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Partly-Immersed Three Phase Hollow Fiber Liquid Phase Microextraction of Semi-Volatile Organic Compounds Combined with High Performance Liquid Chromatography

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A novel method for fast determination of nicotine in tobacco and cigarette was established by using high performance liquid chromatography (HPLC) coupled with a partly immersed three phase hollow fiber liquid phase microextraction (PILPME) technique for sample preparation. The model of partly immersed was introduced for the first time. This study has demonstrated that PILPME constituted a real alternative to the other liquid phase microextraction methods. Parameters related to PILPME (organic solvent, pH of donor and acceptor phase, the proportion of hollow fiber in gas and liquid, extraction time, temperature) were optimized experimentally, for preconcentration and extraction of nicotine in tobacco and cigarette samples has shown many advantages over traditional sample preparation technique, which provided a good linear range (0.05-10 μg/mL) with $r^2 = 0.9996$, a low detection limit (5 ng/mL, S/N = 3) and a satisfactory relative standard deviation (< 8 %). The result showed 3.03 and 3.39 mg/g in tobacco and 1.51, 1.45 and 1.73 mg/g in cigarette, respectively. The recovery was obtained in the range of 95.91-97.95 %.

Key Words: Partly immersed liquid phase microextraction, High performance liquid chromatography, Nicotine, Sample preparation technique, Tobacco, Hollow fiber.

INTRODUCTION

Analyte extraction and pretreatment is the most challenging and time consuming step in an analytical procedure. There are several approaches to accomplish this[1], including superheated water extraction (SHWE), super critical fluid extraction (SFE), microwave assisted extraction (MAE), ultrasonic extraction (USE), liquid-liquid extraction (LLE), solid phase microextraction (SPME), membrane extraction (ME), liquid phase microextraction (LPME), *etc*. Recently, LPME has attracted increasing attention as a novel technique for sample preparation. Since it was first introduced by Jeannot and Cantwell in[2], LPME has drawn more and more attention quickly. The original microextraction model was completed with an organic solvent microdrop suspended on the tip of either a Telflon rod or microsyringe, which was immersed in/upon the stirring aqueous sample solution[3,4]. However, the single microdrop was not robust. It may be lost and evaporated during microextraction process. Lately, Pedersen-Bjergaard and Rasmussen developed a new liquid phase microextraction based on a pierce of hollow fiber to protect and contain solvent micrdrop[5-9]. Until now, models of three phase LPME, two phase LPME, static LPME, dynamic LPME, headspace LPME, surfactant enhanced LPME[10], coneshaped membrane protected-LPME[11], fiberintube microextraction[12], floating organic drop LPME[13] and so on have been proposed and utilized to determine PAHs[14-16], aniline and its derivatives[17,18], phenolic compounds[19-21], organic compounds in pesticides[22-24] and drugs[25-27] coupled with HPLC, CE, GC, LC/MS, GC/MS and MS.

The amount of sample preparation needed depends on the sample matrix and the properties and level of analyte to be determined. As for non-volatile organic compounds (VOC), we can use LLE, immersed LPME[28] and so on. Direct headspace sampling has been widely used for determining VOCs[29] without interference. The classical headspace analysis is done by sealing the sample in gas-tight vial with a septum. After a prescribed extraction time, the analyte vapour is sampled, generally with a gas tight microsyringe. However, such a method is only suitable for highly volatile compounds and requires that the analyte possesses high Henry's Law constant[30]. Thus, its application is limited. Techniques such as purge and trap, headspace solid phase microextration (HSSPME), headspace liquid phase microextraction (HSLPME)[31] have been developed to improve extraction efficiency and widen their application in VOC and semi-VOC analysis. Although the HSSPME and HSLPME can use to preconcentrate main VOC and semiVOC, as for few analytes

can not been concentrated efficiency, time consuming, low recovery[10,32]. Thus, it is the high time and necessary to establish a sample preparation suitable for semi-VOC analysis wholly and efficiently.

