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Automated on-line column-switching HPLC–MS/MS method for the quantification of triclocarban and its oxidative metabolites in human urine and serum

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ABSTRACT

3,4,4′-Trichlorocarbanilide (triclocarban, TCC) is widely used as an antimicrobial agent in a variety of consumer and personal care products. Because of its widespread use, the potential for human exposure to TCC is high. Human exposure to TCC may be assessed by measuring the concentrations of conjugated or free species of TCC and its two oxidative metabolites, 2′-hydroxy-TCC (2′-OH-TCC) and 3′-hydroxy-TCC (3′-OH-TCC), in urine or serum. To assess human exposure to TCC, we developed a method that uses restricted access materials (RAM) on-line solid phase extraction (SPE) coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry with peak focusing (HPLC–MS/MS). Sample clean-up by RAM relies on both size exclusion chromatography, to remove the high-molecular matrix components, and reversed phase partition, to extract and pre-concentrate the target analytes. TCC, 2′-OH-TCC and 3′-OH-TCC present in urine or serum were concentrated on the RAM SPE column, back-eluted from the SPE column, diluted through a mixing tee for peak focusing, separated by HPLC, and detected by isotope dilution-MS/MS. The method required a small amount of sample (50 μL) and minimal sample pretreatment. The limits of detection (LOD) ranged from 0.01 to 0.1 ng/mL. The method was applied to measure TCC and its metabolites in 158 urine and 16 serum samples collected from adults with no known exposure to TCC. TCC was detected in 35.4% of the urine samples (range: <LOD to 401 ng/mL). This sensitive method is rugged as well as labor- and cost-effective, and allows for the analysis of a large number of samples for epidemiological studies.

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1. Introduction

3,4,4′-Trichlorocarbanilide (TCC) is widely employed as an antimicrobial agent in a variety of consumer and personal care products, including bar soaps, detergents, toothpastes, deodorants, and cleansing lotions [1,2]. TCC is also used in cleansing preparations in hospitals and other medical settings where the potential risk for the transmission of infections is high. TCC is classified as a high production volume chemical [3]; the annual production and/or import volume in the United States is at least half a million pounds [2]. The major route for TCC into the environment is through urban waste water [4]. TCC has been detected in waste water, surface waters, municipal waste water treatment effluents, estuarine sediments, and US streams nationwide [5–9].

TCC can impair mammalian reproduction. It has been found to cause decreased birth weight and survival rate in rats [10]. In humans, exposure to TCC has been associated with methemoglobinemia [11]. Furthermore, recent research suggests that TCC can act as an endocrine disruptor, both in cell-based assays and in rats [12–15]. Although potential adverse health effects of TCC in humans are still largely unknown, because of its widespread use, the potential for human exposure to TCC is high.

Early publications on the metabolism of TCC in rats indicated that the major biliary and fecal metabolites of TCC were nonconjugated and conjugated TCC and 2′-hydroxy-TCC (2′-OH-TCC) [16,17]. In a previous study, we identified nonconjugated and conjugated TCC, 3′-hydroxy-TCC (3′-OH-TCC), and 2′-OH-TCC as the major urinary and serum metabolites of TCC in Sprague Dawley rats after administration of TCC once (500 mg/kg body weight) by oral gavage [18]. Data on the metabolism of TCC in humans, albeit limited, suggest that conjugated TCC is the main urinary metabolite [19–21]. Together, all these findings suggest that urinary or serum concentrations of TCC, 3′-OH-TCC, and 2′-OH-TCC may be valid biomarkers for assessment of human exposure to TCC.

Several analytical techniques have been developed for the determination of TCC in environmental samples. Extraction

Abbreviations: CDC, Centers for Disease Control and Prevention; 2′-OH-TCC, 2′-Hydroxy-triclocarban; 3′-OH-TCC, 3′-Hydroxy-triclocarban; LOD, Limit of detection; RAM, Restricted access materials; RSD, Relative standard deviation; TCC, Triclocarban.

