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Development of a Simple and Efficient Method for Preconcentration and Determination of Trace Levels of Fexofenadine in Plasma and Urine Samples

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Abstract

A simple, inexpensive and sensitive three-phase hollow fiber liquid-phase microextraction (HF-LPME) coupled with high performance liquid chromatography (HPLC) with UV detection was successfully developed for determination of trace level of an anti-Histamine drug, fexofenadine, in human plasma and urine. The analyte was extracted into n-octanol that was immobilized in the wall of a porous hollow fiber from 15 mL of aqueous sample, with pH 4 (donor phase), and was back extracted into the acceptor phase with pH 9 located in the lumen of the hollow fiber. The extraction occurred due to a pH gradient between the sides of the hollow fiber. Several parameters affecting the extraction process such as type of extraction solvent, pH, salt concentration, extraction time, and stirring speed were optimized. Under the optimized conditions enrichment factor of 65, linear dynamic range (LDR) of 1.8-200 ng/mL and limit of detection (LOD) 0.6 ng/mL were obtained. The percent relative intraday and interday standard deviations (RSD%) based on 3 replicate determinations were 7.1% and 7.6% respectively.

Key words: *fexofenadine, hollow fiber liquid–phase microextraction, biological samples, high performance liquid chromatography, drug analysis.*

Introduction

Fexofenadine, (±)-4-[1 hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α, α-dimethyl benzeneacetic acid (fig 1), is the second generation selective peripheral H1 blocker of the GI tract, large blood vessel, bronchial smooth muscle preventing the symptoms associated with allergies.

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Approximately, 80% is excreted in the feces and 11% in the urine. It does not cause sedation or other central nervous system effects because it does not cross the blood–brain barrier. After oral administration, fexofenadine is rapidly absorbed from the gastrointestinal tract [1, 2]. fexofenadine is a substrate of the OATP-A uptake transporter [3] and of the efflux transporter p-glycoprotein, which determines absorption, tissue distribution and effects of a broad variety of drugs [4].

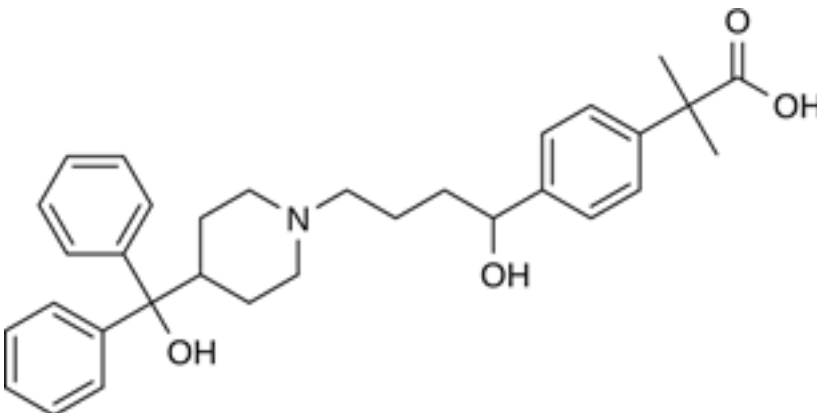


Figure 1. Chemical structure of fexofenadine.

Because fexofenadine disposition does not fit on compartment model, lower concentration is important to precisely define the pharmacokinetic parameters of fexofenadine. Several HPLC methods have been previously reported for the determination of fexofenadine concentrations, most of which used liquid chromatography–mass spectrometry (LC–MS) [2, 5-8]. Although LC–MS methods are enough sensitive, they are very expensive for routine analysis of therapeutic concentration of fexofenadine. Therefore, new methods which are both simple and sensitive for determination of fexofenadine are required. Generally, a pre-concentration step is required prior to determination of trace amounts of the drug. Several extraction and preconcentration techniques such as liquid–liquid extraction (LLE) [9] and solid phase extraction (SPE) [10] have been employed, but each one has their own drawbacks [11].

In the present study, we described a simple, inexpensive and sensitive three-phase hollow fiber microextraction technique which is one of the effective solvent microextraction techniques discussed in several papers [12] combined with a high performance liquid chromatography-ultra violet detection (HPLC-UV) for extraction and determination of fexofenadine in human plasma and urine samples.

