Original Article

Hollow Fiber-based Liquid-phase Microextraction and HPLC-UV Determination of Lovastatin in Biological Fluids

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Abstract

In this study, a hollow fiber liquid-phase microextraction (HF-LPME) method coupled with high-performance liquid chromatography (HPLC) was successfully developed for the determination of trace levels of lovastatin in urine and plasma samples. Lovastatin was extracted from 15 mL of the acidic sample solution with a pH of 2 into an organic extracting solvent (*n*-octanol) impregnated in the pores of a hollow fiber and then back extracted into an acidified aqueous solution in the lumen of the hollow fiber. After extraction, 10 µL of the acceptor phase was injected into HPLC system. To obtain high extraction efficiency, the parameters affecting the HF-LPME, including pH of the sample and extractant phases, type of organic phase, ionic strength, stirring rate, extraction time, and temperature, were studied and optimized. Under the optimized conditions (solvent 1-octanol, pH = 2, 45 min stirring at 45°C with 750 rpm), the relative recovery percentage was 85.2–97, which shows the capability of the method to analyze the analyte concentration. This technique provided preconcentration factor 199, 185, and 170 for water, urine, and plasma, respectively. Good precisions values (with relative standard division ≤ 10.5%) were obtained. The results indicated that the HF-LPME method has an excellent cleanup capacity and a high preconcentration factor and could serve as a simple and sensitive method for monitoring the drug in biological samples.

Keywords: *Biological sample, drug analysis, high-performance liquid chromatography, hollow fiber*

Introduction

Lovastatin (LV), a member of statin family, is a medicine used for lowering cholesterol in those with hypercholesterolemia to reduce the risks of cardiovascular disease.[1] LV is a naturally occurring compound found in low concentrations in food such as oyster mushrooms and red yeast rice. It inhibits 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA), an important step in cholesterol biosynthesis. LV is therapeutically used both in free acid and lactone forms. β-Hydroxic acid forms of LV are insoluble in water, whereas it is soluble in lactone ring form. LV not only reduces blood cholesterol but also has an antifungal property and anticarcinogenic effects.[2-4]

It is commonly used once or twice a day. Studies suggest that less than 5% of the oral dose reaches the general circulation as active inhibitors.[5] Time to peak serum concentration is 2–4 h. LV undergoes extensive first-pass metabolism so the availability of the drug in the system is low and variable.[6]

Several liquid chromatographic methods with ultraviolet and mass spectrometric detection have been reported in the literature for the quantitative determination of LV in biological fluids.[7-10] Techniques using high-performance liquid chromatography (HPLC) alone do not have an adequate lower limit of quantification suitable for monitoring LV in low concentrations. However, most of these methods are tedious and time-consuming, which also considered costly for routine analysis work.

Solid-phase microextraction was first introduced by Arthur and Pawliszyn[11] about 20 years ago and has been used for many years.[12-14]

Pedersen-Bjergaard and Rasmussen[6] suggested using porous hollow polypropylene fibers as a retainer to maintain the extraction phase, a method known as hollow fiber liquid-phase microextraction (HF-LPME). HF-LPME can be performed in two or three microextraction phase modes. The three phases involved in the extraction are the analyte solution (donor), an organic phase, and second aqueous phase into which the extraction is conducted (acceptor phase). During the extraction, the desired analyte under proper

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conditions is first transferred into the organic phase and then into the acceptor phase. The rate of conducting the extraction depends on the rate of mass transfer between the two interfaces of the donor/organic phase and the organic/acceptor phase. The main reported limitation of this method is that it is only applicable for species which are capable of ionization. Solvent extraction has often been used for separating and/or isolating chemical compounds. The efficiency of the extraction process depends on the interface area through which mass is transferred. This interface area is composed of two insoluble phases (organic or aqueous) containing various amounts of the extracted species (solute). In the classical extraction process, dispersing one phase (e.g., in the form of droplets) generates the interface. After the process has been completed, the phases are allowed to separate into the extract and the raffinate. Therefore, differences in density, viscosity, and surface tension between both phases make very important parameters for extraction process realized in conventional equipment including columns, mixers settlers, or centrifuges. In many cases, too little density and/or surface tension differences cause formation of stable emulsions that are difficult to break. In the systems of high surface tension value, creation of a large interface area for mass transfer is rather difficult and implies use of large-size apparatus. A new nondispersive phase contacting technology has been worked out. In the process, hollow fiber membrane modules are used to generate the interfacial area.[12]

