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Extraction of triclosan and methyltriclosan in human fluids by *in situ* ionic liquid morphologic transformation

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ABSTRACT

Herein, we established an ionic liquid (IL)-based liquid-solid transformation microextraction (IL-LTME) combined with HPLC-UV detection for the simultaneous determination of triclosan (TCS) and its methylated product, methyltriclosan (MTCS), in human fluids. The IL-LTME method was based on an *in situ* metathesis between hydrophilic IL and ion-exchange salt to form a solid hydrophobic IL. According to the above principle, a hydrophilic IL, $[C_{12}MIM]Br$, was selected as the extractant, and NH_4PF_6 as ion-exchange salt. The prominent advantages of the newly developed method are: (1) the *in-situ* reaction between the extractant $[C_{12}MIM]Br$ and ion-exchange salt NH_4PF_6 changed the IL from hydrophilic to hydrophobic that avoiding the stick of ionic liquid on the tube wall; (2) bubbling with NH_3 greatly increased the contact area between IL-extractant and analytes resulting in improved extraction recovery; and (3) solidification of the $[C_{12}MIM]$ PF_6 provided a good separation and avoided the use of specialized equipment. A series of main parameters were optimized by single-factor screening and central composite design as follows: 0.9 mL of NaOH, 2.0 min of second ultrasonically time, 10 min of centrifugation time, 21 mg of extractant $[C_{12}MIM]PF_6$, 2.4 min of ultrasonic time, 65 mg of NH_4PF_6 and 13.8 min of cooling time. Under the optimized conditions, the limits of detection for TCS and MTCS were 0.126–0.161 $\mu g\,L^{-1}$ in plasma samples, and 0.211–0.254 $\mu g\,L^{-1}$ in urine samples, respectively. The extraction recoveries for TCS and MTCS were in the range of 94.1–103.8%. The intra-day and inter-day precisions were 1.00–4.74% and 1.02–5.21%, respectively. In general, the IL-LTME method is environment-friendly, time-saving, economical, high efficient and robust with low detection limits and high recoveries. Thus, the newly developed method has excellent prospects for sample pretreatment and analysis of trace TCS and MTCS in blood and urine samples.

1. Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol, TCS), is a non-ionic, broad spectrum antimicrobial ingredient in disinfectants, soap, detergent, toothpaste, mouthwash, fabric, deodorant, shampoo and plastic additives, in addition to innumerable other personal care, veterinary, industrial and household products [1]. TCS was discovered and synthesized over 40 years ago and has been used increasingly over the past 25 years [1,2]. The widespread use of TCS leads to a relatively high content in a variety of environmental matrices (1–50 $\mu g\,L^{-1}$) [3]. Chau and coworkers investigated the waters of Lincun River and Victoria Harbor in Hong Kong, and found that TCS were detected to be 4.1–117.0 $ng\,L^{-1}$ [4]. TCS by UV radiation can be photo-degraded to be *p*-chlorophenol and dioxin, which have the higher toxicity than their parent compound [5]. Methyltriclosan (MTCS) is a biodegradation product of TCS, which is formed under aerobic conditions [6,7]. The bioaccumulation coefficient of TCS and MTCS in adipose tissue can reach up to 2000–8700, suggesting that they have high bioaccumulation [8]. MTCS ($\log^{Kow} = 5.4$) is more lipophilic than TCS ($\log^{Kow} = 4.78$) [9] and is believed to be more persistent in environmental matrices [10]. Currently, TCS is widely used as an additive in food-packaging polymers and surface contact food-processing industries [11]. Canosa et al. proved that in the food-processing industry, TCS can migrate from foodstuffs or food-packaging films containing TCS into food and the environment [12]. If people ingest food that is contaminated with TCS or MTCS, it can change the protein function by forming TCS or MTCS-HSA (human serum albumin) complex [13]. TCS and MTCS are lipophilic compounds, can be oral mucosa, digestive tract, respiratory and skin absorption, after the blood through the liver microsomes rapid metabolism of sulfide and glucuronide. Sandborgh-

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Englund et al. found that when 10 healthy adults in a single swallow with 4 mg TCS mouthwash, their plasma TCS concentration quickly increased to the peak after 1–2 h with the half-life of 13–29 h [14]. TCS was mainly excreted through urine with 24 h discharge up to 50% of the exposure dose [14,15]. Therefore, it is crucial to develop a simple, fast, efficient and sensitive method for TCS and MTCS detection in plasma and urine samples.

