Rapid analysis of chlorinated anilines in environmental water samples using ultrasound assisted emulsification microextraction with solidification of floating organic droplet followed by HPLC-UV detection

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ABSTRACT

The present study demonstrates a simple, rapid and efficient method for the determination of chlorinated anilines (CAs) in environmental water samples using ultrasonication assisted emulsification microextraction technique based on solidification of floating organic droplet (USAEME-SFO) coupled with high performance liquid chromatography-ultraviolet (HPLC-UV) detection. In this extraction method, 1-dodecanol was used as extraction solvent which is of lower density than water, low toxicity, low volatility, and low melting point (24 °C). After the USAEME, extraction solvent could be collected easily by keeping the extraction tube in ice bath for 2 min and the solidified organic droplet was scooped out using a spatula and transferred to another glass vial and allowed to thaw. Then, 10 μL of extraction solvent was diluted with mobile phase (1:1) and taken for HPLC-UV analysis. Parameters influencing the extraction efficiency, such as the kind and volume of extraction solvent, volume of sample, ultrasonication time, pH and salt concentration were thoroughly examined and optimized. Under the optimal conditions, the method showed good linearity in the concentration range of 0.05–500 ng mL^{-1} with correlation coefficients ranging from 0.9948 to 0.9957 for the three target CAs. The limit of detection based on signal to noise ratio of 3 ranged from 0.01 to 0.1 ng mL^{-1}. The relative standard deviations (RSDs) varied from 2.1 to 6.1% ($n=3$) and the enrichment factors ranged from 44 to 124. The proposed method has also been successfully applied to analyze real water samples and the relative recoveries of environmental water samples ranged from 81.1 to 116.9%.

1. Introduction

Chlorinated anilines (CAs) are an important class of environmental water pollutants. They are used worldwide in different industries and could be released from the manufacturing of medicines, personal-care products, dyestuff, polymers and as by-products of energy technologies [1,2]. They can be easily discharged into rivers, lakes and soil by inappropriate waste disposal during their own production. Moreover, CAs are also formed from biodegradation of various phenylcarbamate, acylanilide and phenylurea herbicides [3,4]. Usually, this class of compounds is considered hazardous to human health and implicated in inducing damage to DNA and to cause cancer [5,6]. Especially, acute administration of 4-chloroaniline (4-CA) induced renal and hepatic toxicity and 2,6-dichloroaniline (2,6-DCA) has been identified as toxic to fish, crustaceans and mammals [7,8]. Dicloran or 2,6-dichloro-4-nitroaniline (DCNA) is a fungicide used

for major crops including celery, lettuce and sweet potatoes. The toxicity of DCNA to organs include kidney, liver, spleen and hematopoietic system, particularly red blood cells [9,10]. As a result, these compounds have been included in the list of priority pollutants by the United States (US) Environmental Protection Agency (EPA) and also in European Union (EU) legislation. Accordingly, the surface water chronic estimated drinking water concentration (EDWC) is 2.76 ng mL^{-1} for 4-CA [11] and the EU predicted environmental concentration of 4-CA is 1 ng mL^{-1} [6]. The surface and ground water chronic EDWC for DCNA was estimated at 1.8 ng mL^{-1} and 1.3 ng mL^{-1} respectively [12]. Having known the highly toxic effects of the above mentioned pollutants in the environment and the complex environmental transformations that they undergo at the trace level, we aimed at developing a simple, rapid, reliable and sensitive analytical method for the determination of these compounds in environmental water samples.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are the most commonly employed methods for the determination of CAs [13]. According to method 8131 of US EPA [14] for the analysis of aniline and its derivatives in

extracts prepared from environmental samples by GC method, the detection limits of 4-CA and DCNA are 0.6 ng mL^{-1} and 2.9 ng mL^{-1} respectively. However, owing to the polarity and thermal lability of these compounds, a derivatization step is essential for obtaining good GC performance [15]. Hence, HPLC analysis seems to be a good alternative to GC analysis since no derivatization step is required [16]. Generally, a sample preparation step should be performed to obtain accurate and sensitive results in most analytical procedures. Of the various sample preparation methods established for sample pretreatment and preconcentration of CAs, liquid–liquid extraction (LLE) [17] suffers from the disadvantages of being tedious, time and large amounts of organic solvents consuming. Followed by LLE, solid-phase extraction (SPE) method was developed by Patsias and Mourkidou [18] for the determination of various chlorinated anilines, but SPE is a relatively expensive and laborious technique. Later on, single drop microextraction (SDME) and different modes of liquid-phase microextraction (LPME) techniques were introduced [19,20]. However, long extraction time, instability of the microdrop, and sometimes low precision are the disadvantages of LPME techniques. In 2006, Assadi and coworkers [21] introduced dispersive liquid–liquid microextraction (DLLME) technique and Wang et al. [22] used this method for the determination of halogenated anilines. However, poor detection limits (> 0.8 ng mL^{-1}), usage of dispersive solvents and toxic chlorinated solvents as extraction solvents which is non environment-friendly, are the disadvantages of this method. In general, the variety of solvents that can be used in DLLME is limited. In 2005, Jiang et al. [23] developed ionic liquid based headspace LPME for the determination of CAs, but the disadvantages of this method include long extraction time of 30 min, and poor detection limits (ranged between 0.5–1 ng mL^{-1}). Then, Zhu et al., (2008) [24] developed ionic liquid (IL) based DLLME for the determination of aromatic amines. But this procedure yielded poor sensitivity when compared to other LPME methods. Moreover, usage of high density, low volatile ionic liquids gives overscale peaks in HPLC and GC and also contributes to rapid column degradation.

