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A method for the analysis of 121 multi-class environmental chemicals in urine by high-performance liquid chromatography-tandem mass spectrometry

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Abstract

Biomonitoring of human exposure to environmental chemicals has gained momentum in recent years. Biomonitoring methods often include analysis of a single class of chemicals with similar chemical properties. In this study, we describe a method that involves solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and capable of measuring 121 environmental chemicals comprising plasticizers (PMs; n=45), environmental phenols (EPs; n=45), and pesticides (n=31) through a single extraction of urine. Urine samples were incubated with 20 μ L of β -glucuronidase/arylsulfatase (4000 units/mL urine) (from *Helix pomatia*) buffered at pH 5.5 for 2 h at 37°C for optimal deconjugation conditions. We compared two extraction methods, namely liquid-liquid extraction and SPE, and the latter with ABS Elut NEXUS® cartridges was optimized to yield best extraction efficiencies. For increased resolution and chromatographic separation, two methods involving Ultra AQ C18® and Betasil™ C18® columns were used. The MS/MS analyses were performed under both negative and positive ionization modes. The optimized method yielded excellent intra- and inter-day variabilities (relative standard deviation: 0.40–11%) and satisfactory recoveries (80–120%) for >95% of the analytes. The limits of detection were 0.1 for 101 analytes and between 0.1 and 1.0 ng/mL for 18 analytes. The optimized SPE LC-MS/MS method was validated through the analysis of standard reference materials and proficiency test urine samples and further applied in the analysis of 21 real

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supporting Information (SI)

The SI contains details of analytical standards (Table S1), MS/MS parameters used in the analysis (Tables S2–S4), recoveries (%) of OH-PAHs and PMs under different enzymatic deconjugation conditions (Table S5), concentrations measured in several PT urine samples (Table S6), concentrations (ng/mL) in real urine samples (Table S7), and total ion chromatograms of target analytes injected at a concentration of 1 ng/mL each (Figures S1–S3).

urine samples to demonstrate simultaneous determination of 121 environmental chemicals in urine samples.

Keywords

Endocrine disruptors; Human biomonitoring; Urine; LC-MS/MS; Multi-class method

1. Introduction

Humans are continuously exposed to toxic environmental chemicals via multiple exposure routes (inhalation, ingestion, and dermal uptake) and sources (food, water, indoor and outdoor air) on a daily basis. Chemical exposure in humans has been a topic of public health concern for decades [1]. As increasing number of new chemicals is constantly introduced into the market, there is an ongoing interest in the assessment of these chemicals' exposures and resulting health effects in humans [2]. For instance, ~150,000 chemical substances have been registered in the European Union's regulatory program namely Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) [3], which signifies the broad range of chemicals to which humans can be exposed. Among various methods available for the assessment of chemical exposures, biomonitoring has become the most health-relevant approach. Nevertheless, ultra-trace level (picogram to microgram quantities) determination of environmental chemicals in human specimens can be challenging due to the lack of quantitative analytical methods, analytical standards, and cost among several others. Furthermore, many environmental chemicals, following exposure, undergo biologic transformation in human bodies [4]. Oxidation, reduction, hydrolysis, hydroxylation, sulfation, and alkylation followed by phase II conjugation with glucuronide or other biomolecules can confound the identification of a chemical's exposure biomarker [5]. Lack of complete understanding of toxico-kinetics of xenobiotics, especially that of emerging chemicals, poses challenges in biomonitoring.

Traditionally, biomonitoring methods were developed with a focus on measuring a defined pollutant class in a single analysis. For example, phthalate metabolites, environmental phenols (EPs), and current use pesticides were analyzed in urine using a separate extraction and sample preparation steps for each of the chemical classes. This is due to the fact that chemicals within a class that possess similar physico-chemical properties (such as solubility, polarity, ionizability, and chromatographic properties) are amenable for optimal extraction and quantification in a single method. Nevertheless, considering the fact that humans are exposed to hundreds, if not thousands, of chemicals application of specific analytical methods for each of the chemical classes can be costly and time-consuming. Furthermore, each of the analytical methods would consume an aliquot of human specimen used in extraction, which would necessitate requirement of large volumes for biomonitoring of multiple chemical classes. Therefore, a robust analytical method capable of measuring a wide range of chemical classes in a single extraction would alleviate the need for large sample volumes while reducing the time and cost of analysis [6–8].

One of the resource/labor-intensive steps in bioanalysis is sample preparation, which is required for the removal of bulk of interferences and endogenous compounds (i.e., proteins and phospholipids) from target chemicals prior to instrumental analysis. A robust sample preparation method that offers acceptable accuracy and precision in multi-class chemical analysis can be challenging, as polar functional groups in each chemical class can have different properties and therefore, are not amenable simultaneous extraction. Owing to the improvement in sensitivities and robustness of the latest generation of high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) instruments, a trend towards the development and application of multi-class analytical methods is envisaged in recent biomonitoring studies (Table 1) [9–19].

A recent investigation of the extant data for chemicals not measured by the United States National Health and Nutrition Examination Survey (NHANES), identified those that are used in consumer products for which toxicity data are available and likelihood of human exposure was considerable [20]; this investigation highlighted the need for biomonitoring of chemical classes such as: alternative flame retardants, alternative plasticizers, environmental phenols (EPs) especially bisphenol A (BPA) substitutes, and current-use pesticides. Further details of the selection criteria of these chemical classes are described elsewhere [20]. Thus, we selected the target analytes from the list of emerging chemicals identified by Pellizzari et al [20] and enhanced the list with legacy chemicals that can be analyzed in urine using the same sample preparation method. The target analytes selected for the present study include plasticizers such as phthalates, terephthalates, and organophosphate ester metabolites (all three classes collectively referred here as PMs), EPs including bisphenol analogues, chlorophenols, hydroxy polycyclic aromatic hydrocarbons (PAHs) and benzophenones and current-use pesticides including neonicotinoids. Phthalate plasticizers are substituted with emerging chemicals like terephthalates and organophosphate esters [21]. EPs are used as antimicrobial preservatives (e.g., parabens, triclocarban, and triclosan), sunscreen agents (e.g., benzophenones), and industrial compounds (e.g., chlorophenols and bisphenols and their halogenated derivatives) [10]. Numerous studies suggested that exposures to these chemicals are associated with endocrine disruption, cytotoxicity, genotoxicity, reproductive toxicity, and neurotoxicity [22, 23]. To the best of our knowledge, no previous study reported the analysis of multi-class chemicals comprising plasticizers, phenolic compounds and pesticides in urine using LC-MS/MS methods. In this study, we describe a multi-class targeted LC-MS/MS method using isotopic dilution mass spectrometry for simultaneous identification and quantification of 121 biomarkers, including 45 PMs, 45 EPs, and 31 pesticides in human urine, which can be applied in the assessment of population exposure to environmental chemicals. The method is capable of measuring several classes of chemicals in a single extraction, and would save cost and time in biomonitoring programs.

2. Materials and methods

2.1. Reagents and chemicals

HPLC-grade water, methanol (MeOH), ethyl acetate (EtAc), acetonitrile (ACN), methyl *tert*-butyl ether (MTBE), dichloromethane (DCM), acetone, hexane, ammonium acetate (NH₄Ac), and ACS-grade formic acid (FA) were obtained from J.T. Baker (Phillipsburg, NJ,

USA). ACS-grade acetic acid (HAc) was purchased from Macron Fine Chemicals (Phillipsburg, NJ, USA). HPLC-grade phosphoric acid (PA) was obtained from Burdick & Jackson (Muskegon, MI, USA). HPLC-grade ammonium formate (AF) and ACS-grade sodium phosphate monobasic monohydrate (SPMM) and ammonium hydroxide solution (NH₄OH; 28.0–30.0% NH₃ basis) were purchased from Sigma-Aldrich (St Louis, MO, USA). Three types of β -glucuronidase enzymes, namely, β -glucuronidase/arylsulfatase liquid enzyme from *Helix pomatia* (ALS; β -glucuronidase activity \approx 100,000 units/mL; sulfatase activity \approx 47,500 units/mL; Item #10127698001), and β -glucuronidase from *E.coli*-K12 (K12; β -glucuronidase activity \approx 140 units/mg; Item #03707601001) and *Helix pomatia* (HP; β -glucuronidase activity 68,800 units/mL; sulfatase activity 5000 units/mL; Item #152284) were purchased from Roche Life Science through Sigma Aldrich (Mannheim, Germany) and MP Biomedicals (Santa Cruz, CA, USA), respectively. The enzyme K12 contained only glucuronidase activity whereas ALS and HP contained both glucuronidase and sulfatase activities with a greater sulfatase activity in the former.

Analytical standards (NSs) for 121 target chemicals and 92 isotopically labeled internal standards (ISs) of 95–99.9% purity were purchased from multiple suppliers including Cambridge Isotope Laboratories (Andover, MA, USA), Sigma-Aldrich (St Louis, MO, USA), AccuStandard (New Haven, CT, USA), Dr. Ehrenstorfer GmbH (Augsburg, Germany), Toronto Research Chemicals (North York, ON, Canada), TCI America (Portland, OR, USA), and C/D/N Isotopes (Pointe-Claire, Quebec, Canada) (Table S1 in the Supporting Information; SI). Chemical names, CAS numbers, and their abbreviations used in this study are given in Table 2. Majority of the analytical standards were purchased at a concentration of 1000 μ g/mL or 100 μ g/mL in an organic solvent. Some standards were purchased as neat compounds. Stock solutions of neat chemicals were prepared in ACN or MeOH and a small fraction of that was also prepared in acetone or hexane, at a concentration range of 100–1000 μ g/mL. Known concentrations of individual stock solutions were transferred into 15 mL glass tubes for the preparation of a mixture of NSs or ISs at a concentration of 1 μ g/mL. These solutions were diluted serially with water:ACN (8:2 v/v) for use in method development and analysis. All intermediate and working standard solutions were stored at -20 °C.

2.2 Urine samples and enzymatic treatment

Pooled urine samples collected from healthy adult volunteers, two standard reference materials (SRM 3672 and 3673) purchased from National Institute for Standards and Technology (NIST; Gaithersburg, MD, USA), and nine proficiency test (PT) samples obtained from three different providers were used in method development and validation. In addition, 21 de-identified individual adult urine samples collected from 11 males and 10 females in 2017 in Albany, New York, USA, were analyzed to demonstrate the feasibility of the developed method. All samples were stored frozen at -20 °C until analysis.

Majority of the target analytes were expected to be excreted as glucuronide or sulfate conjugates in urine. Enzymatic deconjugation steps were optimized to yield total concentrations of all target analytes [24, 25]. Further details of optimization of enzymatic deconjugation are given below. Among three different types of β -glucuronidase/arylsulfatase

tested, ALS type from Sigma-Aldrich yielded higher recoveries within 2 h of incubation. The conditions for enzymatic deconjugation were optimized as: 500 μ L of urine taken in a polypropylene (PP) tube, fortified with 2.5 ng each of ISs followed by the addition of 500 μ L of 1 M NH_4Ac buffer (pH 5.5) containing 20 μ L of ALS enzyme (2000 units). After gentle mixing, samples were incubated for 2 h at 37°C with shaking at 100 rpm (Jeio Tech Co., Seoul, Korea). Quality control (QC) samples include analyses of reagent/procedural blanks (HPLC grade water used instead of urine), matrix blanks (pooled urine), matrix spike samples (analytes spiked in pooled urine at three different concentrations: 1, 10, and 20 ng/mL), two SRMs, and nine PT samples..

2.3 Sample extraction

After incubation of samples with β -glucuronidase/arylsulfatase, the reaction was quenched by the addition of 1.0 mL of 0.145 M SPMM buffer (pH 2.0; prepared by dissolving 20 g SPMM and 10 mL of PA in 990 mL of water). The sample was then subjected to ABS Elut NEXUS[®] solid phase extraction (SPE) (60 mg, 3 mL; Agilent Technologies, Santa Clara, CA, USA). Whereas several combinations of conditioning of cartridges followed by elution of analytes were followed, optimal recovery and reproducibility were found under the following conditions: preconditioning with 1.5 mL of ACN followed by 1.5 mL of 0.145 M SPMM buffer (pH 2.0), loading of samples, washing with 1.5 mL of 0.1 M FA in water and 1.5 mL of 5% MeOH in water (to remove matrix interferences) and elution of target compounds with 1.0 mL of ACN, 1.0 mL of EtAc, and 1.0 mL of MeOH:DCM (1:1, v/v). The eluate was collected into a 15 mL PP tube and concentrated to near-dryness under a gentle nitrogen stream. The residue was reconstituted in 250 μ L of water:ACN (8:2, v/v) mixture, vortexed, and transferred into amber glass vials for LC-MS/MS analysis.

