were used in the development of the in-house database. The reference *m/z*, retention time, ion intensity ratio, and up to 10 fragment ions were included in the compound database (Table S1, supporting information). Samples were processed using Thermo Scientific TraceFinder™ 5.1 via a target screening method with settings including a signal-to-noise ratio (S/N) > 5, intensity filter of 10 000, retention time threshold of ±15 s, a minimum of three confirmation ions present, and a mass error of ±5 ppm. The method blank, an unworn wristband prepared following the sample preparation procedure described above, was amplified by a factor of three. Features identified in worn wristbands using the in-house database must be at least three times the level observed in the method blank to be reported.

2.5 | Data processing: unknown screening

A data processing workflow was developed for unknown screening of samples as shown in Figure 1. Samples were injected in triplicate and full-scan EI data were processed using the Deconvolution Plugin 1.5 for TraceFinder™ 5.1. Features defined by retention time and *m/z* were generated using the deconvolution software with initial settings of S/N greater than 3, mass error of ±5 ppm, total ion chromatogram (TIC) intensity threshold of 100 000, search index (SI) threshold of 750, and an ion overlap window of 95%. Feature filtering was then performed requiring at least three ions to be present. Features present in the triplicate injections were then retention time aligned and features also observed in the method blank were excluded. Finally, identification was performed against the NIST 17 library and the vendor-supplied GC/Orbitrap™ contaminants library. The NIST library contains EI spectra for over 260 000 compounds and the vendor-supplied library contains high-resolution spectra for over 700 compounds including pesticides, PAHs, PCBs, flame retardants, dioxins, and furans.

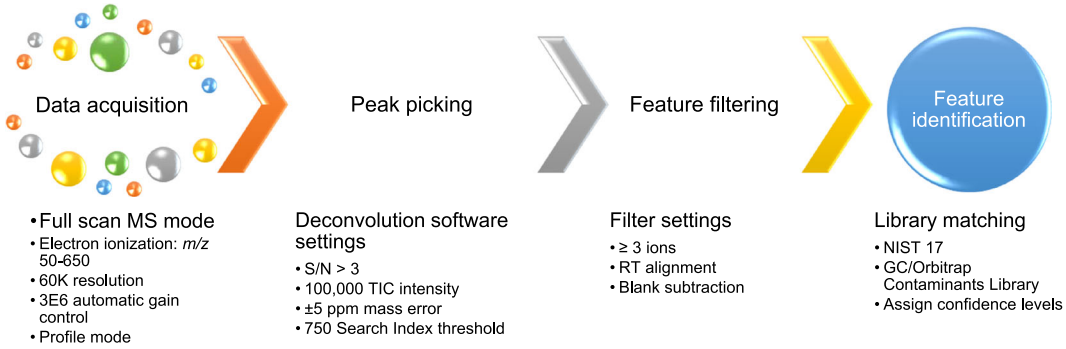

FIGURE 1 Unknown screening workflow for GC/Orbitrap™ MS analysis. Data are acquired using full-scan mode followed by peak picking with the deconvolution software. Feature filtering is then performed and then identification is carried out using the NIST 17 library and vendor-supplied GC-Orbitrap™ contaminants library

Levels of confidence for feature identification are proposed in Table 1 and were assigned to features based on the experimental data obtained and their matched spectra (if available) with MS databases (e.g. NIST 17 library and GC-Orbitrap™ contaminants library). Level 4 identifications have the lowest confidence and are features that contain only an accurate mass for three ions. Level 3 identifications contain a database match with a predicted molecular formula and tentative structure from the accurate mass and the isotope pattern. Level 2 identifications have a higher confidence and include a database match which contains either the molecular ion plus two fragment ions (level 2A) or a database match with three fragment ions (level 2B). Level 1 identifications require a level 2A or level 2B confidence, and match in retention time (± 15 s) and mass spectrum to a reference standard.

3 | RESULTS AND DISCUSSION

3.1 | Investigating efficiency of sample preparation procedure

A vital first step in the analysis of complex environmental samples is to determine a suitable balance between sample cleanup and analyte recovery. Extensive cleanup procedures will reduce matrix interferences, but may also sacrifice analyte recoveries if the extraction conditions are not selective. However, unknown analysis of environmental samples should limit bias towards certain groups of compounds (i.e. polar versus nonpolar) to provide a comprehensive overview of potential environmental exposures. Nevertheless, it is not always possible to eliminate all bias without sacrificing detection limits in unknown analysis. In many instances, the cleanup of complex matrices is necessary to provide a viable sample that produces accurate and reproducible results for compounds that are well recovered in a particular sample cleanup condition. For silicone wristbands, many studies have optimized sample cleanup procedures that involve labor-intensive steps including gel permeation chromatography, or multiple SPE procedures to accommodate a wide range of contaminants for targeted analysis or qualitative screenings.[11,12,17] Therefore, we investigated results from wristbands prepared without cleanup and compared them with samples that were passed through an SPE cartridge to remove some of the unwanted matrix. The MS data for the final extracts were processed following Figure 1 and the total number of features were recorded as well as features after filtering (Table 2).