Experimental

Chemicals and Materials

HPLC grade solvents such as acetonitrile, n-octanol, methanol, n-octane, isobutyl methyl ketone, decanol and benzyl alcohol were from Merck company (Darmstadt, Germany). Fexofenadine was kindly donated by the Food and Drug Organization (Tehran, Iran). Stock solutions containing 100μg mL^{-1} of fexofenadine were prepared in HPLC grade methanol and stored at 4°C. Working solutions were prepared daily by dilution of the stock solutions prior to use. All chemicals were analytical reagent grade. Drug-free plasma samples were obtained from the Clinic of Taleghani Hospital (Tehran, Iran). Urine samples were collected from healthy young volunteers. The samples were diluted using deionized water.

Instrumentation

Chromatographic separations were carried out on a HPLC (Shimadzu, Japan) equipped with a LC-10 AD quaternary pump and a SPD-M10A VP detector. Chromatographic data were recorded and analyzed using Class-VP. The separations were carried out on a C_{18} column (150mm×4.6mm, with 5μm particle size) from Teknokroma (Barcelona, Spain). A mixture of (50:50, v/v) acetonitrile:methanol, with a flow rate of 1.0 mL min-1 was used as the mobile phase. The injection volume was 20μL for all the standards and the samples, and the detection was performed at the wavelength of 210 nm. All of the pH measurements were performed with a GPHR 1400A pH meter (Germany). All of the extractions were carried out using a Q3/2 Accrual polypropylene hollow fiber membrane from Membrana (Wuppertal, Germany) with a 0.2 μm pore size, 600 μm internal diameter and 200 μm wall thickness.

HF-LPME procedure

Each membrane unit was cut into 8.8 cm length pieces and ultrasonically cleaned in acetone for 5 minutes to remove any possible contaminants. They were then dried in air and the solvent was allowed to evaporate completely. In order to avoid any possible memory effects, a new fiber was used for each extraction. The internal volume of the hollow fibers lumen was approximately 24 μL. The end of each fiber was connected to the needle of a 25μL Hamilton microsyringe (Bonaduz, Switzerland). 15 mL of the sample solution with a pH of 4 (HCl, 0.1 M) containing 50 mg L^{-1} of fexofenadine was placed in a 20 mL vial with a 4mm×14mm magnetic stirring bar. The sample vial was placed on a Heidolph MR 3001 K magnetic stirrer (Schwa Bach, Germany) and the Hamilton microsyringe was used to introduce the receiving phase into the hollow fiber. Then, 25 μL of the receiving phase (pH = 9.0) was withdrawn into the microsyringe and its needle was inserted into the lumen of the hollow fiber. The fiber was

inserted into the organic phase (n-octanol) for 30 s and the excess of organic phase was carefully removed by washing the outside of the hollow fiber with ultrapure water. Then, 24 mL of receiving phase (RP) was injected into the lumen of the hollow fiber and the end of the hollow fiber was sealed by a piece of aluminum. The U-shape hollow fiber was immersed into the sample solution. The extraction was performed at room temperature and the sample was stirred at 500 rpm during the extraction time (40 min). After extraction, the fiber was removed from the sample vial, the end of the hollow fiber was opened, and the RP was retracted into the microsyringe. Finally, 20 µL of RP was injected into the HPLC system for subsequent analysis.

Results and discussion

In this study, a three-phase hollow fiber microextraction technique combined with HPLC-UV was applied for the extraction and determination of fexofenadine in human plasma and urine samples. The effect of experimental variables such as the nature of the organic solvent, composition of the donor and acceptor phases, ionic strength, stirring speed and extraction time on the extraction efficiency were investigated and optimized.

Effect of Organic solvent

The effect of the organic solvent on the extraction of fexofenadine by HF-LPME was investigated. Different kinds of solvents including n-octanol, n-octane, isobutyl methyl ketone, decanol and benzyl alcohol were tested. The experimental results demonstrated that n-octanol gave much better extraction efficiency for extraction of fexofenadine (figure 2).

