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Research Article

Development and Validation of an RP-HPLC Method for the Quantitative Analysis of Triclosan in Human Urine

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Triclosan (TCS), a synthesized chlorinated phenolic compound, is commonly utilized in consumable products as an antimicrobial agent. TCS has sparked widespread awareness because of its toxicity and possible negative effect on public health in recent years. In this study, a highly sensitive, fast, and cost-effective isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method coupled with solid-phase extraction for analysis of triclosan in human urine samples was developed. The method utilized methanol and water in a ratio of 90 : 10 as the mobile phase on a Phenomenex Luna 3 μm C18(2) 100 Å, 150 × 4.60 mm stationary phase, with a runtime of 5 minutes. The method showed good resolution of triclosan in the presence of the sample matrix. Validation of the method was performed according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). Linearity was tested over a range of 0.00625 μg/mL to 6.4 μg/mL, as accuracy recorded a recovery of 89.25%, 91.0%, and 92.75%. Limits of detection (LOD) and quantification (LOQ) were obtained to be 0.0173 μg/mL and 0.0525 μg/mL, respectively. The method proved to be robust over a temperature range of 26°C, 30°C, and 35°C and a flow rate of 0.5 ml, 1.0 ml, and 1.5 ml. The developed method was employed to detect and quantify triclosan in 153 urine samples, comprising 60 samples from Ibadan, Nigeria, and 93 samples from Kumasi, Ghana. Triclosan was detected in a total of 52 samples with an average content of 0.054588 μg/ml. This method can therefore be used for the routine analysis of triclosan in urine samples.

1. Introduction

Triclosan (TCS), 5-chloro-2-(2,4-dichlorophenoxy) phenol (Figure 1), is a synthesized chlorinated phenolic substance discovered in the 1960s. It is an aromatic ether which possesses two functional groups, that is, the phenol and ether functional group. It has served as a preservative and antimicrobial agent over the years. TCS is found in most consumable products such as hand sanitizers, soaps, toothpaste, and mouth rinses, due to its broad antimicrobial

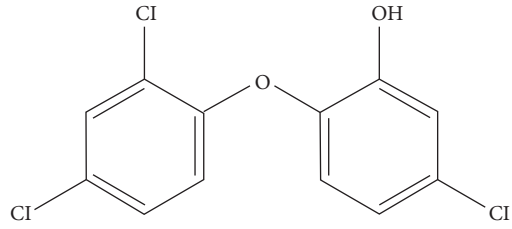

Figure 1: Chemical structure of 5-chloro-2-(2, 4-dichlorophenoxy) phenol (triclosan).

spectrum [1]. Triclosan has a molecular formula of $C_{12}H_7Cl_3O_2$, the molar mass of 289.54 g/mol, melting and boiling point of 55–57°C and 120°C, respectively, and a density of 1.45 g/ml. The standard permissible limit of triclosan in consumable products is 0.01 mg/L to 0.1 mg/L [2].

Human exposure to TCS may be through the mouth or through the skin. TCS is readily absorbed through the skin, mucous membrane of the mouth and gastrointestinal lining due to its lipophilic properties [3]. Mammals as the subject of concern metabolize TCS predominantly through conjugation processes prior to hydroxylation, which result in glucuronide and sulphate conjugates that are eliminated in the faeces, urine, and breast milk [4–6]. At the ecologically relevant doses of 1 to 5 micromolar, triclosan sulphate, and glucuronide are produced in the liver at almost comparable rates, according to pharmacokinetic studies. Sulfonation is likely to be the main metabolic pathway for triclosan removal when concentrations are less than 1 micromolar [2].

An average of 50% of free TCS is excreted in 24 hours with urine being the principal route of excretion [5]. In 2003 and 2004, a detection of 75% of TCS in urine sample was recorded according to Weatherly, among the United States of America's population with a concentration range of 7.9 nM to 13.1 μM [6].

Triclosan, in spite of its broad-spectrum antimicrobial property may impose an adverse effect on human health. Although triclosan is for external use, it still finds its way into human body through the skin. This makes triclosan a public health concern as there could be a potential bioaccumulation of this life-threatening compound. Triclosan can alter hormone regulation, serving as an endocrine disruptor, and contributing to antibiotic resistance [7, 8]. Research by Savage et al. [9] also shows that triclosan is capable of precipitating asthmatic attack in kids. Studies have shown triclosan worsened fatty liver disease in mice. Elevated levels of triclosan in urine have been linked to immunological malfunction, allergic responses, and the development of asthma [10]. TCS has been demonstrated to bind to human serum albumin as well, causing the protein structure to be altered [11]. Toxins bonding to serum albumin can obstruct endogenous chemical transport and create structural changes in the protein complex, which can impair activity or modify its physiological function [12, 13].

In view of the permissible limit of (0.01 mg/L to 0.1 mg/L) of triclosan [2] and its negative effects on humans, many governmental agencies and organizations have raised concerns on the role of various foods and drugs regulatory authorities in their country. Various analytical methods for the analysis of triclosan in environmental, consumable products, and human samples have been developed over the years including enzyme-linked immunosorbent assay (ELISA) [14, 15], cuvette-less microvolume Ultraviolet/visible spectroscopy (UV/Vis) [16], gas chromatography coupled with mass spectrometry (GC-MS) [17, 18], and high-performance liquid chromatography (HPLC) [19–21]. However, most of these methods target TCS in environmental samples or consumable products and not human urine samples.