In this study, we developed a new approach to semi-VOC analysis, that is partly immersed three phase hollow fiber liquid phase microextraction (PILPME) controlled by a syringe. Part of the hollow fiber exposing in gas phase, the other part immersed in liquid phase. Thus, it could simultaneity preconcentrate analytes in gas and liquid phase. Nicotine (*Nicotina* or *Nicotia*), β-pyridyl-α-*n*-methyl-pyrrolidine, boiling at 247 ºC, semivolatile was selected as model compound since it is the main alkaloid in tobacco and cigarette and widely used in industry of fine chemicals, pharmacy, organic synthesis, industry of national defence, agriculture and so on. The newly developed technique was used to evaluate the content of nicotine in tobacco and cigarette. The result showed that the procedure is an efficient, low cost, fast and green sample preparation for determination nicotine in tobacco and cigarette coupled with HPLC. This work provided an alternative method to determine the semivolatile organic compounds.

EXPERIMENTAL

Nicotine was obtained from MERK-Schuchardt (Germany). HPLC-grade acetonitrile and methanol were purchased from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). Other chemicals were of analytical grade and used without further purification. All water used during experiment was doubly deionized water obtained. The Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). Stock solution containing 1 mg/mL of nicotine was prepared in methanol and stored at 4 ºC. Standard solutions of different concentrations were obtained by diluting the 1 mg/mL stock solution with doubly deionized water.

A 25 μL microsyringe (Hamilton,Shanghai). KQ3200 ultrasonic oscillator (Kunshan, China). CL-3A constant temperature-heat magnetic stirring apparatus (AM-3250A, Nanjin). PHS-3C pH meter (Kunshan, China).

Preparation of tobacco and cigarette sample: The tobacco and cigarette were purchased from market, was dryed under vacuum at 100 ºC for 1 h, then making them as powder and filtrated for 80 mesh. Then folded in aluminium foil and sealed by plastic bag. One mg of tobacco or cigarette was mixed with 25 mL doubly deionized water and ultrasonic extraction for 0.5 h. Transferred directly 2 mL as donor phase for further extraction and HPLC analysis.

HPLC determination: The Shimadzu LC-20AT 600 HPLC instrument (Shimadzu, Japan) consisting UV-SPD-20A detector was employed. The detail HPLC parameters were displayed as followings. Analytical column: ODS® dC_{18} (5 μm, 4.6 mm × 150 mm). Mobile phase consists of 10 mM KH_2PO_4, methanol, triethylamine (90 + 10 + 0.1, V + V + V), pH 2.5 (adjust by H_3PO_4), which was filtered by Milli-Q filtering system. Flow rate: 0.6 mL/min. Detection wavelength: 260 nm. Column temperature: room temperature.

PI-LPME procedures: Prior to use, an Accurel Q3/2 hollow fiber (53 cm) was cut into 3 cm segments and these segments were ultrasonically cleaned 15 s in acetonitrile and dried in air and one of the dry segment was immersed into organic solvent to impregnate the wall pores with ultrasonic for another 10 s. Then the fiber segment was removed out and the surface organic solvent was wiped away with dry filter paper. Meanwhile, 20 μL acceptor phase was drawn into a 25 μL HPLC syringe (0.7 mm o.d.), which had been washed by acceptor phase more than 5 times to avoid the analyte carryover and air bubble. The syringe was then inserted through the rubber lid of sample vial (1.2 cm i.d. × 5 cm height) into one end of the prepared fiber segment at the needle tip. After that, the acceptor phase in syringe was pushed out carefully to flush the excess of organic solvent in the lumen of fiber. The other end was sealed with hot nipper to make sure the length of fiber segment was 2.5 cm from the sealed end to the syringe needle tip and the acceptor phase contained in the lumen was about 6 μL. Then the syringe was fixed to immerse fiber segment in the donor phase, let partly of the hollow fiber in liquid and exposure partly of the hollow fiber in the headspace of sample can extract the nicotine in liquid and gas phase synchronously (Fig. 1). A rubber lid was used to cover the vial during extraction to prevent the evaporation of the nicotine and organic phase. Once the position of fiber segment in donor phase fixed, it would not be altered further. After prescribed extraction time with a stirring rate of 1000 rpm, the syringe was taken away and the sealed end was unsealed. 5 μL aliquot of the acceptor phase was drawn out and injected into HPLC without any further treatment. Because of the fiber is very cheaper and in order to avoid the sample carryover, one segment was used only one time.

