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Hollow fiber-liquid phase microextraction combined with gas chromatography for the determination of phenothiazine drugs in urine

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ABSTRACT

A simple method of hollow fiber-liquid phase microextraction (HF-LPME) combined with gas chromatography (GC) was developed for the analysis of four phenothiazine drugs (promethazine, promazine, chlorpromazine and trifluoperazine) in human urine samples. All variables affecting the extraction of target analytes including organic solvent type, stirring rate, extraction time, extraction temperature, pH of sample solution and ionic strength were carefully studied and optimized. Under the optimal conditions, the analytical performance of HF-LPME-GC-flame photometric detector (FPD) and HF-LPME-GC-flame ionization detector (FID) were evaluated and compared. The results showed that the HF-LPME-GC-FID was more sensitive than HF-LPME-GC-FPD for the determination of four target phenothiazine drugs, while the signal peak shape and resolution obtained by HF-LPME-GC-FPD was better than that obtained by HF-LPME-GC-FID. HF-LPME-GC-FPD/FID was successfully applied for the assay of the interested phenothiazine drugs in urine sample, and the excretion of the drugs was also investigated by monitoring the variation of the concentration of chlorpromazine in urine of a psychopath within 8 h after drug-taking. The proposed method provided an effective and fast way for the therapeutic drug monitoring (TDM) of phenothiazine.

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1. Introduction

The use of phenothiazine drugs with well-demonstrated efficacy in psychiatric disorders has become wide spread since 1950s. Presently, these drugs are still being prescribed predominantly for psychosis treatment, such as schizophrenia, and they are also used as antiemetic and antihistaminic medicines. Their action mechanism is based on the blockade of nervous impulses from the central nervous system (CNS), because phenothiazine drugs are antagonists of dopamine receptors. Fig. 1 shows the general structure of 2- and 10-disubstituted phenothiazine drugs. They usually exhibit low concentration level in blood but high- or medium-concentration levels in urine of the medicine taker.

Overdoses of these drugs are common, and are potentially life-threatening for patients. Therapeutic drug monitoring (TDM) is a practical tool that can help the physician to provide an effective and safe drug therapy for the patients who need medication. To help with TDM in phenothiazine medication, simple and sensitive methods are required in the judgment that if the concentration level of phenothiazine in patients is excessive or if the therapeutic effect of the drug is as expected.

Many analytical methods have been reported for the determination of phenothiazine drugs, such as gas chromatography (GC) [1–3], gas chromatography-mass spectrometry (GC-MS) [4], high-performance liquid chromatography (HPLC) [5–7], liquid chromatography/tandem mass spectrometry (LC/MS/MS) [8,9], and capillary electrophoresis (CE) [6,10,11]. For GC analysis of these drugs, the detectors of flame ionization detector (FID) [1], nitrogen phosphorus detector (NPD) [12] and flame photometric detector (FPD) [13] are mostly employed.

Because of the low concentration of these drugs in biological samples (blood or urine), separation and preconcentration steps are usually required before their instrumental analysis. Ohashi et al. [1] developed a method of cloud point extraction (CPE) for the determination of phenothiazine drugs in spiked human serum by GC. The obtained surfactant-rich phase after phase separation was spiked with methanol for the removal of matrix including fat and protein. After centrifugation, the supernatant was passed through a cation exchange column to remove the surfactant (Triton X-114) used in CPE, and then introduced into GC for subsequent analysis of target phenothiazine drugs. Lara et al. [10] combined solid-phase extraction (SPE) and field-amplified sample injection-capillary zone electrophoresis (FASI-CZE) to determine five phenothiazine drugs in human urine. Besides, solid-phase microextraction (SPME) is a recently developed technique, which integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. With a polyacrylate-coated

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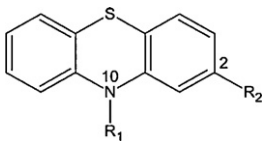


Fig. 1. General structure of 2- and 10-disubstituted phenothiazine drugs.

fiber, SPME was also applied for LC/MS/MS determination of 11 phenothiazine drugs with heavy side-chains in human whole blood and urine samples [8]. However, it should be pointed out that the SPME fiber definitely suffers from the fragility and carry-over effects.