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and/or derivatization followed by detection with gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) have been used to measure TCC in surface water [8,22–27], municipal waste water [28–30], and municipal bio-solids [28,31].

Analytical methods to measure TCC in biological fluids, on the other hand, are rather limited [21,32,33]. A selected ion monitoring (SIM) GC–MS method has been used to quantitate TCC and 2′-OH-TCC in human urine and plasma with limits of detection (LODs) ranging from 1.5 to 3 ng/mL [32]. This method, which required a derivatization step, was time-consuming and labor-intensive. More recently, an HPLC–MS/MS method with turbo flow on-line extraction was developed to measure TCC in urine and plasma [21]. Restricted access material (RAM), one type of special extraction sorbents used for on-line sample clean up and extraction for biological samples, allows direct injection of complex biological matrices into the HPLC system without previous sample treatment [34]. In the present study, we developed and validated a highly sensitive and selective method that uses RAM on-line solid phase extraction (SPE) coupled with HPLC–MS/MS with peak focusing to measure the urinary and serum concentrations of TCC and its two metabolites, 3′-OH-TCC and 2′-OH-TCC.

2. Experimental

2.1. Analytical standards and reagents

HPLC-grade methanol (MeOH), water (H_2O), and acetonitrile (ACN) were obtained from Tedia (Fairfield, OH). Analytical-grade formic acid (98%) was purchased from EM Science (Gibbstown, NJ). TCC, 4-methylumbelliferyl glucuronide, 4-methylumbelliferyl sulfate, β-glucuronidase/sulfatase (*Helix pomatia*, H1), and ammonium acetate (>98%) were purchased from Sigma–Aldrich Laboratories, Inc. (St. Louis, MO). D_7-TCC and $^{13}C_4$-4-methylumbelliferone were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). 2′-OH-TCC and 3′-OH-TCC were provided by the University of California–Davis (Davis, CA).

2.2. Human urine and serum specimen's collection for method validation

We collected 158 urine samples in Atlanta, GA during 2009–2011 from a diverse group of male and female adult volunteers with no documented occupational exposure to TCC. The Centers for Disease Control and Prevention's (CDC) Human Subjects Institutional Review Board reviewed and approved the study protocol. A waiver of informed consent was requested under 45 CFR 46.116(d). Samples were collected anonymously. Except for sex information, no personal or demographic data were available. Sixteen serum samples, collected between 1998 and 2003, were purchased from Tennessee Blood Services (Memphis, TN). No demographic data and no information regarding potential exposure to TCC from the blood donors were available.

2.3. Preparation of standard stock solutions and quality control materials

We prepared the initial stock solutions of analytical standards and stable isotope-labeled internal standards by dissolving measured amounts of the target analytes in MeOH. Ten working standard spiking solutions that contained TCC, 2′-OH-TCC, and 3′-OH-TCC were generated by serial dilution of the initial stock with MeOH. Final concentrations of the ten working standards were such that a 50-μL spike covered a concentration range from 0.01 to 50 ng/mL of the three analytes. The stable isotope-labeled internal standard working solution was prepared by diluting the internal standard stock solutions in MeOH, so that a 25-μL spike would result in a 25 ng/mL concentration of D_7-TCC. All standard stock solutions and spiking solutions were dispensed into glass vials and stored at −70 °C until used.

Quality control (QC) materials were prepared from urine collected anonymously at CDC, or from commercial calf serum (Gibco, Grand Island, NY). The urine or serum was mixed uniformly and divided into two aliquots for QC low (QCL) and QC high (QCH) concentration pools. The QCL and the QCH pools were enriched with different levels of native target compounds. To avoid precipitation of serum proteins when we prepared the serum QC pools, we first added 3 mL of 0.1 M formic acid to 1 mL of the spiking standard solutions before spiking into the serum. All QC materials were stored at −70 °C until used.