In the matrix blanks (unworn wristband extracts), the total number of features was reduced by 133 with SPE cleanup compared to no cleanup. In contrast, there were 29 more total features in WB1 with SPE. While sample cleanup decreases the total number of features present in the matrix, it also reduces the background levels (Figure 2) and allows for many contaminants of concern to have a greater S/N and pass the peak picking criterion (S/N > 3). For instance, in WB1 the number of total features is greater with cleanup (1329) than with no cleanup (1300). However, features identified after filtering (retention time alignment, blank subtraction, and minimum ion count of three) were lower in the sample prepared with cleanup (178) than with no cleanup (236), potentially eliminating a

TABLE 1 Proposed confidence levels for EI-HRMS data. Confidence of identifications of features is based on experimental data and available databases.

Confidence level of compound identification	Accurate *m/z*	Accurate *m/z* and isotopes with database match	Database match to three fragment ions	Database match to molecular ion *plus* two fragment ions	Retention time (±15 s) and spectrum match to reference standard
Level 1: Feature confirmed	✓	✓	✓	✓	✓
Level 2A: Putative structure	✓	✓		✓	
Level 2B: Putative structure	✓	✓	✓		
Level 3: Molecular formula and tentative structure	✓	✓			
Level 4: Exact mass	✓				

Sample	SPE	Total features	Number of features after filtering (% of total features)
Matrix blank	No	1146	427 (37.3%)
Matrix blank	Yes	1013	375 (37.0%)
WB1	No	1300	236 (18.2%)
WB1	Yes	1329	178 (13.4%)

TABLE 2 Number of features identified in samples prepared with and without SPE cleanup. Matrix blank (unworn wristband) and a worn wristband (WB1) were used for comparisons of the total number of features as well as the features present after filtering.

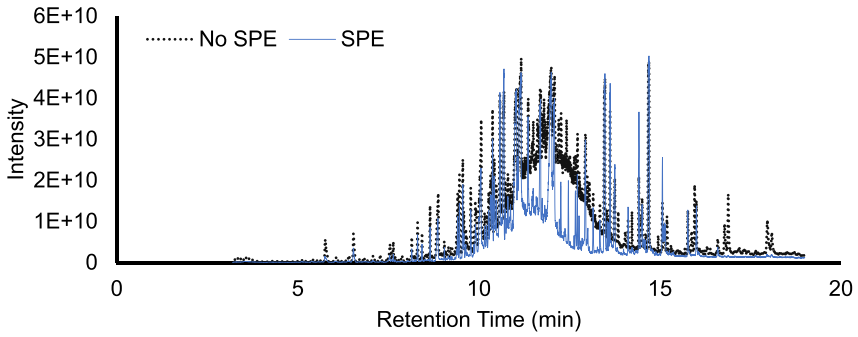

FIGURE 2 Total ion chromatogram comparing a wristband using SPE cleanup (solid line, light blue) and the other without cleanup (dashed line, black). SPE decreases the chromatographic baseline without significant loss in sensitivity, improving S/N ratio and analytical performance

larger number of false positive results. The baseline of the TIC in Figure 2, representing background noise in the sample that underwent an SPE cleanup (solid line, light blue), was reduced by nearly 50%, specifically between 9 and 15 min. In addition, the intensities of the TIC using both sample preparation methods were of similar magnitude (4.9E10), indicating that cleanup had little effect on peak intensity, and in some cases the intensities in the cleaned-up sample were greater than in the sample without cleanup (dashed line, black). With reduced noise, there is an overall gain in S/N as the signal remained the same and baseline was reduced. Such increase in S/N will likely allow for more compounds to be identified, especially at lower concentrations than for wristbands prepared without cleanup.