The current study, therefore, seeks to develop and validate a reliable, cost-effective reversed-phase high-performance liquid chromatographic method coupled with solid-phase extraction (SPE) to analyze triclosan in urine samples. The urine samples were obtained from children aged between 6 and 14 years attending Asthma Clinics at University College Hospital, Ibadan, Nigeria, and Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana.

2. Materials and Methods

2.1. Chemicals and Reagents. Triclosan reference standard was purchased from Sigma-Aldrich with lot number of BCBW3642. HPLC grade methanol was obtained from VWR International (Chromanorm), and trifluoroacetic acid was obtained from Wagtech Projects Ltd. (Table 1). Distilled water was obtained from KNUST Central Laboratory.

2.2. Equipment. Equipment used in this study included the Stuart SMP10 Melting Point Apparatus for the determination of melting point. Analytical balance (Sartorius TE214S) for accurate weighing of chemicals. UV/Vis Spectrometer and Infrared Spectrometer (Bruker) were used for the identification test of reference standard used. An HPLC Chromatographic instrument with the specification of (Perkin Elmer 785A Flexar PDA detector) having a Phenomenex Luna 3 μm C18(2) 100 Å, 150 × 4.60 mm column was used. The HPLC possesses a binary mixing Flexar LC pump and Flexar LC Autosampler, making use of Chromera software and Flexar LC Solvent Manager.

Visiprep™ SPE vacuum manifold 12–port model with Strata™—X 33 μm polymeric reversed phase SPE cartridges (200 mg/6 ml) from Phenomenex was used for sample clean up.

2.3. Methods

2.3.1. Identification Test for Triclosan Standard. The following identification tests were performed to ascertain the integrity of the reference standard obtained (triclosan): infrared spectroscopy, UV-Vis spectrophotometry, and melting point determination.

2.3.2. RP-HPLC Method Development. Based on the preliminary studies conducted, the isocratic RP-HPLC chromatographic mode was used for eluting triclosan. The mobile phase selected (90 : 10, methanol: water) was pumped through the Phenomenex Luna 3 μm C18(2) 100 Å, 150 × 4.60 mm column at a flow rate of 1.0 ml/min with the

Table 1: Profile of triclosan reference standard.

Name	Company	Lot number	Manufacturing date	Expiration date	Assay
Triclosan	Sigma-Aldrich	BCBW3642	February 2018	February, 2022	99.8%
Methanol	VWR International	20G214004		July, 2025	99.8%
Trifluoroacetic acid	Wagtech Project Ltd.	UN 2699		April, 2023	99+%

column kept at an ambient temperature. Equilibration of the column was performed by running the mobile phase through it for 30 minutes preceding injection of triclosan. The detection of triclosan by photodiode array (PDA) was monitored at 280 nm. The working solution of 50 μg/ml was injected at a flow rate of 1 ml/min, and triclosan was eluted at a mean retention time of 2.863 minutes. The chromatogram recorded showed good resolution and a sharp peak. The procedure was repeated three consecutive times and the results were found to be reproducible.

2.3.3. ICH Guided Method Validation

(1) Linearity. To ascertain the relative relationship of the obtained response against analyte concentration, the linearity of the method was tested over a range of calibration points using the calibration point solution prepared. The solutions, whose concentration ranged from (0.00625–6.4) μg/ml, were analyzed in triplicate per concentration with blank analysis between them using the developed method. The results from the analysis were recorded, and a calibration curve of response versus concentration was plotted using a GraphPad prism.

(2) Limit of Detection and Limit of Quantification. Using the statistically approved formulae, LOD and LOQ values were calculated using the following equations:

$$\text{LOD} = \frac{3.3\sigma}{S}, \qquad \text{LOQ} = \frac{10\sigma}{S}, \tag{1}$$

where σ = the standard deviation of the y-intercept of the calibration curve and S means slope of the calibration line [22, 23].

(3) Intraday Precision (Repeatability). A nominal concentration of 1.6 μg/ml of triclosan was injected 9 times at a 15-minute interval. The peak area and relative standard deviation were recorded to ascertain intraday precision.

(4) Interday Precision (Intermediate Precision). In four consecutive days, using a nominal concentration of 1.6 μg/ml of triclosan, the interday precision was evaluated by analyzing the solution in triplicate. The peak areas were recorded, and relative standard deviation (RSD) values were calculated.

(5) Accuracy. The accuracy of the developed method was evaluated by a spiking blank urine sample with three different concentration triclosan reference standards corresponding to 80%, 100%, and 120% of the target concentration of 0.32 μg/mL, 0.40 μg/mL, and 0.48 μg/mL. The obtained solutions were analyzed in triplicate, and the percentage recoveries and relative standard deviation of their responses were calculated according to ICH guidelines.

