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Analysis of triclosan and triclocarban in human nails using isotopic dilution liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this study, we were able to develop a simple analytical procedure to assay the presence of two antimicrobial agents, triclosan (TCS) and triclocarban (TCC), in human nails. Samples were digested using sodium hydroxide (NaOH), extracted using dichloromethane, and analyzed using ultra performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry operating in the negative ion mode. Mean recoveries were performed at three fortification levels ranging from 98.1% to 106.3% with relative standard deviations between 1.8% and 18.1% (n = 6). The limits of quantification (LOQ) for the method were 2.0 and 0.2 μg/kg for TCS and TCC, respectively. Both compounds were ubiquitously found in all real samples (n = 20) with concentrations ranging from μg/kg to several mg/kg.

1. Introduction

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether, TCS) and triclocarban (3,4,4′-trichlorocarbanilide, TCC) are used globally as antimicrobial agents in a variety of cosmetics and personal care products, including toothpastes, mouthwashes, deodorants, soaps, shampoos, detergents, cleaning lotions, textiles and plastic kitchenware [1,2]. The compounds are also used in hospitals and other medical settings, for example, in surgical scrubs and surgical scrub solutions [1]. Toxicity studies using cell-based assays [2–4] and animal experiments [5–10] have indicated that TCS and TCC could function as endocrine system disruptors. TCS is thought to play a role in activating pregnane X receptor (PXR)-regulated steroid hormone metabolism, based on HuH7 cell tests [3], and may also exhibit estrogenic and androgenic activity in breast cancer cells [4]. Though the acute toxicity of TCS in mammals is low, earlier reports have suggested that TCS could alter thyroid hormone-associated gene expression at low levels in tadpoles [5] and affect thyroxine homeostasis in rats [6,7]. Recent reports have documented that TCC at high concentrations could synergistically amplify the effects of estradiol and testosterone in cell-based assays and in rats [2,8]. Acute and chronic toxicities to TCC are observed in fish, noting that the chronic effect threshold is within the environmental concentrations reported for surface waters [9]. TCC has also been shown to impair mammalian reproduction where its presence has been linked to a decrease in the number of rat pups [10]. The adverse health effects of TCS and TCC are still largely unknown; however, human exposure to both compounds will likely be ongoing due to their widespread and continued use in many different applications. Therefore, accurate and quantitative measurements of exposure levels are pivotal to understand the association between exposure and disease.

Many studies have involved the measurement of TCS and TCC levels in different environmental matrices and biological fluids, including surface water [11], waste water [12], sediment [13], human blood [14–16], human urine [17–19], and several other biological matrices [20–22]. Potential health risks are closely linked to the internal exposure levels of TCS and TCC, and biological specimens such as blood, urine and breast milk are the materials often used to study such levels. In previous studies, TCS was found in all of the pooled serum samples of an Australian population of varying age, gender and geographical region [15]. The average TCS levels in Australians were 2-fold higher than those found in the plasma of Swedish nursing mothers [16]. The main disadvantage of using blood specimens is that blood is an invasive matrix, and thus, its acquisition could have an adverse effect on the participants of the study. For studies involving urine samples, approximately 75% and 95% were found to contain TCS for both American [17] and Chinese

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populations [18], respectively. Moreover, TCC was detected in urine at a frequency of approximately 35%, with levels higher than those found in serum [19]. However, urine specimens have some inherent problems of their own, including the fluctuation in its composition throughout the day and the hygienic practice during its collection and handling.

There are several advantages to using nail specimens instead of serum and urine samples, such as cleanliness, ease of storage, portability, and handling, but it is the non-invasive collection of nails, especially, that would benefit research involving children and large populations [23]. Furthermore, the bi-directional (length and thickness) growth of nails results in dual mechanisms of xenobiotic incorporation via the nail matrix and nail bed [24]. Nails take several months to grow out and seem to be well suited for studies of chronic exposure [25]. More importantly, once the analytes of interest incorporate into the keratinic matrix, they remain isolated from the rest of the body with no fluctuation due to changing metabolic activities, whereas those same compounds have been reported to undergo a more rapid decay in blood and urine [26]. Thus, nails are characterized by a larger detection window that could facilitate retrospective analysis, and the analyte content in nail clippings reflects long-term cumulative exposure. Recently, both fingernails and toenails have been extensively used as biomarkers of drug abuse [24,27], occupational exposure [28] and environmental exposure [29]. To the best of our knowledge, there are few reports on the occurrence of TCC and TCS in human nails [30], and we feel it is important to assess the long-term chronic exposure to these chemicals. In this study, we have developed a method for the simultaneous analysis of TCC and TCS in human nails using liquid-liquid extraction and isotopic dilution liquid chromatography–tandem mass spectrometry.

