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Assessment of Environmental Phenolic Endocrine-Disrupting Chemicals in Human Urine and Their Association with Demographic, Lifestyle, and Metabolic Factors in a Bangladeshi Population Using High-Throughput Solid-Phase Extraction Coupled with UHPLC–Tandem Mass Spectrometry

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ABSTRACT

Background: Environmental phenolic endocrine-disrupting chemicals (EDCs) are widely distributed contaminants associated with plastics, food-packaging materials, thermal papers, flame-retardant products, antimicrobial formulations, and a variety of household and personal-care products. Human exposure to these compounds is of increasing public-health concern because several phenolic chemicals can interact with endocrine pathways and may contribute to metabolic, reproductive, developmental, and other adverse biological effects. Biomonitoring of urinary concentrations provides an effective approach for evaluating recent human exposure to these compounds. Nevertheless, simultaneous determination of multiple phenolic EDCs in urine is analytically challenging because of their low concentrations, diverse physicochemical characteristics, biological conjugation, and matrix-related interference. Objective: This study aimed to develop and apply a high-throughput solid-phase extraction coupled with ultra-high-performance liquid chromatography–tandem mass spectrometry (SPE–UHPLC–MS/MS) method for the simultaneous determination of eight environmental phenolic EDCs in human urine from a population in Bangladesh. The investigated compounds were bisphenol A (BPA), bisphenol F (BPF), tetrachlorobisphenol A (TCBPA), tetrabromobisphenol A (TBBPA), bisphenol S (BPS), bisphenol AF (BPAF), bisphenol B (BPB), and triclosan (TCS). The study also establishes an integrated framework for examining potential relationships between urinary phenolic exposure and demographic, lifestyle, and metabolic characteristics. Methods: A total of 64 human urine specimens were analyzed. Urine samples underwent enzymatic hydrolysis followed by high-throughput extraction using a 96-well Oasis HLB solid-phase extraction plate containing 60 mg sorbent per well. A 30% acetonitrile washing solution was used to remove interfering matrix components, followed by methanol elution. Chromatographic separation was achieved using an ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 µm). Detection was performed using tandem mass spectrometry with negative electrospray ionization and multiple-reaction monitoring. Stable isotope-labeled internal standards were incorporated throughout the analytical procedure. Method performance was evaluated in terms of linearity, sensitivity, recovery, precision, matrix effects, and accuracy using certified reference material. Results: The analytical method showed excellent linearity, with correlation coefficients generally exceeding 0.999. Limits of detection ranged from 0.002 to 1.09 µg/L, whereas limits of quantification were approximately 0.007–3.63 µg/L. Mean recoveries ranged from 81.0% to 101.9%. Intra-day relative standard deviations ranged from 0.4% to 19.4%, while inter-day relative standard deviations ranged from 2.5% to 17.8%. In the 64 urine specimens, BPA was detected in 100% of samples, BPS in 96.9%, TCS in 57.8%, TBBPA in 46.9%, TCBPA in 23.4%, and BPF in 21.9%. BPB and BPAF were not detected. Median urinary concentrations were 0.69 µg/L for BPA, 0.086 µg/L for BPS, 1.44 µg/L for TCS, 0.0032 µg/L for TBBPA, and 0.00050 µg/L for TCBPA. Median concentrations for BPF, BPB, and BPAF were zero. Conclusion: The high-throughput SPE–UHPLC–MS/MS method demonstrated strong sensitivity, satisfactory recovery, excellent linearity, and acceptable precision for simultaneous determination of eight phenolic EDCs in human urine. The widespread detection of BPA and BPS and the measurable occurrence of TCS, TBBPA, TCBPA, and BPF demonstrate exposure to multiple phenolic chemicals in the investigated Bangladeshi population. The analytical strategy provides a robust platform for human biomonitoring and supports future epidemiological studies investigating relationships between environmental phenolic exposure, demographic characteristics, lifestyle practices, and metabolic health.

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1.INTRODUCTION

Endocrine-disrupting chemicals are increasingly recognized as an important category of environmental contaminants because of their capacity to interfere with endogenous hormonal signaling [1]. These chemicals may act through several biological mechanisms, including interaction with nuclear hormone receptors, alteration of hormone synthesis and metabolism, modification of intracellular signaling, and disruption of endocrine feedback mechanisms [2]. Human exposure can occur through food, drinking water, household dust, consumer products, occupational environments, and dermal contact [3].

Among the numerous classes of endocrine-active substances, phenolic compounds have attracted considerable scientific attention [4]. Bisphenol A is one of the most extensively investigated phenolic chemicals because of its widespread use in polycarbonate plastics and epoxy resins [5]. BPA may migrate from food-contact materials and other consumer products, resulting in repeated low-level human exposure [6].