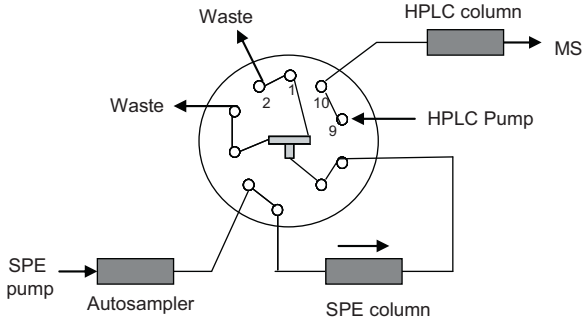
Results from studies on the metabolism of TCC in humans suggest that TCC may undergo phase II metabolism to form conjugates [19–21]. Because standards of conjugates of TCC and its metabolites are not commercially available, these conjugates in urine and serum must be hydrolyzed in order to measure the concentration of the total (unconjugated plus conjugated) species of TCC and its metabolites. We chose to hydrolyze the conjugates enzymatically as commonly done for other environmental chemicals [35,36]. The enzyme solution was prepared daily by dissolving 0.04 g of β-glucuronidase/sulfatase (463 000 U/g solid) in 10 mL of 1 M ammonium acetate buffer (pH 5). To check the efficiency of the enzyme used, we monitored the deconjugation of 4-methylumbelliferyl sulfate and 4-methylumbelliferyl glucuronide (500 ng/mL each), added to each sample, using $^{13}C_4$-4-methylumbelliferone as internal standard. After incubation, 4-methylumbelliferyl sulfate and 4-methylumbelliferyl glucuronide are fully deconjugated to free 4-methylumbelliferone. We used the area ratio of 4-methylumbelliferone/$^{13}C_4$-4-methylumbelliferone for each sample to confirm that the enzyme functioned properly.

2.4. Sample and standard preparation

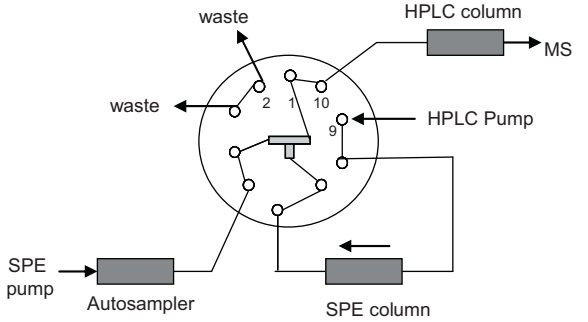
Urine or serum was thawed and vortex-mixed. For the purpose of estimating the concentrations of free species of TCC, 2′-OH-TCC and 3′-OH-TCC, 25 μL of D_7-TCC internal standard solution and 50 μL of urine (or serum) were added to 925 μL of HPLC-grade H_2O in a 1.5 mL conical bottom autosampler vial.

For the purpose of determining the total concentrations of the compounds, the preparation included an enzymatic hydrolysis: 25 μL of D_7-TCC internal standard solution, 50 μL of 4-methylumbelliferyl glucuronide/4-methylumbelliferyl sulfate/$^{13}C_4$-4-methylumbelliferone standard solution (500 ng/mL each), and 50 μL of β-glucuronidase/sulfatase solution were added to 50 μL of urine (or serum) in an autosampler vial. After being gently mixed, the spiked sample was incubated at 37 °C for 4 h. After incubation of the sample, 825 μL of HPLC-grade H_2O was added. Because some precipitation was observed upon incubation, all sample vials were vortex mixed and centrifuged at $812 \times g$ for 15 min before the on-line SPE–HPLC–MS/MS analysis. The autosampler injector needle was programmed to withdraw the sample 4.5 mm above the bottom of the autosampler vial, so that the precipitate would not be drawn into the HPLC system. We prepared QCs and blanks (e.g., reagent blank and matrix blank) by using this same procedure (with enzymatic deconjugation), but we replaced the urine or serum with the same volume of QC materials, HPLC-grade H_2O (reagent blank), or blank urine or serum (matrix blank).