The organic phase flows inside the hollow fibers (in the membrane tube side) and spontaneously wets (fills) membrane pores. To block the flow of organic phase through the membrane pores, another phase (e.g., water) is introduced to the shell side of the hollow fiber module. HF-LPME method has got attention recently due to its beneficial aspects such as reduction of processing time and limit of detection (LOD), and higher enrichment factor, recovery percentage, and extraction throughput.

So, developing a low-cost and precious method for analyzing LV concentration in plasma or other biological fluids would be beneficial in adjusting its dose.

As previous studies in this area need a mixture of organic solvents for extraction,[15] in this method, we try to use as little organic solvents as possible. HPLC method for determination of LV and statins in organic solvents was performed successfully previously. In this method, we combined hollow fiber microextraction with HPLC technique, in order to evaluate a simple, fast, and accurate method with high yield of extraction for determination of LV in biological fluids.[16]

In this study, a simple, inexpensive, and sensitive hollow fiber microextraction technique combined with HPLC-ultraviolet detection (HPLC-UV) is described for the extraction and determination of LV in urine and plasma samples.

Materials and Methods

Lovastatin in organic solvents

LV reference standard was kindly donated by Daroopakhsh Pharmaceutical Company (Tehran, Iran) with 98% purity. For extraction, 10 mL glass container and polypropylene hollow fibers, with an internal diameter of 600 µm and 2.0 µm pore diameter (Enka, Wuppertal, Germany), were used. Also, for injection of solution into fiber, 25 µL Hamilton syringe with 800-µm needle (Hamilton Ilmenau, Germany) was used. Using 2-, 5- and 10-mL pipet, 10-100 and 0.10-100 µL sampler (Eppendorf, Hamburg, Germany), and variable size of disposable plastic interchangeable heads, solution was removed.

For preparation of sample solution, LV powder was dissolved in methanol (HPLC grade) and other concentrations were prepared using this solution. Working solutions were freshly prepared in HPLC grade water by dilution of the sample solution and filtered using a Millipore filter (Merck KGaA, Darmstadt, Germany; 0.45 µm) each day prior to use. The urine and plasma samples were obtained from the Taleghani Clinic (Tehran, Iran). The samples were diluted using deionized water and were used for method development and calibration. The working standards for real sample analysis were prepared by spiking LV to 15 mL of urine samples, which were diluted 1:1 with ultra-pure water.

Deionized water, methanol, and acetonitrile were purchased from Duxson (Seoul, South Korea). All chemicals were of analytical grade unless otherwise stated. Octanol, decanol, hexane, sodium chloride, acetic acid, ammonium acetate, sodium hydroxide, boric acid, hydrochloric acid, and methyl isobutyl ketone were bought from Merck (Hamburg, Germany). Ammonium acetate buffer was prepared by adding ammonium acetate into distillated water and justifying pH= 6 with acetic acid.

For extraction, a solution with LV was prepared from acidic solution with pH = 2. A polypropylene fiber segment with 5.4 cm length and 12 µm internal diameter was prepared. The fiber was immersed in an organic solvent for 10 s (*n*-octanol) to impregnate the fiber pores with the organic solvent. The fiber was then placed in distilled water for 30 s to wash the extra solvent from its surface. This fiber was filled with 15 µL octanol using a 50 µL Hamilton syringe. Then the fiber was put in LV solution and extraction was performed in 45 min, temperature of 45°C and 750 rpm agitation. In order to avoid any possible memory effects, a new fiber was used for each extraction. After extraction, the extractants were collected by a micro-syringe and 10 µL were injected into HPLC.

