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Development and validation of a GC-MS method for the simultaneous determination of bisphenols, phthalate monoesters, and triclosan in human meconium for comprehensive prenatal exposure assessment

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ABSTRACT

Determining in utero exposure to endocrine-disrupting chemicals (EDCs) is crucial for public health, given their widespread presence and high production volumes. Analyzing EDCs, such as bisphenols, phthalate monoesters, and triclosan in meconium is essential for evaluating prenatal exposure and gaining critical insights into potential fetal health hazards throughout gestation. The present work describes a GC–MS method for the determination of bisphenols, phthalate monoesters, and triclosan in meconium samples. Sample preparation involved hydrolysis of glucuronidated analytes followed by liquid–liquid back extraction with dichloromethane. A BSTFA+ 1% TMCS was used for derivatization of compounds prior to the GC-MS analysis. The method was linear over the concentration range 0.25–10 ng/g for all analytes. Intra- and inter-day precision (%RSD) values ranging from 0.39% to 5.79% and 1.07–6.97%, respectively. Accuracy (%) was within 85–115%. Application to real human meconium samples revealed that bisphenols were frequently detected, particularly BPS and BPA, with BPS detected in 96.0% of the samples (0.88–24.98 ng/g) and BPA in 88.0% (0.32–7.72 ng/g), while BPF and BPAF were found less frequently, in 36.0% and 24.0% of the samples, respectively. Among the phthalate monoesters, MMP, MEP, and MEHP were ubiquitous, while MBP was detected in nearly all samples (96.0%). Triclosan was also quantified, though at a lower frequency (12.0%). These findings confirm the prenatal exposure to these EDCs and demonstrate that the developed method provides the sensitivity and robustness required to quantify these contaminants at environmentally relevant concentrations.

1. Introduction

The robust assessment of human exposure to environmental contaminants is an essential component of contemporary toxicology and public health, a pursuit fundamentally reliant upon the sophisticated capabilities of advanced analytical chemistry. Among the wide range of xenobiotics, endocrine-disrupting chemicals (EDCs) notably bisphenols, phthalates, and the antimicrobial agent triclosan elicit considerable concern due to their pervasive presence and potential to perturb sensitive biological pathways. These are high production volume chemicals, integral to the manufacture of ubiquitous polymers and a myriad of consumer goods [1]. Bisphenols, exemplified by bisphenol A (BPA) alongside its increasingly utilized analogues such as bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF), serve as key monomers in polycarbonate plastics and epoxy resins, finding their way into food containers, beverage bottles, and the internal linings of cans [2,3]. Phthalates are primarily employed as plasticizers, imparting flexibility to polyvinyl chloride and thereby permeating an array of products from medical devices and vinyl flooring to personal care items and children's toys [4]. Concurrently, triclosan (TRC), a broad-spectrum antimicrobial,

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is incorporated into numerous personal care products, textiles, and household items [5,6].

The non-covalent incorporation of these compounds into product matrices facilitates their leaching, culminating in ubiquitous human exposure. Critically, upon maternal absorption, these EDCs can traverse the placental barrier, leading to fetal exposure during susceptible windows of organogenesis and physiological programming. This prenatal burden is a significant concern, as these chemical classes are well documented to interfere with intricate hormonal signaling [7]. Bisphenols are recognized for their estrogenic and thyroid-disrupting activities, while phthalates are primarily associated with anti-androgenic effects [8–10], and triclosan has also demonstrated endocrine modulating properties [11]. Significant evidence from epidemiological and toxicological investigations has linked prenatal coexposure to this EDC triad with a constellation of adverse health sequelae in offspring. These include, but are not limited to, an augmented risk of asthma, wheeze, and respiratory infections [12]; cryptorchidism and hypospadias [13]; cardiometabolic disturbances evidenced by associations with increased childhood weight, body mass index, and pericardial fat index [14,15]; neurodevelopmental deficits encompassing symptoms of attention deficit hyperactivity disorder and social impairments pertinent to autism spectrum disorders [16]; compromised bone mineral density and area adjusted bone mineral content linked to maternal BPS exposure [17]; reproductive dysfunctions affecting testosterone levels and semen quality [8]; and an elevated incidence of atopic dermatitis [18], particularly pronounced in females. The widespread acknowledgment of BPA's toxicity has instigated regulatory restrictions and its substitution with analogues, yet emerging evidence suggests these alternatives may harbor comparable, if not augmented, hazardous potential [19].

To accurately gauge this critical in-utero exposure, meconium, the neonate's primordial fecal matrix, has emerged as a superior biological specimen. Its accumulation within the fetal intestine throughout the second and third trimesters provides an integrated, historical record of the chemical entities that have reached the fetal compartment, offering a distinct advantage over matrices reflecting short term exposure, such as urine or blood [20,21]. However, the analytical interrogation of meconium is particularly challenging. Its complex, viscous, and lipid rich constitution creates substantial potential for matrix induced signal suppression or enhancement, diminished analyte recovery, and instrumental interference [22,23].

This analytical challenge is further compounded by the inherent physicochemical properties of the target analytes. Bisphenols and triclosan are phenolic compounds, while the primary metabolites of phthalates are monoesters bearing a free carboxylic acid group. These polar functional moieties impart low volatility, rendering the compounds unsuitable for direct analysis by gas chromatography mass spectrometry (GC-MS). Consequently, a chemical derivatization step is imperative to convert these polar hydroxyl and carboxyl groups into more volatile and thermally stable derivatives, typically silyl ethers/esters.

While liquid chromatography tandem mass spectrometry (LC-MS/MS) is frequently employed for such polar analytes in meconium, GC-MS offers distinct benefits for exposure assessment. These include high confidence in compound identification afforded by electron impact (EI) ionization and access to extensive, well characterized mass spectral libraries (e.g., NIST). Furthermore, the operational robustness and lower maintenance costs of GC-MS instrumentation make it a highly sustainable and accessible platform for large-scale, long-term biomonitoring studies. Several GC-MS methods have been reported for the analysis of phthalate monoesters [24–27], bisphenols [28–32], and TRC [33] in environmental, biological and food matrices. Nevertheless, there is a notable gap in the current literature regarding the simultaneous determination of phthalate monoesters, bisphenols, and TRC in meconium utilizing a single run GC-MS method. Existing methodologies typically focus on a subset of these compounds and primarily employ LC-MS/MS for meconium samples [34–38]. The development of a unified analytical procedure, integrating efficient sample extraction, a single-step derivatization effective for all three chemical classes, and robust GC-MS analysis, would therefore represent a significant methodological advancement. Such an approach promises to enhance analytical throughput, reduce requisite sample volumes, and curtail analytical expenditure, thereby rendering large scale biomonitoring studies of the prenatal chemical exposome more feasible.

