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Short communication

Determination of urinary triclosan by stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry

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ABSTRACT

We have developed an analytical method for the determination of urinary 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan), which utilizes stir bar sorptive extraction (SBSE) and thermal desorption (TD)–gas chromatography–mass spectrometry (GC–MS). Human urine sample is deconjugated by treatment with β-glucuronidase and sulfatase. A stir bar coated with polydimethylsiloxane (PDMS) is added to the urine sample in a vial and the sample is stirred for 60 min at room temperature (25 °C). Then, the PDMS stir bar is subjected to TD–GC–MS. The detection limit of triclosan is 0.05 ng mL^{-1}. The method shows linearity over the calibration range (0.1–10 ng mL^{-1}) and the correlation coefficient (r) is higher than 0.993 for triclosan standard solution. The average recoveries of triclosan in human urine sample are 102.8–113.1% (RSD: 2.4–6.7%). This simple, sensitive, and selective analytical method may be used in the determination of trace amounts of triclosan in human urine samples.

1. Introduction

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, is widely used as a broad-spectrum antimicrobial agent. As an additive in plastics, it prevents growth of microorganisms and suppresses the formation of stain and odor, thereby extending the polymer's shelf life [1]. For this reason, it has been considered that triclosan in polymeric materials come in contact with food [2,3]. As healthy humans may be exposed to triclosan via a variety of daily activities, exposure assessment of triclosan in human is an important issue.

The urinary excretion of orally ingested triclosan as free triclosan and glucuronide and sulfate conjugates has been reported [4]. Thus, it is thought that human exposure can be evaluated by measuring triclosan in urine sample. To this end, high-sensitivity and high-accuracy analytical methods are required.

Many analytical methods for the determination of triclosan in biological samples have been reported, including liquid chromatography (LC) with diode array detection (DAD) [5], electrochemical detection (ECD) [6] or tandem mass spectrometry (MS–MS) [7]. On the other hand, gas chromatography (GC) with MS or MS–MS was initially used for the determination of triclosan in environmental analysis [8–11].

Liquid–liquid extraction (LLE) [6] and online solid-phase extraction (SPE) [7] have been developed for the determination of triclosan in urine sample. Recently, a new sorptive extraction technique that uses a stir bar coated with polydimethylsiloxane (PDMS) was developed [12] and is known as stir bar sorptive extraction (SBSE). Its main advantage is its wide application range that includes volatile aromatics, halogenated solvents, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, endocrine disruptors, preservatives, odor compounds, and organotin compounds [13–15]. Silva and Nogueira determined triclosan in biological and environmental matrices with the SBSE-liquid desorption (LD)-LC-DAD method [5]. In LC analysis, LD was used to desorb the analyte from the PDMS stir bar. On the other hand, thermal desorption (TD) is often used to desorb the analyte from the PDMS stir bar. Because TD enables injection of the entire quantity of desorbed analyte into an analytical instrument such as GC–MS, high-sensitivity analysis is expected.

The aim of this study was to determine trace amounts of triclosan in human urine samples by SBSE and TD–GC–MS.

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2. Experimental

2.1. Materials and reagents

Triclosan was purchased from Wako Pure Chemical, Inc. (Osaka, Japan). Benzophenone-d_{10} (BP-d_{10}, used as internal standard) was purchased from Kanto Chemical Inc. (Tokyo, Japan). *E. coli* β-glucuronidase (25,000 units/0.4 mL, 62,500 units mL^{-1}) and *H. pomatia* sulfatase (3540 units mL^{-1}) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Prior to use, β-glucuronidase was added to 0.1 M ammonium acetate to make a total concentration of 10,000 units mL^{-1}. Other reagents were purchased from Wako Pure Chemical, Inc. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solution

A concentrated solution (1.0 mg mL^{-1}) of triclosan was prepared by dissolving in methanol. Seven-point calibration (0.1, 0.2, 0.5, 1, 2, 5, and 10 ng mL^{-1}) was performed daily in duplicate analysis for all samples containing the internal standard using the SBSE method.