2.4 Instrumentation

The HPLC system comprised ExionLC solvent valve, ExionLC AD pump, ExionLC autosampler, ExionLC controller, and ExionLC AC column oven (SCIEX, Redwood City, CA, USA). Mass spectrometric analysis was performed with an AB SCIEX QTRAP 5500+ triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization source operated in both positive and negative modes. The optimal LC-MS/MS conditions, other method development and quality assurance protocols are described in detail below.

3. Results and discussion

A total of 121 analytes grouped into three categories as PMs, EPs and pesticides were targeted in the present study (Table 2). The method development and validation consisted of the following protocols in that order: (1) optimization of mass spectrometric parameters followed by liquid chromatographic separation, (2) optimization of sample preparation and extraction protocols, (3) method validation through the analysis of SRMs, PT and fortified samples, and (4) demonstration of feasibility through the analysis of real urine samples. Analytical methods for individual classes of the target chemicals studied here were developed, validated and successfully applied in several biomonitoring studies in our

laboratory [11, 26, 27]. The current method combines salient features from several of those earlier methods into a single robust method for extraction and analysis.

3.1 Optimization of LC-MS/MS conditions

The choice of aqueous-organic mobile phase additives and LC columns plays vital roles in chromatographic separation. Various combinations of mobile phases [two organic mobile phase solvents (MeOH and ACN) and five additives (HAc, FA, NH₄Ac, AF, and NH₄OH)] and four reverse-phase columns [Ultra AQ C18[®] (3 μm, 100 mm × 2.1 mm, Restek, Bellefonte, PA, USA), Betasil[™] C18[®] (5 μm, 100 mm × 2.1 mm, Thermo Scientific, West Palm Beach, FL, USA), Eclipse Plus C18[®] (3 μm, 100 mm × 4.6 mm, Agilent, Santa Clara, CA, USA), and Kinetex C18[®] (1.3 μm, 50 mm × 2.1 mm, Phenomenex, Torrance, CA, USA)] were tested in various combinations by analyzing the standard mixture (both NSs and ISs) at 10 ng/mL dissolved in the mobile phase. It was found that peaks of several PMs and EPs did not resolve adequately under a single LC condition. For instance, under the mobile phase compositions of 0.1% HAc in water and MeOH, PMs showed excellent chromatographic separation and peak shapes whereas signal intensities of several EPs were considerably suppressed. We tested MeOH, ACN and a combination of MeOH and ACN as mobile phases for the separation and analysis of EPs and pesticides. We found that ACN, as the organic mobile phase solvent, yielded narrower and well resolved chromatographic peaks with all four LC columns tested. Mobile phase additives such as NH₄Ac or AF enhanced the signals for pesticides. However, it was found that the presence of a buffer in mobile phases affected the retention, signal intensity and resolution of some bisphenols (e.g. BPA and BPF), benzophenones (e.g. BP-3), and bisphenol A diglycidyl ethers (e.g., BADGE, BADGE·H₂O, and BADGE·2H₂O). Enhancement in signal intensities was achieved for some EPs such as BPA and BADGE, with NH₄OH as the mobile phase additive; but analytes such as BPS, 2,4-D, and dinotefuran were not retained efficiently in the analytical column and eluted within 1 min, under those alkaline conditions. On the other hand, poor chromatographic separation and peak shapes of OH-PAHs resulted from acidic mobile phase or the presence of buffer. Among the four LC columns tested, Ultra AQ C18[®] column enabled excellent chromatographic separation of PMs, with symmetric peaks. Good separation and peak shapes were achieved for 2,3,4,6-TeCP, 2,3,5,6-TeCP, and 2,3,4,5-TeCP by Betasil[™] C18[®] column with water and ACN as the mobile phase. Therefore, two separate injections were required with two different LC conditions to accomplish excellent sensitivity, peak shape and chromatographic separation of 121 target compounds.

The first LC method involved chromatographic separation of target analytes using an Ultra AQ C18[®] column. The HPLC mobile phase comprised 0.1% HAc in water (A) and 0.1% HAc in MeOH (B). The initial mobile phase composition was 5% B, held for 1 min, and then increased to 45% B within 0.2 min, and held for 1.3 min. Then, the composition was increased to 70% B within 2.2 min, then to 99% B in 2.0 min, and held for 3.0 min. Return to initial mobile phase conditions and column equilibration were accomplished in the last 2.3 min, with a total run time of 12 min. The MS/MS method was in the negative ionization mode (MSM1_NEG). Curtain gas (CUR), collision activated dissociation gas (CAD), source temperature (TEM), nebulizer gas (GS1), heater gas (GS2), and turbo ion spray voltage (IS) were set at 20 psi, 8 psi, 600°C, 60 psi, 60 psi, and 5500 V, respectively. For the second LC

method, the chromatographic separation of target analytes was achieved with a Betasil™ C18® column. The HPLC mobile phase comprised water (A) and ACN (B) with the following gradient program; 20% B for 1 min, increased to 60% B within 0.2 min, then to 99% A within 4.3 min, held for 1.5 min, decreased to 20% B within 0.5 min, and equilibrated for 1.5 min, with a total run time of 9 min. The MS/MS analysis proceeded under both negative (MSM2_NEG) and positive (MSM2_POS) ionization modes. CUR, CAD, TEM, GS1, GS2, and IS were set at 20 psi, 8 psi, 600°C, 60 psi, 70 psi, and –4500/+4500 V, respectively. All target analytes were grouped based on their peak shape, separation, and sensitivity under the two separate methods. Accordingly, MSM1_NEG (mostly PMs), MSM2_NEG (mostly EPs), and MSM2_POS (mostly pesticides) yielded optimal analytical performance for 45, 45, and 31 of the 121 target compounds, respectively (see Table 2 for specific method classification). The compound specific m/z transitions of multiple reaction monitoring and optimized MS/MS parameters acquired by direct infusion of each analyte at 10 ng/mL using an in-built syringe pump are shown in Tables S2–S4. Typical chromatograms obtained from MSM1_NEG, MSM2_NEG, and MSM2_POS under the optimized mobile phase conditions are shown in Figures S1–S3 (1 ng/mL) and Figures 1–3 (10 ng/mL). Compound specific retention times are listed in Table 2.

3.2 Sample preparation

3.2.1 Enzymatic hydrolysis—Xenobiotics undergo conjugation reactions to form various hydrophilic conjugates, such as *O*-glucuronides (i.e., PMs and EPs) [28, 29], *N*-glucuronides (i.e., TCC) [30], and sulfates (i.e., EPs) [31–33], prior to biliary or urinary excretion. Several factors such as age, gender, race and environmental factors determine the rate of glucuronidation and that can result in dramatic differences in the formation of glucuronidated metabolites. Thus, glucuronide/sulfate deconjugation is an important step in analysis of exposure to environmental chemicals. In this study, glucuronidase reaction was optimized through a full factorial design to examine the effect of enzyme type (ALS, HP, and K12), enzyme amount (10, 20, 30, and 40 μL/sample), buffer pH (5.0, 5.5, 6.0 and 6.5) and incubation time (1, 2, 4, 6, 8, 12, and 24 h), for a total of 54 experiments. The effectiveness of deconjugation reaction was also evaluated by analyzing SRM 3672, for which certified and reference values are available for 17 EPs and 11 PMs targeted in this study. The experiments on enzymatic hydrolysis yielded similar results under all test conditions for OH-PAHs and PMs (except at buffer pH 5.0 for PMs; Table S5), but the results varied considerably among EPs (Figure 4). Based on the recoveries of analytes, ALS enzyme was found superior over the other types (Figure 4A). Satisfactory recoveries were obtained for 7 certified EPs with ALS (80–103%) enzyme in comparison to those of HP (65–97%) and K12 (50–94%) enzymes. Incomplete hydrolysis of MeP (50%), EtP (62%), and PrP (63%) with K12 enzyme may be due to its lack of sulfatase activity. Furthermore, a complete hydrolysis of *N*-glucuronidated TCC was achieved using ALS enzyme than that of HP and K12 enzymes. Measured TCC concentrations in 21 real urine samples incubated with ALS enzyme were 26% and 45% higher than those obtained using HP and K12 enzymes, respectively. We also tested the hydrolysis efficiency of 7 EPs with varying amounts of ALS enzyme (Figure 4B). Incubation with both 10 μL (80–103%) and 20 μL (82–105%) volumes of enzyme yielded similar recoveries for 7 EPs but a volume of 20 μL yielded higher recoveries for TCS than did 10 μL (95% vs 89%). Incubation with 30 and 40

μL volumes of enzymes resulted in inconsistent recoveries of EtP (80%) and TCS (125%). This may be due to alkyl/aryl sulfatase mediated hydrolysis of alkyl-ester bond of EtP to *p*-hydroxybenzoic acid [34]. It was reported that the addition of MeOH before incubation would quench aryl sulfatase activity [35], which was not observed in our study. We then tested deconjugation efficiencies at different pH (5.0, 5.5, 6.0 and 6.5; Figure 4C) and incubation times (1, 2, 4, 6, 8, 12, and 24 h; Figure 4D). Although the manufacturers recommended an optimal pH of 4.5–5.0 for the ALS enzyme activity, we found that pH 5.5 was optimal for MeP and PMs. With regard to the incubation time, complete hydrolysis of 7 EPs was accomplished within 2 h at 37°C. Furthermore, the recoveries of MeP and TCS decreased with increasing incubation time. It has been reported that the sulfatase activity can convert parent phthalate diesters into their monoester metabolites [36]. This was found for mEHP, in that the recoveries significantly increased after incubation beyond 12 h (Table S5). Under the optimized conditions of enzymatic deconjugation, 28 certified chemicals in SRM 3672 yielded recoveries of between 80 and 111% following incubation at 37°C for 2 h with 20 μL ALS enzyme (i.e., 4 units/ μL urine) at pH 5.5.

3.2.2 Sample extraction—Conventional liquid-liquid extraction (LLE) with EtAc and solvent induced phase transition extraction (SPITE) with ACN, MTBE, and EtAc were examined. Fortified urine samples were extracted twice with 2 mL of EtAc or combinations of ACN, MTBE, and EtAc. The urine-solvent mixture was shaken in an orbital shaker (Eberbach Corp., Ann Arbor, MI, USA) at 180 strokes per min for 30 min and centrifuged at $4500 \times g$ for 10 min. The supernatants were combined in a clean PP tube and purified by mixing with water or Q-sep™ QuEChERS dispersive-SPE (dSPE; containing 150 mg MgSO_4 and 50 mg C18; Restek, Bellefonte, PA, USA). Some more polar compounds such as PA, mMP, mCPP, dinotefuran, and 2,4-D were lost in the extraction step. Furthermore, quantification of EPs such as BPA, BP-3, and BADGEs was affected by strong matrix effects even after purification by dSPE. dSPE step also increased the background level of contamination of some target compounds in blanks (e.g., mEHP and BPA). Our target analytes vary in polarity from moderate to very high. Therefore, LLE or SPITE method was not found suitable and we optimized the SPE method that has a broad range of retention for polar to nonpolar compounds at a wide pH range.

To maximize extraction efficiency and minimize matrix effects, three commonly used SPE cartridges in biomonitoring studies [10, 16, 37], namely ABS Elut NEXUS®, Oasis HLB® (60 mg, 3 mL; Waters, Milford, MA, USA) and Strata X-AW® (60 mg, 3 mL; Phenomenex, Torrance, CA, USA) were selected for optimization, by following manufacturers' guidance, with some modifications. Specifically, cartridges were conditioned by passage of solvents - [1.5 mL of ACN followed by 1.5 mL of 0.145 M SPMM buffer (pH 2.0)] and [1.5 mL of MeOH followed by 1.5 mL of water] for ABS/HLB and X-AW cartridges, respectively. As charged molecules are not retained in ABS/HLB cartridges, enzymatically hydrolyzed sample was pH adjusted by the addition of 1 mL of 0.145 M SPMM buffer (pH 2; "pK_a-rule") prior to loading. For X-AW cartridge, 1.0 mL of 25 mM NH_4Ac buffer (pH 6.5) was used. Then, the urine samples were loaded onto cartridges and washed with 2.0 mL of water and 2.0 mL of 25 mM NH_4Ac buffer (pH 6.5) for ABS/HLB and X-AW cartridges, respectively. After dried under vacuum for 5 min, the target analytes were eluted by [1.5 mL

of ACN and 1.5 mL of EtAc] and [1.5 mL of MeOH and 1.5 mL of 5% NH₄OH in MeOH] for ABS/HLB and X-AW cartridges, respectively. The eluate was collected into a 15 mL PP tube and concentrated to near-dryness under a gentle nitrogen stream. The residue was reconstituted in 250 μ L mixture of water:ACN (8:2, v/v), vortexed, and transferred into amber glass vials for LC-MS/MS analysis. Among the three SPE cartridges, ABS showed excellent recoveries for all target analytes except for TBBPA, TCBPA, TeCBPA, and OH-PAHs (<10%), which suggested that ACN and EtAc were not adequate to elute compounds with high log K_{ow} values. Nevertheless, ABS cartridge was selected and the elution and washing steps were further optimized to recover chemicals with high log K_{ow} . A mixture solvent of MeOH and DCM or acetone was used to test the elution and found that recoveries of the target compounds with high log K_{ow} values increased (>90%) with 50% DCM in MeOH. Besides, we found that 1.5 mL of 0.1 M FA in water followed by 1.5 mL of 5% MeOH in water as washing solvents considerably reduced matrix interferences and ion suppression while improving sensitivity and robustness of the method.