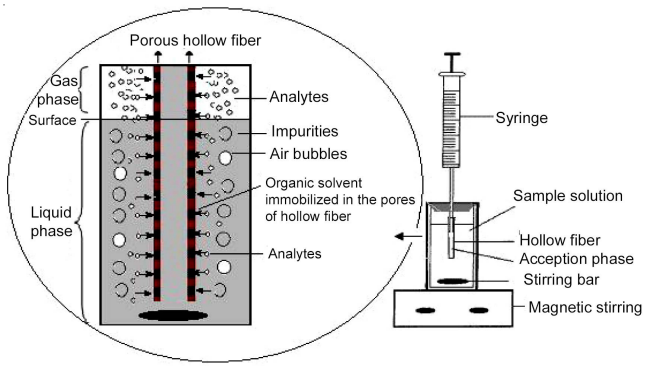

Fig. 1. Experimental setup of PILPME

RESULTS AND DISCUSSION

Basic principle: Partly immersed three phase hollow fiber liquid phase microextraction consists of two three phase system that includes gas and liquid. In gas, includes the analyte of gas phase, organic phase immobilized in the pore of hollow fiber wall and then back extracted into the acceptor phase in the lumen; in liquid, includes the analyte of liquid phase, organic phase immobilized in the pore of hollow fiber wall and then backextracted into the acceptor phase in the lumen. The proportion of hollow fiber in gas and liquid phase, extraction time and temperature are very important. At first, make sure of the analyte been fully extracted into the acceptor phase in the

hollow fiber lumen of liquid part. Then, heated for 3 min at 60 ºC in the thermostatted water bath, in order to drive and accelerate both of the analytes in liquid and gas been preconcentration into the acceptor phase in the hollow fiber lumen of gas part.

It was necessary to convert the analyte by some reactions, such as protonation, complexation, so that the converted analyte had slight affinity for the organic phase and was easy to be back extracted into the acceptor phase[33]. In this study, protonation was used to promote the extraction. Owing to the alkalinity of nicotine, the solubility in water was decreased by basic donor phase. Then it was easily extracted into the organic phase under stirring and further backextracted into acid acceptor phase.

The target analyte in gas and liquid part both were extracted into organic phase immobilized in the pore of hollow fiber wall and then backextracted into the acceptor phase in the lumen of gas and liquid part, respectively.

Optimization of the parameters related to PI-LPME

Selection of organic solvent: Selection of proper solvent was prime key for PI-LPME. The solvent should be strongly immobilized within the pores of the hollow fiber and should provide appropriate solvent polarity, selectivity, volatility, viscosity and low solubility in water[7,34]. The organic solvents used to extract nicotine were dichlorlmethane[4] and ethyl ether, which were not suitable for PI-LPME due to their high volatility. In the study, 1-octanol, tri-*n*-butylphosphate (TBP), tri-*n*-octylamine (TOA), amyl alcohol were investigated (Fig. 2). Experiments were conducted at room temperature for 10 min with 5 mL donor phase (2 mL of 20 µg/mL working solution of nicotine + 2 mL of 0.1M KOH) and 10 mM KH_2PO_4 (pH 2.5, adjusted by H_3PO_4) as acceptor phase. The stirring rate (*ca.* 800 rpm) was fixed to avoid formation of air bubble during extraction and not altered in further study. The results show that 1-octanol had best extraction performance (Fig. 2). Thus, it was selected for subsequent experiments.