The increasing regulatory and scientific concerns surrounding BPA have resulted in the introduction of alternative bisphenol compounds, including BPS, BPF, BPAF, and BPB [7]. These substances have been incorporated into different industrial applications as substitutes for BPA. However, the replacement of one bisphenol by another does not necessarily eliminate endocrine-related concerns. Structural similarities among these compounds may result in overlapping biological activities and exposure pathways [8].

Halogenated bisphenols are another group of environmental phenolic compounds of concern [9]. TBBPA is widely associated with flame-retardant applications and may enter environmental and human exposure pathways during manufacturing, use, recycling, and disposal of treated materials [10]. TCBPA is another halogenated bisphenol that warrants attention because of its environmental persistence and potential biological activity [11].

Triclosan is a chlorinated phenolic antimicrobial compound that has historically been incorporated into numerous personal-care and consumer products [12]. Although its use has been restricted in several applications in different countries, environmental persistence and continued exposure through existing products and environmental reservoirs remain relevant [13].

The assessment of phenolic EDC exposure in humans requires reliable analytical techniques capable of detecting multiple compounds at trace concentrations [14]. Urine is particularly useful for biomonitoring because many phenolic chemicals are rapidly metabolized and excreted, primarily following glucuronidation and sulfation [15]. Measurement of urinary concentrations can therefore provide information concerning recent exposure [16].

However, urinary analysis presents several analytical challenges. Urine contains salts, endogenous metabolites, proteins, and other matrix constituents capable of affecting chromatographic performance and electrospray ionization [17]. Furthermore, target compounds differ in polarity, molecular structure, ionization efficiency, and concentration. Consequently, efficient sample preparation is essential [18].

Solid-phase extraction is widely used for biological matrices because it provides simultaneous concentration and purification of target compounds [19]. The development of 96-well SPE formats has further increased analytical throughput and has made SPE particularly suitable for biomonitoring studies involving multiple human specimens [20].

Coupling SPE with UHPLC–MS/MS offers several advantages. UHPLC provides rapid and efficient chromatographic separation, whereas tandem mass spectrometry provides high selectivity through precursor-to-product ion transitions [21]. The use of multiple-reaction monitoring further improves specificity and sensitivity [22].

In Bangladesh, systematic human biomonitoring of multiple phenolic EDCs remains an important area for environmental-health research. Rapid urbanization, increasing consumption of packaged foods and beverages, widespread use of plastics, and exposure to a broad range of consumer products may contribute to complex patterns of environmental chemical exposure.

Therefore, the present investigation was designed to simultaneously determine eight phenolic EDCs in human urine using a high-throughput SPE–UHPLC–MS/MS procedure. The target compounds included BPA, BPF, TCBPA, TBBPA, BPS, BPAF, BPB, and TCS.

The primary objective of this study was to describe the occurrence of eight environmental phenolic chemicals in 64 human urine specimens using high-throughput solid-phase extraction coupled with UHPLC–tandem mass spectrometry. The analytical assessment considered calibration linearity, detection and quantification limits, extraction recovery, precision, and comparison with a reference material. The biomonitoring component focused on compound-specific detection frequencies and the interpretation of the reported urinary concentration summaries.

Demographic characteristics, lifestyle practices, and metabolic indicators were considered within the broader conceptual framework as potentially relevant determinants or correlates of urinary phenolic exposure. However, the aggregate results presented here do not permit estimation of these associations. The empirical findings are therefore descriptive, while the proposed relationships remain questions for subsequent participant-level investigation. Testing these relationships requires paired exposure and covariate measurements, appropriate control of confounding, and an explicit strategy for urinary dilution and concentrations below analytical reporting limits.

2. Materials and Methods

2.1 Study design

A human biomonitoring investigation was conducted in Bangladesh to evaluate urinary exposure to eight environmental phenolic endocrine-disrupting chemicals.

The analytical investigation included 64 human urine specimens. The study incorporated a high-throughput sample-preparation strategy followed by UHPLC–MS/MS analysis.

The principal analytical outcomes were urinary concentrations and detection frequencies of the eight target compounds.

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The broader epidemiological framework considered potential associations between urinary concentrations and participant characteristics, including demographic, lifestyle, and metabolic variables.

2.2 Target compounds

Eight phenolic EDCs were investigated:

- Bisphenol A (BPA)
- Bisphenol F (BPF)
- Tetrachlorobisphenol A (TCBPA)
- Tetrabromobisphenol A (TBBPA)
- Bisphenol S (BPS)
- Bisphenol AF (BPAF)
- Bisphenol B (BPB)
- Triclosan (TCS)

These compounds were selected because they represent several environmentally important phenolic chemical groups, including bisphenol analogues and halogenated phenolic compounds.