The standards were prepared in pooled urine collected anonymously (or commercial serum). The pooled urine or commercial serum used for standards preparation was prescreened for TCC, 2′-OH-TCC and 3′-OH-TCC, and we did not detect any of the target



1A: Position 1-2 (0–3 min and 5–12 min)



1B: Position 1-10 (3–5 min)

Fig. 1. Schematic diagram of on-line SPE–HPLC–MS/MS set-up.

analytes. To prepare the standards, we added 25 μL of D_7-TCC internal standard solution and 50 μL of matrix (blank urine or serum) into 875 μL of H_2O in a 1.5 mL conical bottom autosampler vial. We then added 50 μL of standard stock solution (in MeOH).

2.5. On-line SPE–HPLC–MS/MS with peak focusing

The on-line SPE–HPLC–MS/MS system used in this study was a modification of the system used for the quantification of phenols in serum [35]. It was assembled from several Agilent 1100 modules (Agilent Technologies, Wilmington, DE) coupled with an API 4000 QTrap™ mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) interface. The on-line SPE–HPLC system consisted of two binary pumps with degassers, an autosampler with a 900 μL injection loop, a high-pressure mixing Tee, and one column compartment with a 10-port switching valve. The mass spectrometer and the Agilent modules were programmed and controlled by use of the Analyst 1.4.1 software (Applied Biosystems). The on-line SPE–HPLC–MS/MS acquisition method was built in 'LC sync' mode (i.e., acquisition was triggered only after completion of the sample injection). The RAM column used for SPE was a LiChrospher™ RP-18 ADS (25 mm × 4 mm, 25 μm particle size, 60 Å pore size; Merck KGaA, Germany). This RAM, a family of special reversed phase sorbents, facilitates the direct extraction and enrichment of hydrophobic, low molecular weight analytes from untreated or minimally treated biological samples. The HPLC column was an Agilent Zorbax Eclipse C18 column (150 mm × 4.6 mm, 5 μm particle size; Agilent Technologies, Wilmington, DE).

The procedure for extracting TCC and its metabolites from the urine or serum involved three periods. During the first period (0–3 min), with the 10-port valve at position 1–2 (Fig. 1A), 500 μL of the sample was loaded onto the SPE column by the SPE pump with 100% H_2O (0–1 min) and washed with 20% ACN:80% H_2O (1.1–3 min) at a flow rate of 1 mL/min. The sample had to be loaded with 100% H_2O so that the serum proteins would not precipitate on the SPE column. During the second period (3–5 min), the 10-port valve was switched to its alternative position (1–10) (Fig. 1B), and the analytes retained on the SPE column were back-eluted by the HPLC pump with 50% ACN:50% H_2O at a flow rate of 0.5 mL/min. At the same time, for peak focusing, we diluted the SPE eluate through a mixing tee with 20% ACN:80% H_2O, provided by the SPE pump, at a flow rate of 0.25 mL/min. During the third period (5–12 min), the 10-port valve was switched to its original position (1–2) (Fig. 1A), and the analytes were transferred to the HPLC column by the HPLC pump by use of a gradient program at a flow of 0.75 mL/min: 5.1–10 min: 70–100% ACN; 10–11 min: 100% ACN; and 11.1–12 min: 50% ACN. During the third period, we performed regeneration of the SPE column by use of the SPE pump with 100% ACN and SPE column equilibration with 100% H_2O.

The negative ion ESI settings were curtain gas (N_2) pressure 20 psig; collision gas (N_2) flow: 9 arbitrary units (au); nebulizer gas (air) pressure: 50 psig; heater gas (air) pressure: 40 psig; heater gas temperature: 500 °C; and ion transfer voltage: −4500 V. Q1 and Q3 were set at unit resolution. Ionization parameters and collision cell parameters were optimized separately for each analyte. The negative fragment ions used for quantification and confirmation and the retention times for the target analytes are listed in Table 1.

