Talanta 136 (2015) 198–203

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of eight pharmaceuticals in an aqueous sample using automated derivatization solid-phase microextraction combined with gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form
25 October 2014
Accepted 1 November 2014
Available online 23 January 2015

Keywords:
Pharmaceutical
Solid-phase microextraction
Derivatization
Automation
Central composite design
The Pearl River Estuary

ABSTRACT

An automated aqueous derivatization solid-phase microextraction (SPME) coupled with a gas chromatography/mass spectrometry (GC/MS) method was developed for simultaneous determination of eight pharmaceuticals in water samples. Dimethyl sulfate and tetrabutylammonium hydrogen sulfate were selected as derivatization and activation reagents for the esterification reaction. An experimental design approach, central composition design (CCD), was employed to investigate and optimize the operative factors influencing the extraction efficiency, including extraction time, extraction temperature and ionic strength. The other parameters such as type of fiber coating, pH and derivatization conditions were also evaluated. SPME was finally carried out in headspace mode at 80 °C for 60 min with the presence of 3.00 g Na_2SO_4, using a home-made 44 μm PDMS fiber. Wide linear ranges and low limits of detection (0.06–1.24 ng L^{-1}) were obtained under the optimized conditions. The relative standard deviations (RSDs) and recoveries ranged from 0.5% to 12.3% and 85% to 110%, respectively. The proposed method was successfully applied to the analysis of the real surface water samples from the Pearl River Estuary.

1. Introduction

"Chemicals of emerging concern" (CECs) or "emerging contaminants" are defined as compounds not covered by existing water-quality regulations that could threaten organisms and human health when they are released into the environment [1]. CECs contain a diverse group of compounds, including algal and cyanobacterial toxins, brominated and organophosphate flame retardants, plasticizers, hormones, pharmaceuticals and personal-care products (PPCPs) and their metabolites, pesticides and their degradation/transformation products, etc. [2] In the last decade, pharmaceuticals such as steroids, lipid regulators, β-blockers, antipyretics, antidepressants resulting from anthropogenic activity and discharged pharmaceutical compounds have been a great concern because of their possibly toxic effects on organisms [3–6].

Pharmaceuticals have been found at very low concentrations ranging from ng L^{-1} to hundreds of μg L^{-1} in aquatic environments. As a consequence, simple, efficient and sensitive analytical methods are required to address the occurrence, concentration and fate of these compounds in the environment, especially in the natural waters.

Due to the complexity of the matrix and very low concentrations of the pharmaceuticals, there is an urgent need for sample preparation and concentration before detection. The methods routinely applied for pretreatment of pharmaceuticals in complex samples are solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [7–9]. However, the LLE methodology requires extensive use of organic solvent and is time-consuming, and the samples should be relatively clean when employing the SPE method. To overcome such problems, microextraction techniques such as solid-phase microextraction (SPME), stir-bar microextraction (SBME), microextraction by packed sorbent (MEPS) and hollow fiber liquid-phase microextraction (HF-LPME) have developed rapidly for drug analysis [10,11]. Solid-phase microextraction (SPME) is a solvent-free sample preparation technique that combines sampling, isolation, concentration and sample introduction into one step, which results in a significant reduction in expenditure of solvent and operation time, as well as the convenience of automation [12–15]. To date, SPME has been widely applied to the sampling and analysis of environmental, clinical, food, biological, forensic and pharmaceutical samples [16–20].

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http://dx.doi.org/10.1016/j.talanta.2014.11.071
0039-9140/

Liquid chromatography (LC) or gas chromatography (GC) coupled to MS or MS/MS are frequently used for the analysis of pharmaceuticals [21–26]. LC–MS/MS is becoming a more suitable technology in pharmaceutical analysis because of its high separation resolution, capability of identifying compounds and low limit of detection (LOD). However, this type of equipment is expensive, and its operation is complex. Therefore, GC/MS is still one of the commonly used methods for the rapid detection of pharmaceuticals. To analyze polar compounds with GC/MS, a derivatization procedure must be performed to increase the volatility and decrease the polarity of the analytes [27,28].

In current work, an automated SPME-GC/MS method with an aqueous derivatization step was developed for simultaneous determination of eight pharmaceuticals in water samples. The parameters influencing the extraction efficiency were optimized with single-factor analysis and central composition design (CCD) methods. The developed method was then successfully applied to the determination of these analytes in the surface water of Pearl River Estuary, South China.