Because of the complicity of human fluid matrices and trace level of TCS and MTCS, the accuracy of quantitative analyses brings some difficulty. Therefore, it is necessary to conduct some suitable extraction and cleanup procedures prior to instrumental determination. So far, several pretreatment methods for TCS and MTCS have been developed including solid-phase extraction (SPE) [16], solid-phase microextraction (SPME) [12], hollow fiber-assisted liquid-phase microextraction (HF-LPME) [17], stir-bar sorption extraction (SBSE) [18], liquid-liquid extraction (LLE) [19] and dispersive liquid-liquid microextraction (DLLME) [20]. Except for DLLME, the disadvantages of the previously reported methods are obvious such as time-consuming, high cost, use of environment-unfriendly solvent, derivatization required prior to GC–MS analysis and so on [21]. Although there are many prominent advantages for traditional DLLME such as only μL-level volume required for extraction and dispersive solvents and short extraction time, some prominent disadvantages lie in the use of organic solvent as the extractant and disperser. Consequently, ionic liquid-based microextraction techniques are becoming much popular in recent years [22,23].

Ionic liquids (ILs), consisting of organic cations and inorganic or organic anions with melting points at or below 100 °C, have been widely applied as “green solvents” to improve extraction and enrichment performance as compared to the traditional use of organic solvents. Currently, ILs are widely used in microextraction procedures as dispersive or extraction solvents according to their different solubility. In general, there are three kinds of ILs-based dispersive liquid-liquid microextraction methods: (1) the traditional DLLME, the use of a small amount of organic solvent as a disperser [24,25]; (2) the whole ILs microextraction, hydrophobic IL as extraction solvent and hydrophilic IL as dispersive solvent [26]; and (3) *in situ* reaction IL microextraction. For the third kind of ILs-based microextraction, a hydrophilic IL (such as $[C_nMIM]BF_4$ or $[C_nMIM]Cl$) is dissolved in water and the analytes enter the hydrophilic IL phase, and then an anion exchanger (such as NH_4PF_6 or $LiNTf_2$) is added for *in situ* reaction to form a hydrophobic IL [27], which makes the IL extractant easily separation from aqueous phase. The most significant advantage of *in situ* reaction IL microextraction is that the extraction time and phase separation time are greatly reduced besides no requirement of organic solvent and environment-friendly properties.

For *in situ* reaction whole IL microextraction, the produced hydrophobic IL is in liquid state and their viscosity is always very large, leading to the difficulty in separation of the sedimented IL phase from aqueous phase. Additionally, the viscous IL is easily adhered to the wall of polyethylene plastic or glass centrifuge tube, resulting in incomplete elution and lower extraction recovery. In order to solve the above problem, we can convert the liquid viscous sedimented phase to solid state in order to facilitate the relatively complete separation from aqueous phase [28–32].