Herein, we report a sensitive, and reliable method for the simultaneous quantification of four bisphenols, four phthalate monoesters and TRC in human meconium using GC-MS. We also present the concentrations of four bisphenol analogues, four phthalate monoesters and TRC real human meconium samples.

2. Materials and methods

2.1. Reagents and standards

Analytical standards, including phthalate monoesters (MMP, MEP, MBP, MEHP), bisphenols (BPA, BPAF, BPF, BPS), triclosan (TRC), and the internal standard BPA-d16, were purchased from AccuStandard, Inc. (New Haven, CT, USA). Derivatization reagents (BSTFA, BSTFA-1% TMCS, MSTFA, MTBSTFA) and various solvents (DCM, HXN, MeOH, MTBE) were obtained from Sigma-Aldrich (Burlington, MA, USA). Ethyl acetate, o-phosphoric acid, and pyridine were supplied by Merck (Darmstadt, Germany), while MSTFA-1%TMCS was sourced from Thermo Scientific (Waltham, MA, USA). Other chemicals, including NaOH, MSA, and β-glucuronidase (E. coli-K12), were purchased from Riedel-de Haën, AppliChem, and Roche Biomedical, respectively. High-purity (99.999%) nitrogen and helium were provided by Ankara Gaz (Ankara, Türkiye). Ultrapure water was obtained from a Human UP 900-UV Scholar system (Seoul, Korea).

2.2. Meconium collection

Meconium samples were collected from newborns born in Ankara University Cebeci Research and Practice Hospital, with the permission of Ankara University Clinical Research Ethics Committee (21–1413–18), Department of Obstetrics and Gynecology, and Department of Pediatrics.

After the meconium was passed, a wooden spatula was transferred directly to a 50 mL centrifuge tube and then refrigerated. Meanwhile, 100 μL 1 M H_3PO_4 was added to the samples to prevent esterase activity [36]. The collected samples were quickly placed in a deep freezer at −20 °C and then stored at −80 °C until analysis.

2.3. Gas chromatography – mass spectrometry analysis

GC-MS analysis was performed on an Agilent 6890 GC equipped with an Agilent 7683 Automatic Liquid Sampler system and coupled with an Agilent 5973 N mass selective detector (Santa Clara,CA, USA). The Restek Rtx-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column was used during the analysis (Bellefonte, PA, USA). Helium was used as a carrier gas and the flow rate was set at 1 mL/min. The injection port and the transfer line temperature were adjusted to 280 °C and the oven temperature was programmed as 100 °C hold for 3 min, increased to 280 °C at 20 °C/min with a final hold time of 5 min, resulting in a total analysis time of 17 min. Following the analysis, a post-run at 300 °C for 5 min was performed to ensure that there was no substance on the column.

The mass spectrometer (MS) was operated at electron impact ionization mode (EI, 70 eV) and the mass range was adjusted at 50–550 amu for scan mode. Three ions for each derivatized analyte were chosen for selected ion monitoring (SIM) acquisition mode to identify and quantify each analyte. The selected quantification and confirmation ions are tabulated in Table 1, with representative SIM chromatograms shown in Fig. S1. Data acquisition and processing were performed using Agilent

Table 1
Measurement parameters for target analytes, including mass, derivatized mass, retention time, quantification ion (*m/z*), and confirmation ions (*m/z*).

Time Segment	Analyte	Mass	Derivatized Mass	Retention Time (min)	Quantification ion (*m/z*)	Confirmation ions (*m/z*)
1	**MMP**	180	252	8.78	237	163, 221
2	**MEP**	194	266	9.16	251	149, 223
3	**MBP**	222	294	10.17	223	221, 149
4	**BPAF**	336	480	11.12	225	315, 190
5	**MEHP**	278	350	11.64	221	149, 239
6	**BPF**	200	344	11.82	344	179, 157
	TRC	288	360	11.84	345	347, 362
7	**BPA-d_{16}**	244	388	12.07	368	369, 386
	BPA	228	372	12.11	357	207, 358
8	**BPS**	250	394	14.89	394	379, 135

MassHunter Qualitative Analysis software (version B.07.00) and MassHunter Quantitative Analysis software (version B.09.00) (Santa Clara, CA, USA).

2.4. Preparation of standard solutions and matrix-matched calibrators

Individual stock solutions of each analyte (1 mg/mL) were prepared by dissolving 10 mg of standard in 10 mL of methanol. From these, a mixed-analyte stock solution was prepared at 10 μg/mL. This mixture was then serially diluted in methanol using volumetric flasks to create intermediate working solutions at 1, 0.1, and 0.01 μg/mL. A separate working solution for the internal standard (IS, BPA-d_{16}) was prepared at 0.5 μg/mL. These standard solutions were subsequently used to prepare the matrix-matched calibrators. This was achieved by spiking aliquots of homogenized meconium with appropriate volumes of the intermediate working solutions to generate a multi-point calibration curve. All prepared standards and calibrators were thoroughly mixed by vortexing to ensure homogeneity. Because meconium inherently contains endogenous levels of the target analytes and a true blank matrix is unavailable, calibrators and QCs were prepared using the ICH M10 [39] standard addition approach. The endogenous concentration quantified in a representative meconium matrix was subtracted from the measured concentration at each spiked level, and the resulting net concentrations were used to construct the calibration curve and assign QC values; selectivity was additionally verified across multiple individual meconium samples.

2.5. Sample preparation

2.5.1. Sample homogenization

Homogenization was performed by adding methanol to weighed meconium samples at a ratio of 1 mL of solvent for every 1 g of sample, followed by mixing with a homogenizer (IKA, Germany). To ensure accurate quantification, 20 μL IS was added to the samples in equal amounts. Vortexing was performed to achieve complete homogenization of the mixtures. Subsequently, the centrifuged meconium mixture was separated, and the liquid phase was carefully transferred to a new tube. Under a gentle stream of nitrogen gas, the liquid phase was evaporated to dryness, leaving behind the residue at the bottom of the tube (Fig. S2).