Liquid phase microextraction (LPME) was another novel microextraction technique introduced by Jeannot and Cantwell in 1996 [14], it is simple, fast and inexpensive. Single drop microextraction (SDME) and hollow fiber-liquid phase microextraction (HF-LPME) are the two sampling modes of LPME. Compared to conventional liquid–liquid extraction (LLE), SDME would provide higher enrichment factor, superior selectivity, and significantly reduced solvent consumption. Nevertheless, the microdrop suspended on the needle of microsyringe is easily dislodged during extraction, especially the case when samples are stirred vigorously. HF-LPME proposed by Pedersen-Bjergaard and Rasmussen [15] could solve the aforementioned problem effectively. In this mode, porous hollow fibers made of polypropylene were used to protect the extraction solvent. The microextract is not in direct contact with the sample solution, so the samples may be stirred or vibrated vigorously without any loss of the microextract. Micro pores of the hollow fiber would prevent large molecules (like proteins) and other impurities from entering into the microextract in the lumen. Moreover, the disposable hollow fiber could avoid cross-contamination. Thus, HF-LPME is a more robust and reliable alternative to SDME, which may provide high preconcentration, short extraction time and excellent sample clean-up capability. It has been applied for the analysis of organic pollutants in a variety of samples, such as soil [16], water [17,18], vegetables [19], fruit juice [20] and drugs [21–25].

Recently, Valcarcel and co-workers [25] developed a method for the analysis of seven phenothiazines derivatives in human urine by using dynamic liquid-phase microextraction-liquid chromatography with 50 μL of the ionic liquid 1-butyl-3-methyl-imidazolium hexafluorophosphate as the extraction solvent. This method is a valuable alternative for the analysis of these drugs in urine. The aim of this work was to develop a new method of HF-LPME-GC for the analysis of phenothiazine drugs in urine samples. Four of the most frequently used phenothiazine drugs (promethazine, promazine, chlorpromazine and trifluoperazine) were selected as the target analytes, and the experimental conditions affecting the extraction of the target analytes by HF-LPME were optimized. The analytical performance of HF-LPME with GC-FPD and GC-FID were evaluated and compared. The proposed method of HF-LPME-GC-FPD/FID was applied for the analysis of the target phenothiazine drugs in urine sample, and the excretion of the drugs was also investigated by the monitoring of the variation of the concentration of phenothiazine drug in the urine of a psychopath.

2. Experimental

2.1. Reagents and materials

Promethazine HCl purchased from Donggang Kangyuan Pharmaceutical Co., Ltd. (Liaoning, China), promazine HCl purchased from Changzhou Nanjiang Pharmaceutical & Chemical Co., Ltd. (Jiangsu, China), chlorpromazine HCl purchased from Shanghai Medicament No. 15 Pharmaceutical Factory Co., Ltd. (Shanghai, China), and trifluoperazine HCl purchased from Taicang Hengyi Medical & Chemical Material Factory (Jiangsu, China) were selected as the target analytes, and their purity are all above 99%. The chemical structure and therapeutic range [26] of these four phenothiazine drugs were presented in Table 1. Tetrachloromethane, toluene, cyclohexane, dodecane and other reagents used in the experiment were of analytical reagent grade or better. Doubly distilled water was used throughout this work.

Individual stock solutions (1 mg/mL) of these drugs were prepared in ethanol. All standard solutions were kept in the dark at 4 °C, and diluted working solutions were prepared daily before the analysis. 0.1 mol/L HCl and NaOH were used to adjust pH of the solution.