In traditional DLLME method, the sedimentary was withdrawn by a syringe and to detected directly without any other concentration. As the extraction agent and the extraction solution cannot be separated directly, the inevitable matrix interference effect would affect the accuracy of determination of the target material. Liquid-solid transformation microextraction is a way to solve such problems. The solidified floating organic droplet microextraction (SFODM) was first reported by Zanjani [28] and uses 1-undecanol with a low-density solvent and room temperature melting point as the extraction solvent to enrich the polycyclic aromatic hydrocarbons (PAHs) from water sample. After centrifugation and freezing, the low-density solvent can be easily collected without the need for specialized equipment. At this time, the solidified 1-undecanol and water phase can be completely separated, which reducing the transfer error, improving the extraction efficiency and also reducing the interference effect of the substrate on the target material. March has reported a dispersive liquid-liquid microextraction based on solidification of the aqueous phase in 2016 to detect the content of nicotine in eggplant [31]. This research was based on solidification (at −18 °C for 40 min) of the aqueous phase obtained after centrifugation, and the decantation, collection and analysis of the extractant toluene layer. With the liquid-solid transformation procedure, the extraction agent and extraction solution can be separated absolutely. However, suitable extraction solvents for such liquid-solid transformation microextraction are limited [33,34], and both the 1-undecanol and toluene have strong toxicities for the environment and experimenter. Therefore, the traditional liquid-solid transformation microextraction still needs to be improved.

According to the physical state of $[C_nMIM]PF_6$, we chose $[C_nMIM]X$ (X = Cl/Br) that the carbon chain length is greater than or equal to twelve as extractant; after *in situ* reaction, the formation of hydrophobic IL ($[C_nMIM]PF_6$) ($n \geqq 12$) is a solid powder at ambient conditions.

Based on the above considerations, this study aimed to develop a novel ionic liquid-based liquid-solid transformation microextraction (IL-LTME) combined with HPLC for the analysis of TCS and MTCS in human fluids. First, a hydrophilic ionic liquid ($[C_{12}MIM]Br$) was dissolved in water and subjected to an *in situ* reaction with an ion-exchanger (NH_4PF_6) [27]. Subsequently, a solid hydrophobic IL [$C_{12}MIM$]PF_6 was formed at room temperature. As a result, the solid-liquid phase separation is easy and complete. The novel method can meet the requirements for making the extraction procedure simplifier, more convenience and low cost owing to the smaller amount of extractant or salt used in the whole experiment process. Optimization of the major experimental parameters was conducted using response surface method (RSM) on the basis of central composite design (CCD). The optimized method was compared with other traditional extraction and microextraction methods to evaluate its advantages and feasibility for the determination of TCS and MTCS in human blood and urine samples. To the best of our knowledge, this is the first application of liquid-solid phase transformation separation for the pretreatment of trace TCS and MTCS in human fluids.

2. Materials and methods

2.1. Chemicals and reagents

The standards of TCS and MTCS (purities > 99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chromatographic-grade methanol and acetonitrile were purchased from Merck Co., Ltd. (Darmstadt, Germany). The six kinds of ILs were purchased from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China), which were 1-dodecyl-3-methylimidazole bromide ($[C_{12}MIM]Br$), 1-tetradecyl-3-methylimidazole bromide ($[C_{14}MIM]Br$), 1-hexadecyl-3-methylimidazole bromide ($[C_{16}MIM]Br$), 1-dodecyl-3-methylimidazolium chloride ($[C_{12}MIM]Cl$), 1-tetradecyl-3-methylimidazole chloride ($[C_{14}MIM]Cl$) and 1-hexadecyl-3-methylimidazole chloride ($[C_{16}MIM]Cl$). Ammonium hexafluorophosphate (NH_4PF_6) and other chemicals were of analytical grade, and purchased from Shanghai Aladdin Reagent Company (Aladdin Industrial Co. Ltd.). Ultrapure water (18.2 MΩ, 25 °C), which was prepared from a Millipore Milli-Q system (Bedford, MA, USA), was used for the mobile phase and sample solutions.

2.2. Instrumentation

An Agilent 1260 HPLC system equipped with a tunable UV detector was used for quantification of TCS and MTCS. A Zorbax Eclipse SB-C_{18} column (5 μm, 4.6 mm × 250 mm) was utilized for separation of analytes, and a 20-μL sample loop was used for injection. The mobile phase was composed of acetonitrile and ultrapure water (75:25, v/v) at a