(6) Suitability. The parameters such as the limit of detection, the limit of quantification, correlation coefficient, and the concentration range were all determined to ascertain that the chosen method is specific and selective.

(7) Selectivity/Specificity. The specificity of the developed method was evaluated by comparison of chromatograms of triclosan standard and triclosan in the urine sample. It was noticed that there was no interference and that there was a good correlation existing between the retention times of the standard and those of the sample.

(8) Robustness

(a) Temperature variation

To validate robustness, the temperature of the column was varied between temperatures of 26, 30, and 35°C for the assay of triclosan in triplicate per sample. The areas of the peaks were recorded, and relative standard deviations were calculated.

(b) Flow rate variation

Using a concentration 0.8 μg/mL, the flow rate of the mobile phase solution was varied between 0.5 min, 1.0 min, and 1.5 min. At each of the stated flow rates, the sample was analyzed in triplicate to assess its robustness. The area under the peaks was recorded and the relative standard deviation was calculated.

2.3.4. Solid Phase Extraction (SPE). To obtain better results after preliminary studies, modification of the SPE protocol on the SPE cartridge purchased (strata X 33 μm polymeric sorbent) was used. The SPE catridges were conditioned with 3 ml of HPLC grade methanol; the cartridge was then equilibrated with 3 ml of distilled water to wet the SPE surface. Two millilitres of the sample were then loaded onto the SPE cartridge, which was then washed with 6 ml of 50 : 50 distilled water and methanol in a series of 3 ml. The cartridges were then dried for 10 minutes following elution with 4 ml of HPLC grade methanol in a series of 2 ml. The eluate was collected and evaporated over nitrogen gas till the final volume reached 2 ml. The obtained eluate was then analyzed using the developed method.

(1) SPE Recovery. To ascertain the performance of the developed SPE method, SPE recovery was performed by first analyzing 0.2 μg/ml of standard TCS in triplicate using the developed RP-HPLC method. The area under the peaks was recorded. Two millilitres of three sets of the same urine

sample was prespiked with 0.2 μg/ml TCS each. The samples were then run following the SPE method developed. The eluates were then analyzed with the RP-HPLC method and the area under the peaks was recorded, the mean area was then computed. The percentage recovery was then calculated using the following formula:

$$\%\ \text{Recovery} = \frac{\text{Pre} - \text{Spike Response}}{\text{Post} - \text{Spike Response}} \text{x } 100\%. \quad (2)$$

2.3.5. Sample Collection. Urine samples were obtained following parental consent and subject assent from 153 children aged 6–14 years attending Asthma Clinics in Ghana (Komfo Anokye Teaching Hospital, Kumasi, $n = 93$) and Nigeria (University College Hospital, Ibadan, Nigeria, $n = 60$) as part of a study to investigate the possible effect of triclosan exposure on asthma symptoms. Ethical clearance was obtained from the Committee on Human Research, Publication, and Ethics, School of Medicine and Dentistry (KNUST, CHRPE/AP/070/20), and the University of Ibadan/University College Hospital, Ibadan Joint Ethics Committee, respectively. The children had no other comorbidities apart from asthma. Urine collection was done under supervision of hospital staff. Each sample was collected into a tight-sealing, amber-coloured glass container, labelled, and stored in a refrigerator at −20°C until they were used. Samples from Nigeria were shipped by air securely in a frozen state from Ibadan to Kumasi.

2.3.6. Sample Preparation and Analysis. The urine samples were placed in an ultrasonic bath and sonicated for 15 min at a frequency of 35 kHz with the temperature function turned off. The samples were filtered using a 0.45 μm nylon membrane filter into a 5 ml falcon tube. The filtered samples were then run through the developed solid-phase extraction method for purification and to concentrate the analyte of interest. The pH of the samples was adjusted to pH 4.00 using trifluoroacetic acid (TFA). The HPLC method developed was used to quantitatively determine amount of triclosan present in the human urine sample in triplicate. The response of each sample was recorded and GraphPad Prism, Excel, and Minitab, statistical software were used to analyze the data.

3. Results and Discussion

3.1. Identification Test for Triclosan Standard. To ascertain the authenticity and integrity of the obtained triclosan reference standard, infrared absorption spectroscopic and ultraviolet-visible spectrophotometric analyses of triclosan were performed according to United Stated Pharmacopoeia 2017 (197E and 197U, respectively) specification, as well as melting point determination. The wavelength of maximum absorbance was obtained from the UV spectrum to be $\lambda_{max} = 281$ nm in methanol, which falls within the USP-specified wavelength range of 280-281 nm [24]. Melting point was determined in the range of 55–57°C [25]. The results of the identification tests are as shown in Table 2, Figure 2, and S1-S2.

Table 2: Identification test for triclosan.