The Q3/2 Accurel polypropylene hollow fiber was purchased from Membrana GmbH (Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. Hollow fiber was cut into several segments, each was 15 mm long and the approximate internal volume was 4 μL.

2.2. Preparation of samples

Drug-free urine (control urine) was obtained from healthy persons with no recent history of drug-taking, and stored at −10 °C. It was used for method development and calibration. Case urine samples were obtained from a psychopath, also a chlorpromazine taker, in Renmin Hospital of Wuhan University (Wuhan, China). It was stored in darkness at −10 °C. After the adjustment of the sample solution pH to 9, drug-free urine samples spiked with target drugs and case urine samples were subjected to HF-LPME according to the procedure given below.

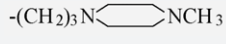
2.3. HF-LPME procedure

Three milliliter sample solution was filled into a 4 mL vial, and then the vial was placed on an 85-2A constant temperature magnetic stirrer (Ronghua, Jiangsu, China) for the subsequent extraction. Four microliter of organic solvent was withdrawn into the microsyringe, and the needle tip was then inserted into a 15 mm length hollow fiber. After the hollow fiber was immersed in the solvent for about 5 s to impregnate the pores with the solvent, the solvent in the syringe was injected completely into the hollow fiber. The microsyringe was then fixed by a stander so that the hollow fiber was immersed into the sample solution together with the microsyringe needle. The magnetic stirrer was switched on at a stirring rate of 1000 rpm, and HF-LPME extraction was maintained at 40 °C for 10 min. After extraction, the solvent in the hollow fiber was retracted back into the microsyringe for GC analysis, and the hollow fiber was discarded.

2.4. GC analysis

GC analysis was performed by an Agilent 6890 gas chromatograph equipped with a flame photometric detector (FPD) and a flame ionization detector (FID). The FPD was operated in the sulphur mode at 200 °C, and the FID was operated at 300 °C. In the optimization process of HF-LPME, FPD was applied as the detector of GC analysis. An HP-5 capillary column, with 30 m × 0.32 mm I.D. and a film thickness of 0.25 μm was used for the separation of the four target phenothiazine drugs. The column oven temperature program was as follows: firstly held at 250 °C for 3 min, then programmed at 20 °C/min to 280 °C and held for 2 min. N_2 (99.999%) was used as the carrier gas with a constant flow rate of 2 mL/min. After HF-LPME, 1 μL analyte-enriched solvent was immediately injected into GC in splitless mode for subsequent analysis.

Table 1
The chemical structure and therapeutic range [26] of examined phenothiazine drugs.

Phenothiazine	R_1	R_2	pK_a	Therapeutic range (ng/mL)
Promethazine	$-CH_2CH(CH_3)N(CH_3)_2$	H	9.1	100–400
Promazine	$-(CH_2)_3N(CH_3)_2$	H	9.24 (9.4)	No data
Chlorpromazine	$-(CH_2)_3N(CH_3)_2$	Cl	9.2 (9.3)	50–500
Trifluoperazine		CF_3	8.4	5–50

3. Results and discussion

3.1. Optimization of HF-LPME

In order to obtain the best analytical performance, experimental parameters that influence on the HF-LPME procedure have been investigated and optimized.

3.1.1. Sample volume and solvent type

The effect of sample volume was investigated, and the results showed that no significant variation was observed for all the target analytes when the sample volume was varied in the range of 3–8 mL. In subsequent experiment, 3 mL of sample solution was used.

In this study, the effect of the extraction solvent on the extraction of four target analytes by HF-LPME was investigated. Four different kinds of solvents including toluene, tetrachloromethane, cyclohexane and dodecane were tested. The experimental results demonstrated that toluene and tetrachloromethane gave much better extraction efficiency for all tested target analytes. Considering that the solubility of toluene in water is lower than that of tetrachloromethane, toluene was chosen as the solvent for the extraction of target phenothiazine drugs by HF-LPME.

