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Extraction of pyridine derivatives from human urine using electromembrane extraction coupled to dispersive liquid–liquid microextraction followed by gas chromatography determination

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ABSTRACT

In the present work, some of pyridine derivatives were analyzed for the first time in complicated biological fluids by coupling electromembrane extraction with dispersive liquid–liquid microextraction (EME–DLLME). 3-Methylpyridine, 2,4-lutidine, quinoline and 4-dimethylaminopyridine (DMAP) were extracted from urine and water samples. Effective parameters on the efficiencies of EME and DLLME were optimized by one variable at a time method and face-centered central composite design (FCCCD), respectively. The supported liquid phase (SLM) employed for the extraction of the analytes was a mixture of 90% 2-nitrophenyl octyl ether (NPOE) and 10% di-(2-ethylhexyl) phosphate (DEHP) which was immobilized in the pores of a piece of hollow fiber. An electric field was applied to carry over the analytes into acceptor solution. The acceptor solution was transferred to 1 mL of an alkaline solution (pH=13) and then DLLME procedure was performed. Preconcentration factors in the range of 40–263 and satisfactory repeatabilities ($2.3 < RSD\% < 5.3$) were obtained in different matrices. The method offered a good linearity with coefficient of determination greater than 0.9948 and was utilized for determination and quantification of pyridine compounds in smokers' urine samples. The proposed technique can be introduced as a simple, fast and inexpensive method for diagnosis of smokers.

1. Introduction

Pyridine derivatives are such compounds which have relatively high toxicities and are particularly hazardous. Most of the pyridine derivatives exist in cigarette smoke. A single puff of a cigarette exposes the body to over 4000 chemical compounds and 600 additives. Pyridine derivatives cause increase in heart rate, heart disease and stroke, blood pressure and lung cancer. So, it is obvious that how much harmful these compounds can be for human health. The presence of these toxic compounds in cigarette smoke has been recognized as an important incentive for development of rapid, sensitive and accurate quantitative methods to analyze them. There are few available techniques for the determination and quantification of 3-methylpyridine (MPY), 2,4-lutidine (LU), quinoline (QUI) and 4-dimethylaminopyridine (DMAP); such as electrostatic precipitation [1], gas chromatography (GC) [2,3], on-line hollow fiber liquid-phase microextraction [4] and gas chromatography–mass spectrometry (GC–MS) [5–9] in various matrices. Most of these methods were conducted in water and not in complex matrices. Due to the high number of interferences occuring in complicated matrices, such as biological fluids, and to improve detection limits, sample preconcentration and cleanup must be carried out before determination of them. A large number of modern sample preparation techniques, including solvent-free extractions or extraction procedures with very high sample to solvent ratio, for such liquid-phase microextraction (LPME) methods have been introduced. Among the LPME methods, dispersive liquid–liquid microextraction (DLLME) and hollow fiber-based liquid-phase microextraction (HF-LPME) have been applied more frequently by analytical chemists. However, these techniques have some drawbacks; DLLME is efficient only for simple matrices, because it creates crowded chromatograms for extracts from complicated matrices, especially biological fluids. Membrane technology has overcome this problem and HF-LPME based on passive diffusion is the major application of this type of technology. This method provides high degree of clean-up, particularly for complex matrices like biological fluids, and also good selectivity by choosing proper organic solvents. But in the case of HF-LPME, the extraction time needed is usually long; so that extraction times of 30–50 min are commonly reported for it [10]. In 2006, Pedersen-Bjergaard et al. reported a novel microextraction technique called electromembrane extraction (EME) [11]. EME can be used to extract ionizable compounds from plasma samples and other complicated biological matrices without protein precipitation

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[12,13]. In this method, an electric voltage is applied which facilitates the extraction of analytes across a hollow fiber membrane. This voltage causes EME to be more efficient than passive HF-LPME and extracts the analytes in a short time relative to the long time required for HF-LPME [14]. In this process, the ionized target analytes are extracted from an aqueous sample into an organic solvent located in the pores of a porous hollow fiber, and then transported into an aqueous acceptor solution inside the lumen of hollow fiber by the force of the electric potential across the SLM. One of the main disadvantages of EME is its incompatibility with gas chromatography (GC) instrument. Moreover, GC instrument is simpler, faster, and less expensive compared to high performance liquid chromatography instrument. It can easily be conjugated with different kinds of sensitive detectors; for example, flame ionization detector (FID) and mass spectrometry (MS). Since the direct injection of aqueous acceptor phase in EME may cause some difficulties for GC instrument, some attempts have been made to transfer analytes into GC-compatible phases [15–18]. Recently, Yamini et al. combined EME with DLLME and in this way, benefited from the high cleanup ability of the EME method as well as the DLLME compatibility with GC instrument [17]. Therefore, extraction of analytes from complex matrices such as biological fluids became possible and the final solution could be analyzed by GC.