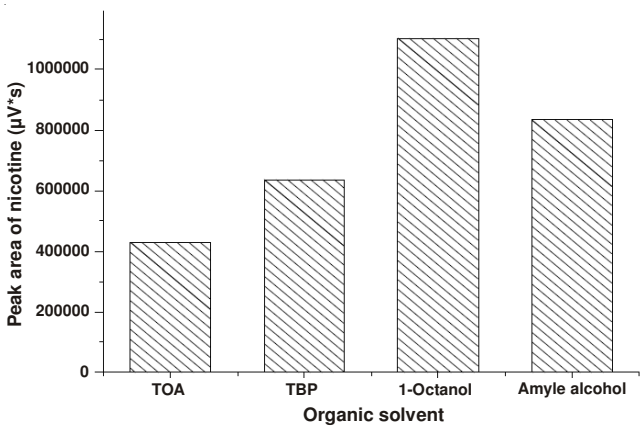

Fig. 2. Selection of organic solvent

Effect of stirring rate: Magnetic stirring rate was used to facilitate the mass transform process and improve the extraction efficiency. The extraction efficiency increased with the stirring rate from 0-1200 rpm, but at 1200 rpm the repeatability was poor. It may be caused by the air bubble under high stirring rate. 1000 rpm was selected as optimum stirring rate.

pH of donor phase and acceptor phase: For a weak organic base, the extraction efficiency depended on both the pH of the donor phase and acceptor phase. The pH of the donor phase was adjusted to appropriate alkalinity prior to extraction so that nicotine was deionized and extracted in organic phase easily. In this experiment, 2 mL of 20 µg/mL standard solutions of nicotine adjusted by 2 mL of KOH at different concentrations were investigated as donor phases (Fig. 3).

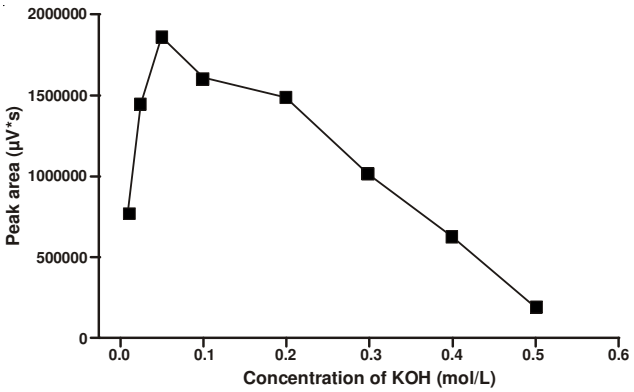

Fig. 3. Effect of pH of donor phase

A series of 10 mM phosphate buffer solutions at different pH was selected as acceptor phase according to the studies of Pedersen-Bjergaard *et al.*[26]. It must provide both sufficient protons consumed during microextraction and low pH to ionize the analytes. The extraction performances at different acceptor phase pH were plotted in Fig. 4. Based on these investigations, a donor phase consisting of 2 mL of 20 µg/mL standard solution of nicotine and 2 mL of 0.05M KOH and an acceptor phase of 10 mM phosphate buffer solution at pH 3.0 were adopted in the following studies.

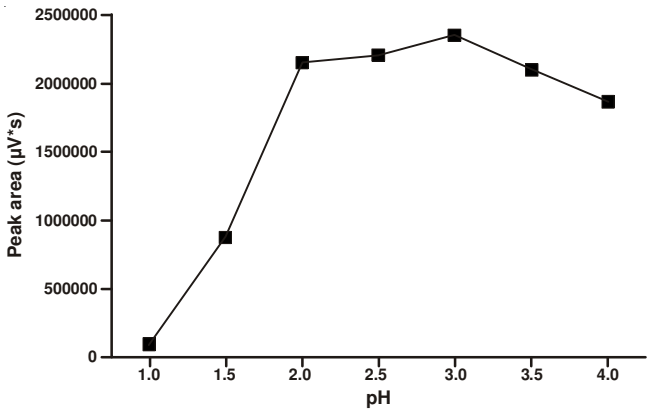

Fig. 4. Effect of pH of acceptor phase

Salt effect: Salt addition in donor phase may have various effects upon extraction: it may enhance, not influence or even limit extraction[35] . The result of enhancement is due to the salting out effect and the contradictory result is due to the electrostatic interaction between polar molecules and salt ions[17]. The increasing viscosity of donor phase by salt addition can also decrease the extraction efficiency. Experiments demonstrated that the peak area of nicotine was not altered significantly by salt (NaCl) addition from 0-50 mg/mL. Therefore, no salt was added into donor phase in the further studies.