3.1.2. Stirring rate and extraction time

To evaluate the effect of stirring rate on the extraction of four target analytes, sample solutions were continuously agitated at different stirring rates and the signal intensities of target analytes in the post-extraction phase were obtained. It was found that the signal intensities of all phenothiazine drugs were increased with the increase of stirring rate from 400 to 1000 rpm. When the stirring rate was further increased from 1000 to 1200 rpm, the signal intensities of the target analytes were slightly decreased. This could be attributed to the dissolution loss of the organic phase induced by the higher speed agitation. Besides, air bubble formation frequently occurred nearby the hollow fiber under the high-speed agitation, which would damage the procedural reproducibility. Consequently, a stirring rate of 1000 rpm was chosen for the further work.

The effect of extraction time on HF-LPME was investigated with the time varying from 0 to 15 min. The experimental results indicated that signal intensities were increased with the increase of extraction time from 0 to 15 min and no extraction equilibrium was observed for the four target analytes. It seems that a very low distribution rate of the target phenothiazine drugs existed between the sample solution and the organic phase. A prolonged extraction time may result in solvent dissolution and ruined analytical accuracy and precision, although improved extraction efficiency was expected. So the extraction time of 10 min was fixed in this work.

3.1.3. pH and temperature

The effect of sample pH on the extraction of four target analytes was investigated by varying the pH values from 4 to 10. It was shown that the signal intensities of all the analytes were increased with increasing the sample solution pH from 4 to 9. When the pH was higher than 9, the signal intensities of chlorpromazine and trifluoperazine were decreased obviously. Considering that four target phenothiazine drugs are all basic compounds, an alkaline medium would be beneficial for their extraction. So, the sample solution pH was adjusted to 9 for the extraction of phenothiazine drugs by HF-LPME.

The effect of temperature on the extraction of four target analytes was studied by varying the temperature from 14 to 40 °C. The experimental results indicated that the signal intensities of all the analytes were increased with the increase of extraction temperature in the whole tested temperature range. To avoid the solvent loss at high temperature, further analysis was performed at the temperature of 40 °C.

3.1.4. Ion strength

The effect of salt concentration on the extraction efficiency of analytes was also investigated. It was found that the signal intensities of all the analytes were decreased with increasing NaCl concentration from 0 to 0.20 g/mL. Therefore, HF-LPME without NaCl addition was employed in the further work.

3.2. Validation of the proposed method

A validation study of the proposed method was carried out by GC-FPD and GC-FID after HF-LPME. To compensate for the quadratic response of the FPD detector, the square root of the peak areas was used as the basis for the calculations. The results concerning linear range, precision, and limits of detection (LODs) were listed in Table 2. As could be seen, good linearity was obtained in urine for all four phenothiazine drugs, with correlation coefficient ranging from 0.9990 to 0.9999 for HF-LPME-GC-FPD and from 0.9997 to 0.9999 for HF-LPME-GC-FID, respectively.

The precision of HF-LPME-GC-FID/FPD was evaluated, and the relative standard deviations (RSDs) obtained by three replicate determinations were between 6.6% and 9.7% (average 8.3%) for HF-LPME-GC-FPD and between 2.9% and 13.0% (average 7.5%) for HF-LPME-GC-FID. Overall, there was no significant difference of RSDs between these two methods of HF-LPME-GC-FPD and HF-LPME-GC-FID, indicating that the sample preparation step was the main affecting factor on the reproducibility of the proposed method.

The limits of detections (LODs) were obtained by adding standards to the drug-free urine, and determining the minimum amount of each analyte required to give an signal of S/N = 3 by GC analysis after HF-LPME procedure. LODs were found to be 26.5 ng/mL (promethazine) to 203.4 ng/mL (trifluoperazine) for HF-LPME-GC-FPD, and 1.4 ng/mL (promethazine) to 12.2 ng/mL (promazine) for HF-LPME-GC-FID, respectively, clearly indicating that HF-LPME-GC-FID provided much better sensitivity than HF-LPME-GC-FPD for the target phenothiazine drugs.