Table 1. Target environmental phenolic endocrine-disrupting chemicals

No.	Compound	Abbreviation	Major exposure relevance
1	Bisphenol A	BPA	Plastics, epoxy resins, food-contact materials
2	Bisphenol F	BPF	BPA substitute, polymers and resins
3	Tetrachlorobisphenol A	TCBPA	Halogenated industrial chemical
4	Tetrabromobisphenol A	TBBPA	Flame-retardant applications
5	Bisphenol S	BPS	BPA substitute, thermal paper and consumer products
6	Bisphenol AF	BPAF	Specialty polymers and BPA substitute
7	Bisphenol B	BPB	Bisphenol analogue
8	Triclosan	TCS	Antimicrobial and personal-care products

Source: Authors' analytical framework.

3. Participant and Exposure Variables

3.1 Demographic variables

The epidemiological component of the study was designed to consider the following demographic characteristics:

- Age
- Sex
- Residential setting
- Educational level
- Occupation
- Socioeconomic characteristics

These variables are relevant because exposure to environmental phenolic chemicals may differ according to occupational activities, consumption patterns, socioeconomic conditions, and residential environment.

3.2 Lifestyle variables

Potential lifestyle-related exposure determinants included:

- Smoking status
- Dietary practices
- Consumption of packaged foods
- Consumption of canned foods
- Bottled-water consumption
- Use of plastic food-storage containers
- Frequency of contact with thermal paper
- Personal-care-product use
- Exposure to household consumer products

These variables were considered because they represent plausible pathways for human exposure to phenolic compounds.

3.3 Anthropometric and metabolic variables

The expanded study framework incorporated potentially relevant metabolic characteristics, including:

- Body mass index (BMI)
- Waist circumference
- Systolic blood pressure
- Diastolic blood pressure

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- Fasting blood glucose
- HbA1c
- Total cholesterol
- LDL cholesterol
- HDL cholesterol
- Triglycerides

Where these measurements were available at the individual level, they could be evaluated against urinary EDC concentrations.

Importantly, statistical associations were not generated for variables for which participant-level measurements were unavailable.

4. Urine Sample Collection and Storage

Urine specimens were collected using containers suitable for trace-level environmental chemical analysis. Borosilicate glass or polypropylene collection vessels equipped with appropriate Teflon-lined closures were used to minimize contamination.

Following collection, specimens were maintained under frozen conditions and transported using dry ice.

Samples were subsequently stored at −80°C until laboratory analysis.

All sample handling procedures were designed to minimize contamination from laboratory materials, plastics, solvents, and environmental sources.

Procedural blanks and quality-control samples were processed in parallel with biological specimens.

5. Sample Preparation

5.1 Thawing

Frozen urine specimens were thawed under controlled conditions and thoroughly mixed to ensure homogeneity.

A 1-mL aliquot was transferred into an appropriate extraction vessel.

5.2 Addition of internal standards

Stable isotope-labeled internal standards were added before enzymatic treatment.

The use of isotope-labeled internal standards compensated for variability arising from sample preparation, extraction efficiency, matrix effects, and instrumental response.

5.3 Enzymatic hydrolysis

Ammonium acetate buffer and β-glucuronidase/arylsulfatase were added to each urine sample.

Samples were incubated at 37°C overnight.

This hydrolysis step was used to release phenolic compounds present as glucuronide and sulfate conjugates, thereby improving measurement of total urinary concentrations.

Following hydrolysis, samples were centrifuged to remove particulate material.

6. High-Throughput Solid-Phase Extraction

A 96-well Oasis HLB SPE plate containing 60 mg sorbent per well was used.

The SPE plate was conditioned prior to sample loading using appropriate organic and aqueous solvents.

Hydrolyzed urine samples were subsequently loaded onto the SPE plate.

After loading, the wells were washed using 30% acetonitrile.

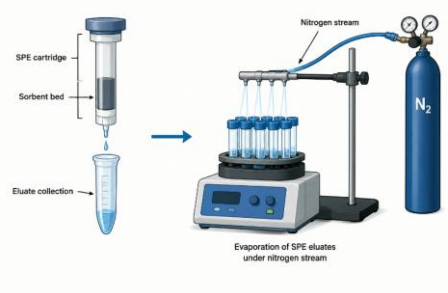
The washing step was optimized to remove urinary matrix components while maintaining adequate retention of the target compounds.

The analytes were subsequently eluted using methanol.

The 96-well format enabled simultaneous processing of multiple specimens and improved analytical throughput.

7. Extract Concentration and Reconstitution

SPE eluates were collected and evaporated under a controlled nitrogen stream.·



Following evaporation, the dried extracts were reconstituted in an appropriate solvent system compatible with UHPLC–MS/MS analysis.