2.5.2. Extraction of free bisphenols, triclosan, and phthalate metabolites in meconium

The remaining residue was then diluted using a 0.2 mL solution of 0.5 M HCl, followed by liquid-liquid extraction with 0.6 mL of DCM. The aqueous phase was discarded, and back-extraction was carried out using 0.2 mL of 0.3 M NaOH solution to minimize the matrix effect and interference from endogenous substances. After discarding the organic phase, the aqueous phase was acidified with 0.2 mL of 0.5 M HCl and subjected to re-extraction with 1.2 mL of DCM. The resulting organic phase was separated, transferred to a new tube, and evaporated to dryness under a gentle flow of nitrogen gas, ensuring the removal of any residual organic solvent and water. The schematic of the meconium sample preparation pipeline used in this study is summarized in Fig. 1.

2.5.3. Extraction of total bisphenols, triclosan, and phthalate metabolites in meconium

In the subsequent stage of the procedure, the evaporated MeOH extracts (Section 2.5.1) were carefully treated to facilitate analysis. A total of 100 μL of β-glucuronidase (Roche, Switzerland) in acetate buffer (1:9; v/v) was added to the extracts, which were then subjected to an incubation period of 3 h at a controlled temperature of 37 °C. This incubation step aimed to enhance the enzymatic hydrolysis of conjugated compounds present in the meconium samples. Subsequently, the solution was acidified through the addition of concentrated HCl, effectively adjusting its pH to an acidic range. By doing so, the subsequent extraction step with DCM could proceed as per the previously described protocol (Section 2.5.2), allowing for the selective extraction of target analytes from the meconium matrix.

2.5.4. Derivatization procedure

To perform the derivatization process, 20 μL of BSTFA containing 1% TMCS was added to the dry DCM extracts. The vials containing the mixture were tightly closed and vigorously mixed for 30 s using a vortex mixer to ensure thorough mixing of the derivatization reagent with the samples. The derivatization reaction was then carried out at a temperature of 70 °C for 40 min, allowing for the desired chemical transformations to occur. Following the completion of the derivatization, the samples were left to cool at room temperature. For analysis, 1 μL of the derivatized sample was taken and analyzed using GC-MS in the splitless mode, enabling the identification and quantification of the target analytes.

2.6. Screening of collection materials and field blanks

During the study, special care was given to the selection of glass materials, ensuring their utilization for various purposes. To ensure as a key precaution, all plastic consumables utilized, including pipette tips, Pasteur pipettes, and centrifuge tubes, underwent thorough scanning before experimentation. The exclusion of products containing phthalates and bisphenol analogs further exemplified the commitment to maintaining the integrity of the study. Additionally, the purity of the ultrapure water and other solvents utilized throughout the research was confirmed to be free of, demonstrating their complete absence of the target analytes. These measures aimed to mitigate potential sources of contamination and uphold the reliability of the study's findings.

2.7. Method validation

In adherence to ICH (M10) [39] and FDA [40] guidelines, the method underwent rigorous validation. The selectivity and specificity were verified against potential matrix interferences, and sensitivity was defined through LOD and LLOQ determinations. Method performance

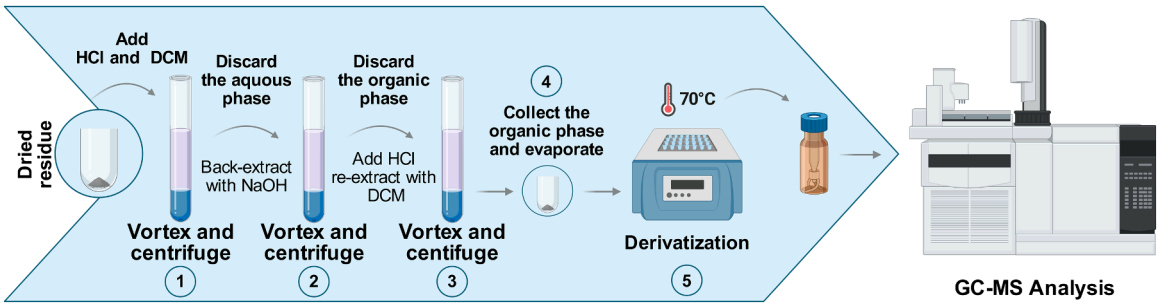

Fig. 1. Schematic overview of the sample preparation workflow for the extraction and derivatization of target EDCs from meconium samples.

was further characterized by evaluating linearity, recovery, and both intra- and inter-day precision and accuracy to ensure robust quantification of the target analytes.

3. Results and discussion

This study presents a comprehensive analytical method for quantifying key EDCs in meconium samples. The method enables the measurement of total analyte concentrations through enzymatic hydrolysis of glucuronidated metabolites using β-glucuronidase. The following sections describe the systematic optimization of analytical parameters required to achieve accurate and reliable quantification.