As we know, FID is suitable for the analysis of carbon-containing compounds while FPD is very sensitive to those compounds containing S or P. With the same HF-LPME pretreatment process, HF-LPME-GC-FID and HF-LPME-GC-FPD may provide different analytical performance for the determination of target drugs because of different sensitivity for the target analytes by FID and FPD detection. The analytical results obtained in this work demonstrated that

Table 2
Analytical performance data for phenothiazines by HF-LPME-GC-FPD/FID.

Phenothiazines	Linearity (ng/mL)		*R*		LODs (ng/mL)		RSD ($n=3$) (%)	
	1	2	1	2	1	2	1	2
Promethazine	100–1000	10–2000	0.9999	0.9998	26.5	1.4	9.7	13.0
Promazine	120–1200	50–2000	0.9998	0.9999	35.7	12.2	8.8	5.1
Chlorpromazine	250–2500	50–2000	0.9991	0.9998	75.9	9.9	8.2	8.9
Trifluoperazine	700–7000	20–2000	0.9990	0.9997	203.4	5.4	6.6	2.9

1: GC-FPD; 2: GC-FID.

Table 3
Comparison of detection limits found in the literature for the determination of phenothiazines in biological samples.

Phenothiazines	Sample	Detection technique[a]	LODs (ng/mL)	Ref.
Promethazine	Urine	HF-LPME-GC-FPD	26.5	This work
Promazine			35.7	
Chlorpromazine			75.9	
Trifluoperazine			203.4	
Promethazine	Urine	HF-LPME-GC-FID	1.4	This work
Promazine			12.2	
Chlorpromazine			9.9	
Trifluoperazine			5.4	
Promazine	Blood and urine	LLE-HPLC-UV	10	[6]
		LLE-NACE-UV	70	
Chlorpromazine		LLE-HPLC-UV	130	
		LLE-NACE-UV	130	
Promethazine	Human urine	LPME-HPLC-UV	47.7	[25]
Chlorpromazine			30.6	
Trifluoperazine			33.6	
Promazine	Whole blood	LLE-GC-NPD	100[b]	[27]
Chlorpromazine			50[b]	
Promethazine			100[b]	
Trifluoperazine	Blood, vomitus and	LLE/SPE-GC-FID	100	[28]
Promethazine	gastric juice		100	
Chlorpromazine			100	
Promazine	Human urine	HPLC-ECL sensor	93.3	[29]
Chlorpromazine			265.6	
Trifluoperazine			814.0	
Promazine	Whole blood	LLE-NACE-UV	80	[30]
Chlorpromazine			150	
Promethazine			150	

[a] HF-LPME, hollow fiber-liquid phase microextraction; LLE, liquid–liquid extraction; SPE, solid-phase extraction; GC, gas chromatography; FPD, flame photometric detector; FID, flame ionization detector; NPD, nitrogen-phosphate detector; HPLC, high-performance liquid chromatography; ECL sensor, electrogenerated chemiluminescence sensor; UV, ultraviolet; NACE, non-aqueous capillary electrophoresis.
[b] Limit of quantification (LOQ).

GC-FID is more sensitive than GC-FPD for analysis of the target analytes. It was also found that much sharper signal peaks of target analytes were observed in the chromatogram obtained by GC-FPD than that obtained by GC-FID, indicating that GC-FPD has a much better selectivity for target drugs than GC-FID.

Table 3 also listed the LODs data reported in literatures for a comparison. As could be seen, the LODs obtained by HF-LPME-GC-FID are lower than those obtained by LLE-HPLC-UV [6], LLE-GC-NPD [27], LLE/SPE-GC-FID [28], HPLC-electrogenerated chemiluminescence sensor (ECL sensor) [29], LLE-non-aqueous capillary electrophoresis (NACE)-UV [6,30] and LPME-HPLC-UV [25]. And the LODs obtained by HF-LPME-GC-FPD are still lower than those reported in the literatures [27–30] and comparable with the LODs obtained by LLE-HPLC/NACE-UV [6] and LPME-HPLC-UV [25].