3.1.1 | Full-scan versus full-scan data-dependent fragmentation acquisition

Full-scan data-dependent fragmentation (ddMS^2) acquisition mode was also investigated as a potentially beneficial option for unknown screening experiments. Full-scan ddMS^2 acquisition fragments features with high signal intensity to aid with structural elucidation of unknown compounds. This acquisition mode has been useful in identifying compounds in LC/HRMS applications using MS/MS libraries[34]; however, the existing libraries for GC/HRMS using EI do not currently contain MS/MS data. Therefore, there is a need for high-resolution GC/MS/MS libraries similar to those that have been developed for LC/HRMS systems.[43]

Identification of EI-MS data utilizes full-scan spectra; and the additional ddMS^2 acquisition could potentially decrease the number of full-scan data points aquired. Fewer full-scan data points may negatively affect the corresponding match factors and therefore decrease the number of positive identifications using EI-MS databases. We investigated both full-scan and full-scan ddMS^2 acquisition modes by analyzing the 123-compound reference mixture containing pesticides, PAHs, flame retardants, PCBs, and OPEs. Samples were acquired in both modes and then subjected to the processing workflow in Figure 1. Compound candidates were given SI and high-resolution filtering (HRF) scores based on the match to spectral databases and experimental spectra; SI and HRF score thresholds were used for peak filtering. The SI score evaluates how well the experimental spectra matches the entry in the library, while the HRF score is the percent of all unique combinations of atoms from the tentative match that are observed in the experimental spectra.[46] For this experiment, an SI threshold of 500 was applied to accommodate a larger range of analytes. Total score, SI, and HRF values were recorded for each analyte and compared between full-scan and full-scan ddMS^2 modes (Table 3).

Average total score, SI, and HRF values all decreased with the addition of ddMS^2 acquisition. The total score, which accounts for both the SI and the HRF values,[41] decreased by 4% for all compounds

TABLE 3 Compound identifications and match factors for full-scan and full-scan ddMS^2 acquisition modes. A reference mixture containing 123 standards composed of pesticides, PAHs, flame retardants, PCBs, and OPEs was spiked into wristband extract. Total score and high-resolution filter values are out of 100, while search index scores are out of 1000. A higher value represents a better match for both parameters.

	Compounds identified (%)		Total score		Search index (SI)		High-resolution filter (HRF)	
Standards (20 ng/g) spiked in wristbands	Full scan	Full scan ddMS^2	Full scan	Full scan ddMS^2	Full scan	Full scan ddMS^2	Full scan	Full scan ddMS^2
Pesticides (n = 58)	43 (77)	34 (59)	88.8 ± 7.6	86.1 ± 8.5	815 ± 108	752 ± 122	83.4 ± 13.9	78.2 ± 15.8
PAHs (n = 24)	22 (92)	15 (63)	91.1 ± 5.2	88.0 ± 7.7	830 ± 65	779 ± 101	86.2 ± 10.2	81.0 ± 15.2
Flame retardants (n = 19)	8 (42)	6 (32)	85.1 ± 5.2	81.4 ± 7.7	755 ± 107	759 ± 68	74.9 ± 12.2	65.4 ± 17.0
PCBs (n = 14)	13 (93)	13 (93)	83.8 ± 7.7	78.7 ± 8.6	840 ± 86	781 ± 110	67.5 ± 15.2	57.8 ± 16.1
OPEs (n = 8)	4 (50)	3 (38)	93.5 ± 5.3	90.9 ± 5.4	774 ± 126	721 ± 111	95.0 ± 7.3	91.2 ± 8.5
All compounds (n = 123)	90 (74)	71 (58)	88.9 ± 7.2	84.9 ± 8.0	815 ± 97	763 ± 109	81.5 ± 14.4	74.3 ± 17.7

and the mean values for total score, SI, and HRF of all compounds were all significantly lower with full-scan ddMS^2 acquisition (total score, $t(159) = 3.183$, $p < 0.005$; SI, $t(159) = 3.178$, $p < 0.005$; HRF, $t(134) = 2.794$, $p < 0.01$). When grouped by class of compounds, the only match factor significantly lowered using full-scan ddMS^2 mode was SI for pesticides ($t(75) = 2.334$, $p < 0.05$). No other statistical differences were observed for any of the other groups or factors. However, with ddMS^2 acquisition, 19 fewer compounds were identified than when no ddMS^2 was acquired, probably as a result of the lower match scores. The lower scan rate for the full-scan ddMS^2 (approximately 4.0 scans/s acquired) than for the full scan (6.8 scans/s) is most likely the reason for fewer identifications. For environmental samples, the lower scan rate would presumably increase the number of false negatives and therefore decrease method sensitivity.