3.3 Method validation

The method was validated by determining precision, accuracy, linearity (dynamic range), limit of detection (LOD), and matrix effect (Table 3). In this study, 92 of the 121 target compounds had corresponding IS available for isotopic dilution method of analysis. Alternative ISs, with properties similar to those of the target compounds, were used in the quantification of remaining 29 analytes. Instrument calibration range (linearity) was verified based on a 12-point matrix-matched calibration curve prepared in synthetic urine (Cerilliant, Round Rock, TX, USA), at concentrations ranging from 0.01 to 100 ng/mL (with 10 ng/mL ISs). More than 90% of the analytes showed excellent linearity with regression coefficient (r^2) values >0.99 except for PA, mBzTP, TPhP, BP-6, BPB, BPP, Tetra-CBPA, BADGE-H₂O, BADGE-2H₂O, cis-DCCA, metribuzin, and propiconazole for which r^2 values were >0.95 (Table 3).

The LODs were calculated as $3 \times S_b/a$ (S_b : intercept standard deviation; a: slope) from the regression equation obtained for low concentration range (ranging from 0.01 to 5 ng/mL) of spiked urine. The LODs ranged from 0.1 for 101 and from 0.1–1.0 ng/mL for 18 of the 121 target compounds. These values are comparable to bioanalytical methods reported previously (Table 1). The LODs for PA (5.0 ng/mL) and mEHP (2.0 ng/mL) were relatively high due to the fact that these two chemicals have high background values (many laboratory products and reagents contain these chemicals).

The accuracy is expressed as the relative error (RE, %) of an expected value, and its variation in repeatability (precision) is determined using the relative standard deviation (RSD). According to the guidance for bioanalytical method validation, acceptable accuracy and precision ranges are divided at two fortification levels [38]. First, from –20% to 20% with RSD \leq 20% at the limit of quantitation (LOQ) level. Second, from –15% to 15% with RSD \leq 15% at higher concentrations [44]. The accuracy and precision were determined at three fortification levels of 1, 10, and 20 ng/mL and the determination of intra- and inter-day variances of five measurements of three different levels injected daily for 6 days. At 1 ng/mL level, RE for the 121 analytes were –13% to 9.7% with RSDs ranging from 1.7% to 11.0%

for intra-day measurements and -13% to 9.8% with RSDs from 0.90% to 10% for inter-day measurements (Table 3). At 10 and 20 ng/mL, RE ranges were -14% to 12% with RSDs of 0.6% to 10% for intra-day and -13% to 14% with RSDs of 0.4% to 10% for inter-day variances. Thus, the analytical method satisfied the criteria for accuracy and precision analyzed using fortified urine samples.

The extraction efficiency of the analytical method was considered excellent when the recovery rate of a target compound was near or equal to 100%. Analyte recovery and its variation (RSD) were also used in the determination of accuracy and precision in bioanalytical methods. Generally, a recovery rate of 80–120% with RSD < 20% is an acceptable range for those parameters [39]. The target analytes were fortified in QC samples (i.e., urine pool) at three different levels (i.e., 1, 10, and 20 ng/mL), to represent low, medium, and high concentrations. The QC samples were passed through the optimized sample preparation method described above (i.e., enzymatic incubation followed by SPE extraction). The recovery ranges of target analytes were 72–156% (RSD; 0.25–12%), 62–122% (RSD; 0.52–11%), and 68–117% (RSD; 0.34–12%) at the fortification levels of 1, 10, and 20 ng/mL, respectively (Table 3). Among the 121 analytes, 115, 117, and 120 analytes showed the recovery range of 80–120% with RSD values of <20% at fortification levels of 1, 10, and 20 ng/mL, respectively. Slightly lower recoveries for BADGE-2H₂O (62% and 68% at medium and high levels) and higher recoveries for flonicamid (156% at low level) and N-Dmt (138% at low level) were found but the repeatability (RSD; 0.72–6.8%) was acceptable for these compounds.

The matrix effect (ME) was calculated using the following Equation (1):

$$ME/\% = \left(\frac{A-B}{A} \right) \times 100 \quad (1)$$

where A is the peak area of an analyte in a pure solvent and B is the peak area of an analyte spiked in synthetic urine that has undergone sample preparation steps, at the same concentration as the standard solution. Matrix effect of each compound is expressed as the percentage enhancement (<0%) or suppression (>0%).

The results of matrix effect calculated for the 121 target analytes are shown in Table 3. The matrix effects ranged from soft (matrix effect within -20% to 0% or 0% to 20%), moderate (-50% to -20% or 20% and 50%), and strong (below -50% or above 50%) [40]. After optimization of SPE washing step, matrix effect for most compounds (83; 68.2% of total) fell within moderate range (Figure 5). Nevertheless, inclusion of IS as well as matrix-matched calibration curve enabled accurate analysis of target chemicals in urine.

The method was further validated through the analysis of NIST SRM 3672 and 3673 as well as nine different PT urine samples from the German-External Quality Assurance Scheme (G-EQUAS; n=4; 65/2020 9A/B and 14/15 A/B), External Quality Assessment Scheme for Organic Substances Program (OSEQAS; n=3; OS-U-E 2004, 2005, and 2006), and Centers for Disease Control and Prevention-Biomonitoring Quality Assurance for State Programs (CDC-BQASP; n=2; 201802001UPSU and 201802002UPSU). Our measurement of 121 target chemicals analyzed by the developed method is shown in Table S6. For those analytes

that have been certified in the samples, our method yielded acceptable concentrations, further validating quantitative accuracy, precision and traceability.

3.4. Method application

The method was applied for the analysis of 21 urine samples collected from healthy adults from Albany area of New York state in 2017 (Table S7 and Figure 6). Forty target analytes including 20 PMs, 15 EPs, and 5 pesticides were found in >70% of the urine samples. The sum concentrations of the 121 compounds ranged from 82 to 629 ng/mL, with mean and median values of 215 and 192 ng/mL, respectively. The concentrations were found in the decreasing order of: PMs (sum of 45 PMs; mean: 146 ng/mL) > EPs (sum of 45 EPs; 42 ng/mL) > pesticides (sum of 31 pesticides; 26 ng/mL). PA, mEP, mIBP, mBP, mBzP, mECPP, and mEHHP made up a vast majority of PMs detected in urine samples. BP-3 (mean: 13 ng/mL), 1-Nap (11 ng/mL), and MeP (9.7 ng/mL) were the major EPs found in urine. Among pesticides, TCPY (100%), thiamethoxam (95%), 4-nitrophenol (95%), and 2,4,5-T (90%) were frequently detected in samples. These results were similar to those reported in previous studies [41–43].

4. Conclusions

A rapid, cost-effective, and high-throughput method for simultaneous analysis of several classes environmental chemicals in human urine using SPE coupled with LC-MS/MS was developed and validated. The target analytes belonged to diverse chemical classes with a wide range of polarity which required a robust sample preparation condition (i.e., incubation at 37 °C for 1 h with 20 µL ALS enzyme at pH 5.5), extraction and cleanup protocol (i.e., Agilent ABS Elut NEXUS SPE cartridge), and instrumental analysis (i.e., two LC and MS methods). The optimized method was validated through multiple approaches for calibration range, accuracy/precision, detection limits, and matrix effects. The method was also validated through the analysis of standard reference materials and external quality assurance scheme proficiency test samples. The method enabled analysis of 121 chemicals with a total instrument run time of 21 min and requires a urine volume of 0.5 mL. The method was sensitive to detect trace levels of target chemicals found in urinary samples. This rapid method will support analysis of a wide range of pollutants from diverse chemical classes for large scale human biomonitoring programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A SPE-LC-MS/MS method to determine 121 chemicals in a single extraction was developed
- The method was sensitive for the analysis of 45 plasticizers, 45 phenolics and 31 pesticides
- Single extraction followed by two instrumental conditions were required for analysis
- The method was validated through the analysis of reference materials and real urines

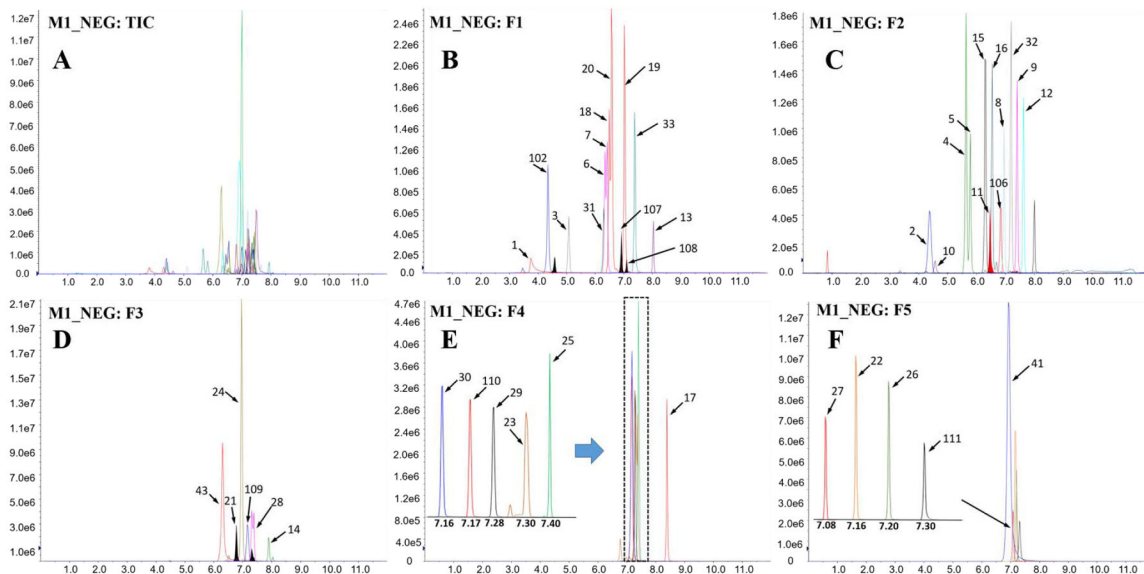


Figure 1. Total ion chromatogram (TIC; A) and extracted ion chromatograms (B–F) of 43 target compounds injected (5 μ L) at a concentration of 10 ng/mL measured using mass spectrometric method 1 under negative mode (MI_NEG).

For the sake of distinction of individual compounds, ion chromatograms were extracted and divided into five fractions (i.e., F1–F5) depending on their retention times and signal intensities. Compound numbers assigned on the peaks are shown in Table 1. The x- and y-coordinates are retention time (min) and signal intensity (cps), respectively.

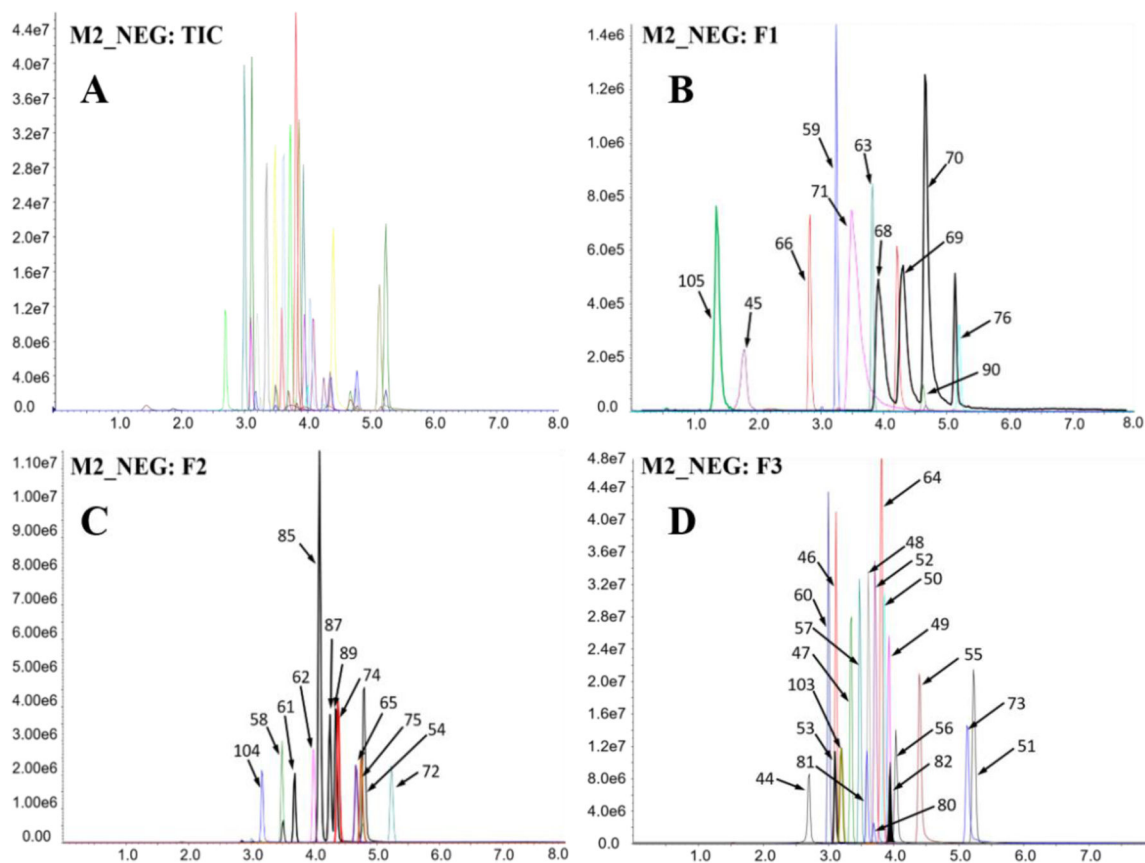


Figure 2. Total ion chromatogram (TIC; A) and extracted ion chromatograms (B–D) of 47 target compounds injected (5 μ L) at a concentration of 10 ng/mL measured using mass spectrometric method 2 under negative mode (M2_NEG).