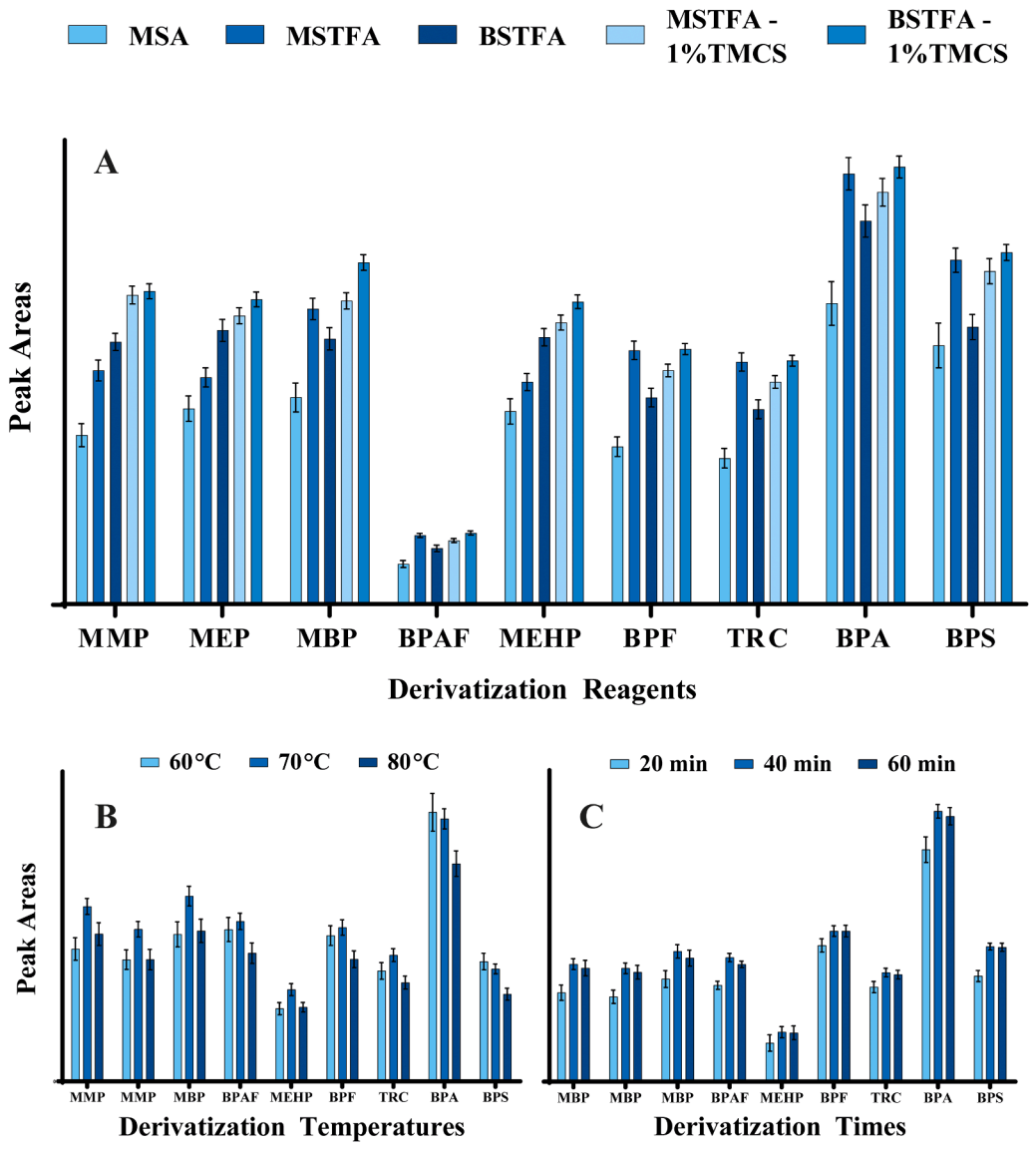

Fig. 2. Systematic optimization of silylation for GC–MS of bisphenols, phthalate metabolites, and triclosan. (A) Reagent comparison. (B) Temperature (60/70/80 °C) under BSTFA + 1% TMCS. (C) Time (20/40/60 min) at 70 °C (n = 3).

3.1. Optimized derivatization

A silylation reagent screen (Fig. 2A) was undertaken for GC–MS of bisphenols, phthalate metabolites, and triclosan, using chromatographic peak area as the response metric. As anticipated for a less reactive silyl donor, MSA yielded uniformly depressed signals, consistent with incomplete trimethylsilylation. MSTFA and BSTFA afforded higher responses; however, inclusion of 1% TMCS in either reagent produced a pronounced, catalyst-enabled enhancement in signal across the analyte suite. This TMCS-catalyzed facilitation is attributable to activation of the silylating milieu and improved conversion of sterically encumbered phenolic substrates (e.g., BPA, BPS). Among all conditions, BSTFA + 1% TMCS delivered the highest and most consistent responses and was therefore selected as the derivatization reagent of record.

Subsequent thermal optimization (Fig. 2B) established 70 °C as the operational optimum: seven of nine analytes including all phthalate metabolites, BPAF, BPF, and TRC achieved maximal responses at this setpoint. In contrast, 60 °C was kinetically insufficient for several targets, while 80 °C induced universal signal depression consistent with onset of thermolysis of the TMS derivatives (notwithstanding BPA/BPS exhibiting maxima at 60 °C). Temporal profiling under the selected chemistry (Fig. 2C) indicated that 20 min was suboptimal (incomplete conversion), 40 min yielded plateaued maxima for all analytes, and extension to 60 min conferred no additional benefit and occasionally elicited slight declines, suggestive of marginal degradative processes or volatility losses.

Accordingly, the finalized derivatization conditions, BSTFA + 1% TMCS, 70 °C, 40 min, provide high-yield conversion with maximal analytical sensitivity across the full panel.

3.2. Optimized conditions for GC–MS analysis

GC parameters were optimized to balance vaporization efficiency for silylated phenols with the thermal lability of phthalates. Inlet temperature (250–300 °C) optimization showed 280 °C as the optimal condition sufficiently high to ensure instantaneous transfer of low-volatility phenolic derivatives while minimizing the thermal degradation of phthalates, thereby maximizing overall response. Oven programming was then tuned for throughput and resolution by varying the initial temperature (60/80/100 °C) and ramp (5/10/20 °C/min); 100 °C with a 20 °C/min ramp produced sharp early peaks and a short cycle suitable for high-throughput screening.

Under the finalized GC–MS conditions, all targets eluted well after the solvent front (t_R 8.77–14.87 min, k′ 3.4–6.4). System suitability met common criteria (Table S1): peak shape acceptable (T_f 1.0–1.5; asymmetry typically 0.81–1.00, minor MMP fronting), and high efficiency (0.96–2.81 × 10^5 plates; 3.2–9.4 × 10^4 plates/m). Baseline resolution ($R_s \geq 1.5$) was achieved for adjacent pairs except TRC/BPF, which co-eluted chromatographically. Selectivity for this pair was secured at the MS level in SIM mode using distinct quantifier/qualifier ions with verified ion-ratio tolerances, enabling identification and accurate quantification despite co-elution. Representative overlaid