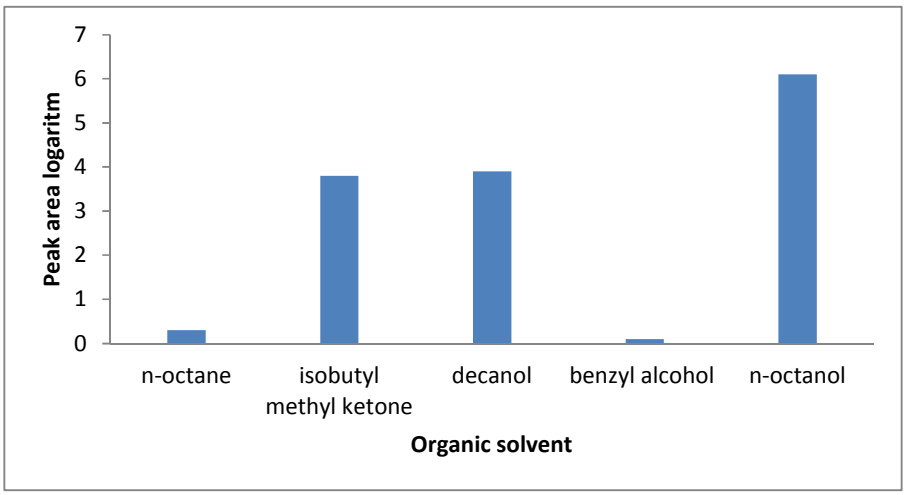


Figure 2. The effect of organic solvent on extraction efficiency of fexofenadine.

Effect of the pH of donor and acceptor phases

To obtain high extraction efficiency for acidic compound (fexofenadine pK_a is 4.5), the sample solution should be acidic to effectively deionise the analyte and consequently reduce their solubility in the sample solution. Different pH of sample solution (pH 3.0–6.0) was tested. The extractions were performed within 40 min at 500 rpm. The results showed that in pH 4, extraction efficiency was high because in this pH fexofenadine has molecular structure and transfer easily into the organic phase. In the acceptor phase pH between (8-10) was tested. At pH 9 extraction efficiency was high because in this pH fexofendine has ionic structure and transfer easily into aquatic phase.

Effect of ionic strength

Salt addition may increase, decrease or not impact the extraction efficacy [13]. Therefore, the effect of salt concentration on the extraction efficiency of analyte was also investigated. It was found that the signal intensities of the analyte were constant with increasing NaCl concentration from 0 to 0.20 g/mL. Therefore, HF-LPME without NaCl addition was employed in the further work.

Effect of stirring rate

Increasing stirring rate can speed up the thermodynamic equilibrium, thus it can enhance the extraction efficiency [14]. Different stirring rate from 250 to 750 rpm was investigated. The extraction recovery increased with higher stirring speed. However, over high speed would produce excessive air bubbles and lose solvent that may affect the precision [15]. Therefore, 500 rpm was chosen as a suitable stirring speed in the experiments.

Effect of extraction time

Extraction time ranging from 20 to 60 min at a stirring rate of 500 rpm was investigated. The results indicated that the recoveries of fexofenadine enhanced with increasing extraction time and reached equilibrium at 40 min. After 40 min, extraction efficiency decreased because after reaching equilibrium, fexfenadine molecules would have enough time to extract back into donner phase. Thus, 40 min was chosen for all subsequent experiments.

Method validation

Evaluation of the method performance

In order to proceed with the current evaluation of the proposed HF- LPME technique, The figures of merit including dynamic linear range (DLR), limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD%) and preconcentration factor (PF) (Table 1) were investigated under the optimum conditions (Table 2).

Table.1. Analytical characteristics of the proposed HF- LPME method.

LOD (ng mL^{-1})	LOQ (ng mL^{-1})	DLR (ng mL^{-1})	R^2	PF	Intra day RSD% (*n*=3)	Inter day RSD% (*n*=3)
0.6	1.8	1.8-200	0.999	65	7.1	7.6

LOQ: limit of quantification; LOD: limit of detection; DLR: dynamic linear range;
PF: preconcentration factor; RSD%: relative standard deviation.

Table. 2. Optimum conditions of HF-LPME of fexofenadine.

