APPLIED SCIENCES

The International Journal of Applied Sciences.
Vol. 2, No.2, 2026, pp. 184-206
ISSN 3104-8935

https://international-journal-of-applied-sciences.jo/#

# 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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DOI: https://doi.org/10.64440/IJAS/IJAS0022

## ARTICLE INFO

**Article history**
Received May 11, 2026
Revised May 17, 2026
Accepted Aug 25, 2026

**Keywords**
phenolic endocrine-disrupting chemicals; ;
bisphenol A;
bisphenol S;
triclosan;
human urine;
Bangladesh;
Biomonitoring;
solid-phase extraction;
UHPLC–MS/MS.

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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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ISSN 3104-8935

## 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.

In addition to analytical characterization, the study framework considers demographic, lifestyle, and metabolic characteristics as potential determinants of urinary exposure. These include age, sex, body mass index, dietary practices, smoking behavior, use of plastic containers, consumption of packaged foods and beverages, personal-care-product use, and metabolic indicators where available.

The principal objective was therefore to characterize the occurrence of multiple environmental phenolic EDCs in human urine and establish a comprehensive biomonitoring approach suitable for evaluating exposure patterns in the Bangladeshi population.

## 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.

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

### 2.2 Target compounds

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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
- Fasting blood glucose
- HbA1c
- Total cholesterol

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- 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.

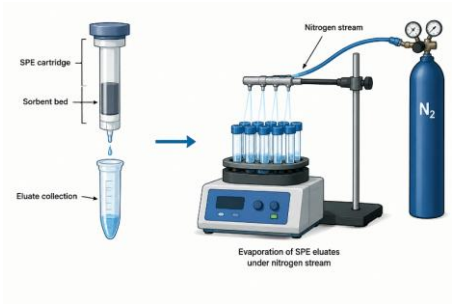
The washing step was optimized to remove urinary matrix components while maintaining adequate retention of the target compounds.

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

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.

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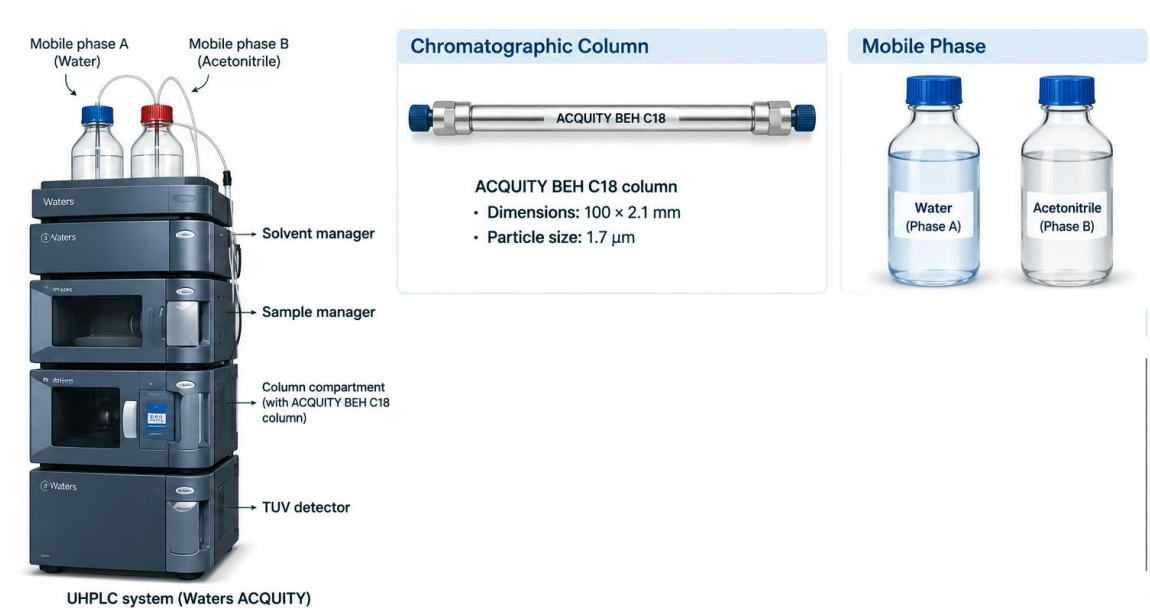


Gradient elution was used to provide adequate separation of the eight target compounds.

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.