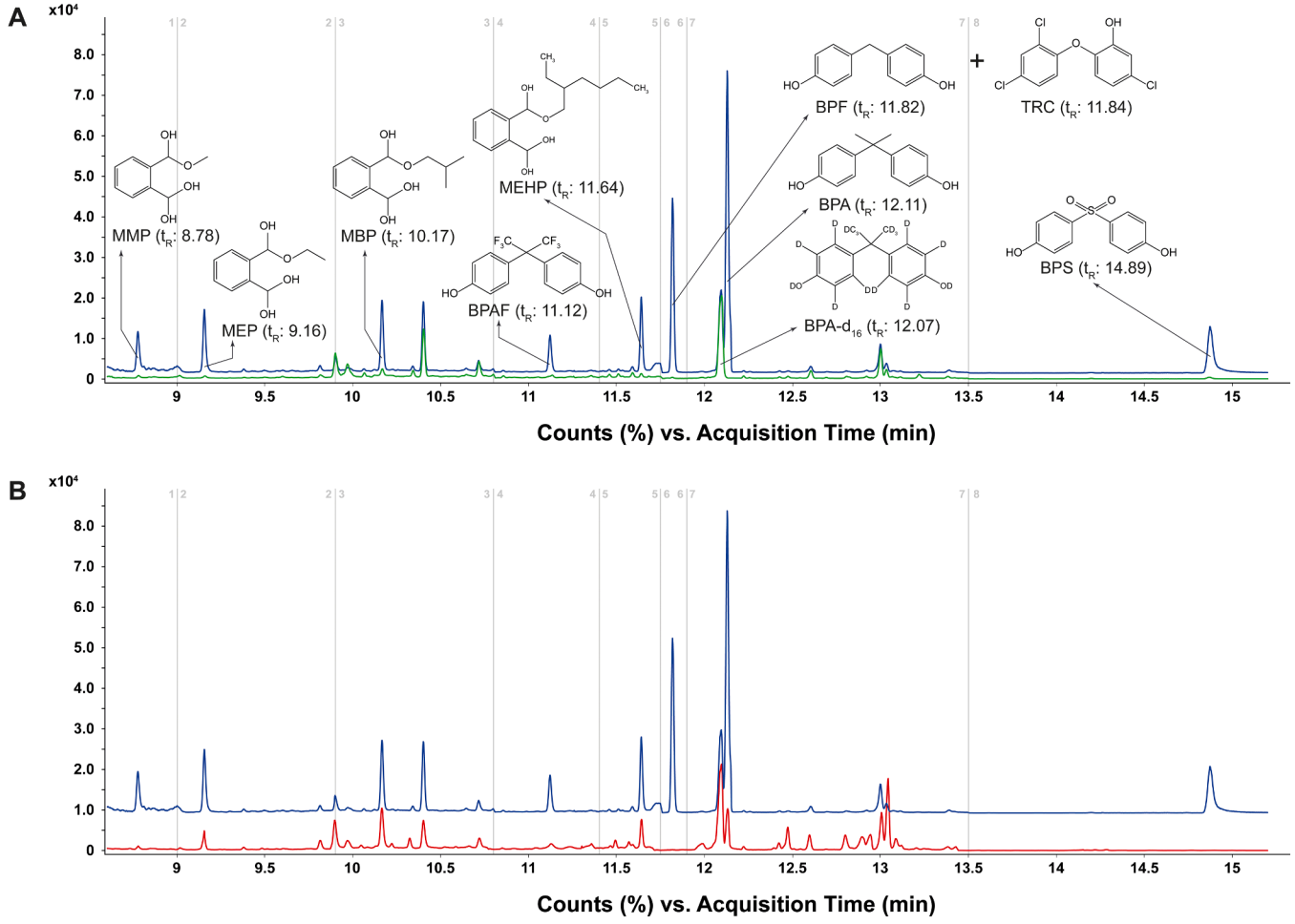

Fig. 3. Representative GC–MS chromatograms acquired under the optimized derivatization and analysis conditions. (A) Blue trace: matrix spiked with 10 ng per analyte; green trace: green trace: surrogate blank matrix (pre-screened meconium with lowest background). (B) Blue trace: matrix spiked with 10 ng per analyte; red trace: real sample analysis.

chromatograms acquired under these conditions are shown in Fig. 3.

3.3. Optimization of the LLE conditions

The selection of an appropriate extraction solvent is one of the most critical parameters determining the overall recovery and efficiency of an analytical method. To identify the most effective solvent for the simultaneous extraction of the target analytes, five organic solvents with varying polarities hexane, ethyl acetate, diethyl ether, MTBE, and DCM were evaluated. The results, presented in Fig. 4A, highlight a strong correlation between solvent polarity and extraction efficiency, governed by the "like dissolves like" principle. Non-polar hexane and moderately polar ethyl acetate proved largely ineffective, particularly for the more polar phenolic compounds. While the ethers (DEE and MTBE) performed well due to their intermediate polarity, DCM proved to be the most effective solvent, yielding the highest peak areas for most of the target analytes.

The performance of DCM can be attributed to several key chemical properties. As a polar aprotic solvent with a significant dipole moment, DCM can establish strong dipole-dipole interactions with the polar moieties of all target compounds. Its versatility allows it to effectively solve a broad spectrum of compounds, from the moderately polar phthalate esters to the highly polar, hydrogen bond capable bisphenols and triclosan. Unlike ethers or esters, DCM's balanced solvating power appears to be optimal for this specific mixture of analytes, which possess a wide range of polarities and functionalities.

It is noteworthy that MTBE yielded a slightly higher response for BPS, the most polar analyte in the suite. This single exception is likely attributable to a more favorable specific interaction between the ether oxygen of MTBE and the highly polar sulfonyl group of BPS. Despite this, based on its comprehensive and superior ability to extract nearly all target compounds with the highest efficiency, DCM was confidently selected as the optimal extraction solvent for the final multi-residue method.

Following the selection of DCM as the most effective extraction solvent, a multi-step optimization was performed to refine the LLE conditions. First, the effect of the sample-to-solvent volume ratio was investigated across a range from 1:1–1:5 (v/v). As shown in Fig. 4B, increasing the solvent volume from a 1:1 to a 1:3 ratio resulted in a substantial increase in extraction efficiency for all analytes. While