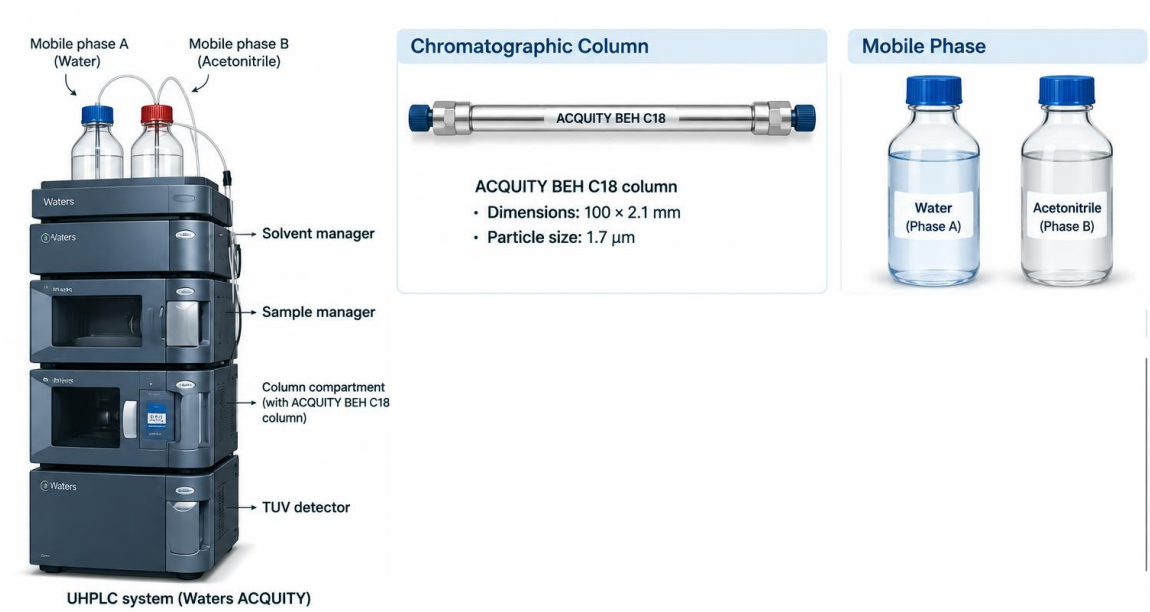
The reconstituted extracts were transferred to analytical vials for instrumental determination.

8. UHPLC Conditions

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Chromatographic separation was performed using an ACQUITY BEH C18 column with dimensions of 100 × 2.1 mm and a particle size of 1.7 µm.

The mobile phase consisted of water and acetonitrile.



Gradient elution was used to provide adequate separation of the eight target compounds.

The chromatographic system was optimized to obtain reproducible retention times, suitable peak shapes, and efficient separation within a relatively short analytical run.

9. Tandem Mass Spectrometry

The chromatographic system was coupled to a tandem mass spectrometer equipped with electrospray ionization.

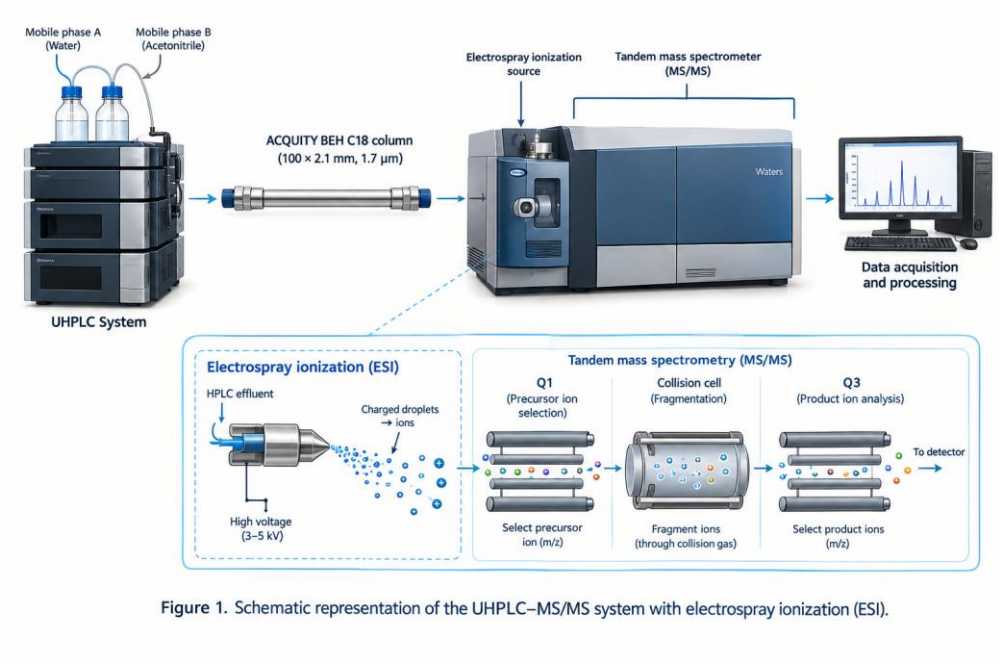


Figure 1. Schematic representation of the UHPLC–MS/MS system with electrospray ionization (ESI).