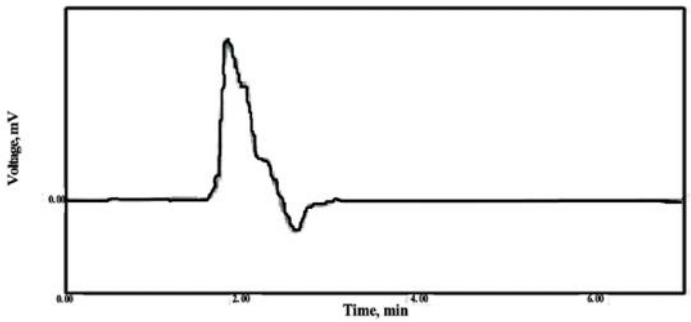
Salt (w/v%)	Time (min)	stirring rate (rpm)	pH of AP	pH of DP	Solvent
0	40	500	9	4	n-Octanol

AP: acceptor phase; DP: donor phase.

Extractions of fexofenadine from biological matrices

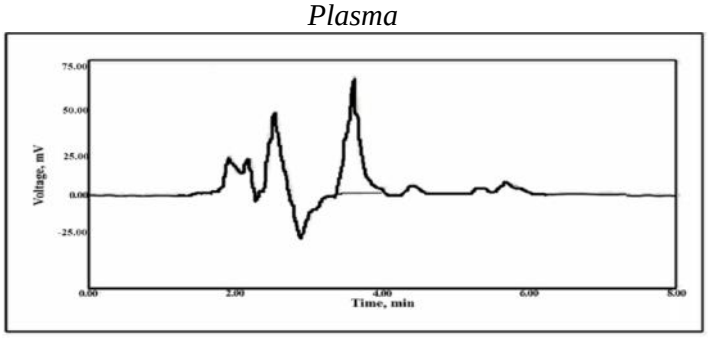
In order to test the applicability of the proposed HF-LPME method for extraction and determination of fexofenadine, the developed technique was utilized for the analysis of purposed drug in human plasma and urine samples (Figure 3.). Table 3 shows that results obtained from three replicate analysis of each sample in which a certain amount of fexofenadine was added by the proposed method are in satisfactory agreement.

(a)



(b)

Plasma



Urine

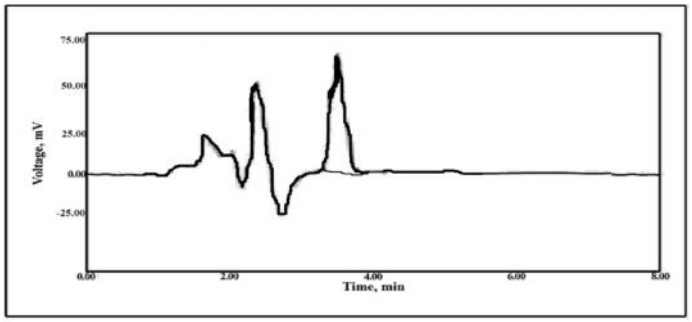


Figure 3. HPLC-UV Chromatograms of (a) blank human plasma and urine and (b) human urine and plasma samples spiked with 10 ng/ml of fexofenadine after HF-LPME at optimum conditions.

Table. 3. Results for determination of fexofenadine in spiked plasma and urine samples subjected to the HF-LPME and analyzed using HPLC.

Sample	C_{added} (ng mL^{-1})	C_{found} (ng mL^{-1})	RSD% (n=3)
Plasma	10.0	8.3	6.6
	15.0	14.4	7.1
	20.0	18.7	6.0
Urine	10.0	10.2	5.5
	15.0	14.8	6.7
	20.0	19.4	6.3

A comparison between the analytical characteristics of the developed method and those of the published methods is shown in Table 4. The proposed method has high sensitivity, wide linearity range, simplicity and a suitable repeatability.

Table. 4. Comparison of the proposed method with other developed methods for determination of fexofenadine in biological samples.

Method	sample	Linearity (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})	Ref.
HPLC-UV	Plasma, Urine	1.8-200	0.6	1.8	Present study
HPLC–MS/MS [1]	Plasma	1.0-200	--	1.0	5
HPLC/ESI-MS/MS[2]	Plasma	1.0-500	--	1.0	6
HPLC–MS/MS	Plasma	--	--	0.5	7
LC-MS/MS	cell lysates	1.0-500	--	1.0	8
LC-MS	Plasma	--	1.0	3.0	2

1- high performance liquid chromatography-mass spectrometry/mass spectrometry
2- high-performance liquid chromatography/positive ion electrospray tandem mass spectrometry

Conclusion

The results indicate that the proposed method is a simple, inexpensive and relatively fast extraction technique with little consumption of organic solvent for the extraction and determination of the fexofenadine in complex matrices.

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