3.1.2 | Data processing optimization

To determine the optimum data processing parameters, a mixture of reference standards containing 123 compounds, including pesticides, flame retardants, PCBs, and PAHs, was spiked into an extracted blank wristband matrix, with concentration of each compound at 20 ng/g wristband. The data obtained were processed using the workflow depicted in Figure 1. Prior to filtering, data processing settings resulted in a feature list containing 1134 reference m/z values. Features from the solvent blank were excluded, reducing the list to 913 reference m/z values, which were then used to determine the optimum SI and HRF thresholds. Threshold values were applied to the data in increments of 10 for the HRF threshold and 50 and 100 for the SI threshold. The filtered feature list generated at each setting was manually inspected for positive hits matching with known analytes in the standard mix. Retention times and annotation of ions were used for final confirmation. Method sensitivity and selectivity defined below in Equations (1) and (2), respectively, were calculated for each filter in Figure 3 to determine the optimum threshold values:

$$\text{Method sensitivity} = \frac{\text{No. of true positives}}{\text{No. of true positives} + \text{no. of false negatives}}. \quad (1)$$

$$\text{Method selectivity} = \frac{\text{No. of true positives}}{\text{No. of true positives} + \text{no. of false positives}}. \quad (2)$$

The method sensitivity is a measure of the ability of the method to identify all of the compounds present in the sample, while the method selectivity is the measure of how well the method accurately identifies compounds present in the sample and distinguishes them from noise.[47] Prior to applying the filters but after blank subtraction, the method sensitivity was 80.5%, given that 99 of the 123 analytes were present among the 913 features identified. Six analytes from the spiked reference mix (1-methylpyrene, pyrene, phenanthrene, tris (2-chloroethyl)phosphate, tris(1-chloro-2-propyl)phosphate, tris (2-butoxyethyl)phosphate) were removed from the feature list during blank subtraction as they were also present in the matrix blank. Twenty-four false negative results were generated due to some compounds not meeting initial parameters (low abundant features

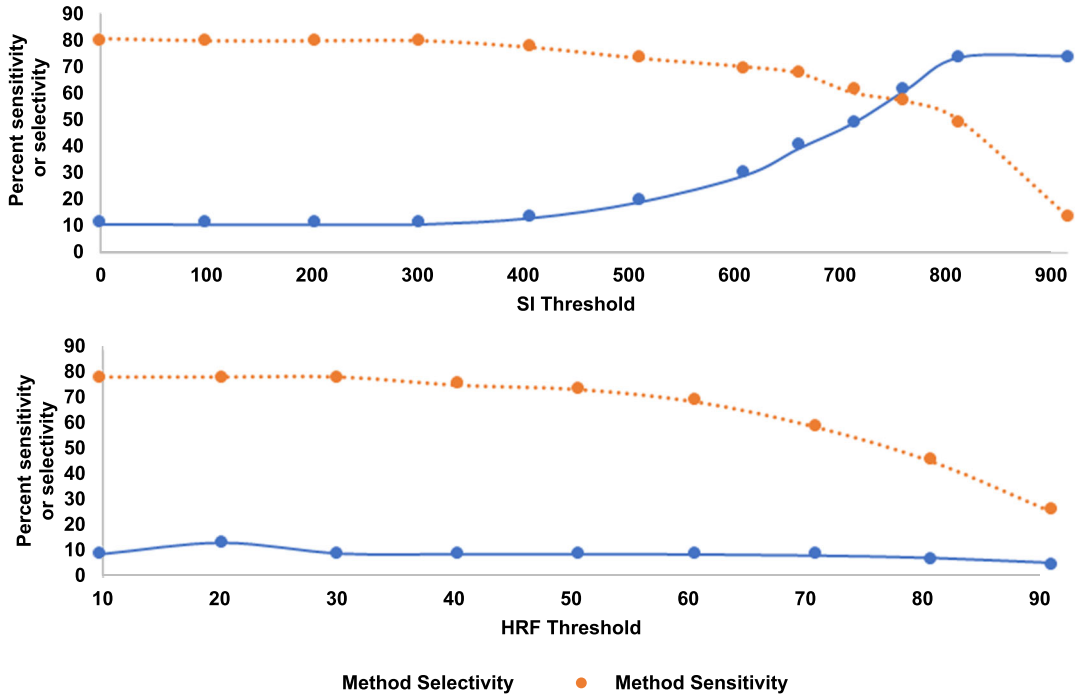

FIGURE 3 Method performance, derived from Equations (1) and (2), at various SI thresholds (top) and HRF thresholds (bottom). Method sensitivity (dashed orange line) and method selectivity (solid blue line) are plotted against each threshold

(below 100 000 intensity) and/or insufficient number of ions (<3 ions)) for inclusion in the feature list.