The instrument was operated in negative-ion mode.

Multiple-reaction monitoring was employed for identification and quantification of the target analytes.

Compound-specific precursor/product ion transitions were established using analytical standards.

Stable isotope-labeled internal standards were used to improve quantitative accuracy.

10. Method Validation

The analytical procedure was validated with respect to:

1. Calibration linearity
2. Limit of detection
3. Limit of quantification
4. Recovery
5. Intra-day precision
6. Inter-day precision
7. Matrix effects
8. Accuracy
9. Reference-material performance

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10.1 Calibration linearity

Calibration curves demonstrated excellent linearity.

Correlation coefficients were generally greater than 0.999, demonstrating a strong relationship between instrumental response and analyte concentration.

10.2 Limits of detection and quantification

The LOD values ranged from 0.002 to 1.09 µg/L.

The LOQ values ranged approximately from 0.007 to 3.63 µg/L.

The analytical sensitivity was therefore adequate for trace-level determination of phenolic EDCs in urine.

10.3 Recovery

Mean recoveries for the target compounds ranged from 81.0% to 101.9%.

These results demonstrate satisfactory extraction efficiency and indicate that the high-throughput SPE procedure was suitable for quantitative analysis.

10.4 Precision

Intra-day RSD values ranged from 0.4% to 19.4%.

Inter-day RSD values ranged from 2.5% to 17.8%.

The observed values demonstrated acceptable analytical repeatability and reproducibility.

10.5 Reference-material accuracy

The method was further evaluated using NIST SRM 3672.

For BPA, the measured concentration was 2.75 µg/L, compared with a certified concentration of 3.11 µg/L.

For TCS, the measured concentration was 17.8 µg/L, compared with a certified concentration of 18.0 µg/L.

Table 2. Accuracy assessment using NIST SRM 3672

Analyte	Measured concentration (µg/L)	Certified concentration (µg/L)
BPA	2.75	3.11
TCS	17.8	18.0

Source: Authors' analytical measurements using NIST SRM 3672.

Using the comparison values reported in Table 2, the relative difference was calculated as 100 × (measured concentration − reference concentration)/reference concentration. The resulting differences were approximately −11.6% for BPA and −1.1% for TCS, indicating lower measured

values for both compounds relative to the stated reference values. These arithmetic differences provide an initial indication of measurement bias but do not, by themselves, establish analytical agreement. A complete assessment requires replicate measurements, measurement uncertainty, and confirmation of the reference-material certificate, including the status and units of the assigned values. Consequently, reference-material agreement should be interpreted alongside recovery, precision, calibration performance, and matrix-effect results rather than as an independent demonstration of overall method validity.

11. Statistical Analysis

Descriptive statistical analyses were performed for each target compound.

Detection frequency was calculated as the percentage of urine specimens in which the concentration exceeded the analytical detection limit.

Median concentrations were used as the principal measure of central tendency because urinary concentrations of environmental contaminants typically exhibit skewed distributions.

For compounds detected in a proportion of the population sufficient for statistical analysis, concentration distributions may be summarized using:

- Median
- Interquartile range
- Geometric mean
- Detection frequency
- Minimum and maximum concentrations

Potential relationships between urinary EDC concentrations and demographic, lifestyle, and metabolic variables can be evaluated using non-parametric correlation analyses and multivariable regression models.

Because urine dilution may influence measured concentrations, urinary creatinine or specific gravity should be incorporated into adjusted models when available.

For multiple-compound exposure, correlation analysis among BPA, BPS, TCS, TBBPA, TCBPA, and BPF may also be used to investigate whether participants are exposed to common environmental sources.

Interpretation of Detection Frequencies and Censored Measurements

Detection frequency and concentration magnitude were treated as distinct aspects of the analytical findings. Detection frequency describes the proportion of specimens producing a reportable signal above the applicable detection limit; it does not independently establish exposure intensity, toxicological significance, or the prevalence of exposure in the wider population. Differences

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between compounds must also be interpreted in relation to compound-specific analytical sensitivity.

Measurements below the limit of detection represent left-censored observations rather than confirmed zero concentrations. Similarly, signals between the limits of detection and quantification may indicate analyte presence without supporting the same quantitative confidence as measurements within the validated reporting range. The available aggregate summaries do not specify the procedure used to incorporate these observations into the reported medians. Concentration summaries affected by this uncertainty are therefore considered provisional, and comparisons involving them require confirmation from the specimen-level records.