2. Materials and methods

2.1. Chemicals and reagents

Standard triclosan (99.5%) and $^{13}C_{12}$-triclosan (99%) were purchased from Dr. Ehrenstorfer Gmbh (Augsburg, Germany) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Triclocarban (99%) and $^{13}C_6$-triclocarban (99%) were obtained from Toronto Research Chemicals (North York, Ontario, Canada). All standards were stored at −20 °C. HPLC-grade solvents such as methanol and dichloromethane were supplied by Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was produced via an in-house Milli-Q ultrapure system (Millipore, Bedford, MA, USA). Analytical-grade sodium hydroxide, potassium phosphate, hydrochloric acid (36%, HCl) and ethanol were obtained from Beijing Chemical Works (Beijing, China).

Standard stock solutions of analytical standards and stable isotope-labeled internal standards were prepared in methanol at concentrations of 1000 mg/L and stored at −20 °C in amber glass vessels. Working standards were generated through serial dilutions of the stock standard with methanol–water (80:20, v/v). Working standard solutions were stored at 4 °C. Potassium phosphate buffer (0.1 mol/L; pH 7.0 ± 0.1) was prepared from a mixture of potassium phosphate monobasic and deionized water.

2.2. Sample collection

Fingernail and toenail clippings were collected using cosmetic nail clippers. 10 subjects were asked to combine nail clippings at the same time; fingernail and toenail samples were stored separately in sealed plastic tubes at room temperature until analysis. All subjects provided written informed consent before entering the study. Samples were collected anonymously, and no personal or demographic data were available.

2.3. Sample preparation

Nail samples (approximately 100 mg) were rinsed for 10 min using deionized water combined with ultrasonication to remove visible surface particulates and then soaked in ethanol three times at room temperature in plastic tubes to remove loosely adhering external TCS and TCC. After washing, the fingernails or toenails were dried in a drying oven at 50 °C. The nail clippings were cut into small segments and weighed, then transferred to 15 mL polypropylene tubes containing 4.0 ng of $^{13}C_{12}$-TCS and 0.4 ng of $^{13}C_6$-TCC as the internal standard. Samples were digested with 1 mL of 2 mol/L sodium hydroxide (NaOH) overnight in a water bath shaker set at 50 °C. At the end of the digestion, 3 mL of potassium phosphate buffer solution (0.1 mol/L; pH 7.0 ± 0.1) was added and the pH was adjusted to 7.0 with hydrochloric acid. Then, 5 mL of dichloromethane was added to the mixture followed by vortexing for 2 min and centrifugation (6500 rpm, 10 min). The separated organic layer was evaporated to dryness under a gentle stream of high purity nitrogen at 37 °C. The dry residue was re-dissolved in 200 μL of methanol–water (80:20, v/v) for the analysis.

2.4. LC–MS/MS analyses

LC separation was carried out on a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) coupled with a Waters Acquity UPLC™ HSS T3 column (2.1 × 100 mm, 1.8 μm) maintained at 40 °C. The mobile phase used in the chromatographic separation consisted of a binary mixture of methanol (A) and water (B) at a flow rate of 0.3 mL/min. The gradient elution was begun with 75% A, then raised to 100% A after 4 min, held at 100% A for 1 min and then returned to the initial composition for 0.1 min and equilibrated for 3 min before the next injection. The injection volume was 10 μL.

MS/MS acquisition was conducted on a Waters Xevo™ TQ MS triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an ESI interface operating in negative ion mode with multiple-reaction monitoring (MRM). The source and desolvation gas temperatures were 135 °C and 300 °C, respectively. Nitrogen gas (99%) was used as the desolvation gas at a flow rate of 650 L/h. The collision gas used was ultra-high-purity argon, and the pressure of the collision cell was kept at 1.12×10^{-3} mbar. The capillary voltage was 2.4 kV. Ion energy 1 and ion energy 2 were set to 0.6 and 1.0, respectively. For each analyte, two transitions were selected for quantification and confirmation, but only one transition was monitored for the internal standards. Optimized MS/MS acquisition parameters and the retention times (RT) are listed in Table 1.

2.5. Quality control

Due to the lack of availability of blank human nail and enough low concentration samples, several pieces of pig hoofs were used as a surrogate matrix for method development and validation, considering human nail and pig hoof both are mainly composed by keratin. To avoid contamination, detergents containing TCS and TCC were not used to wash experimental utensils. Each batch of multiple test samples included a procedural blank to check for contamination during sample preparation. Additionally, a reagent blank was injected after every ten sample injections to check for carry-over and for basic cleaning of the chromatographic system. An isotopic dilution method using $^{13}C_{12}$-labeled TCS and $^{13}C_6$-labeled TCC as internal standards was introduced for more accurate quantification. Calibration curves were obtained by carrying out a linear regression analysis on the ratio of standard solution areas to