In determining the method sensitivity and selectivity, the SI threshold had a greater effect than the HRF threshold. SI values greater than 400 showed larger changes to each of the performance parameters. An SI threshold of 750 yielded the greatest selectivity (61%) while still maintaining method sensitivity (57%). These parameters resulted in positively identifying 70 out of 123 compounds in the reference mixture and reduced the amount of data to manually interrogate by 90%. Although the method sensitivity and selectivity did not vary greatly with changes in the HRF threshold, at an HRF value above 60, the sensitivity began to decline due to an increase in the number of false negative results. On the other hand, the selectivity increased to greater than 70% using SI thresholds above 800, but this compromised method sensitivity. Consequently, the SI threshold of 750 was used as an optimum parameter for a balanced method sensitivity and selectivity.

3.2 | Results from worn wristband samples

3.2.1 | Unknown screening analysis

The three wristband samples from volunteers were analyzed using the workflow optimized above (Figure 1) with an SI threshold of 750. Confidence levels were then assigned to features according to Table 1, where on average 920 ± 20 total features were present in each wristband (Table 4). Most of the total features had a level 4 confidence – they had an accurate mass and retention time, but no database match. Since this analysis was focused on providing a streamlined workflow using EI data where the molecular ions may not be present, it is more likely that the level 4 unknowns will be identified using chemical ionization with MS^2 fragmentation. Features with a confidence level 3 and above (Tables S2–S4, supporting information) contained a spectral database match to either the NIST 17 library or GC-Orbitrap contaminants library. Level 2 and above are high confidence identifications containing at least three ions (molecular ion and two fragment ions, or three fragment ions). The basis for these requirements is to have similar identification requirements to those in targeted analysis using a precursor and two fragments (quantifying and qualifying ion). Out of the average 23 ± 5 level 2 features, 13 of these were selected for verification using reference standards. Of these 13, seven features matched the retention time (±15 s) and spectra of the reference standard and therefore were elevated to level 1 confidence (Table 5).

Figure 4 shows an example of a confirmed library match from the unknown analysis that was validated with a reference standard. The five most abundant ions in the spectrum in WB2 (top) that correspond to the library spectrum of 1-methylnaphthalene (bottom) are within the 5 ppm mass error threshold. The retention time of the standard was used to verify the analyte as well as to validate the ions matching the experimental and library spectra. This feature at retention time of 7.19 min resulted in database matches to isomers 1-methylnaphthalene and 2-methylnaphthalene; however, the retention times of the reference standards were used to rule out 2-methylnaphthalene and confirm 1-methylnaphthalene.

Using the unknown screening workflow, a total of seven analytes listed in Table 5 were confirmed using standards. Four of these were included in the 123-compound reference mix (1-methylnaphthalene, 2-methylnaphthalene, naphthalene, and triethyl phosphate) and three additional compounds (2-phenylnaphthalene, lilial, and octrizole) were confirmed after purchasing standards. Lilial is an aromatic compound used in personal care products[48,49] and octrizole is a UV stabilizer also referred to as UV-329.[50,51] Four phthalate standards were purchased (diethyl, dibutyl, di-*n*-octyl, and di-isononyl phthalate) and their fragmentation patterns matched the experimental and library spectra, although the retention times did not match. Based on the observed matching fragmentation patterns, it is hypothesized that the tentative identifications belong to the phthalate compound class but may contain different carbon chain lengths. By confirming tentative identifications with reference standards, further expansion of the target compounds, including those not confirmed, can be implemented in future analysis of wristband samples.

3.2.2 | Suspect screening

Unknown analysis has proven to be useful through the above workflow in identifying unforeseen analytes; however, one of the limitations of reducing the data to a manageable level is that a bias is introduced towards high-abundance features. Therefore, to obtain a comprehensive analysis, a suspect screening workflow was performed using an in-house compound database containing 123 relevant environmental contaminants. Worn wristbands were processed using

TABLE 4 Number of features in wristbands worn by volunteers after each step of the unknown screening workflow, as well as the compounds confirmed with reference standards.

				Level 2		
Wristband sample	Total features	Level 4	Level 3	A	B	Level 1 confirmed with reference standards
WB1	917	737	161	2	15	1-methylnaphthalene, octrizole (UV-329)
WB2	901	725	145	10	15	1-methylnaphthalene, 2-methylnaphthalene, 2-phenylnaphthalene, lilial, naphthalene, triethyl phosphate
WB3	941	750	163	7	20	1-methylnaphthalene

TABLE 5 Confirmed identifications from unknown screening analysis (level 1), suspect screening, and retrospective suspect screening. Reference standards were used to confirm retention time, reference *m/z* values as well as ions characteristic of the compound's structure.