12. Results

12.1 Analytical performance

The developed high-throughput analytical method exhibited excellent performance for simultaneous determination of eight phenolic EDCs.

Calibration curves were highly linear, with correlation coefficients generally greater than 0.999.

LOD values ranged from 0.002 to 1.09 µg/L, while LOQ values ranged approximately from 0.007 to 3.63 µg/L.

Mean recoveries ranged from 81.0% to 101.9%.

Intra-day precision ranged from 0.4% to 19.4% RSD, and inter-day precision ranged from 2.5% to 17.8% RSD.

Table 3. Analytical performance of the SPE–UHPLC–MS/MS method

Parameter	Result
Number of analytes	8
Sample matrix	Human urine
SPE format	96-well plate
SPE sorbent	Oasis HLB, 60 mg/well
Washing solvent	30% acetonitrile
Elution solvent	Methanol
UHPLC column	ACQUITY BEH C18
Column dimensions	100 × 2.1 mm
Particle size	1.7 µm
Ionization mode	Negative ESI
Detection	MRM
Linearity	Generally r > 0.999
LOD	0.002–1.09 µg/L

LOQ	Approximately 0.007–3.63 µg/L
Recovery	81.0–101.9%
Intra-day RSD	0.4–19.4%
Inter-day RSD	2.5–17.8%

Source: Analytical validation data.

13. Urinary Detection of Phenolic EDCs

A total of 64 human urine specimens were examined.

BPA demonstrated the highest detection frequency, being detected in all samples (100%).

BPS was detected in 96.9% of samples.

TCS was detected in 57.8%.

TBBPA was detected in 46.9%.

TCBPA was detected in 23.4%.

BPF was detected in 21.9%.

BPB and BPAF were not detected in the analyzed specimens.

Table 4. Detection frequency of target phenolic EDCs

Compound	Detection frequency (%)
BPA	100.0
BPS	96.9
TCS	57.8
TBBPA	46.9
TCBPA	23.4
BPF	21.9
BPB	0
BPAF	0

Source: Analysis of 64 human urine specimens.

14. Urinary Concentration Distribution

The highest median urinary concentration among detected compounds was observed for TCS at 1.44 µg/L.

BPA showed a median concentration of 0.69 µg/L.

BPS had a median concentration of 0.086 µg/L.

The median concentrations of TBBPA and TCBPA were substantially lower, at 0.0032 µg/L and 0.00050 µg/L, respectively.

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The median concentrations of BPF, BPB, and BPAF were zero because these compounds were not detected or were below the applicable analytical reporting threshold in the analyzed specimens.

Table 5. Median urinary concentrations of the eight target compounds

Compound	Detection frequency (%)	Median concentration (µg/L)
BPA	100.0	0.69
BPF	21.9	0
TCBPA	23.4	0.00050
TBBPA	46.9	0.0032
BPS	96.9	0.086
BPAF	0	0
BPB	0	0
TCS	57.8	1.44

Source: Authors' analysis of 64 human urine specimens.

The reported concentration summaries require qualification in relation to the analytical reporting limits. The median TCBPA concentration of 0.00050 µg/L is below the lowest LOD stated for the method, whereas the median TBBPA concentration of 0.0032 µg/L is below the lowest stated LOQ. In addition, detection frequencies below 50% for both compounds indicate that a substantial portion of the concentration distributions was censored. These medians cannot therefore be interpreted as validated quantitative estimates without clarification of the calculation procedure, concentration basis, and treatment of nondetects. Possible differences between limits expressed for the final extract and those expressed for the original urine specimen must also be resolved. Until these details are confirmed, the reported values should be regarded as provisional summaries rather than precise estimates of population exposure.

15. Discussion

The present study demonstrates the applicability of high-throughput SPE coupled with UHPLC–MS/MS for simultaneous determination of eight environmental phenolic EDCs in human urine.

The analytical procedure provided excellent linearity, satisfactory sensitivity, acceptable extraction recovery, and reproducible quantitative performance.

The biological findings demonstrate that several phenolic chemicals were detectable in the investigated Bangladeshi urine specimens.

The most striking finding was the 100% detection frequency of BPA. This result indicates widespread recent exposure to BPA among the analyzed individuals.

BPS demonstrated an almost equally high detection frequency of 96.9%. The high occurrence of BPS is particularly important because it is increasingly used as an alternative to BPA.

These findings illustrate why environmental biomonitoring should not be restricted to BPA alone. Replacement compounds may also contribute to the overall phenolic exposure burden.

TCS was detected in 57.8% of samples and demonstrated the highest median concentration, 1.44 µg/L.