2.3. Instrumentation

TD was performed with a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel Cooled Injection System (CIS) 4 programmable temperature vaporization (PTV) inlet. GC–MS was performed with an Agilent 6890N gas chromatograph equipped with a 5973N mass-selective detector (Agilent Technologies).

Stir bars coated with a 0.5-mm thick PDMS layer (24 μL; Twister™: a magnetic stirring rod is placed inside a glass jacket and coated with PDMS) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 1 h at 300 °C in a flow of helium. Then, the PDMS stir bars were kept in new 2 mL vials until immediately prior to use. The stir bars could be used more than 50 times with appropriate re-conditioning. For the extraction, a 10 mL headspace vial from Agilent Technologies (Palo Alto, CA, USA) was used.

2.4. TD–GC–MS conditions

TDS 2 temperature was programmed to increase from 20 °C (held for 1 min) to 240 °C (held for 5 min) at 60 °C min^{-1}. The desorbed compounds were cryofocused in CIS 4 at −150 °C. After the desorption, CIS 4 temperature was programmed to increase from −150 °C to 300 °C (held for 10 min) at 12 °C s^{-1} to inject the trapped compounds into the analytical column. CIS 4 is a type of PTV device. Once an analyte is trapped by means of temperature control, the entire quantity is subjected to GC–MS. Injection was performed in the solvent vent mode. Separations were conducted on a DB-5ms fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). Oven temperature was programmed to increase from 40 °C (held for 1 min) to 190 °C at 5 °C min^{-1} and from 190 °C to 280 °C (held for 3 min) at 15 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (ionization voltage: 70 eV). Monitoring ions are shown in Table 1.

2.5. Human urine samples

Urine was collected from six healthy volunteers (I, II, III, IV, V, and VI). All samples were stored at 4 °C prior to use.

2.6. Sample preparation

One milliliter of urine sample spiked with BP-d_{10} was pipetted into a 10 mL vial. Then, 1.0 M ammonium acetate (100 μL, pH 6.8) was added. After β-glucuronidase (10 μL, 10,000 units mL^{-1}) and sulfatase (10 μl, 3540 units mL^{-1}) were added, the sample was gently mixed. Glucuronidase and sulfatase hydrolysis to release free triclosan was accomplished by incubating at 37 °C for 3 h. After enzymatic de-conjugation, 1 mL of purified water was added to the vial. A PDMS stir bar was added and the vial was crimped with a Teflon-coated silicone septum cap. SBSE was performed at room temperature for 60 min while stirring at 500 rpm. After the extraction, the stir bar was easily removed, rinsed with purified water, dried with lint-free tissue, and placed inside a glass TD tube. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and the desorbed analyte was subjected to GC–MS thereafter.

3. Results and discussion

3.1. Optimization of GC–MS conditions

In the mass analysis of standard solutions using electron impact ionization (EI)-MS, *m*/*z* 288 was observed as the main peak of triclosan. The mass spectrometer was operated in the SIM mode. Three ions were monitored (*m*/*z* <u>288</u>, 218 for triclosan; and *m*/*z* <u>192</u> for BP-d_{10}. The underlined and none underlined number are the *m*/*z* of the ion used for quantitation and qualitative analysis, respectively).

3.2. Optimization of SBSE conditions

One important parameter affecting SBSE was the extraction time. To determine the optimum extraction time, 1 mL of human urine sample (5 ng mL^{-1} triclosan standard solution) and 1 mL of purified water were mixed and used. The extraction time profile (0–180 min) of triclosan in the human urine sample that was subjected to SBSE and TD–GC–MS is shown in Fig. 1. Triclosan reached equilibrium after approximately 60 min. Therefore, this condition was used for the determination of triclosan in human urine samples.

Table 1
Figures of merit of SBSE and TD–GC–MS.