3.3. Comparison of HF-LPME and SDME

For comparison, SDME was also employed for the extraction of four target phenothiazine drugs. The extraction conditions for SDME were the same as for HF-LPME, except that 2 μL toluene as the extraction solvent (4 μL for HF-LPME) and the stirring rate of 500 rpm (1000 rpm for HF-LPME) were applied. With GC-FPD as the subsequent detection technique, the enrichment factors and RSDs of both HF-LPME and SDME for four target phenothiazine drugs were investigated, and the results were given in Table 4. The enrichment factor, defined as the concentration ratio of the concentration obtained after extraction to the original concentration in the sample, were found to be between 98 and 141 folds for HF-LPME and between 68 and 94 folds for SDME, and RSDs were from 2.6% to 8.6% for HF-LPME and from 11.9% to 14.2% for SDME.

Table 4
Comparison of the enrichment factor obtained by HF-LPME and SDME combined with GC-FPD detection.

Phenothiazines	Enrichment factor		RSD ($n=3$) (%)	
	HF-LPME	SDME	HF-LPME	SDME
Promethazine	141	94	4.3	14.2
Promazine	126	85	2.6	13.4
Chlorpromazine	128	91	8.6	11.9
Trifluoperazine	98	68	5.7	12.2

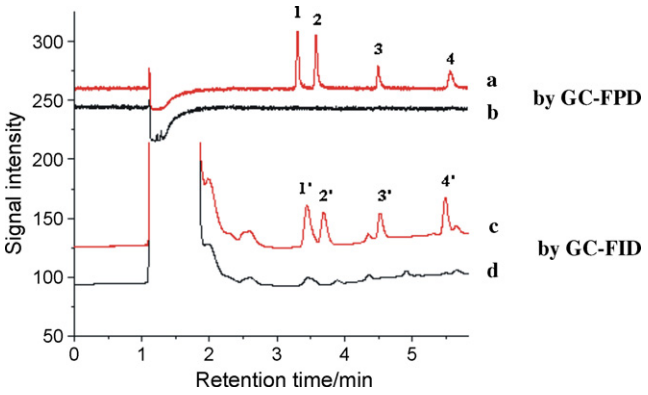


Fig. 2. Chromatograms of the healthy urine sample obtained by HF-LPME-GC-FPD/FID. (a and c) Spiked urine sample, (b and d) blank urine sample. (1 and 1′) 0.2 μg/mL promethazine, (2 and 2′) 0.2 μg/mL promazine, (3 and 3′) 0.2 μg/mL chlorpromazine, (4 and 4′) 0.4 μg/mL trifluoperazine; conditions: sample solution pH of 9 and temperature of 40 °C, toluene as the extraction solvent, 10 min extraction with 3 mL of spiked urine sample at a stirring rate of 1000 rpm, injection volume 1.0 μL.