Unknown screening												
Retention time (min)	Reference *m/z*	Mass error (ppm)	Molecular formula	Component name	CAS no.		Molecular ion	*m/z* 1	*m/z* 2	*m/z* 3	*m/z* 4	*m/z* 5
5.61	98.9842	0.5	$C_6H_{15}O_4P$	Triethyl phosphate	78–40-0	*m/z*	NA	98.9842	155.0468	127.0155	109.0049	81.9815
						Formula		H_4O_4P	$C_4H_{12}O_4P$	$C_2H_8O_4P$	$C_2H_6O_3P$	H_3O_3P
						Ppm error		0.5	0.0	0.1	0.2	0.3
6.33	128.0621	0.4	$C_{10}H_8$	Naphthalene	91–20-3	*m/z*	128.0621	102.0464	126.0464	127.0543		
						Formula	$C_{10}H_8$	C_8H_6	$C_{10}H_6$	$C_{10}H_7$		
						Ppm error	0.4	0.0	0.1	0.1		
7.20	141.0699	0.0	$C_{11}H_{10}$	1-Methylnaphthalene	90–12-0	*m/z*	142.0777	141.0699	115.0543	139.0542	113.0386	
						Formula	$C_{11}H_{10}$	$C_{11}H_9$	C_9H_7	$C_{11}H_7$	C_9H_5	
						Ppm error	0.2	0.0	0.2	0.5	0.2	
7.32	141.0699	0.0	$C_{11}H_{10}$	2-Methylnaphthalene	91–57-6	*m/z*	142.0778	141.0699	115.0543	139.0542	113.0386	
						Formula	$C_{11}H_{10}$	$C_{11}H_9$	C_9H_7	$C_{11}H_7$	C_9H_5	
						Ppm error	0.2	0.0	0.2	0.5	0.2	
8.70	189.1274	0.0	$C_{14}H_{20}O$	Lilial	80–54-6	*m/z*	204.1509	189.1274	131.0856	91.0543	147.1169	117.0700
						Formula	$C_{14}H_{20}O$	$C_{13}H_{17}O$	$C_{10}H_{11}$	C_7H_7	$C_{11}H_{15}$	C_9H_9
						Ppm error	0.1	0.0	0.7	1.0	0.6	1.0
11.33	204.0933	0.2	$C_{16}H_{12}$	2-Phenylnaphthalene	612–94-2	*m/z*	204.0933	202.0778	203.0855			
						Formula	$C_{16}H_{12}$	$C_{16}H_{10}$	$C_{16}H_{11}$			
						Ppm error	0.2	0.7	0.3			
13.90	252.1132	0.8	$C_{20}H_{25}N_3O$	Octrizole (UV-329)	3147-75-9	*m/z*	323.1995	252.1132	133.0649			
						Formula	$C_{20}H_{25}N_3O$	$C_{15}H_{14}N_3O$	C_9H_9O			
						Ppm error	0.8	0.8	1.1			

(Continues)

TABLE 5 (Continued)