Compound	SIM[a] (*m*/*z*)	LOD[b] (ng mL^{-1})	LOQ[c] (ng mL^{-1})	Range (ng mL^{-1})	Correlation coefficient (*r*)	Amount spiked			
						0.5 ng mL^{-1}		5 ng mL^{-}	
						Recovery (%)	RSD (%)[d]	Recovery (%)	RSD (%)[d]
Triclosan	<u>288</u>, 218	0.05	0.1	0.1–10	0.993	102.8	2.4	113.1	6.7

[a] The underlined number is the *m*/*z* of the ion used for quantification.
[b] LOD: limit of detection (S/N = 3).
[c] LOQ: limit of quantification (S/N > 10).
[d] Recoveries and precision were also examined by replicate analysis ($n = 6$) of human urine samples.

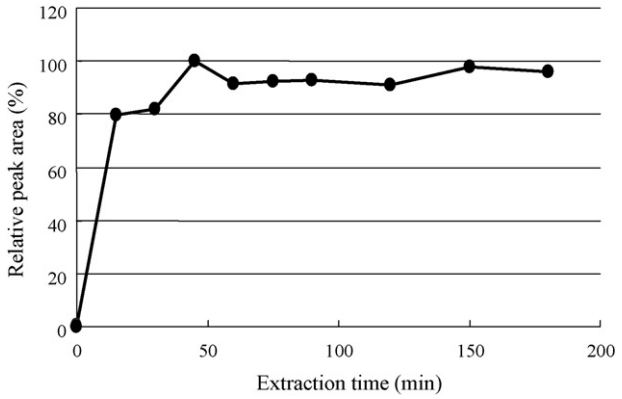

Fig. 1. Optimization of extraction time.

3.3. Figures of merit of SBSE and TD–GC–MS for determination of triclosan

The calculated detection limit (LOD) is 0.05 ng mL^{-1} for SBSE and TD–GC–MS, with the ratio of the compound's signal to the background signal (S/N) being 3. In addition, the limit of quantification (LOQ) when S/N > 10 is 0.1 ng mL^{-1} for triclosan. Some different lots of human urine were used for calculation of LOD and LOQ. The blank and internal standard only samples were checked. The interference was not observed. The method shows linearity over the calibration range (0.1–10 ng mL^{-1}) and the correlation coefficient (r) is higher than 0.993 for triclosan standard solution. The figures of merit of the present method are summarized in Table 1. The LOD of triclosan in urine sample previously determined by LLE-LC-ECD [6] and the online SPE–LC–MS–MS method [7] was 1.0 and 2 ng mL^{-1}, respectively. Compared to those studies, approximately 10–20 times higher sensitivity was achieved in this study.

The recovery and within-day precision of the method were assessed by replicate analysis (n = 6) of human urine samples fortified at 0.5 and 5 ng mL^{-1} levels. Non-spiked and spiked samples were subjected to SBSE and TD–GC–MS. Recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using calibration curves obtained from standard solutions with internal standard. The recovery and precision were 102.8–113.1% (RSD: 2.4–6.7%) for human urine samples (Table 1). Therefore, the method enables the precise determination of standards and may be applicable to the

Table 2
Concentrations of triclosan in human urine samples.

Compound	De-conjugation	Human urine					
		I	II	III	IV	V	VI
Triclosan (ng mL^{-1})	Without de-conjugation	0.17	0.15	0.15	0.15	0.52	0.15
	De-conjugation	2.62	1.35	1.13	4.30	13.98	1.26
Ratio of conjugate (%)[a]		93.5	89.2	86.8	96.4	96.3	87.9

[a] Ratio of conjugate was calculated by (Total-Free)/Total*100.