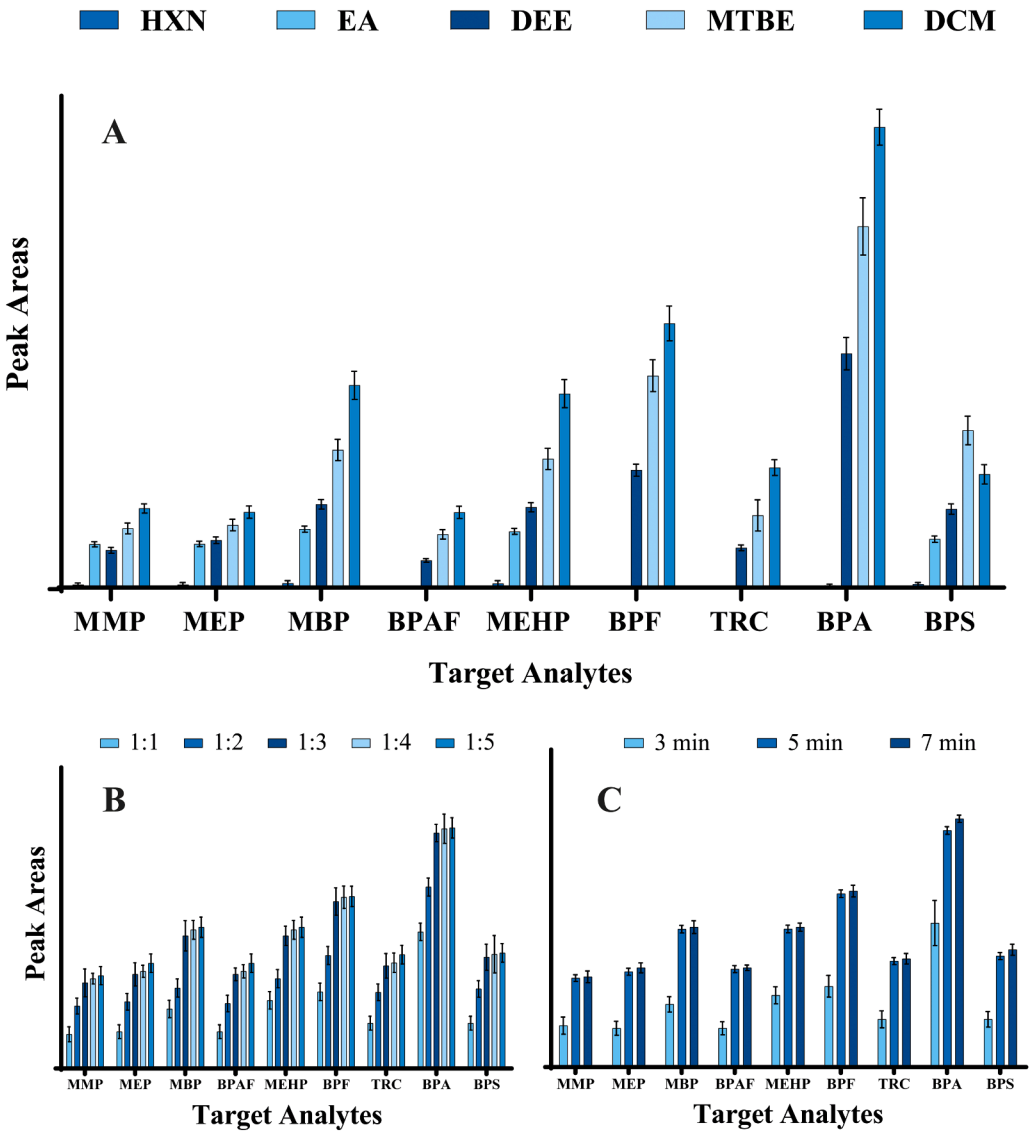

Fig. 4. Optimization of the extraction procedure for the target analytes. **(A)** Effect of five different organic solvents on extraction recovery. **(B)** Comparison of different sample-to-solvent volume ratios (1:1, 1:2, 1:3, 1:4, 1:5, v/v) using the solvent that provided the highest recovery. **(C)** Investigation of the effect of different vortexing times (3, 5, and 7 min) on efficiency, using the optimal solvent and ratio. (n = 3).

slightly higher recoveries were observed at 1:4 and 1:5 ratios, these gains were marginal and did not justify the increased solvent use. Therefore, a 1:3 ratio was selected as the optimal condition, representing a robust compromise between achieving high extraction recovery and minimizing solvent consumption, in line with green analytical chemistry principles.

Subsequently, using the optimized 1:3 solvent ratio, the vortexing time was evaluated at 3, 5, and 7 min to ensure sufficient phase contact and analyte partitioning. The data, presented in Fig. 4C, indicate that extending the vortexing time from 3 to 5 min led to a significant improvement in analyte recovery. However, a further extension to 7 min did not yield any additional significant increase in efficiency. Consequently, 5-minute vortexing time was determined to be optimal, ensuring thorough extraction without unnecessarily prolonging the sample preparation time.

3.4. Validation results

The analytical method developed for the simultaneous quantification of MMP, MEP, MBP, BPAF, MEHP, BPF, TRC, BPA and BPS in meconium samples was successfully validated. The validation parameters demonstrate that the method is robust, reliable, and fit-for-purpose for its intended application in biomonitoring studies.

The method showed excellent linearity for all target analytes over a working concentration range of 0.25–10 ng/g. The coefficients of determination for all calibration curves were consistently greater than 0.999, confirming a strong and predictable linear relationship between analyte concentration and instrumental response (Table 2). This high degree of linearity is a prerequisite for accurate quantification. Furthermore, the distinct retention times for each compound indicated that the chromatographic conditions provided excellent separation, which is crucial for minimizing the risk of coelution and ensuring the accurate identification of each analyte in a complex biological matrix like meconium.

Method sensitivity was characterized in line with FDA and ICH guidance using the reportable range. A uniform calibration range of 0.25–10 ng was established for all analytes, with 0.25 ng set as the LLOQ and 10 ng as the ULOQ. All analytes met the guideline acceptance criteria at and above the LLOQ. This low LLOQ and defined upper range are particularly advantageous for assessing prenatal exposure, where ECDs are often present at very low concentrations.

Matrix effect (ME) inherent to the complex meconium matrix were mitigated using matrix-matched calibration and IS, as per ICH M10 guidelines. This strategy effectively compensated for matrix induced response enhancement where matrix components minimize analyte adsorption within the injector. The robustness of our extraction protocol was confirmed by consistent recoveries and high precision. These results indicate that the optimized cleanup isolated the target bisphenols, phthalate metabolites, and triclosan from matrix interferences, establishing a reliable framework for trace level neonatal biomonitoring.

Precision and accuracy were assessed at four different QC concentration levels. The intra-day precision (%RSD) values ranged from 0.39% to 5.79%. While inter-day precision ranged from 1.07 to 6.97%. For all analytes at QC concentration levels were significantly below the internationally accepted limit of $\leq$ 15% for bioanalytical method validation. The accuracy of the method expressed as the percentage was within 95.51–103.89% (Table 3).

In summary, the validation data provides that the developed analytical method is highly suitable for the simultaneous determination of the target compounds in meconium. The method exhibited excellent linearity, sensitivity, precision, and accuracy. These robust performance characteristics ensure that the method can produce reliable data, making the method a valuable tool for future studies investigating prenatal exposure to these environmental contaminants.

3.5. Application to real meconium samples

To demonstrate the practical applicability and fitness-for-purpose of the validated method, a cohort of 25 authentic meconium samples, collected from newborns at Ankara University Cebeci Hospital, were analyzed. The demographic, obstetric, and neonatal characteristics of the study cohort are summarized in Table S2. The results, summarized in Table 4, reveal widespread and complex exposure profiles to several of the targeted EDCs among the studied population.