Apparatus and chromatographic conditions

HPLC analysis was performed on a Knauer (Berlin, Germany) isocratic system consisting of a K-1001 pump, a K-2501 UV detector, sample loop injector and a 250 × 4.6 mm Eurospher-100 C18, 5-µm particle size column with integrated precolumn. The mobile phase was acetonitrile and 10-mM ammonium acetate (75 to 25) with pH = 6. The flow rate was maintained on 1 mL/min throughout the run and the detection carried out at 240 nm. The sensitivity of this assay was 1 µg/mL.

Lovastatin in biological fluids

Plasma and urine samples were prepared and stored at −20°C. For sample preparation, 4 mL plasma with 100 µL hydrochloric acid and 100 µL trifluoro acetic acid was deproteinated and agitated for 10 min in 4000 rpm. The volume was set to 10 mL and used as donating phase. Plasma samples with LV concentrations of 25, 50, and 200 mg/L were prepared.

For urine samples, urine with hydrochloric acid (1:1) was mixed for 5 min and 4000 rpm. Different concentrations of LV were prepared using the above solution (25, 50, and 200 µg/L).

Results and Discussion

Extraction parameters

In this study, a three-phase hollow fiber microextraction technique combined with HPLC-UV was used for the extraction and determination of LV in organic solvents and biological samples.[5] Parameters that affect the extraction and identification of LV were investigated. The volume of extraction was kept constant in these procedures.

Solvent

Figure 1 shows the effect of solvent which fills the microfiber pours on extraction of LV. As shown in the figure, 1-octanol had the highest efficacy in LV extraction.

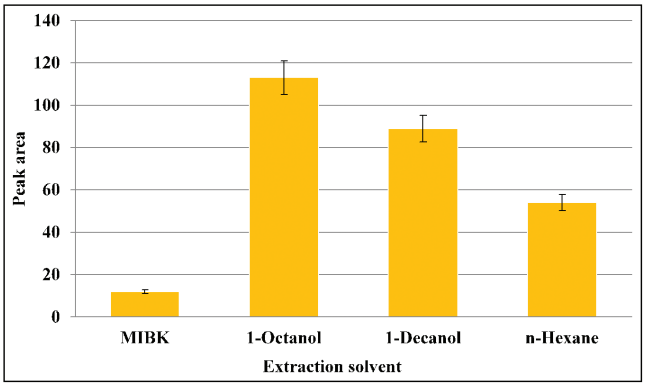

Figure 1: Effect of solvent on lovastatin extraction

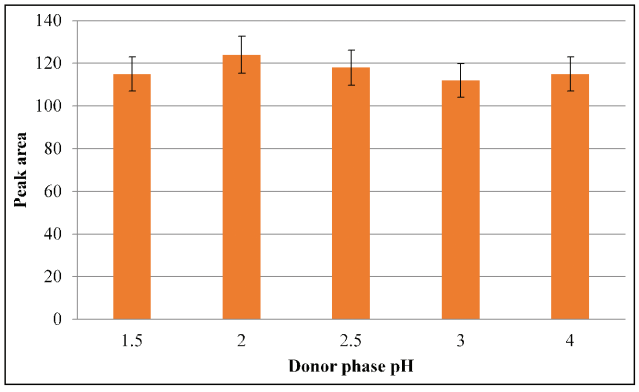

Figure 2: pH affection on extraction

pH

The pH values of the donor and acceptor solutions are important factors in a three-phase LPME. For ionizable analytes, acid–base dissociation is the most common reaction used to facilitate the extraction of the analyte from the donor to the acceptor phase.[14] For finding the optimum donor phase pH for extraction, a range of 1.5–4 was prepared and examined. The results showed that the maximum extraction was occurred in pH = 2 [Figure 2].

Time

Extraction is a balanced procedure and it needs time for completion, so a range of 15–75 min for extraction was tested and the optimum time was 45 min. After this time, the extraction was not carried out any more [Figure 3].

Stirring rate

Stirring the sample during extraction reduces the thermodynamic equilibrium time and increases the extraction efficiency as stirring provides a fresh donor solution for the organic phase to extract and enhances analyte transport from the donor phase to the organic solvent. Finding the best rate for stirring, a range of 250–1000 rpm was tested and the best rate was 750 rpm [Figure 4].