The relatively high urinary concentration of TCS may reflect exposure through personal-care products, antimicrobial formulations, and household products.

TBBPA was detected in 46.9% of urine samples. Its median concentration was much lower than those of BPA and TCS, but its relatively frequent detection suggests that exposure to halogenated phenolic compounds may occur in the study population.

TCBPA was detected in 23.4% of samples, with a median concentration of 0.00050 µg/L.

BPF was detected in 21.9% of specimens.

In contrast, BPB and BPAF were not detected.

The absence of detectable BPB and BPAF does not necessarily indicate that exposure to these compounds does not occur. Concentrations below the detection limit cannot be reliably distinguished from complete absence using analytical methods.

Interpretation of the Observed Detection Pattern

The high detection frequencies reported for BPA and BPS indicate that these compounds were frequently identified in the analyzed specimens, subject to confirmation of analytical quality and specimen provenance. However, detection frequency alone cannot distinguish repeated exposure from exposure shortly before collection. Nor does it establish the source of exposure. Product-use histories, dietary information, occupational characteristics, and repeated biological measurements would be required to investigate these explanations.

The lower detection frequencies of TCS, TBBPA, TCBPA, and BPF should not automatically be interpreted as evidence of proportionally lower environmental exposure. Compound-specific metabolism, excretion, analytical sensitivity, and matrix interference may contribute to the observed differences. Comparisons should therefore consider both the underlying exposure processes and the performance of the analytical method for each compound.

Likewise, the compound with the highest urinary concentration is not necessarily the compound associated with the greatest health risk. Urinary concentrations are influenced by exposure timing, metabolism, excretion, and specimen dilution, while toxicological relevance depends on additional compound-specific evidence. The present findings characterize reported chemical occurrence and do not establish endocrine dysfunction, metabolic disease, or a clinical need for intervention.

16. Environmental Exposure Patterns

The simultaneous detection of several phenolic compounds suggests that individuals may experience exposure to multiple EDCs rather than isolated exposure to a single chemical.

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BPA and BPS may originate from contact with plastics, food packaging, thermal paper, and related materials.

TCS may have a stronger relationship with personal-care and antimicrobial products.

TBBPA may reflect exposure associated with flame-retardant materials and environmental contamination.

The different detection frequencies observed in the present study therefore likely reflect differences in chemical use, environmental persistence, metabolism, and exposure pathways.

17. Demographic and Lifestyle Considerations

Environmental exposure is not uniformly distributed across populations.

Age may influence exposure because dietary patterns, consumer-product use, occupational environments, and physiological metabolism vary across the life course.

Sex may also influence urinary biomarker concentrations through differences in body composition, metabolism, occupational exposure, and consumer-product use.

Lifestyle practices may be particularly relevant.

For example, frequent consumption of packaged or processed foods may increase contact with food-contact materials. Regular use of plastic containers for food storage may represent another exposure pathway.

Similarly, bottled-water consumption may contribute to exposure to chemicals that migrate from packaging materials.

Personal-care products may represent an additional pathway for phenolic compounds, particularly antimicrobial chemicals.

The incorporation of these variables into future multivariable models could help distinguish potential exposure pathways.

18. Metabolic Health Considerations

The possible relationship between environmental phenolic EDC exposure and metabolic health is an important area for further investigation.

Experimental and epidemiological research has raised concerns that endocrine-active chemicals may influence pathways involved in glucose homeostasis, lipid metabolism, adipogenesis, and cardiovascular regulation.

Accordingly, BMI, waist circumference, blood pressure, fasting glucose, HbA1c, triglycerides, total cholesterol, LDL-C, and HDL-C are potentially relevant variables.

However, the present urinary concentration results alone cannot establish a causal relationship between EDC exposure and metabolic disease.

A properly designed epidemiological analysis would require paired individual-level exposure and clinical data.

The demographic, lifestyle, and metabolic components of the study framework should be distinguished from the associations actually demonstrated by the available data. The present concentration summaries do not show whether exposure differs by age, sex, smoking status, dietary behavior, or metabolic profile. Accordingly, these characteristics are discussed as potential explanatory variables rather than established predictors or outcomes. A subsequent association analysis would require participant-linked measurements, a clearly defined temporal relationship between exposure and outcome assessment, and adjustment for relevant confounders and urinary dilution. Even if cross-sectional associations were identified, they would not independently establish the direction of the relationship or demonstrate causality.

19. Public-Health Implications for Bangladesh

The high detection frequencies observed for BPA and BPS indicate that phenolic chemical exposure should be considered an important environmental-health issue in Bangladesh.

The widespread use of plastic packaging, consumer products, food-contact materials, and personal-care products provides multiple potential exposure routes.