2. Experimental

2.1. Chemicals and materials

Eight analytes including flufenamic acid (FLUF), mefenamic acid (MEF), flurbiprofen (FLUB), clofibrate (CLO), ketoprofen (KET), naproxen (NAP), tolfenamic acid (TOL) and gemfibrozil (GEM) were purchased from J&K Scientific Ltd. (Beijing, China). The derivatization reagent dimethyl sulfate (DMS) was obtained from Ai Keda Chemical Technology Co., Ltd. (Chengdu, China). Tetrabutylammonium hydrogen sulfate (TBA–HSO_4) was obtained from J&K Scientific Ltd. (Shanghai, China). A stock standard solution of 1000 mg L^{-1} of each compounds was prepared in chromatographic grade CH_3OH (Sigma-Aldrich, St. Louis, USA). The collected water samples were stored at 4 °C in the presence of NaN_3 (Tianjin Fuchen Chemical Reagents Factory, Tianjin, China).

Two types of commercial fiber, 65 μm PDMS/DVB and 85 μm PA, were supplied by Supelco (Bellefonte, PA, USA). The homemade 44 μm PDMS fibers were prepared based on the reported method [29].

2.2. Instrumentation

An Agilent 6890N GC/5975 MS system equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies) was used for the analysis. A MultiPurpose Sampler (Gerstel, Germany) was utilized for the automation of the method. Helium was used as the carrier gas, and the flow rate was set at 1.2 mL min^{-1}. The oven temperature was maintained at 80 °C for 1 min and then increased to 180 °C by 8 °C min^{-1}, held for 5 min, then reached 260 °C by 10 °C min^{-1}, held for 3 min. The total run time was 29.5 min. The MS system was operated in the electron ionization (EI) mode. The EI was set to turn on at 10 min (after the solvent delay). Quantification was performed using SIM mode and the m/z ratios for the target compounds are given in Table S1.

2.3. Solid-phase microextraction procedures

The SPME fibers were conditioned under a nitrogen atmosphere in an old GC injector at 250 °C for 30 min prior to use. Headspace mode was employed for the extraction of analytes from 10 mL of sample solution contained in a 20 mL sample vial. The concentration of the sample solution for the SPME optimization was 100 μg L^{-1} of each analyte. Before SPME, 3.0 g of Na_2SO_4 was dissolved in 10 mL of sample solution using ultrasonication, and then the sample vial was sealed with a magnetic crimp cap furnished with PTFE-faced septa. Subsequently, 20 μL of DMS and 40 μL of TBA–HSO_4 were injected into the sample with the autosampler. After incubation and reaction at 80 °C for 10 min, the analytes were extracted with the SPME fiber at 80 °C for 60 min at 500 rpm in the headspace mode. Finally, the SPME fiber was introduced into the GC injector for desorption at 250 °C. All of the extraction procedures, including the vial transfer, incubation, agitation, extraction and injection were auto-performed with the Gerstel autosampler using the Gerstel Maestro 1 software.

2.4. Experimental design

The SPME method requires optimization of large numbers of parameters to achieve the highest sensitivity for the all analytes, including extraction time, extraction temperature, stirring speed, desorption time, desorption temperature, pH, ion strength and derivatization conditions. However, it is very difficult to obtain the best extraction efficiency for each analyte because target analytes with different physicochemical properties may need different experimental conditions. Therefore, multivariate methods of optimization, including factorial design and response surface methods, have been employed to evaluate the main and interactive effects of the variables related to the analytical response [20,30–32]. Meanwhile, the experimental design can optimize the factors simultaneously with a reduced number of experiments.

In this study, the optimization experiments included three sections such as extraction, desorption and derivatization conditions. It is supposed that the influences on setting desorption temperature and time could be illustrated by a carryover experiment and they did not interact with extraction conditions. In addition, the derivatization parameters like sample pH, volume of DMS and volume of TBA–HSO_4 could be optimized using "one variable at a time" approach to make the esterification reactions were complete. Last, we considered the effects of extraction parameters such as extraction time, temperature, salting-out effect and agitation speed. Considering that the agitation speed of MPS autosampler varied from 250 to 750 rpm. To ensure the fast mass transfer process from water to bulk air then to the fibers and enough lifetime of fibers, we chose a medium agitation speed of 500 rpm. Therefore, the remaining extraction parameters, extraction time, temperature and mass of Na_2SO_4 were optimized using a central composition design method. The response surface was modeled by fitting the second-order polynomial models. The data obtained from the optimization procedures during the CCD experiments were analyzed by the Design-Expert 8.0.1 software.

3. Results and discussion

3.1. Development of SPME procedures

3.1.1. Optimization of the derivatization conditions

In a derivatization step, silylation and acylation derivatization 3reagents such as N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), hexamethyldisilazane (HMDS) and N-(*t*-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) are deactivated in an aqueous environment [33]. Dimethyl sulfate [34] and ethanol/pyridine/ethyl chloroformate [35] have been reported to react with pharmaceuticals to produce the corresponding derivatives directly in aqueous samples. The derivatization reagent DMS reacted with H_2O producing H_2SO_4 and CH_3OH, and then the CH_3OH reacted with the target acidic analytes producing the corresponding methyl esters. TBA–HSO_4 acted as the catalyst in the esterification reaction [34].