Taking the above disadvantages into account, an approach of utilizing the cavitation phenomenon of ultrasonic radiation to achieve dispersion has been proposed recently [25]. A novel DLLME method coupled with ultrasound radiation was introduced by Regueiro et al. in 2008 [26] and was termed ultrasound-assisted emulsification microextraction (USAEME). In this method, a micro-liter amount of water-immiscible extraction solvent is dispersed into water sample by ultrasound-assisted emulsification without using any dispersive solvent [27]. This method is simple, efficient and contributes to fast analyte extraction. However, this method requires high-density or highly toxic extraction solvents, such as chlorobenzene, carbon tetrachloride, chloroform and tetrachloroethylene etc., all of which are toxic and non environment-friendly [28].

Solidification of floating organic droplet (SFO) technique was first introduced by Yamini et al. [29] and was coupled with LPME for the determination of polycyclic aromatic hydrocarbons in water samples using GC. This is one of the effective LPME techniques that has been explored in recent research papers [30,31]. One of the main conditions for SFO technique is that the organic solvent must have melting point near room temperature (in the range of 10–30 °C), so that the droplet could be collected easily by solidifying it in lower temperature after the USAEME procedure. However, the extraction time is longer, thereby failing to satisfy the demand of rapid analysis. More recently, in order to achieve the merits of both USAEME and LPME-SFO techniques, a combination technique was introduced, termed as USAEME-SFO, that includes the advantages of large contact surface between the aqueous solution and the droplets of

extraction solvent, thus speeding up mass transfer, so that the method was as fast as USAEME and had shorter extraction time than LLME-SFO [32,33].

The goal of the present study was to explore the potential application of USAEME-SFO for the fast analysis of CAs in environmental water samples by HPLC-ultraviolet (HPLC-UV) detection. To the best of our knowledge, this may be the first report about the application of USAEME-SFO method for the determination of CAs using 1-dodecanol as the extraction solvent (melting point: 24 °C). Various parameters affecting the extraction efficiency were examined and optimized. The present method was proven to be simple and had good analytical performance in terms of accuracy, linearity, repeatability, and limits of detection (LODs).

2. Experimental

2.1. Reagents and solutions

4-chloroaniline (98%) and 2,6-dichloroaniline (98%) were obtained from ACROS Organics, (New Jersey, USA) and dicloran (2,6-dichloro-4-nitroaniline, 95%) was purchased from Fluka Chemika, (Buchs, Switzerland). 1-dodecanol (density, 0.83 g/mL) and undecanol (density, 0.82 g/mL), were obtained from Merck (Hohenbrunn, Germany). HPLC-grade acetonitrile (ACN), acetone, ethanol, hydrochloric acid (HCl), methanol and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). All chemicals used in this work were of ACS reagent grade. Ultrapure water for all aqueous solutions was produced in the laboratory using Barnstead Nanopure water system (Barnstead, New York, USA). Stock solutions (1 mg/L of each analyte) were prepared by dissolving the CAs in methanol and stored in brown glass bottles with PTFE-lined caps and kept at 4 °C. Working standard solutions were obtained daily by diluting the stock solutions with ultrapure water.

2.2. Instrumentation

A liquid chromatograph (Knauer, Germany) with a UV/vis detector system (Knauer, Germany), was used for separation and determination of CAs. Extraction solvent collection and injections were carried out using a 50 μL HPLC microsyringe (SGE, Ringwood, Australia). Chromatographic separations were accomplished using a LiChrospher 100RP-18 (5 μm, 125 mm × 4 mm ID) column (Merck, Darmstadt, Germany) and all injections were performed manually with a 10 μL sample loop. Data acquisition and process were accomplished with a Euro-chrom Workstation (Knauer, Germany). The mobile phase was water and acetonitrile (30:70, v/v) at a flow rate of 0.7 mL/min. Detection was set at 240 nm. Under these chromatographic conditions, baseline separation could be obtained for the target compounds.

2.3. USAEME-SFO procedure

10 mL of aqueous sample was placed in a 15 mL screw-cap glass test tube with conical bottom. Then, 60 μL of 1-dodecanol (as extraction solvent) was rapidly injected into the above mentioned aqueous sample by a 0.5 mL syringe (SGE, Australia, Ringwood, Australia) and the resulting mixture was immersed in an ultrasonic bath (model D80, Delta, Taiwan) at 43 KHz frequency (80 W power) for 2 min (at 25 °C), following which a turbid cloudy solution was formed in the test tube. In this step, the analytes in aqueous sample were extracted into the fine droplets of 1-dodecanol. The formed emulsion was centrifuged for 6 min at 4000 rpm and the dispersed fine particles of the extraction phase were collected at the top of conical test tube which