For the sake of distinction of individual compounds, ion chromatograms were extracted and divided into three fractions (i.e., F1–F3) depending on their retention times and signal intensities. Compound numbers assigned on the peaks are shown in Table 1. The x- and y-coordinates are retention time (min) and signal intensity (cps), respectively.

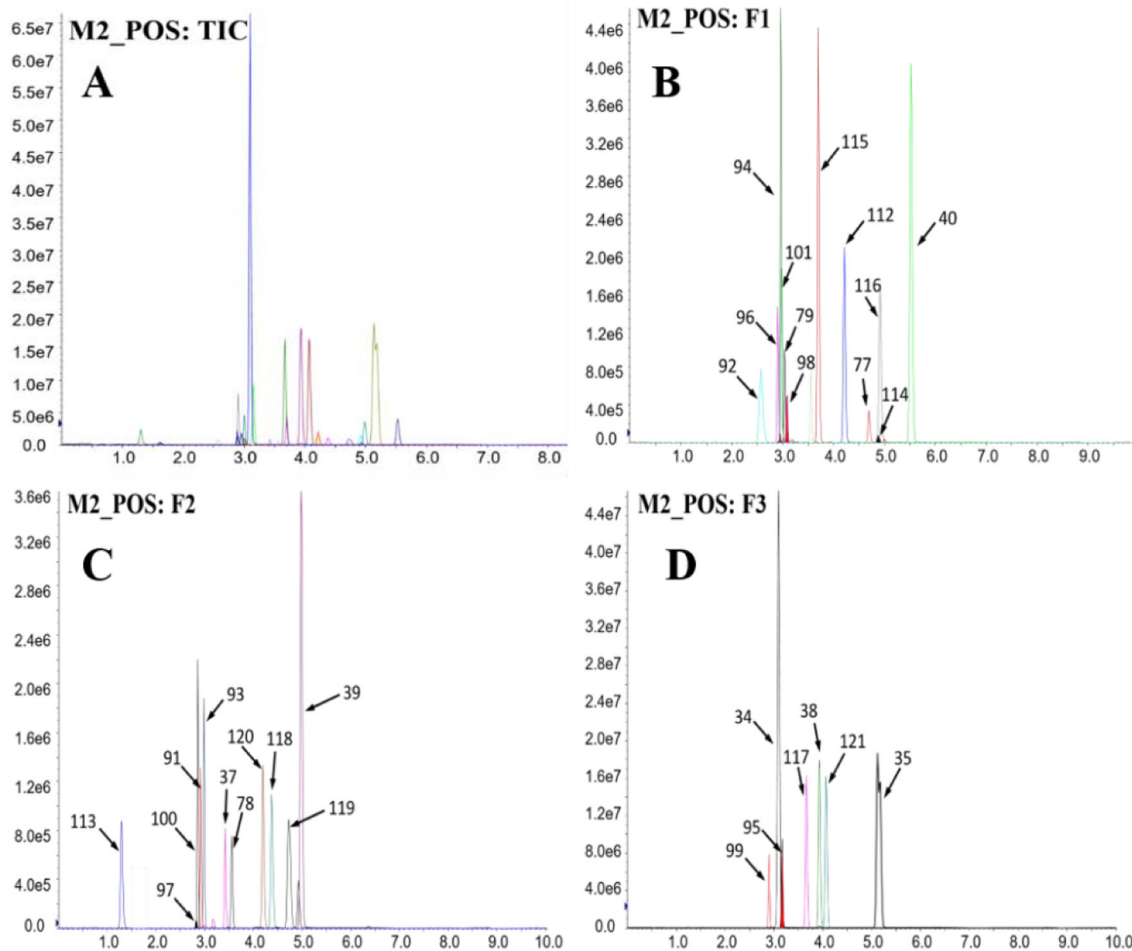


Figure 3. Total ion chromatogram (TIC; A) and extracted ion chromatograms (B–D) of 31 target compounds injected (5 μ L) at a concentration of 10 ng/mL measured using mass spectrometric method 2 under positive mode (M2_POS).

For the sake of distinction of individual compounds, ion chromatograms were extracted and divided into five fractions (i.e., F1–F3) depending on their retention times and signal intensities. Compound numbers assigned on the peaks are shown in Table 1. The x- and y-coordinates are retention time (min) and signal intensity (cps), respectively.

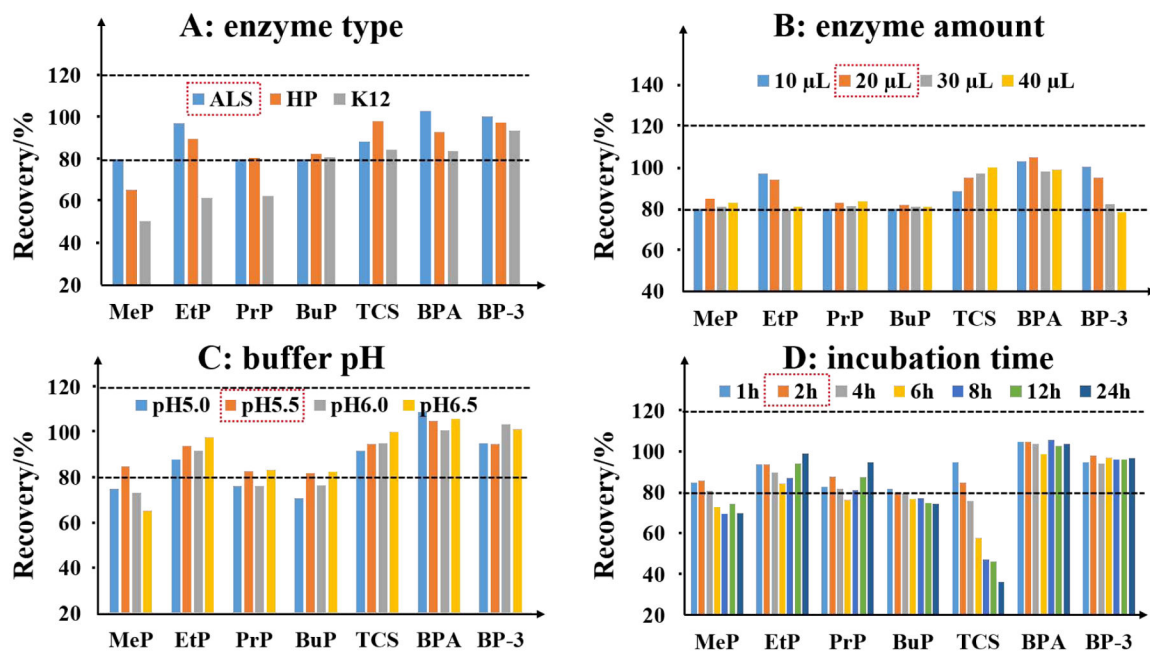


Figure 4.

Recoveries of seven phenolic compounds certified in standard reference material 3672 (SRM 3672) with different enzymatic deconjugation conditions including enzyme type (A), enzyme amount (B), buffer pH (C), and incubation time (D).

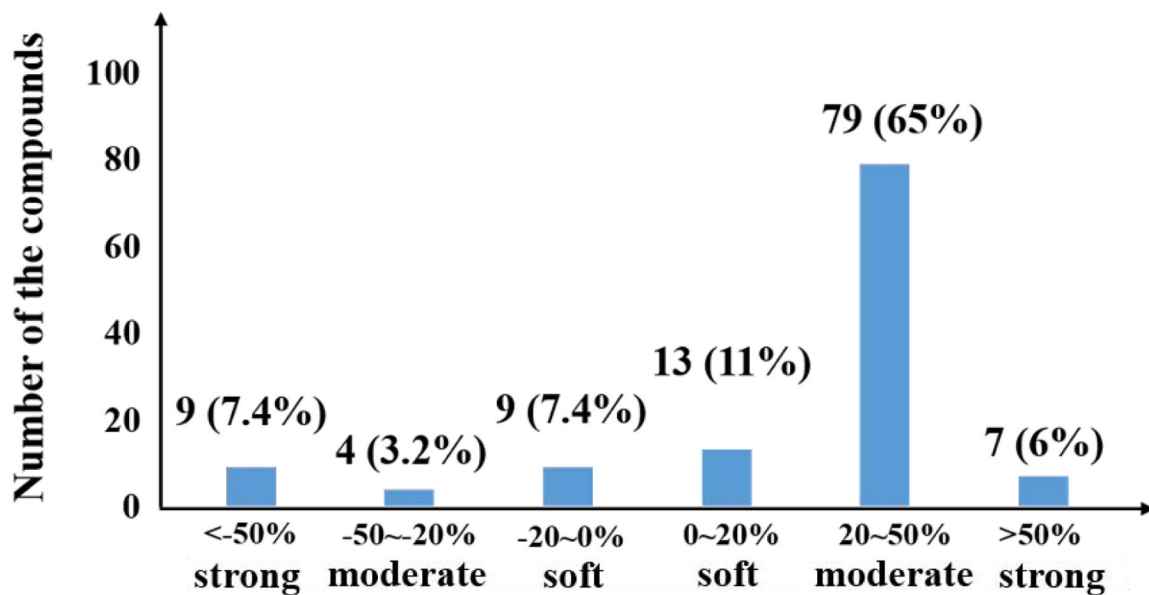


Figure 5. Summary of matrix effects (ME) determined for 121 analytes analyzed in the study. Matrix effects are classified into soft effect (−20% to 20%), moderate effect (−50% to −20% and 20% to 50%), and strong effect (<−50% and >50%). If ME ~0%, there is no matrix effect. ME > 0% indicates an ion-suppression and, ME < 0% indicates an ion-enhancement.