3. Results and discussion

3.1. On-line extraction of urine and serum with RAM

Direct injection of biological fluids to a HPLC system is complicated because of the presence of protein and endogenous compounds. Proteins could precipitate with the organic content of the HPLC mobile phase gradient, leading to high backpressure and resulting in column blockage. Furthermore, endogenous matrix compounds may co-elute with target analytes and cause interferences. SPE is a useful technique for sample pretreatment before HPLC analysis. Among the SPE sorbents, RAM is a porous chromatographic support specifically designed to decrease the matrix content of protein and endogenous macromolecules by limiting access only to small molecules to the interaction sites within the packing material pores. The use of RAM on-line-SPE allowed direct injection of biological fluids onto the HPLC system without previous sample clean up. As a result, the sample pre-treatment for both urine and serum by the current method was minimal and simply involved the addition of internal standard and dilution with HPLC-grade H_2O, followed by centrifugation.

3.2. Matrix effects

Due to its selectivity and sensitivity, HPLC–MS/MS is a good choice for bioanalytical analyses. However, matrix effects have become one of the recognized challenges for developing HPLC–MS/MS methods for analysis of biological specimens [27,37]. Matrix effects can suppress or enhance the ionization of target compounds, resulting in considerable quantification errors, especially when stable isotope-labeled internal standards are not available. We used a matrix factor (MF), defined as the ratio of analyte peak area in the presence of matrix ions to the analyte peak area in the absence of matrix ions [38], to evaluate the matrix effects. We calculated the matrix factors for 2′-OH-TCC and 3′-OH-TCC in urine and serum at three concentration levels (0.5, 5, 50 ng/mL). For 2′-OH-TCC, the matrix effects were negligible for both matrices: MF

Table 1
Analyte retention time (RT) and precursor ion → product ion transitions monitored for quantitation and confirmation.

Analyte	RT (min)	Precursor ion → product ion (*m*/*z*)[a]	
		Quantitation	Confirmation
TCC	10.0	313 → 160	313 → 126
2′-OH-TCC	9.6	329 → 168	329 → 142
3′-OH-TCC	8.5	329 → 168	329 → 142

[a] For D_7-TCC, used as the internal standard for all three analytes, we monitored the precursor → product ion transition (*m*/*z*) 320 → 163.

was 1.01 (SD: 0.09) in urine and 0.94 (SD: 0.07) in serum. However, we observed some matrix effects, especially in serum, for 3′-OH-TCC, the earliest eluted compound: MFs in urine and serum were 0.90 (SD: 0.08) and 0.56 (SD: 0.06), respectively. These results suggest ion suppression of 3′-OH-TCC in serum. Because we did not have isotope labeled standards for 2′-OH-TCC and 3′-OH-TCC, we prepared the calibration curves in the appropriate matrix (urine or serum) to compensate for potential matrix effects for these two compounds.

3.3. Method validation and quality control

Blank urine (serum) spiked with standard and isotope-labeled standard solutions was analyzed repeatedly to determine the limit of detection (LOD), accuracy, and precision of the method. The LOD was calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero [39]. S_0 was determined from five repeated measurements of three low-level standards prepared in the matrix. The calculated LODs ranged from 0.01 to 0.10 ng/mL (Table 2). The limits of quantification (LOQs) were calculated as 3 * LODs, and those limits ranged from 0.03 to 0.3 ng/mL. These values reflect the good sensitivity of the method, especially considering the relatively low sample volume (50 μL) used and the simplicity of the sample preparation procedures. The LOD for TCC (0.1 ng/mL both in urine and serum) obtained using the current method was about ten times more sensitive than the LOD achieved using a similar approach for measuring TCC in breast milk (LOD: 0.91 ng/mL) [33]. Moreover, the sample volume (50 μL) needed for the current method was half the amount required for measuring TCC in breast milk before [33].

RAM on-line SPE used in the current method is one type of special reversed phase sorbent. Its two different surfaces allow for simultaneous size-exclusion and reversed phase chromatographic separations. High molecular weight matrix components (e.g., protein and endogenous macromolecules) elute through size exclusion chromatography, while low molecular weight analytes retain and concentrate by reversed phase chromatography. Therefore, the RAM used in this current method can eliminate proteins with molecular weight larger than 15 kDa (versus 100 kDa provided by turbo flow on-line SPE). Compared with a turbo flow on-line extraction high throughput method described before [21], the RAM used in the current method could provide a more efficient clean up of biological samples. However, the sensitivities of both methods were comparable [21].