Test name	Experimental results	Literature value
Melting point	55°C-56°C	55°C–57°C
UV	281 nm	280 nm
IR	S1	S2

3.2. RP-HPLC Method Development. This study aimed at developing and validating the most reliable, cost-effective, adaptive, and fast, high-performance liquid chromatographic method coupled with solid-phase extraction (SPE) to analyze triclosan in urine samples with high sensitivity, selectivity, accuracy, and precision. A C18 octadecyl silane, Phenomenex Luna 3 μm C18(2) 100 Å, 150 × 4.60 mm stationary phase was used due to its suitability for the separation and resolution of intermediate polar-to-polar analytes, and also based on the outcomes from preliminary studies, and the nature of the sample matrix. The mobile phase solution of methanol to water in a ratio of (90 : 10) v/v, respectively, was used for effective separation in an isocratic elution following preliminary studies. The selection of mobile and stationary phases was based on the continuous experimental study, as it was able to give the best chromatographic response factor, and insignificant tailing effect at a run time of 5.0 min [20], made use of mobile phase composition of 35 : 35 : 30, v/v of acetonitrile: methanol: water, respectively [26], also made use of a mobile phase combination of (50 : 50) acetonitrile: methanol [27], on the other hand, used a mobile phase composition of 99 : 1, methanol: water, respectively, with 0.1% formic acid in the ultrapure water. The cited pieces of literature indicate a significant difference in the mobile phase used in this study. Concerning Yang et al. and Alshishani, et al. as they both employed Acetonitrile, which is relatively costly as compared to methanol and water. However, Luo's mobile phase composition is similar in terms of chemical composition to the mobile phase used in this study but differs in the proportion of methanol and water used, making Luo's mobile phase a little more costly than that of this study. An isocratic elution system where the mobile is kept constant was employed, with an instrument flow rate of 1.0 mL/min per 20 μl injection volume of sample. A run time of 5 minutes was set, where a mean retention time of 2.863 minutes was achieved (Figures 3 and 4). Comparing this chromatographic condition to [28], who recorded a retention time of 5.35 minutes for HPLC analysis of triclosan in cream and spray. Tohidi and Cai [17] had a retention time of 17.22 min for GC-MS analysis of triclosan. Aminu et al. [29] also obtained a retention time of 12.47 min for HPLC analysis of triclosan in stimulated saliva. The retention times, as well as the total run time for these studies, are relatively longer in comparison to this study, making this method relatively faster.

3.3. HPLC Method Validation. To evaluate the overall performance of a developed method, the ICH method validation protocol plays an important role. The developed method was validated to test its integrity and applicability (Table 3). The linearity of the method was tested within a calibration range of 0.00625 μg/mL (Figure S3) to 6.4 μg/mL for triclosan. An r^2 value of 0.9999 was obtained which indicated good linearity (Figure 5). Table S4 shows the

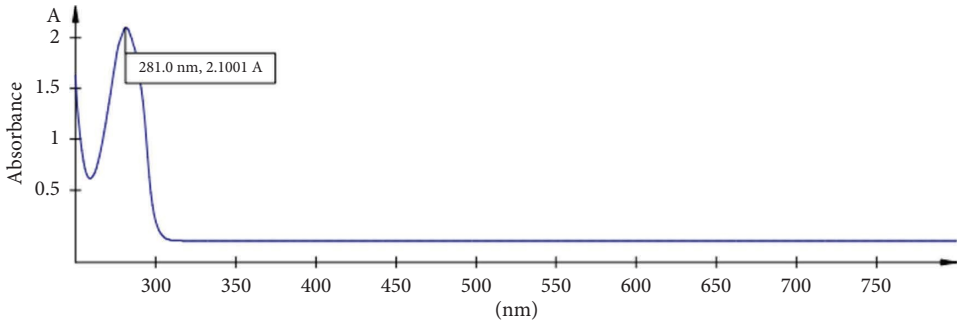

Figure 2: UV spectrum of triclosan.

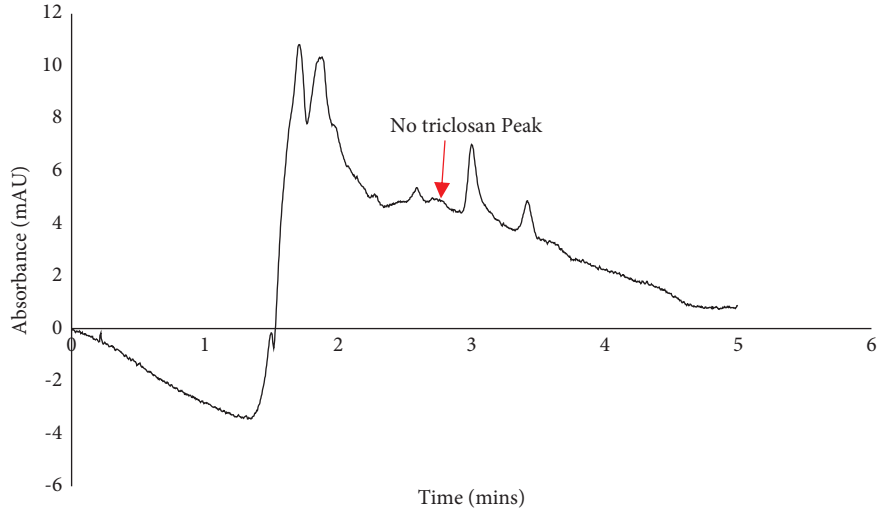

Figure 3: Chromatogram of blank urine sample, with no triclosan peak at time of 2.70–2.90 min.