Among the bisphenols, BPA was a frequently detected analyte, found in 88.0% of the samples, with concentrations ranging from 0.32 to 7.72 ng/g wet weight. Notably, the BPA-analogue BPS, often used as a substitute, was detected at an even higher frequency of 96.0%, underscoring the importance of monitoring for replacement chemicals. For the phthalate monoesters, MMP, MEP and MEHP were ubiquitous (100% detection), and MBP was also found in nearly all samples (96.0%). TRC was also quantified, albeit at a lower frequency of 12.0%. These findings confirm the prevalence of prenatal exposure to a mixture of these EDCs and affirm that the developed method possesses the requisite sensitivity and robustness to quantify these contaminants at environmentally relevant concentrations.

The strength of this multi-analyte method lies in its ability to reveal individual exposure patterns that would otherwise be missed. A key observation from our cohort is the direct evidence of the "regrettable substitution" phenomenon. For instance, a participant exhibited a non-quantifiable BPA concentration (<LLOQ) while simultaneously having one of the highest BPS concentrations in the cohort (24.98 ng/g). This profile strongly suggests a lifestyle with conscious avoidance of BPA-containing products, leading to high exposure to its chemical

Table 2
Calibration, sensitivity, and precision metrics for MMP, MEP, MBP, BPAF, MEHP, BPF, TRC, BPA, and BPS in the meconium samples.

Analyte	Range (ng/g)	Equation	R^2	LOD (ng/g)	LLOQ (ng/g)	RSD% at LLOQ level
MMP	0.25–10	y = 0.0037x - 0.0004	0.9996	0.06	0.25	3.3
MEP	0.25–10	y = 0.0107x - 0.0001	0.9997	0.06	0.25	3.3
MBP	0.25–10	y = 0.0155x - 0.001	0.9999	0.04	0.25	3.5
BPAF	0.25–10	y = 0.0229x - 0.0004	0.9997	0.06	0.25	2.5
MEHP	0.25–10	y = 0.004x – 0.00008	0.9997	0.08	0.25	9.7
BPF	0.25–10	y = 0.1055x - 0.0039	0.9993	0.08	0.25	3.9
TRC	0.25–10	y = 0.044x + 0.0038	0.9992	0.08	0.25	3.9
BPA	0.25–10	y = 0.2099x + 0.0094	0.9998	0.05	0.25	2.5
BPS	0.25–10	y = 0.0154x - 0.0007	0.9997	0.06	0.25	2.9

Table 3
Summary of intraday and interday precision and accuracy for MMP, MEP, MBP, BPAF, MEHP, BPF, TRC, BPA, and BPS across four QC levels.

Analyte	Intra RSD% (min - max)	Inter RSD% (min - max)	Accuracy% (min - max)	Mean Bias% (vs 100%)
MMP	2.81–5.79	3.09–4.28	95.51–103.07	-0.39
MEP	0.96–2.54	1.11–4.77	98.84–103.75	0.6
MBP	1.19–3.06	2.26–6.97	96.09–100.20	-2.04
BPAF	0.46–2.92	1.63–4.52	96.76–102.62	-0.65
MEHP	1.41–9.7	2.36–6.25	99.00–101.66	-0.29
BPF	1.52–5.11	1.07–6.41	97.43–100.85	-1.22
TRK	1.43–4.08	3.23–3.53	100.04–103.36	1.44
BPA	0.74–2.55	1.14–2.71	100.56–103.89	1.71
BPS	0.39–3.28	1.53–5.19	103.38–110.02	5.63

Table 4
Detection frequencies and concentration distributions (ng/g wet weight) of target EDCs in human meconium samples (n = 25).

Analyte	Detection Frequency (%) (n)	Geometric Mean (95% CI)†	Mediana	25th Percentile (Q1)†	75th Percentile (Q3)†	Range†† (Min–Max)
Bisphenols						
BPA	88.0% (22)	1.14 (0.77 – 1.69)	1.62	0.66	3.35	0.32 – 7.72
BPS	96.0% (24)	3.49 (2.29 – 5.33)	4.57	1.91	9.28	0.88 – 24.98
BPF	36.0% (9)	0.32 (0.22 – 0.46)	<LLOQ	<LLOQ	1.55	0.51 – 5.74
BPAF	24.0% (6)	0.28 (0.20 – 0.40)	<LLOQ	<LLOQ	0.99	0.51 – 3.85
Phthalates						
MMP	100% (25)	15.61 (13.38 – 18.22)	16.82	10.63	25.49	6.92 – 37.56
MEP	100% (25)	2.5 (2.05 – 3.05)	2.46	1.43	3.6	0.79 – 9.91
MBP	96.0% (24)	4.95 (3.11 – 7.89)	5.7	2.72	16.59	0.80 – 38.02
MEHP	100% (25)	4.68 (2.78 – 7.86)	4.69	2.05	9.87	0.31 – 42.12
Antimicrobial						
TRC	12.0% (3)	0.22 (0.19 – 0.26)	<LLOQ	<LLOQ	<LLOQ	1.83 – 4.97

† Statistical calculations (Geometric Mean, 95% Confidence Interval, Median, Q1, Q3) were performed on the full dataset (N = 25). For concentrations below the LLOQ (0.25 ng/g), a substitution value of LLOQ/$\sqrt{2}$ (≈ 0.177 ng/g) was used. †† The range (Min–Max) is reported only for samples with concentrations detected above the LLOQ.

substitute. Similarly, three of the samples had no detectable BPA, yet all showed exposure to BPS, with one of those three samples also being exposed to BPF and BPAF. These cases highlight that "BPA-free" labeling does not equate to a lack of bisphenol exposure and validates the necessity of monitoring for a broader range of analogues.

Conversely, the data also reveal that a simple inverse relationship between BPA and its analogues does not hold for the entire population, pointing to a more complex, co-exposure reality. Another sample, for example, carried the highest burden of BPA in the cohort (7.72 ng/g) while also having a high concentration of BPS (6.66 ng/g). This coexposure pattern, also observed in other participants, suggests simultaneous exposure to both traditional BPA sources (e.g., certain food can linings) and newer products containing BPA substitutes. Such mixed exposures to compounds that may act on similar biological pathways could pose unique health risks that are critical to investigate in future toxicological and epidemiological studies.

Finally, the results underscore the significant inter-individual variability in exposure levels, emphasizing the need for sensitive methods to identify highly exposed individuals. While median concentrations provide a central tendency, the observed ranges were vast, with the maximum concentration of MBP (~38 ng/g) being nearly 48 times its lowest detected value, and the maximum for BPS (~25 ng/g) being 28 times its minimum. The ability of this single, robust GC-MS method to capture this wide dynamic range from low level ubiquitous exposures to high-level burdens in specific individuals makes it an invaluable tool for future research aimed at linking specific prenatal chemical profiles to child health outcomes.