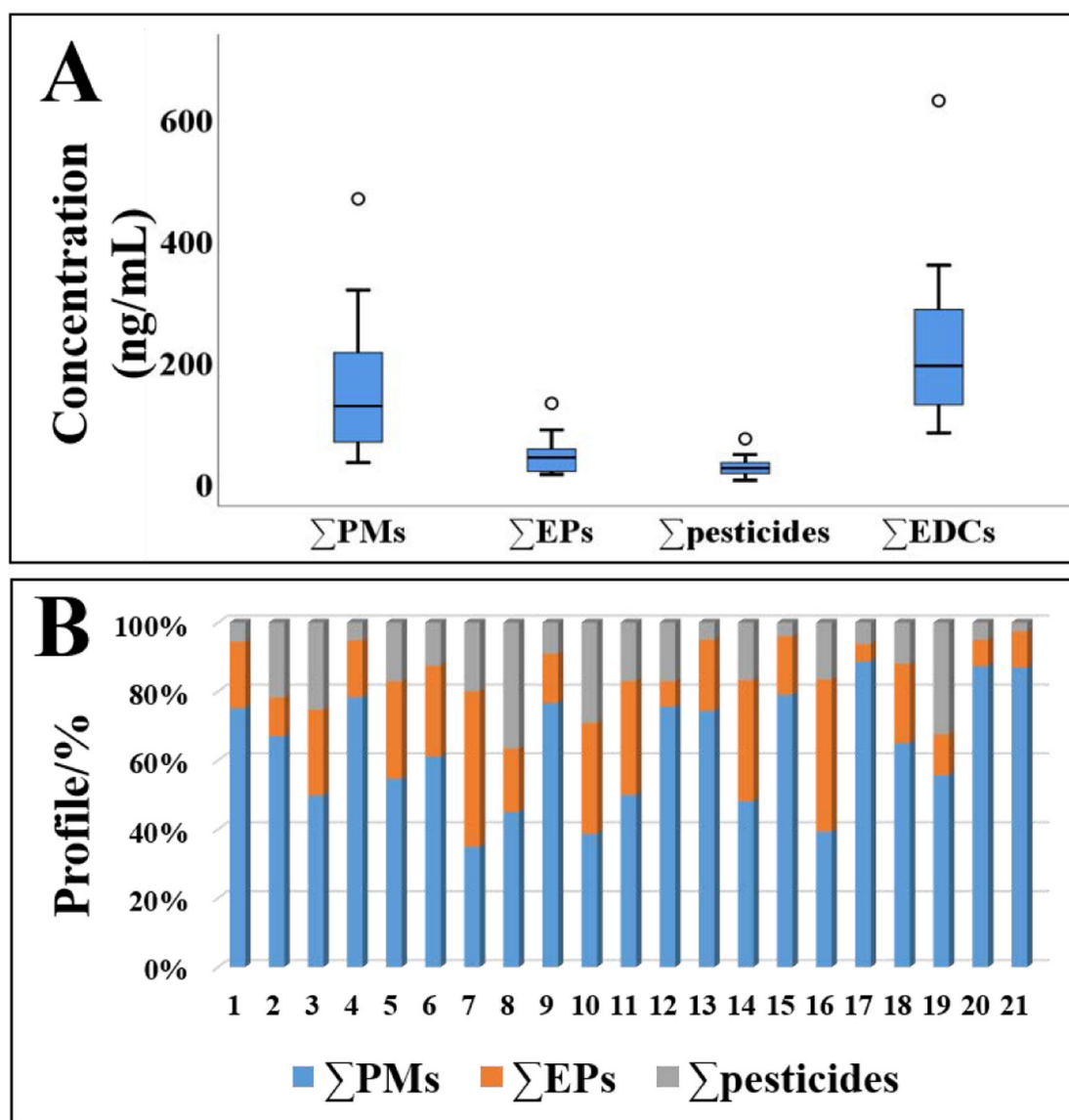


Figure 6. Concentrations (ng/mL; A) and profiles (%; B) of target chemicals found in 21 real urine analyzed.

PMs: plasticizers and metabolites; EPs: environmental phenols; The black horizontal line inside each box represents median, the boxes represent 25th and 75th percentiles, whiskers represent a value of 1.5*SD and the dots represent outliers.

Table 1.

Published multiple-class analytical methods for endocrine disrupting chemicals in human urine

Analyte	Urine volume	Enzyme Type	Sample preparation	Sample extraction	Instrumental method	LOD (ng/mL)	Reference
Total: 35 analytes 16 BRPs, 12 OH-PAHs, 5 OH-PBDEs, TCS, and TBBPA	2.0 mL	β -glucuronidase/arylsulfatase enzyme (<i>Helix pomatia</i> , β -glucuronidase activity \approx 100,000 U/mL; sulfatase activity \approx 47,500 U/mL)	enzyme amount: 10 μ L (i.e., 50 U/100 μ L urine); buffer: 1 mL of 1 M NaOAc (pH 5.5); incubation time: overnight at 37 °C	Oasis HLB SPE cartridge (500 mg, 6 mL)	Single injection: Instrument: LC-MS/MS; LC column: Poroshell 120 EC-C18 (100 mm \times 4.6 mm, 2.7 μ m); Mobile phase: 5 mM NH ₄ Ac in water and ACN; Run time: 27 min; Injection volume: 10 μ L;	0.008–0.161	[10]
Total: 21 analytes 7 BRPs, 7 parabens, 5 benzophenones, TCC, and TCS	1.0 mL	β -glucuronidase enzyme (Type HP-2 from <i>Helix pomatia</i> , β -glucuronidase activity 100,000 U/mL; sulfatase activity \approx 7500 U/mL)	enzyme amount: 2.0 μ L (i.e., 20 U/100 μ L urine); buffer: 0.1 mL of 1 M NH ₄ Ac (\approx 6.0); incubation time: 24 h at 37 °C	vortex-assisted dispersive liquid-liquid microextraction	Single injection: Instrument: LC-MS/MS; LC column: Brownlee Aq C18 column (100 mm \times 4.6 mm, 5 μ m); Mobile phase: water and MeOH; Run time: 12 min; Injection volume: 10 μ L;	0.01–0.20	[9]
Total: 19 analytes 5 BADGEs, 5 benzophenones, 7 parabens, TCC, and TCS	0.5 mL	β -glucuronidase/arylsulfatase enzyme (<i>Helix pomatia</i> , β -glucuronidase activity \approx 145,700 U/mL; sulfatase activity \approx 887 U/mL)	enzyme amount: 0.15 μ L (i.e., 4.4 U/100 μ L urine); buffer: 0.3 mL of 1 M NH ₄ Ac (no pH adjustments); incubation time: 12 h at 37 °C;	liquid-liquid extraction with EtAc	Two injections: Instrument: LC-MS/MS; LC column: Betasil C18 (2.1 mm \times 100 mm, 5 μ m); 1st injection for 5 BADGEs: Mobile phase: water and MeOH; Run time: 30 min; Injection volume: 10 μ L; 2nd injection for remaining analytes: Mobile phase: 0.1% FA in water and MeOH; Run time: 30 min; Injection volume: 10 μ L;	0.06–0.6	[11]
Total: 21 analytes 7 BRPs, 7 parabens, 5 benzophenones, TCS, and TCS	5.0 mL	β -glucuronidase enzyme (type HP-2 from <i>Helix pomatia</i> , β -glucuronidase activity \approx 197,000 U/mL)	enzyme amount: 20 U/100 μ L urine; buffer: not provided; incubation time: overnight at 37 °C;	air-assisted liquid-liquid microextraction	Single injection: Instrument: LC-MS/MS; LC column: Atlantis T3 dC18 column (75 mm \times 2.1 mm, 3.0 μ m); Mobile phase: water and MeOH; Run	0.01–0.30	[12]

Analyte	Urine volume	Enzyme Type	Sample preparation	Sample extraction	Instrumental method	LOD (ng/mL)	Reference
					time: 10 min; Injection volume: 10 µL;		
Total: 12 analytes 7 PhMs, 4 parabens, and BP-3	3.0 mL	β-glucuronidase enzyme (Type HP-2 from <i>Helix pomatia</i> , β-glucuronidase activity 200,000 U/mL)	enzyme amount: 25 µL (i.e., 167 U/100 µL urine); buffer: 0.75 mL of 1 M NaOAc (pH 4.5); incubation time: overnight at 37 °C;	Bond Elut Certify SPE cartridge (130 mg, 10 mL)	Single injection: Instrument: LC-MS/MS; LC column: Kinetex Phenyl-Hexyl column (2.1 mm × 100 mm, 1.7 µm); Mobile phase: 0.1% HAc in both water and ACN; Run time: 20 min; Injection volume: 5 µL;	0.09–0.37	[13]
Total: 23 analytes 8 BPs and 15 OH-PAHs	0.5 mL	β-glucuronidase enzyme (from <i>E. coli</i> K12, β-glucuronidase activity ≈ 140 U/mL)	enzyme amount: 20 µL (i.e., 0.6 U/100 µL urine); buffer: 1 mL of 0.5 M NH ₄ Ac (pH 6.0); incubation time: 14 h at 37 °C;	liquid-liquid extraction with mixture of EtAc:pentane:toluene (5:4:1, v:v)	Two injections: Instrument: LC-MS/MS; 1st injection for 8 BPs: LC column: Betasil C18 (2.1 mm × 100 mm, 5 µm); Mobile phase: 0.1% NH ₄ OH in water and MeOH; Run time: 20 min; Injection volume: 10 µL; 2nd injection for 15 OH-PAHs: LC column: Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm); Mobile phase: water and MeOH; Run time: 20 min; Injection volume: 2 µL;	0.003–0.36	[14]
Total: 19 analytes 14 PhMs and 5 BPs	0.05 mL	β-glucuronidase enzyme (from <i>E. coli</i> K12, β-glucuronidase activity ≈ 200 U/mL)	enzyme amount: 0.5 µL (i.e., 0.2 U/100 µL urine); buffer: 0.025 mL of 1 M NH ₄ Ac (pH 6.5); incubation time: 1.5 h at 37 °C;	Online Strata X SPE cartridge (20 mm × 2.0 mm, 25 µm)	Single injection: Instrument: LC-MS/MS; LC column: Synergi MAX-RP column (150 mm × 3.0 mm; 4 µm); Mobile phase: 0.05% HAc in both water and ACN; Run time: 13 min; Injection volume: 50 µL;	0.005–0.39	[15]
Total: 6 analytes 4 PhMs, BPA, and 4-n-NP	0.9 mL	β-glucuronidase enzyme (from abalone, β-glucuronidase activity > 100,000 U/mL,	enzyme amount: 0.5 U/100 µL urine; buffer: 0.25 mL of 1 mM NH ₄ Ac (no pH	ABS Elut NEXUS SPE cartridges (60 mg, 3 mL)	Two injections: Instrument: LC-MS/MS; LC column: Acquity UPLC BEH Phenyl column (100 mm × 2.1	0.3–0.5	[16]

Analyte	Urine volume	Enzyme Type	Sample preparation	Sample extraction	Instrumental method	LOD (ng/mL)	Reference
		arylsulfatase activity < 20,000 U/mL)	adjustment); incubation time: 2 h at 37 °C;		mm, 1.7 μm); 1st injection for PhMs: Mobile phase: 0.1% FA in water and MeOH; Run time: 22 min; Injection volume: 5 μL; 2nd injection for BPA and 4-n-NP: Mobile phase: 0.05% NH4OH in water and MeOH; Run time: 14 min; Injection volume: 5 μL;		
Total: 260 analytes pesticides	0.1 mL	no enzymatic deconjugation step		Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method	Single injection: Instrument: LC-MS/MS; LC column: Kinetex C18 column (100 mm × 2.1 mm, 2.6 μm); Mobile phase: 5 mM AF and 0.1% FA in both water and MeOH; Run time: 15 min; Injection volume: 4 μL;	10	[17]
Total: 13 analytes pesticides	1.0 mL	acidic hydrolysis using 5 mL of HCl (50%) and heating in a 90 °C water bath for 45 min for deconjugation		Liquid-liquid extraction with hexane followed by derivatization with <i>N-tert</i> -butyldimethylsilyl- <i>N</i> -methyltrifluoroacetamide	Single injection: Instrument: GC-MS; GC column: Restek RTX-35 column (30 m × 0.25 mm, 0.25 μm); Run time: 30 min; Injection volume: 1 μL;	0.025–0.10	[18]
Total: 10 analytes pesticides	1.0 mL	β-glucuronidase enzyme (type H-1 from <i>Helix pomatia</i> with a specific activity of ~500 U/mg)	enzyme amount: 56 units/100 μL urine; buffer: 0.75 mL of 0.2 M NaOAc; incubation time: > 6 h at 37 °C;	Oasis HLB SPE cartridge (30 mg, 1 mL)	Single injection: Instrument: LC-MS/MS; LC column: Betasil C18 column (100 mm × 2.1 mm; 3 μm); Mobile phase: 0.1% HAc in water and ACN; Run time: 20 min; Injection volume: 30 μL;	0.1–0.6	[19]
Total: 121 analytes 45 plasticizers and metabolites, 34 environmental phenols,	0.5 mL	β-glucuronidase/arylsulfatase enzyme (<i>Helix pomatia</i> , β-glucuronidase activity ≈ 100,000 U/mL;	enzyme amount: 20 μL (i.e., 400 units/100 μL urine); buffer: 0.5 mL of 1 M NH4Ac (pH 5.5);	ABS Elut NEXUS SPE cartridge (60 mg, 3 mL)	Two injections: Instrument: LC-MS/MS; 1st injection for 43 analytes: LC column: Ultra AQ C18 column (2.1 mm	0.1 for 101 analytes 0.1–1.0 for 18 analytes 1.0	the present study

Analyte	Urine volume	Enzyme Type	Sample preparation	Sample extraction	Instrumental method	LOD (ng/mL)	Reference
31 pesticides, 11 OH-PAHs;		sulfatase activity \approx 47,500 U/mL)	incubation time: overnight at 37 °C		\times 100 mm, 3 μ m); Mobile phase: 0.1% HAc in both water and MeOH; Run time: 12 min; Injection volume: 5 μ L; 2nd injection for 78 analytes: LC column: Betasil C18 column (100 mm \times 2.1 mm, 5 μ m) Mobile phase: water and ACN; Run time: 9 min; Injection volume: 5 μ L;	for 2 analytes	

OH-PAHs: monohydroxylated polycyclic aromatic hydrocarbons; BRPs: brominated phenols; OH-PBDEs: hydroxyl polybrominated diphenyl ethers; TCC: triclocarban; TCS: triclosan; TBBPA: tetrabromobisphenol A; BPs: bisphenols; BADGEs: bisphenol A diglycidyl ethers; PhMs: phthalate metabolites; BPA: 4,4'-(1-methylethylidene)bisphenol; NH₄Ac: ammonium acetate; FA: formic acid; HAc: acetate acid; NH₄OH: ammonium hydroxide; AF: ammonium formate; ACN: acetonitrile; MeOH: methanol; EtAc: ethyl acetate; NaOAc: sodium acetate; LOD: limit of detection.