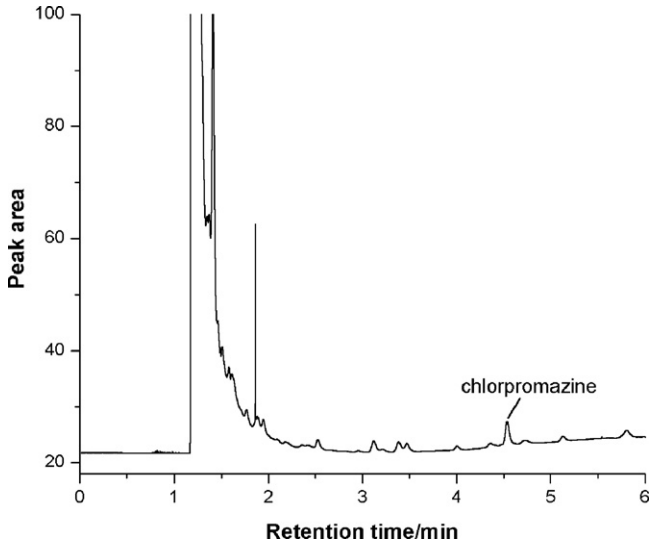


Fig. 3. Chromatogram of the case urine sample obtained by HF-LPME-GC-FID. Conditions: sample solution pH of 9 and temperature of 40 °C, toluene as the extraction solvent, 10 min extraction with 3 mL of urine sample at a stirring rate of 1000 rpm, injection volume 1.0 μL.

Additionally, it should be pointed out that filtration of the urine sample before SDME operation was necessary to prevent the losses of solvent drop because precipitates could be observed after urine sample pH was adjusted to 9, and significantly affect the stability of the single drop.

Based on the above facts, it could be concluded that HF-LPME was more suitable for the extraction of these four phenothiazine drugs than its counterpart of SDME, due to its better stability and precision, and a higher preconcentration capability. Moreover, HF-LPME could be directly performed for more complex matrix samples like human urine.

3.4. Applications

Fig. 2 is the typical chromatograms for the spiked healthy human urine sample obtained by HF-LPME-GC-FPD and HF-LPME-GC-FID. As could be seen, the peak shape and resolution of the signal obtained by GC-FPD is better than that obtained by GC-FID, probably due to a good selectivity of FPD to the target phenothiazine drugs (sulphur containing compounds), while the sensitivity of GC-FID for the four target analytes is higher than that of GC-FPD as specified in Section 3.2.

A case urine sample of a psychopath (male), taking chlorpromazine as his therapeutic medicine, was analyzed as soon as possible by HF-LPME-GC-FID after sampling, and the chromatogram was presented in Fig. 3. The concentration of chlorpromazine found in the urine sample was 303.5 ng/mL, while the other three phenothiazine drugs, promethazine, promazine and trifluoperazine, were not detected. After stored in the refrigerator for about three days, the case urine sample was also analyzed by HF-LPME-GC-FPD, but none of the target analytes was detected. This could be attributed to the degradation of the analytes due to long time storage before analysis. Nevertheless, a much better selectivity of GC-FPD for the four target phenothiazines over GC-FID, along with the LODs of 26.5–203.4 ng/mL obtained by HF-LPME-GC-FPD, also revealed an application potential of the proposed HF-LPME-GC-FPD for the rapid analysis of drug poisoning in real samples.

Additionally, the spiked case urine sample with each analyte spiking at two different concentration levels was analyzed by both HF-LPME-GC-FID and HF-LPME-GC-FPD, and the analytical results along with the recovery were given in Table 5. The recovery was defined as the percentage ratio between the found concentration and spiked concentration of the target analyte. It should be pointed out that the case urine sample analysis by HF-LPME-GC-FID was processed immediately after sampling, while its analysis by HF-LPME-GC-FPD was carried out after three days storage. The

Table 5
Analytical results of the case urine sample obtained by HF-LPME-GC-FPD/FID.