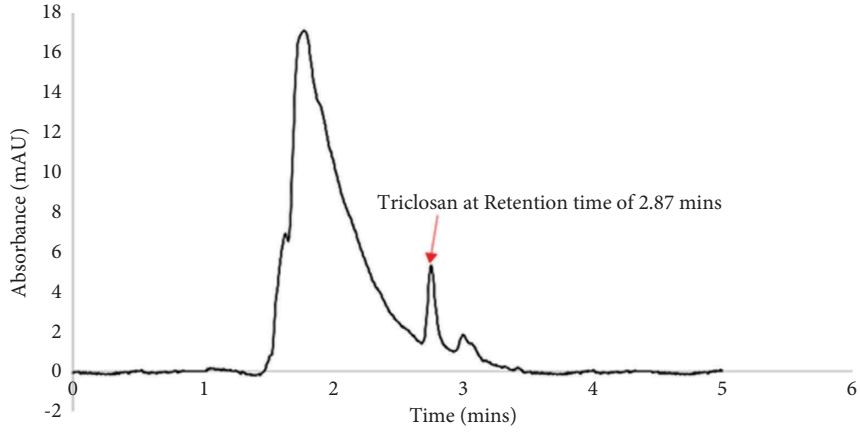

Figure 4: Chromatogram of blank urine spiked with triclosan (at a concentration of 0.100 μg/ml) eluting at a retention time of 2.87 min during method development.

concentrations and their corresponding peak areas used in the linearity test.

The precision of the developed method was evaluated by both intraday and interday precision. The intraday precision gave a percentage relative standard deviation (%RSD) of 1.723, while the interday precision was 0.106, 1.489, 0.071, and 1.326 for four different days. Based on the results obtained, it can be concluded that the proposed method is precise as all the results fall within the ICH acceptable limit of $\leq \pm 2.0\%$.

The accuracy of the developed method was evaluated by spiking a blank urine sample with three different concentrations of triclosan reference standard, corresponding to 80%, 100%, and 120% of the target concentration of

Table 3: HPLC method validation parameters.

Parameters	Results			
Mean retention time (minutes)	2.863			
	$Y = 14247x + 122.77$			
Range (μg/mL)	0.00625–6.400			
Coefficient of determination (r^2)	0.9999			
Limit of detection (μg/mL)	0.0173			
Limit of quantification (μg/mL)	0.0525			
	Concentration (μg/mL)	(Mean peak area ± SD)		RSD (%)
Intraday precision	1.6	26159.12 ± 450.78		1.723
Interday precision	Day			
	1	25962.063 ± 27.54		0.106
	2	25721.940 ± 382.96		1.489
	3	25379.707 ± 18.13		0.071
	4	26167.183 ± 346.91		1.326
Robustness (flow rate)	Flow rate (ml/min)	Mean peak area		
	0.5	11512.3		1.492
	1.0 (original condition)	11724.3		0.866
	1.5	9999.9		1.346
Robustness (temperature)	Temperature (°C)	Mean peak area		
	26	21236.8		1.447
	30	21652.3		0.223
	35	21603.7		0.828
Accuracy	Amount of triclosan added (μg)	Mean amount recovered (μg)	Mean % recovery	
	0.32	0.3011	91.00	0.874
	0.40	0.3670	92.75	1.255
	0.48	0.4284	89.25	0.725

Mean of 3 determinations; acceptance criteria for mean % recovery (accuracy): 80–120%; acceptance criteria for RSD: ≤2.0.

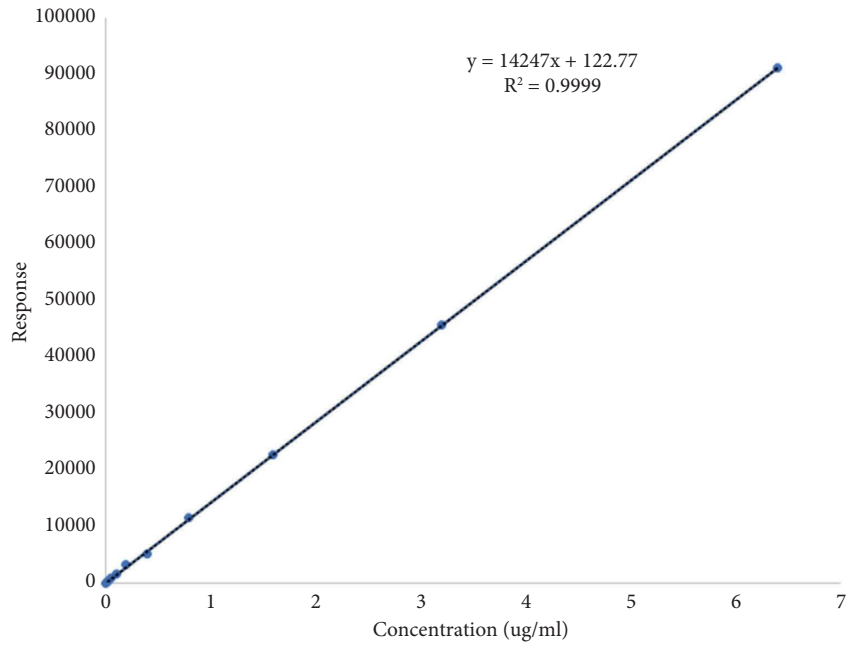

Figure 5: Calibration curve for triclosan reference standard.