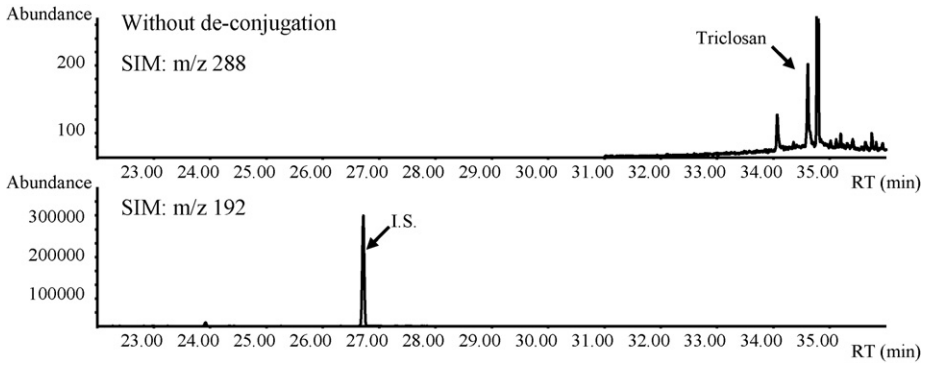

Fig. 2. Typical SIM chromatogram of triclosan in human urine sample (I) by SBSE–TD–GC–MS without de-conjugation. Triclosan: 0.17 ng mL^{-1}.

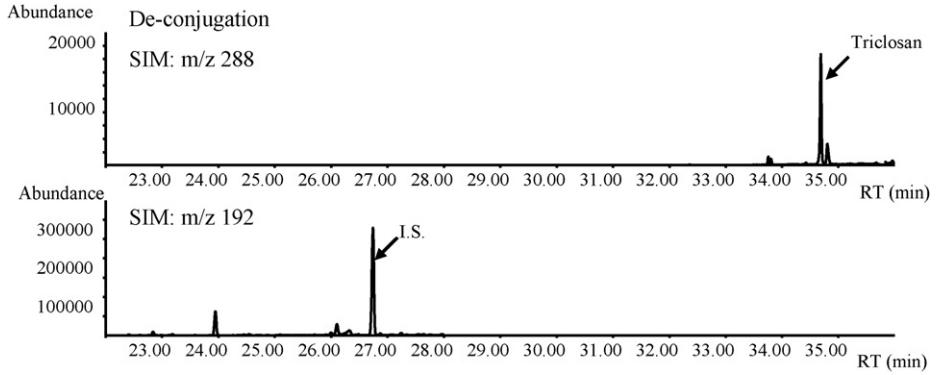

Fig. 3. Typical SIM chromatogram of triclosan in human urine sample (I) by SBSE–TD–GC–MS with de-conjugation. Triclosan: 2.62 ng mL^{-1}.

determination of trace amounts of triclosan in human urine samples.

3.4. Determination of triclosan in human urine samples

A total of six human urine samples were analyzed for triclosan using the present method and the results are shown in Table 2. For quality control, the urine samples spiked with standard were analyzed between the measurements of six human urine samples. In the human urine samples without de-conjugation, 0.15–0.52 ng mL^{-1} triclosan was detected. On the other hand, in the human urine sample treated with β-glucuronidase and sulfatase, 1.13–13.98 ng mL^{-1} triclosan was detected by the present method. Typical chromatograms of human urine sample (Volunteer I) by SBSE–TD–GC–MS without de-conjugation and with de-conjugation are shown in Figs. 2 and 3, respectively. The ratio of conjugate in human urine sample was 86.8–96.4%. Most triclosan existed in human urine sample as conjugate. The comparatively high concentration of triclosan was determined in the sample. It was thought that the volunteers may be exposed to triclosan via a variety of daily activities.

SBSE and TD–GC–MS enabled the successful determination of trace amounts of triclosan in human urine sample. Because the previous methods [6,7] were low sensitivity, it was considered that triclosan in human urine sample might not be determined. The proposed method has many practical advantages, including small sample volume (1 mL) and simplicity of extraction; it is also solvent-free and has high sensitivity.

4. Conclusions

The determination of trace amounts of triclosan in human urine samples using SBSE and TD–GC–MS was performed. The detection limit for triclosan was 0.05 ng mL^{-1}. In addition, the present method showed good linearity and high correlation coefficient using the internal standard. The recovery was high (102.8–113.1%) and the precision was good (RSD: 2.4–6.7%) for human urine samples fortified at 0.5 and 5 ng mL^{-1} levels. This simple and highly sensitive method is expected to have potential applications in various aqueous samples.

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