Table 2.

Compound name, abbreviation, CAS number, MRM (quantification) transition, collision energy (CE), method classification (MC), and retention time (RT) for 121 target compounds analyzed in the present study

No.	Compound name	Synonym	CAS number	QT	CE	MC	RT
Plasticizers and metabolites							
1	phthalic acid	PA	88-99-3	165>77	-25	MSM1_NEG	3.77
2	mono-methyl phthalate	mMP	4376-18-5	179>77	-25	MSM1_NEG	4.37
3	mono-ethyl phthalate	mEP	2306-33-4	193>77	-25	MSM1_NEG	5.06
4	mono-iso-propyl phthalate	mIPrP	4376-18-5	207>77	-25	MSM1_NEG	5.59
5	mono-n-propyl phthalate	mPrP	4376-19-6	207>77	-25	MSM1_NEG	5.77
6	mono-iso-butyl phthalate	mIBP	30833-53-5	221>77	-25	MSM1_NEG	6.34
7	mono-butyl phthalate	mBP	131-70-4	221>77	-25	MSM1_NEG	6.40
8	mono-pentyl phthalate	mPeP	24539-56-8	235>77	-25	MSM1_NEG	6.95
9	mono-hexyl phthalate	mHxP	24539-57-9	249>77	-25	MSM1_NEG	7.40
10	mono-(3-carboxypropyl) phthalate	mCPP	66851-46-5	251>103	-25	MSM1_NEG	4.56
11	mono-benzyl phthalate	mBzP	2528-16-7	255>183	-16	MSM1_NEG	6.47
12	mono-2-heptyl phthalate	mHpP	129171-03-5	263>77	-25	MSM1_NEG	7.62
13	mono-octyl phthalate	mOP	5393-19-1	277>125	-20	MSM1_NEG	8.03
14	mono-(2-ethylhexyl) phthalate	mEHP	4376-20-9	277>134	-21	MSM1_NEG	7.89
15	mono-(2-ethyl-5-oxohexyl) phthalate	mEOHP	40321-98-0	291>121	-25	MSM1_NEG	6.29
16	mono-(2-ethyl-5-hydroxyhexyl) phthalate	mEHHP	40321-99-1	293>121	-26	MSM1_NEG	6.52
17	mono-iso-decyl phthalate	mIDP	31047-64-0	305>155	-25	MSM1_NEG	8.39
18	mono-(2-ethyl-5-carboxypentyl) phthalate	mECP	40809-41-4	307>159	-25	MSM1_NEG	6.39
19	mono-[2-(carboxymethyl)hexyl] phthalate	mCMHP	82975-93-7	307>159	-25	MSM1_NEG	7.00
20	mono-(7-carboxyheptyl) phthalate	mCHpP	856869-57-3	307>159	-25	MSM1_NEG	6.49
21	mono-carboxy-iso-octyl phthalate	mCIOP	898544-09-7	321>173	-20	MSM1_NEG	6.77
22	mono-carboxy-iso-nonyl phthalate	mCINP	1373125-93-9	335>187	-21	MSM1_NEG	7.16
23	2-(((9-hydroxydecyl)oxy)carbonyl) benzoic acid	mHiDP	not available	321>121	-35	MSM1_NEG	7.30
24	monohydroxy-iso-nonyl phthalate	mHiNP	898544-10-0	307>121	-25	MSM1_NEG	6.95
25	cyclohexane-1,2-dicarboxylic acid-mono (hydroxy-isononyl) ester	mHNCH	1637562-52-7	313.3>153	-25	MSM1_NEG	7.40
26	cyclohexane-1,2-dicarboxylic acid-mono (oxo-isononyl) ester	mONCH	1588520-62-0	311.4>153	-25	MSM1_NEG	7.20
27	mono-2-(propyl-6-oxoheptyl)-phthalate	mPOHP	1373125-92-8	319.3>121.1	-22	MSM1_NEG	7.08
28	mono-2-(propyl-6-hydroxy-heptyl)-phthalate	mPHHP	1372605-11-2	321.2>121	-35	MSM1_NEG	7.31
29	cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester	mCOCH	1637562-51-6	327.4>173.1	-22	MSM1_NEG	7.28
30	mono-2-(propyl-6-carboxy-hexyl)-phthalate	mPCHP	1412411-10-9	335.3>187.3	-15	MSM1_NEG	7.16
31	mono-ethyl terephthalate	mETP	713-57-5	192.9>119.9	-28	MSM1_NEG	6.29
32	mono-tert-butyl terephthalate	mTBTP	20576-82-3	221>119.8	-30	MSM1_NEG	7.18
33	mono-benzyl terephthalate	mBzTP	18520-63-3	255.3>119.9	-25	MSM1_NEG	7.38

No.	Compound name	Synonym	CAS number	QT	CE	MC	RT
34	triethyl phosphate	TEP	78-40-0	183>99.1	35	MSM2_POS	3.09
35-1	tri-n-butyl phosphate	TNBP	126-73-8	267.1>99	22	MSM2_POS	5.12
35-2	tri-iso-butyl phosphate	TIBP	126-71-6	267.1>99	22	MSM2_POS	
37	tris(2-chloroethyl) phosphate	TCEP	115-96-8	284.9>63.1	40	MSM2_POS	3.42
38	tripropyl phosphate	TPP	513-08-6	225.1>99	35	MSM2_POS	3.93
39	triphenyl phosphate	TPhP	115-86-6	327.1>77.1	46	MSM2_POS	4.98
40	tris(2-butoxyethyl) phosphate	TBOEP	78-51-3	399.1>199	20	MSM2_POS	5.51
41-1	di-n-butyl phosphate	DNBP	107-66-4	209>78.9	-35	MSM1_NEG	6.92
41-2	di-iso-butyl phosphate	DIBP	6303-30-6	209>78.9	-35	MSM1_NEG	
43	diphenyl phosphate	DPhP	838-85-7	248.9>93.1	-40	MSM1_NEG	6.28
44	bis(2-methylphenyl) phosphate	BMPP	35787-74-7	277>107	-40	MSM2_NEG	2.66
45	bis(1,3-dichloro-2-propyl) phosphate	BDCIPP	72236-72-7	316.9>35	-30	MSM2_NEG	1.74
Environmental phenols							
46	methyl paraben	MeP	99-76-3	151>92	-32	MSM2_NEG	3.11
47	ethyl paraben	EtP	120-47-8	165>92	-32	MSM2_NEG	3.34
48	n-propyl paraben	PrP	94-13-3	179>92	-32	MSM2_NEG	3.61
49	n-butyl paraben	BuP	94-26-8	193>92	-40	MSM2_NEG	3.93
50	benzyl paraben	BzP	94-18-8	227>92	-32	MSM2_NEG	3.85
51	heptyl paraben	HeP	1085-12-7	235>92	-40	MSM2_NEG	5.24
52	benzophenone-1	BP-1	131-56-6	213>91	-35	MSM2_NEG	3.71
53	benzophenone-2	BP-2	131-55-5	245>91	-40	MSM2_NEG	3.10
54	benzophenone-3	BP-3	131-57-7	227>211	-35	MSM2_NEG	4.78
55	benzophenone-6	BP-6	131-54-4	273>123	-24	MSM2_NEG	4.40
56	benzophenone-8	BP-8	131-53-3	243>93	-38	MSM2_NEG	4.04
57	4-hydroxybenzophenone	4-OH-BP	1137-42-4	197>92	-42	MSM2_NEG	3.47
58	4,4'-(1-methylethylidene)bisphenol	BPA	80-05-7	227>212	-26	MSM2_NEG	3.48
59	4,4'-di-hydroxydiphenylmethane	BPF	620-92-8	199>77	-34	MSM2_NEG	3.25
60	4,4'-sulfonyldiphenol	BPS	80-09-1	249>108	-35	MSM2_NEG	2.99
61	2,2-bis(4-hydroxyphenyl)butane	BPB	77-40-7	241>211	-39	MSM2_NEG	3.69
62	4,4'-cyclo-hexylidenebisphenol	BPZ	843-55-0	267>173	-35	MSM2_NEG	3.99
63	4,4'-(1-phenylethylidene)bisphenol	BPAP	1571-75-1	289>273	-43	MSM2_NEG	3.82
64	2,2-bis(4-hydroxyphenyl) hexafluoropropane	BPAF	1478-61-1	335>265	-31	MSM2_NEG	3.81
65	4,4'-(1,4-phenylenediisopropylidene) bisphenol	BPP	2167-51-3	345>330	-36	MSM2_NEG	4.68
66-1	2,4,5-trichlorophenol	2,4,5-TCP	95-95-4	195>35	-35	MSM2_NEG	2.85
66-2	2,4,6-trichlorophenol	2,4,6-TCP	88-06-2	195>35	-35	MSM2_NEG	
68	2,3,5,6-tetrachlorophenol	2,3,5,6-TeCP	935-95-5	231>35	-60	MSM2_NEG	3.94
69	2,3,4,6-tetrachlorophenol	2,3,4,6-TeCP	58-90-2	231>35	-60	MSM2_NEG	4.34
70	2,3,4,5-tetrachlorophenol	2,3,4,5-TeCP	4901-51-3	231>35	-60	MSM2_NEG	4.77
71	pentachlorophenol	PCP	87-86-5	265>35	-60	MSM2_NEG	3.65

No.	Compound name	Synonym	CAS number	QT	CE	MC	RT
72	triclocarban	TCC	101-20-2	313>160	-25	MSM2_NEG	5.14
73	triclosan	TCS	3380-34-5	287>35	-43	MSM2_NEG	5.24
74	3,3',5-trichlorobisphenol A	TCBPA	40346-55-2	329.1>278	-35	MSM2_NEG	4.36
75	2,2',6,6'-tetrachlorobisphenol A	TeCBPA	79-95-8	365.1>314	-37	MSM2_NEG	4.76
76	3,3',5,5'-tetrabromobisphenol A	TBBPA	79-94-7	542.8>420	-57	MSM2_NEG	5.21
77	bisphenol A diglycidyl ether	BADGE	1675-54-3	358>191	16	MSM2_POS	4.70
78	bisphenol A (2,3-dihydroxypropyl) glycidyl ether	BADGEH2O	76002-91-0	376>209	20	MSM2_POS	3.56
79	bisphenol A bis(2,3-dihydroxypropyl) glycidyl ether	BADGE2H2O	5581-32-8	394>209	20	MSM2_POS	3.00
80	1-hydroxynaphthalene	1-Nap	90-15-3	143>115	-38	MSM2_NEG	3.69
81	2-hydroxynaphthalene	2-Nap	135-19-3	143>115	-38	MSM2_NEG	3.58
82-1	2-hydroxyfluorene	2-Fluo	2443-58-5	180.9>179.9	-30	MSM2_NEG	3.94
82-2	3-hydroxyfluorene	3-Fluo	6344-67-8	180.9>179.9	-30	MSM2_NEG	
82-3	9-hydroxyfluorene	9-Fluo	1689-64-1	180.9>179.9	-30	MSM2_NEG	
85-1	2-hydroxyphenanthrene	2-Phen	605-55-0	193.1>164.9	-45	MSM2_NEG	4.09
85-2	3-hydroxyphenanthrene	3-Phen	605-87-8	193.1>164.9	-45	MSM2_NEG	
87-1	1-hydroxyphenanthrene	1-Phen	2433-56-9	193.1>164.9	-45	MSM2_NEG	4.25
87-2	9-hydroxyphenanthrene	9-Phen	484-17-3	193.1>164.9	-45	MSM2_NEG	
89	4-hydroxyphenanthrene	4-Phen	7651-86-7	193.1>164.9	-45	MSM2_NEG	4.35
90	1-hydroxypyrene	1-Pyr	5315-79-7	217.1>188.9	-45	MSM2_NEG	4.63
Pesticides							
91	nitenpyram	nitenpyram	150824-47-8	271.1>126	50	MSM2_POS	2.88
92	thiamethoxam	thiamethoxam	153719-23-4	291.8>211.1	18	MSM2_POS	2.57
93	imidacloprid	imidacloprid	138261-41-3	256>209	32	MSM2_POS	2.95
94	acetamiprid	acetamiprid	135410-20-7	222.8>126	23	MSM2_POS	3.00
95	thiacloprid	thiacloprid	111988-49-9	253>126	27	MSM2_POS	3.15
96	clothianidin	clothianidin	210880-92-5	250>169.1	20	MSM2_POS	2.90
97	flonicamid	flonicamid	158062-67-0	230>203	20	MSM2_POS	2.83
98	N-desmethyl thiamethoxam	N-Dmt	171103-04-1	278>132	45	MSM2_POS	3.02
99	N-desmethyl-acetamiprid	N-Dma	190604-92-3	209>126	35	MSM2_POS	2.90
100	thiacloprid-amide	Ta	676228-91-4	271.1>126.1	36	MSM2_POS	2.88
101	imidaclothiz	imidaclothiz	105843-36-5	262>181	32	MSM2_POS	3.00
102	6-chloronicotinic acid	6-Cn	5326-23-8	155.9>111.9	-15	MSM1_NEG	4.33
103	sulfoxaflor	sulfoxaflor	946578-00-3	275.9>213	-21	MSM2_NEG	3.19
104	4-nitrophenol	4-nitrophenol	100-02-7	138>108	-24	MSM2_NEG	3.17
105	2,4-dichlorophenoxyacetic acid	2,4-D	94-75-7	219>161	-20	MSM2_NEG	1.31
106	3,5,6-trichloro-2-pyridinol	TCPY	6515-38-4	196>35	-40	MSM1_NEG	6.83
107	trans-3-(2,2-di-chlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid	trans-DCCA	55701-07-0	207>35	-40	MSM1_NEG	6.92
108	cis-3-(2,2-di-chlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid	cis-DCCA	55667-40-8	207>35	-40	MSM1_NEG	7.10