3.6. Comparative assessment of analytical performance and methodological strategies

The role of meconium in prenatal toxicology has transitioned from a niche interest to a primary tool for assessing cumulative fetal exposure, largely because it offers a much broader window into the intrauterine environment than the transient snapshots provided by maternal urine or blood. Since the early work of Kato et al. [36], the field has struggled with the inherent complexity of this matrix, a dense mixture of lipids and proteins that often compromises analytical sensitivity. While recent literature has seen a decisive pivot toward LC-MS/MS platforms to handle these challenges (Table S3), our study demonstrates that a modernized GC-MS approach is not only viable but offers distinct advantages. By optimizing a silylation protocol using BSTFA and 1% TMCS, we were able to leverage the qualitative certainty of EI ionization and NIST spectral libraries. This provides a level of structural confirmation for emerging analogs that are often difficult to replicate with the ESI techniques seen in studies by Mathew et al. [37] and Xie et al. [38].

Managing the meconium matrix often feels like a tradeoff between exhaustive cleanup and analyte recovery. For instance, the multistage procedure recently proposed by Domínguez-Liste et al. [35], involving keratinase liquefaction and EMR-Lipid cartridges, showcases the extreme measures sometimes required to mitigate matrix effects. In contrast, our findings suggest that a carefully optimized liquid–liquid back extraction (LLBE) can achieve similar analytical robustness without the need for such complex and expensive enzymatic steps. The resulting precision of our method is particularly noteworthy; with RSD values tightly controlled between 0.39% and 6.97%, we have significantly reduced the variability seen in earlier foundational works [36] and large-scale surveys like the Canadian study [41], where fluctuations often exceeded 20%. For a laboratory focused on routine clinical or forensic biomonitoring, this level of reproducibility is essential for making meaningful comparisons across different birth cohorts.

Beyond technical optimization, a primary motivation for this work was to address the "regrettable substitution" phenomenon now dominating the plasticizer industry. As regulatory pressure mounts against BPA, it is being replaced by structural analogs like BPS, BPF, and BPAF compounds that may carry equivalent or even higher endocrine-disrupting potential. Although Cai et al. [42] and Tillotson et al. [43] have recently characterized BPF and BPS in meconium, the present study provides the first evidence of BPAF in this specific matrix to the best of our knowledge. The fact that we detected BPS in 96% of our samples and traditional phthalates like MEHP in 100% highlights a disturbing ubiquity. This detection frequencies align with trends reported in high-density urban centers in China [42] and the USA [37], confirming that the prenatal chemical burden is not a localized issue but a globalized reality.

A critical consideration in modern biomonitoring is the balance between extreme sensitivity and practical accessibility. High resolution platforms, such as the ESI-HRMS system utilized by Mathew et. al. [37], undoubtedly offer remarkable ultra trace detection limits. However, such instrumentation often requires prohibitive capital investment and

specialized technical support that remains out of reach for many laboratories. Our method's LOD of 0.04–0.08 ng/g strikes a strategic balance, providing a much more accessible and cost-effective alternative while remaining highly competitive with these more advanced techniques. Despite the lower operational costs, our results are sensitive enough to capture environmental exposure baselines with rigorous precision. Furthermore, our data from a Turkish cohort fills a significant geographic gap in a literature historically dominated by North American and East Asian populations. By documenting these exposure levels in the Mediterranean region, this research offers a sustainable and high-performance framework for future epidemiological studies seeking to connect complex chemical mixtures to long-term health outcomes.

Although the sample size (n = 25) and single-center recruitment at a tertiary hospital in Ankara limit the geographic and socioeconomic representativeness of the reported profiles, it is important to emphasize that this research was primarily designed for the technical optimization and rigorous validation of a unified GC-MS framework. Within the current meconium literature, study designs typically diverge based on their primary objectives; while expansive multi-center cohorts focus on epidemiological power [37], foundational method-development works and high-precision pilots frequently utilize smaller, highly controlled cohorts to prioritize analytical rigor and methodological stability (Kato et al. [36], n = 5; Domínguez-Liste et al. [35], n = 20; Tillotson et al. [43], n = 22). In our case, the single-center framework was a strategic choice to ensure maximum oversight of pre-analytical variables such as standardized collection protocols, immediate acidification, and uniform cold-chain maintenance all of which are critical for minimizing confounding factors that could compromise trace-level quantification. Ultimately, these findings establish a robust methodological and baseline foundation for the region, and a larger, multi-center epidemiological investigation is currently underway to assess broader population-level trends and clinical outcomes in greater detail.

4. Conclusion

This study reports the development and validation of a novel, unified GC-MS method for the concurrent determination of a comprehensive suite of nine EDCs in human meconium. To the best of our knowledge, this is the first GC-MS method of its kind. A central achievement of this work is the highly optimized, single-step derivatization protocol that successfully overcomes the common analytical challenge of simultaneously converting disparate functional groups, both phenolic hydroxyls and carboxylic acids, in a single reaction. This technical advance enabled the creation of a unified procedure and, to the best of our knowledge based on the current literature, represents the first report of BPF and BPAF analysis in this critical biological matrix. The method was validated according to international guidelines, demonstrating strong analytical performance with high linearity ($R^2 > 0.999$), a low limit of quantification of 0.25 ng/g, high precision with intra- and inter-day relative standard deviations below 7.0%, and accuracy results falling within the required 85–115% acceptance range. Crucially, its practical utility and fitness-for-purpose were confirmed through its successful application to a cohort of authentic meconium samples.

In conclusion, this validated method offers a sensitive, reliable, and efficient tool for biomonitoring studies focused on prenatal EDC exposure. By reducing requisite sample volumes and enhancing analytical throughput, it is well-suited for large-scale projects and facilitates broader epidemiological research aimed at understanding the relationship between the in-utero chemical environment and child health outcomes. Future applications could involve applying this high-throughput method to large, geographically diverse birth cohorts to investigate potential links between specific EDC mixtures and neurodevelopmental or metabolic disorders.

CRediT authorship contribution statement

Kenan Can TOK: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mehmet Gumustas:** Writing – review & editing, Supervision, Resources, Conceptualization. **Khaled Mohamed Masoud:** Formal analysis, Data curation. **Saadet Arsan:** Resources, Investigation. **Aslihan Gurbuz Bolkan:** Writing – review & editing, Supervision, Project administration. **H. Sinan Suzen:** Writing – review & editing, Supervision, Resources, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2026.117556.

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