Unknown screening								
Suspect screening								
Compound name	Reference *m/z*	Mass error (ppm)	Retention time (min)	*m/z* 1	*m/z* 2	*m/z* 3	*m/z* 4	*m/z* 5
1-Propanol, 2,3-dichloro-, phosphate (3:1) (TDCPP)	98.9841	−2.4	12.76	158.9607	208.9530	192.9395	74.9995	190.9424
Acenaphthene	153.0698	−0.4	8.52	151.0542	154.0736	152.0620	154.0777	155.0811
Acenaphthylene	152.0619	−1.0	8.31	126.0464	151.0541	153.0652	150.0463	76.0307
Anthracene, 1-methyl-	192.0934	0.1	11.06	190.0776	165.0699	191.0855		
Anthracene, 2-methyl-	192.0932	−0.8	10.97	190.0777	193.0966	189.0697	191.0854	165.0698
Azinphos-methyl	132.0443	−0.9	13.88	71.0855	77.0386	57.0699	105.0335	
BDE-47	325.8755	−1.5	13.70	487.7097	483.7118	327.8734	323.878	326.8796
BDE-99	403.7855	−2.3	14.72	401.7892	201.8928	202.8921	405.7844	406.7878
Chlorpyrifos	196.9195	−0.9	11.18	207.9480	315.9537	96.9507	259.8912	313.9566
Chrysene	228.0933	−0.3	13.56	224.0622	229.0960	226.0777	200.0619	202.0777
Dibenzothiophene	184.0340	−0.6	10.17	183.0264	185.0373	139.0542	152.0619	186.0297
Dibutyl phthalate	149.0232	−1.1	11.03	105.0334	205.0858	121.0284	150.0266	65.0386
Dichlorvos	127.0155	−0.6	6.66	146.9785	144.9815	186.9733	78.9943	
Diethyl phthalate	149.0233	−0.4	9.02	150.0265	177.0544	176.0466	121.0284	65.0386
Ethanol, 2-butoxy-, phosphate (3:1) (TBEP)	124.9998	0.0	12.99	101.0961	127.0155	153.0310	199.0728	98.9842
Ethylhexyldiphenyl phosphate (EHDPHP)	251.0467	−0.3	13.17	252.0500	83.0855	97.1012	249.0310	95.0855
Fluoranthene	202.0775	−0.8	12.00	101.0386	201.0697	203.0808	200.0619	100.0307
Fluorene	165.0697	−0.8	9.15	166.0732	164.0619	163.0541	166.0776	167.0810
Lilial	189.1273	−0.4	8.68	131.0855	147.1168	119.0856	117.0699	91.0542
Naphthalene	128.0620	−0.3	6.33	127.0542	102.0464	126.0464	129.0654	75.0229
Naphthalene, 1-methyl-	141.0698	−0.3	7.19	142.0733	139.0542	115.0543	142.0777	143.0811
Naphthalene, 1,6-dimethyl-	156.0932	−0.7	8.10	152.0620	115.0542	153.0697	155.0854	141.0698
Naphthalene, 2-methyl-	141.0698	−0.3	7.32	89.0386	143.0811	142.0733	139.0542	115.0542
Octrizole (UV-329)	252.1131	−0.1	13.87	224.0816	105.0699	133.0649	253.1164	79.0542
Parathion	109.0049	0.2	11.18	119.0855	132.0933	96.9507		
PCB 180	393.8020	0.1	13.62	251.9293	325.8603	323.8640	395.7990	

(Continues)

TABLE 5 (Continued)

Unknown screening								
PCB-153	359.8408	−0.4	12.74	363.8345	291.9003	361.8380	289.9033	
PCB-28	255.9612	1.4	10.74	151.0541	186.0231	257.9578		
Pebulate	128.1069	−0.3	8.14	57.0699	72.0443	58.0732	61.0104	
Phenanthrene	178.0775	−1.0	10.33	177.0697	179.0808	152.0619	176.0619	151.0542
Phenanthrene, 2-methyl-	192.0932	−0.9	10.93	190.0776	193.0965	165.0698	189.0696	191.0854
Pyrene	202.0775	−0.9	11.80	201.0697	203.0808	200.0619	101.0385	199.0541
Tri(2-chloroethyl) phosphate (TCEP)	142.9659	−1.6	9.99	160.9764	248.9844	186.9921	222.9687	116.9503
Tributyl phosphate (TBP)	98.9841	−0.2	9.30	137.0361	124.9998	211.1094	127.0155	110.9842
Triethyl phosphate (TEP)	98.9841	−0.2	5.61	124.9998	155.0466	127.0154	81.9814	
Triphenyl phosphate (TPhP)	325.0622	−0.7	13.11	215.0255	233.0362	169.0646	83.0855	123.0440
Tris(3-chloropropyl) phosphate (TCPP)	124.9998	−2.0	10.18	156.9814	201.0075	116.9505	98.9841	139.0157
Retrospective suspect screening								
Dibutyl phthalate	149.0232	−1.1	11.03	105.0334	205.0857	121.0283	150.0264	65.0385
Diethyl phthalate	149.0233	−0.4	9.02	150.0265	177.0543	176.0466	121.0283	65.0385
Lilial	189.1272	−0.9	8.69	131.0855	147.1167	119.0855	117.0698	91.0542
Octrizole	252.1131	−0.1	13.87	224.0816	105.0699	133.0649	253.1164	