No.	Compound name	Synonym	CAS number	QT	CE	MC	RT
109	3-phenoxybenzoic acid	3-PBA	3739-38-6	213>93	-30	MSM1_NEG	7.15
110	4-fluoro-3-phenoxybenzoic acid	4F-3PBA	77279-89-1	231>93	-35	MSM1_NEG	7.17
111	2,4,5-trichlorophenoxyacetic acid	2,4,5-T	93-76-5	255>197	-20	MSM1_NEG	7.30
112	pyrimethanil	pyrimethanil	53112-28-0	200.1>107	34	MSM2_POS	4.21
113	dinotefuran	dinotefuran	165252-70-0	203>129.1	20	MSM2_POS	1.30
114	metribuzin	metribuzin	21087-64-9	215.1>187	10	MSM2_POS	4.88
115	atrazine	atrazine	1912-24-9	216>174	25	MSM2_POS	3.70
116	cyprodinil	cyprodinil	121552-61-2	226>93	50	MSM2_POS	4.92
117	metalaxyl	metalaxyl	57837-19-1	280.2>220	20	MSM2_POS	3.67
118	tebuconazole	tebuconazole	107534-96-3	308.2>70	45	MSM2_POS	4.37
119	propiconazole	propiconazole	60207-90-1	342.1>159	42	MSM2_POS	4.72
120	tetraconazole	tetraconazole	112281-77-3	372>159	38	MSM2_POS	4.19
121	azoxystrobin	azoxystrobin	131860-33-8	404>372.1	20	MSM2_POS	4.07

QT: quantification ion transition; CE: collision energy (volt); MSM1_NEG: method 1 under negative mode; MSM2_NEG: method 2 under negative mode; MSM2_POS: method 2 under positive mode; MC: method classification; RT: retention time (min); There is no chromatographic resolution between compounds with same ID; therefore, sum concentrations are expressed for these isomers by using the present developed method.

Table 3.

Regression coefficient (linearity of calibration; r^2), accuracy and precision from intra-day and inter-day measurements, recoveries, matrix effect results, and limit of detection (LOD) for 121 target compounds analyzed in this study.

No.	Synonym	r^2	Accuracy and Precision (Intra-Day)						Accuracy and Precision (Inter-Day)						Recovery			
			1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL	
			RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %
1	PA	0.9875	-6.2	6.7	1.9	7.5	-3.6	5.1	-2.8	4.7	5.6	4.8	-0.85	1.8	106	7.9	112	
2	mMP	0.9923	-3.7	6.2	-11	9.5	0.55	2.5	-0.90	5.7	-7.2	4.0	1.7	1.5	94	1.0	92	
3	mEP	0.9993	-8.8	8.3	-6.0	5.7	-5.5	6.0	-3.6	4.9	-3.5	1.8	-3.4	3.3	106	0.67	99	
4	mIPrP	0.9991	-3.7	3.3	-12	9.6	-1.4	4.8	-2.3	1.7	-8.7	3.4	0.73	2.5	99	2.1	93	
5	mPrP	0.9991	-5.9	6.2	-10	8.2	-1.8	5.2	-4.4	5.2	-7.2	4.3	0.27	3.8	98	4.1	94	
6	mIBP	0.9985	-4.3	7.8	-13	9.1	9.6	8.4	0.91	4.4	-10	7.0	14	4.2	96	3.5	98	
7	mBP	0.9985	-4.8	8.6	-13	9.0	-3.0	4.7	1.4	6.8	-11	6.7	-2.6	5.1	92	0.69	92	
8	mPeP	0.9999	-3.6	5.8	-4.3	8.1	-9.7	9.0	-2.3	5.5	-2.2	7.9	-6.5	4.5	93	12	96	
9	mHxP	0.9997	-1.3	5.3	-10	8.3	-5.5	7.2	-2.1	4.5	-7.0	4.7	-4.3	3.6	102	5.2	96	
10	mCPP	0.9956	-10	5.6	-2.8	3.8	-1.1	2.0	-10	5.6	-1.2	2.3	-1.5	2.0	101	1.4	101	
11	mBzP	0.9997	-11	8.9	-3.6	4.8	-2.5	7.4	-10	9.7	-2.0	3.8	-0.96	6.1	94	8.8	86	
12	mHpP	0.9963	-4.5	5.8	-12	9.7	-5.0	5.6	-0.72	3.9	-9.5	4.0	-2.5	2.3	97	7.1	97	
13	mOP	0.9983	-1.0	4.8	-6.5	5.6	-7.4	8.1	1.2	3.3	-4.4	3.9	-5.0	4.6	101	3.7	94	
14	mEHP	0.9912	-4.8	5.1	-14	6.9	-4.6	7.9	-1.9	2.5	-10	3.9	-0.62	3.2	114	8.9	106	
15	mEOHP	0.9996	-3.7	4.0	-11	8.6	-1.3	4.1	-1.8	1.7	-8.0	3.3	0.56	2.3	101	4.2	96	
16	mEHHP	0.9995	0.98	5.6	-9.6	8.4	-0.74	4.0	1.6	6.0	-6.2	5.1	0.99	2.8	101	2.2	98	
17	mIDP	0.9998	-2.0	6.2	3.1	5.0	0.73	3.8	-0.62	5.3	2.2	4.0	2.0	3.1	94	2.2	84	
18	mECPP	0.9972	-9.6	9.4	-5.4	3.7	-12	8.0	-3.5	3.6	-3.9	3.1	-12	7.3	107	0.66	86	
19	mCMHP	0.9948	2.3	7.5	4.6	6.1	-2.4	4.9	3.1	3.6	-1.6	4.6	1.2	5.1	105	4.7	98	
20	mCHpP	0.9992	-9.4	6.3	-4.4	7.0	-0.46	6.6	-7.2	4.9	-2.0	5.8	3.2	3.1	103	7.5	102	
21	mCIOP	0.9998	-2.1	5.3	-14	4.6	-3.7	6.7	1.0	4.0	-8.5	5.3	-1.9	5.3	97	4.3	96	
22	mCINP	0.9993	-4.6	4.5	-12	4.9	-3.3	5.8	-2.3	2.3	-7.1	4.7	-0.73	2.8	92	1.7	98	
23	mHiDP	0.9982	-1.8	3.2	-11	7.4	-1.2	4.0	-0.17	1.8	-7.7	3.9	0.38	2.9	101	1.9	98	
24	mHiNP	0.9970	-4.0	3.4	-6.5	8.6	-3.8	5.5	-3.8	2.7	-3.7	7.4	-0.87	3.3	88	3.1	97	
25	mHNCH	0.9997	-6.4	5.4	-11	8.8	-7.6	8.3	-3.5	3.1	-8.5	4.1	-4.5	4.6	102	0.70	98	
26	mONCH	0.9994	-4.3	6.2	-9.8	8.9	-10	7.4	-1.9	4.1	-5.4	3.6	-8.7	2.9	101	6.7	94	
27	mPOHP	0.9998	-3.5	4.3	-11	6.7	-3.3	5.3	-0.56	2.9	-7.3	4.4	-1.4	3.1	100	1.2	97	
28	mPHHP	0.9989	0.05	3.3	-9.1	8.3	-3.2	4.9	2.2	2.1	-5.6	3.0	-1.0	2.2	102	1.3	96	
29	mCOCH	0.9984	-3.5	4.5	-12	5.1	1.0	4.4	-1.2	3.8	-7.0	4.3	3.5	2.2	94	2.0	96	
30	mPCHP	0.9990	-1.9	4.2	-13	4.7	-3.4	5.4	-0.19	3.7	-8.4	5.3	-0.91	3.1	95	3.8	97	
31	mETP	0.9992	-2.2	6.5	-10	9.2	0.15	5.9	2.3	3.2	-7.4	3.3	2.6	4.5	109	2.5	105	
32	mTBTP	0.9982	-1.6	7.0	-12	9.2	-0.09	3.1	1.0	7.0	-9.0	5.1	-1.1	3.0	90	9.0	96	