The current method also incorporated a peak focusing feature achieved from diluting the SPE eluate through a mixing tee with solvent of low organic content before reaching the HPLC column. As a result, peak shapes of TCC and the other two analytes were shaper (peak width at half height from 0.25 min to 0.1 min) compared with the method without peak focusing. Typical chromatograms of matrix blanks and low concentration standards are shown in Fig. 2.

The method accuracy was assessed by five replicate analyses of pooled urine and serum spiked at four different concentrations; accuracy was expressed as the percentage of expected levels (Table 2). The intra-day variability, reflected in the method accuracy, ranged from 83% to 120% (urine) and 89% to 120% (serum) for all three analytes at the four spike levels (Table 2). We determined the method precision from 30 repeated measurements of QCL and QCH materials over a period of two weeks (Table 2). The relative standard deviations (RSDs), which reflect the intra- and inter-day variability of the method, ranged from 7.0% to 15.3% (urine) and 8.8% to 25% (serum).

The SPE recoveries of the three target analytes from urine or serum were calculated by use of a method described previously [35]. SPE recoveries ranged from 61% to 67% in urine and 77% to 86% in serum. We compensated for the relatively low SPE recoveries of TCC, 2′-OH-TCC, and 3′-OH-TCC in urine by using a matrix matched calibration curve. As a result, the accuracy

Table 2
Solid-phase extraction (SPE) recoveries (*N* = 3), spiked standard concentration (*N* = 5) accuracies, limits of detection (LOD), and inter- and intra-day precision (RSD %) of concentration measurements in spiked urine QCs and spiked serum QCs (*N* = 30).

Analyte	SPE recovery (%)	Accuracy (%)				LOD (ng/mL)	QC low		QC high	
		Standard concentration (ng/mL)					Mean (ng/mL)	RSD %	Mean (ng/mL)	RSD %
		1	2.5	5	10					
Urine										
TCC	64	112	120	105	104	0.10	2.8	7.9	9.9	7.0
2′-OH-TCC	67	83	94	94	98	0.01	2.7	15.0	9.8	11.3
3′-OH-TCC	61	102	112	94	97	0.10	2.7	10.4	10.6	15.3
Serum										
TCC	78	93	120	101	90	0.10	2.9	12.8	10.4	8.8
2′-OH-TCC	77	90	102	105	97	0.01	2.5	20.9	9.7	17.4
3′-OH-TCC	86	89	97	98	89	0.10	2.8	25.0	9.7	24.7