0.32 μg/mL, 0.40 μg/mL, and 0.48 μg/mL. A recovery of 91.00% was obtained for 0.32 μg/mL, 92.75% was obtained for 0.40 μg/mL, and 89.25% was obtained for 0.48 μg/mL. They all fall within the ICH acceptable recovery values of 80–120%, indicating the method was accurate. Specificity or selectivity of the method was justified as there was no significant interference from the constituent of the urine sample in any peak region, demonstrating that the method is selective.

Table 4: SPE recovery for triclosan.

Samples	Mean response sample 1	Mean response sample 2	Mean response sample 3	Overall mean
Postspike blank urine	3736.15	3946.84	4010.86	3893.95
Prespike blank urine	3628.72	3424.59	3579.97	3544.43

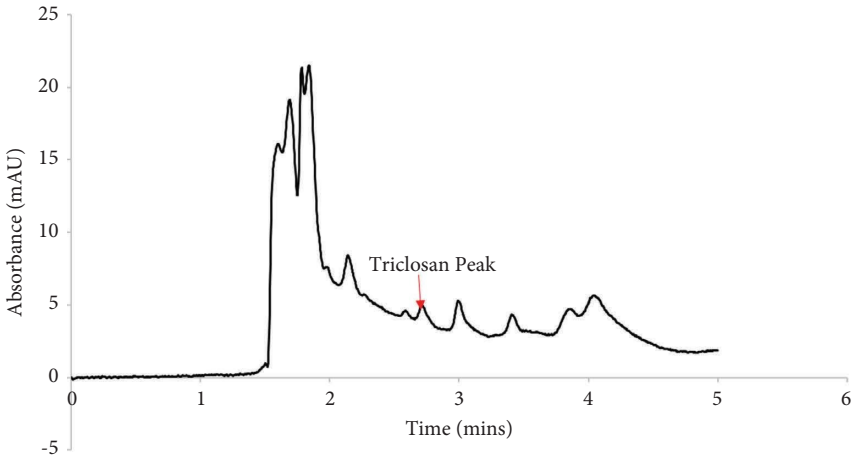

Figure 6: Chromatogram of one of the urine samples from the patient (with code 02GO25), showing triclosan at a concentration of 0.03 μg/mL eluting at a retention time of 2.81 min.

Table 5: RP-HPLC representative Results for TCS determination in urine samples.

Sample ID	Average peak area	Concentration (μg/ml)
02G001	2,002.19	0.04
02G002	3,836.47	0.07
02G003	BDL	BDL
02G004	4,800.43	0.09
02G005	3,550.20	0.07
02G006	2,026.83	0.04
02G007	BDL	BDL
02G009	2,150.03	0.04
02G010	BDL	BDL
02G011	BDL	BDL
02G013	3,711.30	0.07
02G015	BDL	BDL
02G016	1,934.41	0.04
02G017	BDL	BDL
02G018	BDL	BDL
02G019	3,814.45	0.07
02G020	BDL	BDL
02G021	2,966.07	0.06
02G022	3,532.09	0.07
02G023	BDL	BDL
02G024	BDL	BDL
02G025	1,878.09	0.03
02G026	BDL	BDL
02G027	BDL	BDL
02G028	2,381.03	0.04
02G029	BDL	BDL
02G030	1,824.20	0.03
02G031	BDL	BDL
02G032	2,790.83	0.05
02G033	BDL	BDL
02G034	2,119.07	0.04
02G036	BDL	BDL

Table 5: Continued.

Sample ID	Average peak area	Concentration (μg/ml)
02G037	1,846.07	0.03
02G038	BDL	BDL
02G039	BDL	BDL
02G040	2,880.50	0.05

BDL = below detection limit.

A purposeful change in temperature of the column at 26°C, 30°C, and 35°C, as well as flow rate, did not have any significant effect on the result. Giving an indication that the method is robust. For sensitivity evaluation of the analytical method, the limit of detection (LOD) and limit of quantification (LOQ) as validation parameters are key. The LOD and LOQ of this proposed method were calculated to be 0.0173 μg/mL and 0.0525 μg/mL, respectively.

3.4. SPE Method Development and Validation. A solid-phase extraction method was developed to concentrate the analyte of interest and for purification. The method made use of strata X 33 μm polymeric sorbent SPE cartridge which is a surface-modified styrene divinylbenzene. Due to its balance between lipophilic and hydrophilic retention characteristics, it enhances the retention of neutral, acidic, or basic aromatic compounds. Moreover, urine samples usually contain a lot of highly polar metabolites, and these can easily be washed off on such columns. Triclosan molecules, which pose lipophilic and hydrophilic moieties, and are therefore classified as moderately polar, can therefore be selectively retained whiles getting rid of the several highly polar metabolites in the urine matrix. A double elution with concentrated methanol as mobile phase was employed as it gave better recovery. Although acetonitrile, *n*-hexane, MeOH-DCM, or acetone could have been used, as used by [17, 30], keeping the method simple, less costly, and less time-consuming was what informed the choice. The SPE recovery process was undertaken and a recovery of 91.02% was obtained, which represents a good recovery (Table 4).