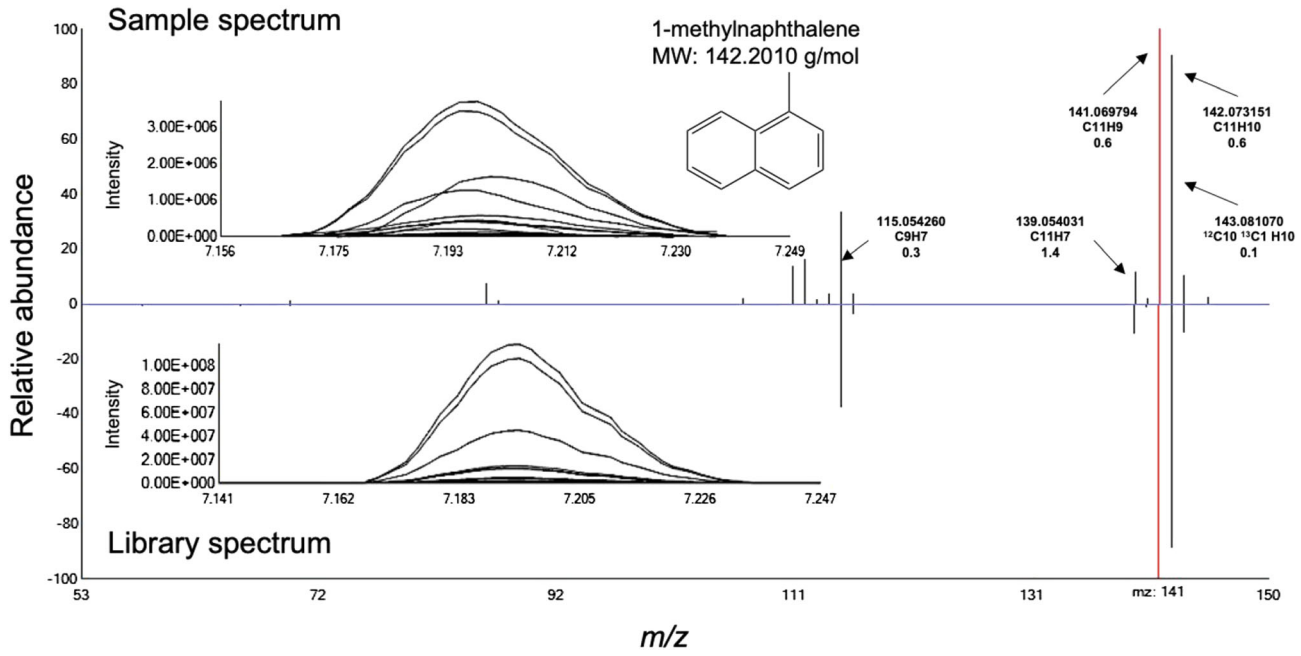

FIGURE 4 Positive identification of 1-methylnaphthalene in WB2 at retention time of 7.19 min. Top shows the spectrum in the sample as well as the analyte peak in the sample. Bottom shows the spectrum of the library match and the peak of the corresponding reference standard. The five most abundant ions matching the library spectra are shown with corresponding formula and mass error (ppm). A certified reference standard was used to confirm the retention time and most abundant ions

the suspect screening workflow outlined above. Across all three wristbands, 32 unique compounds were identified including 15 PAHs, 8 OPEs, 5 pesticides, 3 PCBs, and 2 PBDEs (Table 5). All four analytes confirmed in the unknown analysis that were included in the in-house compound database were also detected through the suspect screening workflow.

Furthermore, a retrospective analysis was also performed by expanding the in-house database with the standards acquired for confirmation of the unknown analysis, and reanalyzing using the suspect screening workflow. Four of the analytes, lilial, UV-329, diethyl phthalate, and dibutyl phthalate, were confirmed in all three worn wristbands. Lilial and UV-329 were confirmed as level 1 in the unknown screening and the two phthalates had been rejected because they did not match the retention times of the standards tentatively identified. In future studies, results from unknown analysis should be used to supplement targeted suspect screening methods and compound databases. Combining suspect screening with unknown analysis will allow for continual exploration and identification of the exposome.

4 | CONCLUSIONS

A workflow for unknown screening of silicone wristbands was optimized to obtain the best method selectivity while retaining sensitivity for the discovery of unforeseen compounds present in samples. Method parameters were optimized for common environmental contaminants including pesticides, flame retardants, and PAHs and resulted in a method sensitivity of 57% and a selectivity of 61%. In addition, comparison of sample preparation resulted in 58 fewer tentative identifications in samples that were cleaned up but displayed an improved S/N. Furthermore, full-scan MS acquisition was observed to produce better matching to mass spectral databases than full-scan ddMS^2 acquisition. The unknown screening workflow was then applied to worn wristband samples, along with proposed confidence levels for EI data, to produce an average of 26 ± 6 high-confidence identifications. Of those, seven were identified using reference standards. Suspect screening and retrospective suspect screening using an in-house compound database were performed as complementary tools to identify 32 and 4 targeted organic contaminants, respectively. Together, these workflows work in tandem to continually unlock the full potential of silicone wristbands in assisting in developing exposomics research.

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PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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