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No.	Synonym	r ²	Accuracy and Precision (Intra-Day)						Accuracy and Precision (Inter-Day)						Recovery			
			1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL	
			RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %
33	mBzTP	0.9846	-3.4	3.8	-10	9.6	-5.0	4.3	-1.5	3.7	-6.9	4.7	-3.3	2.4	109	5.5	109	2
34	TEP	0.9973	-3.3	5.0	5.2	3.2	-2.5	4.4	-4.5	4.8	4.9	3.6	-2.3	3.4	106	5.3	109	2
35-1	TNBP	0.9953	2.0	2.8	3.3	2.5	-0.63	3.0	0.89	2.2	3.1	1.6	-2.2	2.0	96	0.44	94	2
35-2	TIBP																	
37	TCEP	0.9995	-4.8	6.1	2.1	4.5	-6.2	5.1	-4.1	6.0	0.82	3.6	-5.4	4.1	97	8.3	104	3
38	TPP	0.9979	1.0	2.3	-0.34	3.0	-0.61	3.0	-0.32	2.0	-1.4	2.7	-2.4	0.90	109	2.5	105	4
39	TPhP	0.9664	-0.55	3.3	1.1	2.6	-1.0	2.4	1.3	2.4	0.38	1.6	-1.4	2.4	95	3.8	81	2
40	TBOEP	0.9978	4.3	3.5	2.1	2.5	-0.12	3.0	3.1	3.3	2.0	1.8	-2.0	0.90	96	3.7	92	2
41-1	DNBP	0.9998	-4.6	3.0	-6.30	5.6	-3.2	4.3	-3.5	1.1	-3.9	2.4	-1.4	2.4	101	1.8	99	6
41-2	DIBP																	
43	DPhP	0.9980	-3.2	2.8	-7.7	6.9	-2.1	3.8	-1.8	0.90	-4.7	2.4	-0.52	2.0	98	3.9	101	0
44	BMPP	0.9990	3.7	4.4	-2.0	3.7	5.6	5.3	0.93	3.5	-3.7	1.8	3.9	4.8	103	2.7	94	8
45	BDCIPP	0.9997	8.9	8.6	-3.0	2.3	-0.29	2.6	4.4	2.8	-2.3	1.8	-0.38	2.7	97	5.2	103	0
46	MeP	0.9957	3.1	5.4	-1.6	2.0	-0.06	0.90	0.78	4.6	-2.3	1.8	0.28	0.70	84	3.7	88	6
47	EtP	0.9964	0.78	7.2	-0.86	1.6	-0.44	2.3	0.41	7.4	-1.5	0.70	-0.94	2.1	95	1.2	96	3
48	PrP	0.9969	-0.64	8.9	-1.5	1.9	-2.6	1.6	0.04	9.0	-2.4	1.2	-2.8	1.8	99	0.93	95	4
49	BuP	0.9920	0.97	3.4	1.4	1.3	-0.94	1.3	-0.64	3.3	1.1	0.60	-0.86	1.3	80	2.6	95	3
50	BzP	0.9989	5.8	3.7	-1.1	1.6	-1.1	1.3	4.5	3.6	-0.93	1.8	-1.1	1.3	97	2.1	97	4
51	HeP	0.9983	-6.3	8.6	-0.96	3.0	0.19	1.1	-2.5	4.6	-1.2	3.0	0.43	0.80	72	3.6	84	2
52	BP-1	0.9991	3.4	7.5	3.2	2.4	-3.2	3.8	1.4	7.7	3.3	2.3	-3.2	4.2	107	1.9	93	3
53	BP-2	0.9987	-4.7	4.7	1.6	1.5	-0.75	1.8	-2.6	4.4	1.4	1.6	-0.71	2.0	107	3.4	86	5
54	BP-3	0.9986	-9.3	8.8	-0.53	4.9	-3.3	1.8	-4.2	4.9	1.7	2.8	-3.2	1.7	96	1.6	92	2
55	BP-6	0.9857	0.10	2.9	1.1	2.5	-1.3	1.8	1.6	2.0	2.4	1.8	-1.4	1.8	88	1.5	100	5
56	BP-8	0.9994	6.79	4.5	-0.09	3.1	-0.11	1.6	7.5	4.5	1.2	2.2	-0.10	1.7	101	3.2	98	4
57	4-OH-BP	0.9929	-0.75	3.1	-0.85	2.6	-0.45	1.7	-2.0	2.3	-2.2	1.5	-1.2	1.2	106	1.3	100	2
58	BPA	0.9953	0.79	5.8	1.4	4.8	-0.45	2.0	-1.3	2.6	-0.89	3.4	-0.34	2.2	85	6.4	94	9
59	BPF	0.9943	2.2	9.7	0.30	1.8	1.1	3.0	8.6	5.6	-0.07	1.6	-0.04	2.6	112	5.7	94	9
60	BPS	0.9935	-4.2	4.4	1.0	2.0	-2.5	2.7	-3.3	4.0	1.5	1.9	-3.4	2.1	81	8.2	96	0
61	BPB	0.9533	-8.5	6.5	-2.3	2.6	0.59	2.6	-1.6	6.8	-1.5	2.2	-0.37	2.0	119	4.3	88	2
62	BPZ	0.9947	-4.2	8.2	-0.14	4.0	-2.5	2.0	0.87	4.7	2.3	1.3	-1.8	0.80	103	6.2	95	6
63	BPAP	0.9945	-8.2	9.8	2.2	2.3	-1.5	2.0	-2.4	7.0	3.1	1.7	-1.1	1.3	98	3.1	97	5
64	BPAF	0.9996	-2.6	4.9	0.33	1.8	-1.2	1.6	-0.05	3.0	0.36	2.0	-1.8	1.2	105	3.3	93	2
65	BPP	0.9745	-8.1	10	-0.76	4.8	-1.4	1.6	-0.27	9.4	1.3	3.7	-1.2	1.1	88	0.32	84	5
66-1	2,4,5-TCP	0.9968	0.56	6.5	-4.8	8.1	4.8	6.6	2.3	5.7	-6.8	7.3	1.8	4.5	103	4.1	101	1
66-2	2,4,6-TCP																	
68	2,3,5,6-TeCP	0.9961	1.6	4.4	2.6	2.8	-0.69	0.50	1.5	4.1	3.0	2.2	-0.53	0.40	96	2.5	106	3
69	2,3,4,6-TeCP	0.9942	-2.7	5.0	1.5	3.0	1.5	1.8	-2.8	5.3	1.7	3.0	1.6	2.0	106	6.3	94	5

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No.	Synonym	r ²	Accuracy and Precision (Intra-Day)						Accuracy and Precision (Inter-Day)						Recovery			
			1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL	
			RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %
70	2,3,4,5-TeCP	0.9937	-0.43	4.2	2.2	2.7	-1.3	1.9	-1.0	4.5	2.5	2.6	-1.4	2.0	104	4.0	91	0
71	PCP	0.9978	6.9	5.6	-0.63	2.7	-1.7	1.3	4.9	4.7	-0.36	2.9	-1.8	1.3	109	5.8	97	0
72	TCC	0.9966	-6.4	6.2	-2.4	4.7	-2.2	2.2	-3.9	4.7	-2.5	3.6	-2.0	2.2	85	0.25	84	1
73	TCS	0.9935	-5.8	4.7	-1.7	4.2	-0.97	1.9	-6.5	2.8	-1.7	4.1	-0.49	1.5	109	3.8	108	5
74	TCBPA	0.9912	1.1	4.9	-3.3	3.5	-4.4	2.1	2.9	4.2	-4.4	3.5	-4.9	1.4	109	6.7	104	2
75	TeCBPA	0.9875	3.9	9.8	-5.0	5.7	-1.3	3.9	-1.4	4.3	-4.4	3.2	-3.2	2.7	112	5.7	105	6
76	TBBPA	0.9964	-0.95	6.3	0.15	4.0	0.28	2.9	-2.7	6.6	6.7	5.8	-1.2	2.0	98	7.9	96	2
77	BADGE	0.9979	-2.7	6.7	3.0	2.7	-1.3	2.6	-4.2	6.3	2.3	2.1	-1.9	2.1	93	2.1	88	7
78	BADGE·H ₂ O	0.9568	-7.0	6.7	1.1	1.8	-1.6	2.4	-9.1	4.5	0.96	1.8	-1.0	1.9	118	1.1	78	7
79	BADGE·2H ₂ O	0.9690	1.4	6.1	1.2	3.3	2.6	3.5	0.29	6.8	1.0	2.4	1.8	2.8	128	9.4	62	0
80	1-Nap	0.9909	3.8	8.0	2.6	2.2	-1.2	3.0	-1.5	5.4	2.0	1.8	-1.5	2.4	86	4.6	94	2
81	2-Nap	0.9967	6.2	7.5	-0.92	1.7	-2.0	1.2	6.9	7.7	-0.17	1.4	-2.1	1.2	75	5.0	92	2
82-1	2-Fluo	0.9996	0.29	7.9	1.2	1.8	1.3	1.3	-3.3	5.5	1.5	1.8	1.6	1.3	103	7.8	90	7
82-2	3-Fluo																	
82-3	9-Fluo																	
85-1	2-Phen	0.9986	-4.0	6.7	1.1	3.1	0.42	1.3	-1.7	7.4	2.0	2.9	-0.17	0.90	97	2.3	91	2
85-2	3-Phen																	
87-1	1-Phen	0.9987	4.2	8.4	3.2	4.7	-0.95	2.6	-1.9	3.7	4.6	3.2	-2.3	1.6	112	8.8	96	3
87-2	9-Phen																	
89	4-Phen	0.9995	3.2	7.0	3.3	2.9	1.1	1.7	0.75	6.2	2.6	2.4	1.2	1.7	103	1.9	92	2
90	1-Pyr	0.9991	0.56	4.7	9.7	10	6.1	10	-0.50	4.9	11	7.4	1.8	7.6	106	6.3	103	2
91	nitenpyram	0.9993	-2.3	5.9	6.8	3.8	1.1	6.0	-2.8	6.2	5.3	2.4	-0.74	4.6	111	0.64	101	6
92	thiamethoxam	0.9989	9.0	7.6	2.4	3.4	1.8	4.4	6.1	3.4	4.1	2.6	0.49	4.2	106	1.3	90	3
93	imidacloprid	0.9986	9.6	5.5	2.7	3.9	0.05	3.2	9.7	4.9	1.1	1.9	-1.1	2.4	95	5.2	94	4
94	acetamiprid	0.9995	4.6	4.8	3.2	2.8	0.56	4.1	4.2	4.4	2.4	1.8	-1.1	2.9	108	0.66	96	0
95	thiacloprid	0.9995	0.88	1.7	0.79	1.4	1.4	2.8	0.69	1.4	0.53	1.4	-0.17	1.5	106	1.3	95	4
96	clothianidin	0.9989	-6.0	4.1	6.3	3.2	0.19	5.7	-7.0	4.2	5.3	2.1	-3.2	1.8	105	2.0	91	2
97	flonicamid	0.9973	3.3	6.2	-2.4	8.0	-0.89	7.6	1.4	6.7	-3.4	8.5	0.99	7.7	156	5.3	107	4
98	N-Dmt	0.9920	1.1	4.7	2.9	4.4	3.1	7.4	-1.7	2.1	0.77	2.5	0.08	6.6	138	5.8	102	1
99	N-Dma	0.9996	0.82	3.7	-0.12	3.3	2.8	4.1	-0.85	3.0	-1.2	2.7	1.0	1.7	114	8.6	100	9
100	Ta	0.9995	-0.07	3.9	2.4	2.6	4.5	6.2	-2.8	2.7	1.8	2.2	1.7	4.2	106	2.6	99	5
101	imidaclothiz	0.9976	7.8	4.4	6.0	3.4	2.7	3.8	5.3	2.7	7.3	2.9	1.9	1.6	95	2.1	104	4
102	6-Cn	0.9991	-6.8	5.9	-12	6.4	-1.9	4.8	-4.1	3.4	-8.3	4.4	0.29	2.1	103	6.9	95	1
103	sulfoxaflor	0.9998	-0.09	6.6	2.1	2.5	-0.40	2.0	0.05	5.0	2.1	2.6	0.12	1.4	106	1.3	95	8
104	4-nitrophenol	0.9992	0.15	7.6	1.4	3.2	1.8	1.8	5.6	3.5	0.54	2.6	0.96	1.2	98	6.7	100	6

No.	Synonym	r^2	Accuracy and Precision (Intra-Day)						Accuracy and Precision (Inter-Day)						Recovery			
			1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL		20 ng/mL		1 ng/mL		10 ng/mL	
			RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %	RE/ %	RSD/ %
105	2,4-D	0.9995	8.4	3.9	1.0	2.1	-4.0	4.9	7.1	3.3	0.50	2.2	-6.7	2.4	109	0.6	100	4
106	TCPY	0.9974	-4.2	5.1	-3.3	6.3	-2.1	2.4	-1.4	2.0	0.05	2.7	-3.0	1.7	107	3.3	102	4
107	trans-DCCA	0.9990	-2.3	5.3	1.3	4.3	-0.69	2.9	-1.9	3.6	-2.0	4.2	0.98	1.9	102	1.3	103	2
108	cis-DCCA	0.9617	-2.5	7.1	-8.2	8.9	-3.3	5.0	0.82	5.1	-4.6	4.7	-1.2	2.2	98	0.72	93	4
109	3-PBA	0.9992	-4.2	5.1	-12	6.4	-4.1	4.9	-1.9	2.8	-8.0	5.6	-2.1	1.9	98	0.72	93	4
110	4F-3PBA	0.9990	-3.2	4.1	-13	3.7	-4.0	4.9	-1.1	2.2	-9.4	5.4	-1.8	2.6	102	1.3	94	5
111	2,4,5-T	0.9950	-7.5	3.8	-9.8	8.3	-3.0	4.1	-7.0	2.7	-6.9	3.6	-1.4	1.7	111	6.3	100	1
112	pyrimethanil	0.9992	1.7	3.2	2.4	1.9	1.3	3.5	2.4	3.1	1.8	1.6	-0.73	1.4	107	4.6	94	3
113	dinotefuran	0.9998	-12	11	3.9	3.8	-0.45	5.8	-7.4	10	4.0	2.9	-1.7	4.8	100	3.0	100	0
114	metribuzin	0.9690	-12	9.5	2.4	4.7	0.44	5.3	-13	9.0	2.7	4.7	-0.95	4.2	97	3.4	94	6
115	atrazine	0.9989	5.8	3.1	2.9	2.7	3.0	2.9	4.9	2.9	2.3	2.2	1.6	2.4	127	0.38	122	4
116	cyprodinil	0.9994	4.6	5.2	3.6	3.8	-0.97	3.0	3.6	3.9	2.4	1.2	-2.4	2.1	110	5.8	91	2
117	metalaxyl	0.9994	3.8	2.8	1.4	1.6	0.36	2.5	4.0	1.7	1.3	1.4	-1.0	1.2	107	1.3	99	2
118	tebuconazole	0.9992	-1.1	3.9	4.6	2.8	-1.8	2.5	-2.7	4.0	3.5	1.7	-2.7	2.4	94	10	92	5
119	propiconazole	0.9743	2.8	2.1	1.0	3.7	-2.3	3.2	1.9	1.8	-0.40	2.1	-4.0	1.7	107	2.7	77	4
120	tetraconazole	0.9991	3.9	6.0	1.2	2.7	-1.2	3.2	5.5	5.1	0.27	2.2	-2.8	2.2	112	7.1	106	6
121	azoxystrobin	0.9998	1.6	2.7	1.7	2.6	1.6	2.4	0.75	2.7	1.0	2.0	0.51	1.6	104	4.7	97	2

RE: relative error; RSD: relative standard deviation; ME: matrix effect; LOD: limit of detection; There is no chromatographic resolution between compounds with same ID; therefore, sum concentrations are expressed for these isomers by using the present developed method.