Effect of extraction time: The optimization of extraction time was conducted at the above optimized parameters. The result displayed in Fig. 5, showed that the highest peak area was obtained at 17 min. Further increasing of extraction time resulted in decreasing of extraction efficiency due to the solubility of 1-octanol in donor phase. According to this investigation, 17 min was chosen as the optimum extraction time.

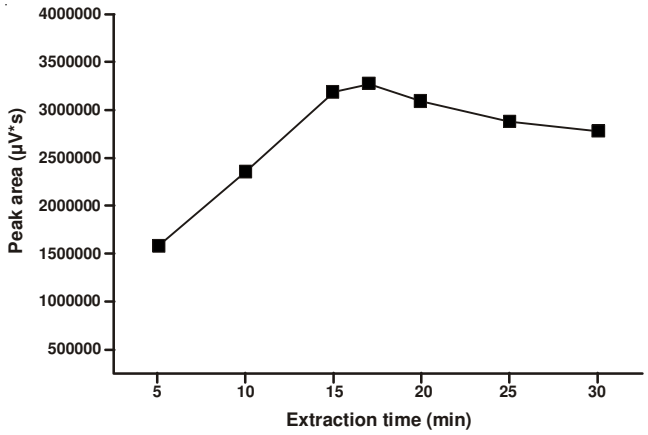

Fig. 5. Effect of extraction time

Proportion of the hollow fiber in gas and liquid: According to the definition of the US Environmental Protection Agency[36], the boiling point of nicotine is 247 ºC,belongs to semi-VOC, can vapourize with water vapour. So let partly of the hollow fiber in liquid and exposure partly of the hollow fiber in the headspace of sample can extract the nicotine in liquid and gas phase synchronously. In present study, we let a bit of hollow fiber (*ca.* 6 mm) exposured fully over the liquid surface, after extracted for a suitable time, then drawing out the accepter phase injected into HPLC. The effect is showed that 296611.344 peak area of nicotine was obtained. The experimental result showed firmly that the hollow fiber exposured fully over the liquid surface have a good extraction effect. This indicate that establishing partly immersed LPME is feasible. In order to attain the best proportion of the hollow fiber in gas and liquid,the different proportion have been choosed to experiment, the result of proportion in air and liquid on extraction efficiency are presented in Fig. 6. The experimental results showed that the changes in proportion affected the extraction strongly. The best proportion is 1/5, when the proportion more than 1/5, the extraction effect is not good because of most of the nicotine are existing in liquid, only a fat lot volatilization. On the other hand, when the proportion less than 1/5, the extraction effect is also not good because of the hollow fiber in gas is too hot that can not extract primely the nicotine in gas. The 1/5 was accepted as best proportion and used throughout the remaining experiment.

Effects of temperature: The influence of temperature on the extraction was examined over a range of 30-100 ºC. It will have a bad extraction if the whole extraction process was heated in the thermostatted water bath. There are two reasons, (1) the main nicotine are exist in liquid. (2) Heated will cause many bubble cling to the hollow fiber affecting the mass transform process and debasing the extraction efficiency. In present

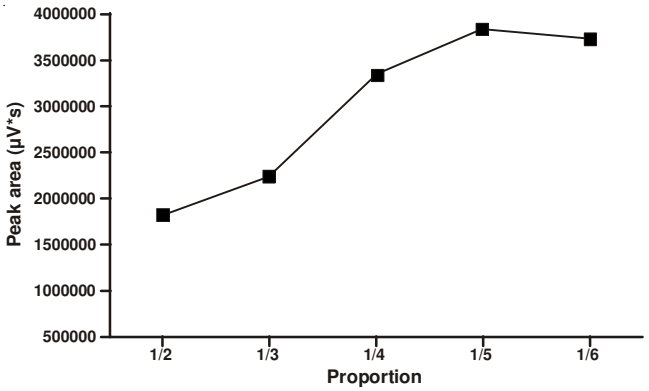

Fig. 6. Effect of proportion in air and liquid on extraction efficiency

study, heated in the thermostatted water bath of 60 ºC for 3 min after extracted for 17 min without thermostatted water bath. 17 min can ensure the nicotine of liquid achieving extraction balance, thermostatted water bath for 3 min was used to facilitate the mass transform process in gas phase.