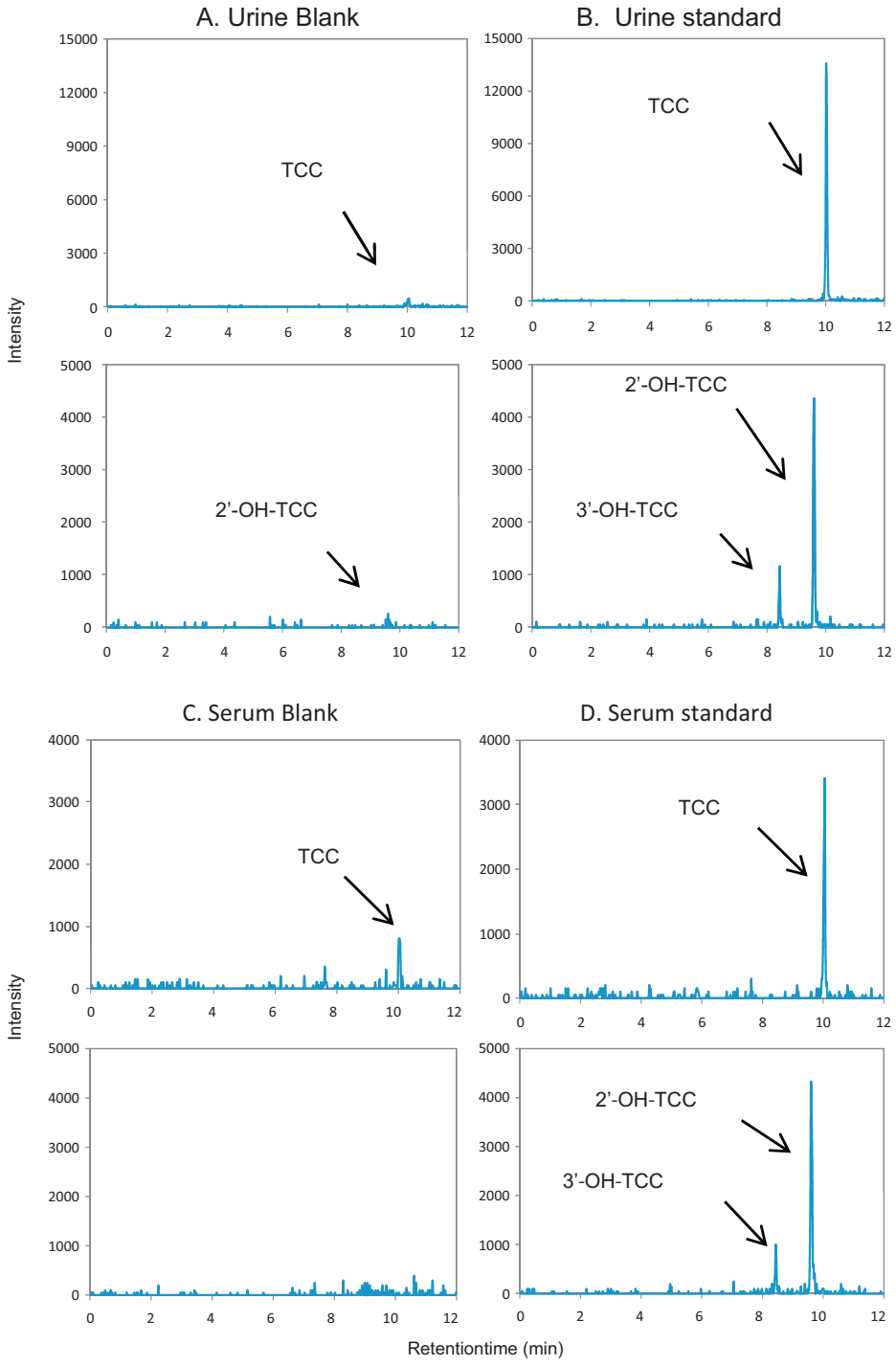


Fig. 2. Typical HPLC–MS/MS extracted ion chromatograms for pooled urine. (A) and pooled serum (C) blanks, and low concentration calibration standards (0.1 ng/mL) prepared in pooled urine (B) and pooled serum (D). The calculated concentrations for all target analytes in the blanks were <LOD.

of the three analytes in urine (range: 83–120%) was acceptable (Table 2).

3.4. Quantification of TCC and its metabolites in human urine and serum

We tested the usefulness of the current method by analyzing 158 urine samples collected between 2009 and 2011 from a diverse group of 79 male and 79 female adult volunteers with no documented occupational exposure to TCC. We also analyzed 16 commercially available serum samples collected between 1998 and 2003. The total concentrations of TCC and its metabolites were measured after enzymatic hydrolysis of the conjugated species in serum or urine. Because analytical standards of conjugated TCC, 2′-OH-TCC, and 3′-OH-TCC were not available, we determined the concentration of these conjugates by subtracting the respective concentrations of free species from the total concentrations. The mean, median, range of concentrations, detection frequency and the mean percentage of conjugated species (calculated as the ratio of concentrations of conjugated and total species) of the three compounds are presented in Table 3. For each analyte, to calculate the mean percentage of conjugated species,

Table 3
The mean and median concentrations (ng/mL) of total and free TCC, 2′-OH-TCC, and 3′-OH-TCC from 158 urine and 16 serum samples.[a,b].