SPE Recovery of Triclosan

$$\begin{aligned} \%\ \text{Recovery} &= \frac{\text{Pre} - \text{Spike Response}}{\text{Post} - \text{Spike Response}} \text{x } 100\% \\ &= \frac{3544.43}{3893.95} \text{X } 100\% \\ &= \mathbf{91.02}\%. \end{aligned} \tag{3}$$

3.5. Determination of Triclosan in Urine Samples. The developed and validated method was used to assay 153 urine samples (Figure 6), among which triclosan was detected and quantified in 52 samples, whereas the remaining 101 were below the limit of detection. The least detected concentration of free triclosan was 0.0246 μg/mL, while the maximum was 0.1595 μg/mL, giving a range of (0.0246–0.1595) μg/ml. The mean concentration of TCS among the detected samples was obtained to be 0.054588 μg/ml (Table 5). This data suggests a significant number of people in the study area are exposed to triclosan.

4. Conclusion

A highly sensitive, fast, cost-effective, and accurate isocratic reversed phase high-performance liquid chromatography (RP-HPLC) and solid-phase extraction (SPE) methods were developed for the analysis of triclosan in human urine samples. The HPLC method was validated according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The RP-HPLC method was shown to be linear, precise, accurate, and robust. The developed method was used to assay 153 urine samples, among which triclosan was detected and quantified in 52 samples with 101 were below the limit of detection. The concentration of detected samples was within a range of 0.0246 μg/mL–0.1595 μg/mL with a mean concentration of 0.054588 μg/ml.

Data Availability

The data used to support the findings of this study are included within the article and additional information is available in the supplementary material of this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Supplementary Information. Figures S1 and S2 are infrared spectrum of triclosan reference standard and that reported in literature, respectively. Figure S3 depicts the HPLC chromatogram of the TCS at concentration of 0.00625 μg/mL. Table S4 shows the concentrations and their corresponding peak areas used in determining the linearity of the

method. Table S5 shows the quantitative determinations of triclosan in all urine samples explored in the study. (*Supplementary Materials*)

References

[1] C. F. Wang and Y. Tian, "Reproductive endocrine-disrupting effects of triclosan: population exposure, present evidence and potential mechanisms," *Environmental Pollution*, vol. 206, pp. 195–201, 2015.

[2] M. Yueh and R. H. Tukey, "Triclosan: a widespread environmental toxicant with many biological effects," *Annual Review of Pharmacology and Toxicology*, vol. 56, no. 1, pp. 251–272, 2016.

[3] Y. J. Lin, "Buccal absorption of triclosan following topical mouth rinse application," *American Journal of Dentistry*, vol. 13, no. 4, pp. 215–217, 2000.

[4] P. J. McNamara and S. B. Levy, "Triclosan: an instructive tale," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 12, pp. 7015-7016, 2016.

[5] G. Sandborgh-Englund, M. Adolfsson-Erici, G. Odham, and J. Ekstrand, "Pharmacokinetics of triclosan following oral ingestion in humans," *Journal of Toxicology and Environmental Health, Part A*, vol. 69, no. 20, pp. 1861–1873, 2006.

[6] L. M. Weatherly and J. A. Gosse, "Triclosan exposure, transformation, and human health effects," *Journal of Toxicology and Environmental Health, Part A B*, vol. 20, no. 8, pp. 447–469, 2017.

[7] Federal Drug Administration (Fda), "5 Things to Know about Triclosan," 2019, https://www.fda.gov/consumers/consumer-updates/5-things-know-about-triclosan.

[8] P. I. Johnson, E. Koustas, H. M. Vesterinen et al., "Application of the Navigation Guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan," *Environment International*, vol. 92-93, no. 93, pp. 716–728, 2016.

[9] J. H. Savage, C. B. Johns, R. Hauser, and A. A. Litonjua, "Urinary triclosan levels and recent asthma exacerbations," *Annals of Allergy, Asthma, & Immunology*, vol. 112, no. 2, pp. 179–181, 2014.

[10] E. M. Rees Clayton, M. Todd, J. B. Dowd, and A. E. Aiello, "The impact of bisphenol A and triclosan on immune parameters in the U.S. Population, nhanes 2003–2006," *Environmental Health Perspectives*, vol. 119, no. 3, pp. 390–396, 2011.

[11] R. J. Bertelsen, M. P. Longnecker, M. Løvik et al., "Triclosan exposure and allergic sensitization in Norwegian children," *Allergy*, vol. 68, no. 1, pp. 84–91, 2013.

[12] K. Nishi, K. Yamasaki, and M. Otagiri, "Serum albumin, lipid and drug binding," *Subcellular Biochemistry*, vol. 94, pp. 383–397, 2020.

[13] B. Spanier, "Transcriptional and functional regulation of the intestinal peptide transporter PEPT1," *The Journal of Physiology*, vol. 592, no. 5, pp. 871–879, 2014.