Method validation: Validation of the method was evaluated by estimating the limit of detection, linearity, precision and enrichment. The method exhibited good linearity over the calibration range of 0.05-10 μg/mL of nicotine in 4 mL donor phase with squared regression coefficient (r^2) = 0.9996, which was investigated by PI-LPME under the optimum conditions. Limit of detection (LOD) of nicotine in this study, calculated on the ratio of signal to noise at 3 (S/N = 3), was 0.005 μg/mL. The precision estimated at a concentration level of 10 μg/mL nicotine in donor phase had satisfied result with the relative standard deviation (RSD) = 3.4 % (n = 5).

The enrichment factor (EF) of nicotine, which was defined as the ratio of the final concentration of the analyte in acceptor phase after LPME to its initial concentration in acceptor phase[37], was about 200 at a level of 2 mL of 2 μg/mL standard adjusted by 2 mL of 0.05 mM KOH as donor phase in this study. The value was not so high as other liquid phase microextractions of other analytes reported in references[38,39] due to the relatively high solubility of nicotine in water.

Analysis of tobacco and cigarette samples: The tobacco (2.3 mL) and cigarette (2 mL) samples were treated 2 mL of 0.05 mM KOH as donor phase, respectively. The method of internal standard addition calibration was used to determine the nicotine content of samples (Table-1). The relative recovery was calculated as the ratio of the peak area of spiked sample after LPME to the peak area of the same concentration of standard solution after LPME. The results of different sample determination are listed in Table-1. The relative recoveries of spiked sample were all over 95 %.

Conclusion

A novel procedure for determination of nicotine in tobacco and cigarette by HPLC with partly immersed three phase hollow fiber based liquid phase microextraction (PI-LPME) as sample preconcentration was developed in this study. The TP-HF-LPME method reduced the extraction time of nicotine from tobacco and cigarette dramatically and simplified the conventional sample preparation of LLE into one step. It also provided good linear range (0.05-10 μg/mL, r^2 = 0.9996), low detection limit (0.005 μg/mL, S/N = 3) and excellent precision (RSD <

TABLE-1
CONTENT OF NICOTINE IN DIFFERENT TOBACCO AND CIGARETTE

Samples	Spiked concentration (μg/mL)	Actual concentration of nicotine (mean ± SD, μg/mL) (n = 5)	Nicotine content (m/m, %)	Recovery (mean ± SD) (n = 5)
Tobacco 1	0	2.42 ± 1.26	3.03	–
	0.5			96.57 ± 4.26
	1.0			97.95 ± 4.18
Tobacco 2	0	2.71 ± 0.15	3.39	–
	0.5	3.09 ± 0.17		96.26 ± 5.29
	1.0	3.61 ± 0.20		96.52 ± 5.54
Cigarette 1	0	1.21 ± 0.11	1.51	–
	0.5	1.64 ± 0.13		95.91 ± 7.93
	1.0	2.12 ± 0.13		95.93 ± 6.13
Cigarette 2	0	1.16 ± 0.12	1.45	–
	0.5	1.61 ± 0.12		96.98 ± 1 7.45
	1.0	2.11 ± 0.14		97.69 ± 1 6.63
Cigarette 3	0	1.38 ± 0.11	1.73	–
	0.5	1.82 ± 0.13		96.81 ± 7.14
	1.0	2.30 ± 0.13		96.64 ± 5.65

8 %, n = 5). Therefore, it might be considered as fast, effective, solvent free and low cost alternative sample preparation for extraction of nicotine from tobacco and cigarette samples. This work provided an alternative method to determine the semivolatile organic compounds.

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