	Total TCC (free TCC)	Total 2′-OH-TCC (free 2′-OH-TCC)	Total 3′-OH-TCC (free 3′-OH-TCC)
Urine (N = 158)			
Mean conc. (ng/mL)	15.3 (<LOD)	0.10 (0.02)	0.12 (<LOD)
Median conc. (ng/mL)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)
Conc. range	<LOD–401 (<LOD–0.34)	<LOD–1.88 (<LOD–0.37)	<LOD–1.33 (<LOD–0.16)
Detection frequency (%)	35 (8)	16 (8)	14.5 (1)
Mean conjugate %[c]	99	94	99
Serum (N = 16)			
Mean conc. (ng/mL)	0.46 (0.26)	<LOD (<LOD)	<LOD (<LOD)
Median conc. (ng/mL)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)
Conc. range	<LOD–3.16) (<LOD–2.00)	<LOD (<LOD)	<LOD (<LOD)
Detection frequency (%)	44 (31)	0 (0)	0 (0)
Mean conjugate %[c]	62	NA	NA

[a] Concentrations <LOD were imputed a value of LOD divided by the square root of 2 for the statistical calculations.
[b] Urine samples were collected between 2009 and 2011. Serum samples were collected between 1998 and 2003. Because TCC can be used in a variety of consumer and personal care products and we had no information on the procedures used for collection, processing, and storage of the serum analyzed, the possibility of external contamination with TCC cannot be ruled out.
[c] To calculate the mean percent of the conjugated species, we included only samples with total concentration values above the LOD.

Table 4
Mean and median concentrations (ng/mL), concentration range, and frequency of detection of total TCC in male and female urine samples.[a]

	Male	Female
Mean conc. (ng/mL)	28.3	2.3
Median conc. (ng/mL)	<LOD	<LOD
Conc. range (ng/mL)	<LOD–401	<LOD–101
Detection frequency (%)	51	20

Concentrations <LOD were imputed a value of LOD divided by the square root of 2 for the statistical calculations.
[a] N = 158 (50% female).

we included only samples with total concentrations above the LOD.

In urine, the detection frequency of TCC, 2′-OH-TCC and 3′-OH-TCC ranged from 14.5% to 35.4% (Table 3). Of the three compounds measured, TCC had the highest detection rate and concentrations. The mean conjugate percentage ranged from 94% to 99%, depending on the compound (Table 4). These data suggest that the conjugated forms were the major species in urine. We also checked the mean and median concentrations and the frequency of detection of total TCC in urine based on sex (Table 4). The frequency of detection [mean concentration] of total TCC in males (50.6% [28.3 ng/mL]) was higher than in females (20% [2.3 ng/mL]) (Table 4). However, for both male and female samples, median concentrations of total TCC were < LOD (Table 4). These data suggest that exposure to TCC might be related to a person's lifestyle. The fact that TCC is used in some shaving creams [2] may explain the higher TCC urinary concentrations in men than in women in our sample population.

In serum, we did not detect 3′-OH-TCC or 2′-OH-TCC in any of the samples tested, but we detected trace levels of TCC in about 50% of them (Table 3). However, these TCC serum results must be interpreted with caution because TCC can be used in a variety of consumer and personal care products; moreover, we had no information on the procedures used for collection, processing, and storage of the serum analyzed. Therefore, we could not rule out the possibility of external contamination with TCC.

4. Conclusions

We developed a highly sensitive on-line SPE–HPLC–MS/MS method with peak focusing for the concurrent quantification of TCC and its two oxidative metabolites, 2′-OH-TCC and 3′-OH-TCC, in human urine and serum. The method requires a small amount of sample (50 μL) and minimal sample pretreatment. This method is rugged as well as labor- and cost-effective. Importantly, it allows for the analysis of a large number of samples for epidemiological studies. Our preliminary research also suggests that human exposure to TCC may be assessed by measurement of the total concentrations of TCC in urine.

Disclaimer

The use of trade names is for identification only and does not constitute endorsement by the US Department of Health and Human Services or the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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