Phenothiazines	Added (ng/mL)	GC-FPD		GC-FID	
		Found[a] (ng/mL)	Recovery[b] (%)	Found[a] (ng/mL)	Recovery[b] (%)
Promethazine	0	n.d.[c]	–	n.d.[c]	–
	200	181.0 ± 10.0	90.5 ± 5.5	216.7 ± 16.2	108.3 ± 7.5
	500	485.9 ± 43.7	97.2 ± 9.0	516.5 ± 21.7	103.3 ± 4.2
Promazine	0	n.d.[c]	–	n.d.[c]	–
	200	194.9 ± 9.9	97.4 ± 5.1	171.2 ± 8.0	85.6 ± 4.7
	500	489.1 ± 40.1	97.8 ± 8.2	572.9 ± 25.2	114.6 ± 4.4
Chlorpromazine	0	n.d.[c]	–	303.5 ± 23.6	–
	200	212.6 ± 13.0	106.3 ± 6.1	505.7 ± 22.2	101.1 ± 4.4
	500	486.6 ± 26.8	97.3 ± 5.5	846.7 ± 89.8	108.6 ± 10.6
Trifluoperazine	0	n.d.[c]	–	n.d.[c]	–
	400	403.9 ± 39.6	101.0 ± 9.8	397.6 ± 33.8	99.4 ± 8.5
	1000	836.4 ± 24.2	83.6 ± 2.9	1167.3 ± 138.9	116.7 ± 11.9

[a] Mean ± SD (n = 3).
[b] Mean ± RSD (n = 3).
[c] Not detected.

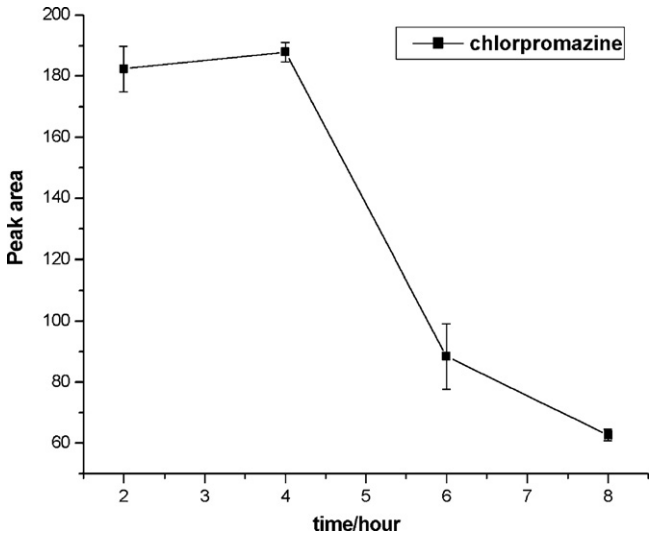


Fig. 4. Excretion of chlorpromazine in urine by HF-LPME-GC-FID. Conditions: sample solution pH of 9 and temperature of 40 °C, toluene as the extraction solvent, 10 min extraction with 3 mL of urine sample at a stirring rate of 1000 rpm, injection volume 1.0 μL.

recoveries obtained by HF-LPME-GC-FID were between 85.6% and 116.7% with the RSDs ranging from 4.2% to 11.9% for three replicate analyses; while the recoveries between 83.6% and 106.3% with the RSDs of 2.9–9.8% at two different spiking levels were obtained by HF-LPME-GC-FPD.

The excretion of chlorpromazine was also investigated with HF-LPME-GC-FID by monitoring the changes of the concentration of chlorpromazine after drug-taking. Fig. 4 exhibited a significant variation of the concentration of chlorpromazine in urine within 8 h after drug-taking. The concentration of chlorpromazine in urine was kept constant at a high level from 2 to 4 h after drug-taking, and was then decreased gradually after 4 h. However, it should be stressed that there may be large difference in the concentration variation of the drug for different drug-takers because the drug excretion is greatly affected by the renal function of the drug-taker.

4. Conclusions

In this work, a new method of HF-LPME-GC-FID/FPD was developed for the assay of four phenothiazine drugs in human urine samples. Compared with SDME, HF-LPME could provide better precision, higher enrichment factor, and was more suitable for the analysis of more complex matrix samples like human urine. HF-LPME-GC-FID was more sensitive than HF-LPME-GC-FPD, while the signal peak shape and resolution obtained by GC-FPD was better than GC-FID. Both HF-LPME-GC-FID and HF-LPME-GC-FPD could be used for real-world sample analysis and they are simple, fast, inexpensive, and provide appropriate alternative techniques for TDM.

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