[14] A. Ranganathan, S. J. Gee, and B. D. Hammock, "An immunoassay for the detection of triclosan-O-glucuronide, a primary human urinary metabolite of triclosan," *Analytical and Bioanalytical Chemistry*, vol. 407, no. 24, pp. 7263–7273, 2015.

[15] E. M. Brun, E. Bonet, R. Puchades, and Á. Maquieira, "Selective enzyme-linked immunosorbent assay for triclosan. Application to wastewater treatment plant effluents," *Environmental Science and Technology*, vol. 42, no. 5, pp. 1665–1672, 2008.

[16] N. Cabaleiro, F. Pena-Pereira, I. de la Calle, C. Bendicho, and I. Lavilla, "Determination of triclosan by cuvetteless UV–vis micro-spectrophotometry following simultaneous ultrasound assisted emulsification–microextraction with derivatization: use of a micellar-ionic liquid as extractant," *Microchemical Journal*, vol. 99, no. 2, pp. 246–251, 2011.

[17] F. Tohidi and Z. Cai, "GC/MS analysis of triclosan and its degradation by-products in wastewater and sludge samples from different treatments," *Environmental Science and Pollution Research*, vol. 22, no. 15, pp. 11387–11400, 2015.

[18] A. M. Casas Ferreira, M. Möder, and M. E. Fernández Laespada, "GC-MS determination of parabens, triclosan and methyl triclosan in water by in situ derivatisation and stir-bar sorptive extraction," *Analytical and Bioanalytical Chemistry*, vol. 399, no. 2, pp. 945–953, 2011.

[19] L. Martín-Pozo, M. D. C. Gómez-Regalado, I. Moscoso-Ruiz, and A. Zafra-Gómez, "Analytical methods for the determination of endocrine-disrupting chemicals in cosmetics and personal care products: a review," *Talanta*, vol. 234, pp. 122642–642, 2021.

[20] A. Alshishani, M. Saaid, C. Basheer, and B. Saad, "High-performance liquid chromatographic determination of triclosan, triclocarban, and methyl-triclosan in wastewater using mini-bar micro-solid phase extraction," *Microchemical Journal*, vol. 147, pp. 339–348, 2019.

[21] T. Liu and D. Wu, "High-performance liquid chromatographic determination of triclosan and triclocarban in cosmetic products," *International Journal of Cosmetic Science*, vol. 34, no. 5, pp. 489–494, 2012.

[22] Ich, *Validation of Analytical Procedures: Text and Methodology Q2 (R1)*, ICH Harmonised Tripartite Guideline, Rockville, MD, USA, 2005.

[23] N. A. Bhaskaran, L. Kumar, M. S. Reddy, and G. K. Pai, "An analytical "quality by design" approach in RP-HPLC method development and validation for reliable and rapid estimation of irinotecan in an injectable formulation," *Acta Pharmaceutica*, vol. 71, no. 1, pp. 57–79, 2021.

[24] R. Lavecchia and A. Zuorro, "Experimental study of the inclusion of triclosan in hydroxypropyl-beta-cyclodextrins," *Chemical Engineering Transactions*, vol. 17, pp. 1083–1088, 2009.

[25] C. L. Domínguez-Delgado, I. M. Rodríguez-Cruz, J. J. Escobar-Chávez, I. O. Calderón-Lojero, D. Quintanar-Guerrero, and A. Ganem, "Preparation and characterization of triclosan nanoparticles intended to be used for the treatment of acne," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 79, no. 1, pp. 102–107, 2011.

[26] D. Yang, S. Kong, F. Wang et al., "Urinary triclosan in south China adults and implications for human exposure," *Environmental Pollution*, vol. 286, Article ID 117561, 2021.

[27] Q. Luo, H. Zhang, Y. Zhou, Z. Liu, and Z. Cai, "Simultaneous determination of triclosan, triclocarban, triclocarban metabolites and byproducts in urine and serum by ultra-high-performance liquid chromatography/electrospray ionization tandem mass spectrometry," *Rapid Communications in Mass Spectrometry: Rapid Communications in Mass Spectrometry*, vol. 35, no. 14, p. e9117, 2021.

[28] H. Swart, J. L. Du Preez, M. De Villiers, A. P. Lötter, and W. Liebenberg, "Correlation between in vitro release from topical delivery vehicles and microbicidal activity of triclosan," *Die Pharmazie*, vol. 61, no. 1, pp. 35–40, 2006.

[29] N. Aminu, S. Chan, N. H. Khan, and S. Toh, "Concurrent determination of triclosan and flurbiprofen by high-performance liquid chromatography in simulated saliva and its application in dental nanogel formulation," *Acta Chromatographica*, vol. 30, no. 4, pp. 219–224, 2018.

[30] J. Chen, E. M. Hartmann, J. Kline, K. Van Den Wymelenberg, and R. U. Halden, "Assessment of human exposure to triclocarban, triclosan and five parabens in U.S. indoor dust using dispersive solid phase extraction followed by liquid chromatography tandem mass spectrometry," *Journal of Hazardous Materials*, vol. 360, pp. 623–630, 2018.