In this work, EME joined to DLLME (EME–DLLME) was exploited for extraction and determination of pyridine derivatives in urine samples. To this end, ionized forms of the analytes were first extracted into an aqueous acceptor phase, located inside the lumen of a hollow fiber, under an electrical field using EME technique. Subsequently, DLLME was employed to transfer the target analytes into a final organic phase which was GC compatible. This simple and cost-effective method can be utilized straightforwardly in clinical centers to recognize smokers which may be a useful test for some worried families or employers.

2. Experimental

2.1. Equipments for EME–DLLME technique

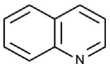
A 24-milliliter vial with an internal diameter of 2.5 cm and height of 5.5 cm was used as the sample compartment. Electrodes applied in this research were platinum wires with diameter of 0.25 mm, and were obtained from Pars Pelatine (Tehran, Iran). The electrodes were coupled to a power supply model 8760T3 with programmable voltages in the range of 0–600 V and current outputs in the range of 0–500 mA from Paya Pajoohesh Pars (Tehran, Iran). During the extraction, EME unit was stirred with speeds in the range of 0–1250 rpm via a heater-magnetic stirrer model 3001 from Heidolph (Kelheim, Germany) using a 1.5×0.3 cm^2 magnetic bar.

A 40-kHz, 0.138-kW (Tecno-Gaz SpA, Italy) ultrasonic water bath with temperature control was exploited to emulsify the organic solvent in the aqueous solution. A Selecta lab model Tl320 centrifuge (Barcelona, Spain) was employed for phase separation of the cloudy solution.

2.2. Chemicals and materials

MPY, LU, QUI, and DMAP were acquired from Sigma (St. Louis, MO, USA). Chemical structures and amounts of log P and pK_a of the analytes are presented in Table 1. Compounds 2-nitrophenyl octyl ether (NPOE), 2-nitrophenyl pentyl ether (NPPE), tris-(2-ethylhexyl) phosphate (TEHP) and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). Acetone, chloroform, methanol, acetonitrile, dichloromethane, trichlorobenzene, carbon tetrachloride and carbon disulfide were supplied by Merck.

Table 1
Chemical structures and amounts of pK_a and log P for the analytes.

Compound	Abbreviation	Chemical structure	pK_a	Log P
3-Methylpyridine	MPY		5.63	1.11
2,4-Lutidine	LU		6.46	1.65
Quinoline	QUI		4.81	2.03
4-Dimethylaminopyridine	DMAP		9.53	−0.9

All of the chemicals used were of analytical-reagent grade. The porous hollow fiber utilized for the SLM was a PPQ3/2 polypropylene hollow fiber from Membrana (Wuppertal, Germany) with inner diameter of 0.6 mm, wall thickness of 200 μm, and pore size of 0.2 μm. Ultrapure water was prepared by a Younglin aquaMAx purification system 370 series (Kyounggi-do, Korea).

2.3. Biological and standard solutions

Urine samples were collected from three smokers and one person who had not smoked at all (as a match matrix for plotting the calibration curves). All samples were stored at −4 °C, thawed and shaken before extraction. A stock solution, containing 1 mg mL^{-1} of the analyte in methanol, was prepared and kept at −4 °C protected from light. Working standard solutions were prepared by diluting the above stock solutions with methanol.

2.4. Gas chromatography apparatus

Separation and detection of MPY, LU, QUI, and DMAP were performed by an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) equipped with a split-splitless injector and a flame ionization detector (FID). A 30-m HP-5 Agilent fused-silica capillary column (0.32 mm i.d. and 0.25 μm film thickness) was applied to the separation of the target compounds. Helium (purity 99.999%) was used as carrier gas at constant flow rate of 2.0 mL min^{-1}. Temperatures of the injector and the detector were set at 280 °C and 300 °C, respectively. Oven temperature program was as follows: 40 °C for 2 min, increasing to 130 °C with a ramp of 15 °C min^{-1}, increasing to 280 °C with a ramp of 100 °C min^{-1}, and holding at 280 °C for 3 min.

2.5. EME–DLLME procedure

The equipment exploited for EME–DLLME procedure is shown in Fig. 1. Twenty four milliliters of the sample solution, containing the target analytes, was transferred into the sample vial. To impregnate the pores of hollow fiber with the organic solvent, a piece of the hollow fiber was cut out (4 cm) and dipped in the solvent for 5 s and then the excess amount of solvent was gently wiped away by air blowing using a medical syringe. The upper end of the hollow fiber was connected to a medical needle tip as guiding tube which was inserted through the rubber cap of the vial. A 100 mmol L^{-1} HCl solution (as an acceptor phase) was introduced into the lumen of hollow fiber by a microsyringe and then the lower end of hollow fiber was mechanically sealed. One platinum cathode was introduced into the lumen of the fiber. The fiber, containing the cathode, the SLM and the acceptor solution,