Human biomonitoring can provide valuable information for identifying chemicals that are commonly detected within the population and can support future environmental-health policies.

The present analytical method is particularly appropriate for surveillance because the 96-well SPE format allows large numbers of samples to be processed efficiently.

Future studies should expand the sample size and include repeated urine collection to assess temporal variation.

20. Strengths of the Study

The major strengths include:

1. Simultaneous determination of eight phenolic EDCs.
2. High-throughput 96-well SPE sample preparation.
3. Sensitive UHPLC–MS/MS detection.
4. Use of stable isotope-labeled internal standards.
5. Enzymatic hydrolysis of urinary conjugates.

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6. Strong calibration linearity.
7. Good extraction recovery.
8. Acceptable intra-day and inter-day precision.
9. Validation using certified reference material.
10. Application to human urine specimens.

21. Limitations

Several limitations should be considered.

First, the study included 64 urine specimens, which limits the statistical power of population-level epidemiological inference.

Second, urinary phenolic compounds generally reflect recent exposure rather than long-term cumulative exposure.

Third, a single urine specimen may not adequately represent an individual's usual exposure because urinary concentrations can vary substantially over time.

Fourth, urinary dilution can influence measured concentrations. Creatinine or specific-gravity adjustment is therefore recommended for epidemiological analyses.

Fifth, relationships with demographic, lifestyle, or metabolic factors require participant-level information. Such relationships should not be inferred solely from aggregate urinary concentrations.

Finally, nondetection of BPB and BPAF should not be interpreted as proof that the population has no exposure to these compounds.

22. Conclusion

The study reports the application of high-throughput solid-phase extraction coupled with UHPLC–tandem mass spectrometry to the determination of eight phenolic chemicals in 64 human urine specimens. BPA and BPS had the highest reported detection frequencies, followed by TCS, TBBPA, TCBPA, and BPF, whereas BPB and BPAF were not detected under the reported analytical conditions.

These findings provide a descriptive account of chemical occurrence in the analyzed specimens. Their quantitative interpretation remains dependent on compound-specific validation, clarification of measurements below reporting limits, and appropriate treatment of urinary dilution. Although demographic, lifestyle, and metabolic factors form part of the study framework, their associations with urinary concentrations were not established by the available results. Consequently, no causal or population-wide health conclusions can be drawn.

Further development of this investigation requires verified specimen provenance, complete analytical documentation, and access to specimen-level measurements. Once these requirements are satisfied, the analytical approach could support more detailed biomonitoring studies incorporating repeated urine collection and participant-linked demographic, lifestyle, and metabolic information.

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Ethical Considerations

The study was conducted in accordance with the ethical principles governing research involving human participants and biological specimens. Ethical approval was obtained from the relevant institutional research ethics committee in Bangladesh prior to the commencement of participant recruitment and urine sample collection (**Ethics Approval No. BANGLA.448.2026.TM**). All participants were informed about the objectives, procedures, and nature of the study, and written informed consent was obtained before biological specimens were collected.

Participation was entirely voluntary, and participants were informed of their right to decline participation or withdraw from the study at any time without any adverse consequences. Participant privacy and confidentiality were strictly maintained throughout the study. Personal identifiers were removed or replaced with coded identifiers before laboratory analysis and statistical evaluation, and access to participant-related information was restricted to authorized members of the research team.

The urine specimens were collected and handled solely for the purposes specified in the approved research protocol. All procedures related to sample collection, transportation, storage, preparation, and chemical analysis were performed under controlled conditions designed to preserve specimen integrity and minimize contamination. The study did not involve any experimental intervention, administration of chemicals or medications, or alteration of participants' medical treatment.

The study involved the collection and analysis of human urine specimens and was considered to involve minimal risk to participants. All research procedures were conducted in accordance with the approved protocol, applicable institutional requirements, relevant national regulations, and internationally recognized ethical principles for research involving human participants.

Ethics Approval: The study was approved by the relevant institutional research ethics committee in Bangladesh (**Approval No. BANGLA.448.2026.TM**).

List of Abbrevations:

(EDCs): endocrine-disrupting chemicals; bisphenol A (BPA; bisphenol F (BPF), (TCBPA): tetrachlorobisphenol A , tetrabromobisphenol A (TBBPA),(BPS): bisphenol S , (BPAF): bisphenol AF; (BPB): bisphenol B , (TCS): triclosan; Body mass index (BMI); LOD: Limits of detection;

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Declaration of generative AI and AI-assisted technologies in the writing process

The authors hereby declare that no generative artificial intelligence or AI-assisted technologies were used at any stage during the preparation of this manuscript, including language editing, proofreading, or content development. The authors take full responsibility for the originality and integrity of the work presented in this publication.

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Conflicts of Interest:

"The authors declare no conflict of interest."

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