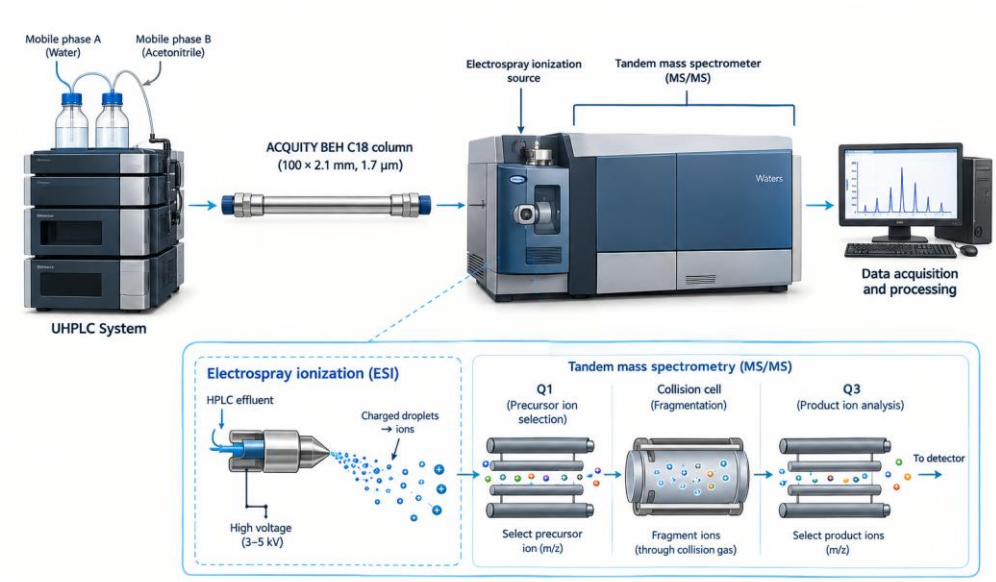


Figure 1. Schematic representation of the UHPLC–MS/MS system with electrospray ionization (ESI).

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

### 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.

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### 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.

## 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.

## 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.

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## 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.

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.

## 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 targeted analytical methods.

## 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.

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.

## 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.
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 present study developed and applied a high-throughput 96-well SPE–UHPLC–MS/MS method for simultaneous determination of eight environmental phenolic endocrine-disrupting chemicals in human urine.

The analytical procedure demonstrated excellent linearity, with correlation coefficients generally exceeding 0.999. The method showed LODs of 0.002–1.09 µg/L, LOQs of approximately 0.007–3.63 µg/L, recoveries of 81.0–101.9%, intra-day RSDs of 0.4–19.4%, and inter-day RSDs of 2.5–17.8%.

Among the 64 analyzed urine specimens, BPA was detected in 100% of samples and BPS in 96.9%. TCS was detected 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.

The findings demonstrate widespread exposure to several phenolic environmental chemicals within the investigated Bangladeshi population. In particular, the very high detection frequencies of BPA and BPS highlight the importance of monitoring both established EDCs and their replacement compounds.

The analytical strategy provides a suitable foundation for future population-based biomonitoring studies in Bangladesh. Expanded investigations incorporating demographic, dietary, lifestyle, occupational, anthropometric, and metabolic information may provide further insight into the sources of exposure and the potential health significance of phenolic EDC mixtures.

## 23. Declarations

**Ethics Approval and Consent to Participate**

The study involving human urine specimens should be conducted in accordance with the ethical principles governing research involving human biological materials and should receive approval from the relevant institutional ethics committee. Written informed consent should be obtained from participants where required.

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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;

## Acknowledgment:

The authors sincerely acknowledge the participants who provided biological specimens for this investigation. The authors also thank the laboratory personnel who contributed to sample preparation, instrumental analysis, quality assurance, and analytical validation.

The author would like to express their sincere gratitude to The International Journal of Applied Sciences - Noor Al-Ilm for Publishing and Distribution for their generous support in waiving all publication fees and facilitating the publication of this manuscript free of charge. Their commitment to promoting scientific research and supporting researchers is highly appreciated.

## Author Contribution:

All authors contributed equally to the main contributor to this paper. All authors read and approved the final paper.

## 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.

## Funding:

This research received no external financial funding. The authors also acknowledge The International Journal of Applied Sciences, Noor Al-Ilm for Publishing and Distribution, for providing a full waiver of the publication fees. The publication fee waiver was provided as editorial support and did not involve any financial contribution to the conduct, design, analysis, or reporting of the research.

**Conflicts of Interest:**

"The authors declare no conflict of interest."

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APPLIED SCIENCES

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Vol. 2, No. 2, 2026, pp. 184-206

ISSN 3104-8935

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