Temperature

Temperature has two distinct effects on liquid microextraction. By increasing the temperature, more and more molecules

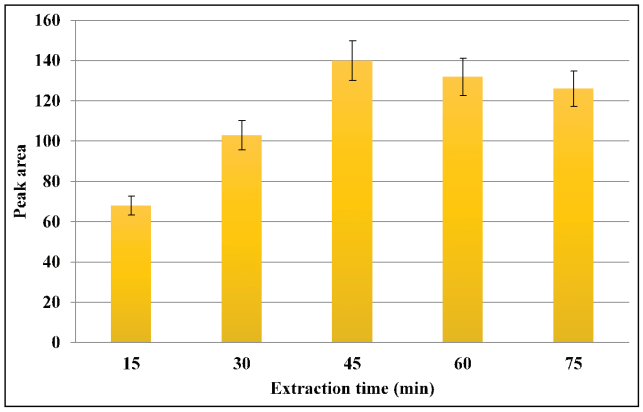

Figure 3: Time of agitation affection on extraction

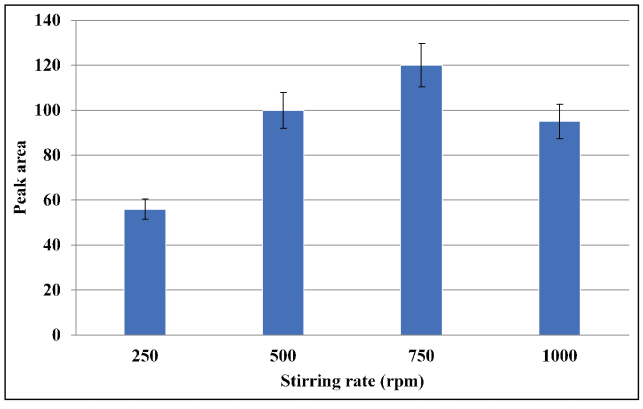

Figure 4: Rate of stirring affection on extraction

transfer from aqueous phase into organic phase, but high temperature leads to solvent evaporation and decreasing extraction. So, finding an optimum temperature can help to achieve a best ratio between water and organic solvent. A temperature range 25°C–55°C was tested [Figure 5] and the best result was obtained at 45°C.

Validation procedure

The corresponding regression equation, coefficient of determination (R^2), dynamic linear range, LOD, and preconcentration factor (PF) of LV were calculated and are summarized in Table 1.

In the three-phase HF-LPME, an analyte transfers from an aqueous donor phase to an organic solvent and finally to an acceptor phase existing in the fiber during an emission process. PF in this process is calculated as follows:

$$PF = \frac{100 V_d R}{V_a} \quad (1)$$

where V_d and V_a are the volumes of the donor phase and acceptor phase, respectively, and R is the extraction recovery.

The LOD was calculated at a signal-to-noise ratio of 3 and was obtained by adding the standard LV solution to drug-free samples. The results indicated that the proposed method displayed good reproducibility to determine LV with intraday relative standard division (RSD) value of 5.4%, 7.5%, 8.1%, and interday RSD value of 7.2%, 9.35%, and 10.5% for water, urine, and plasma samples, respectively [Table 1].

Extraction of lovastatin from urine and plasma samples

Under the optimized conditions, the developed technique was applied to the preconcentration and determination of LV in urine and plasma samples. Typical chromatograms for spiked and nonspiked urine and plasma samples obtained by the method are shown in Figures 6 and 7, respectively.

In order to evaluate the precision and accuracy of the method, relative recovery percentage (RR%) and RSD% was calculated. RR% was obtained by

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

C_{found}, C_{real} and C_{added} are the obtained concentration of LV in samples, the real concentration of LV, and the concentration of LV which was added to the samples, respectively. RR% was 85.2–97, which shows the capability of the method to analyze the analyte concentration. The results are shown in Table 2.

Conclusion

The present method was compared with other studies in terms of the method of extraction, validation, and precision. The method compares well with those mentioned in Table 3.[17]

As can be seen, the LOD value of this method is comparable with those obtained in previous studies, and even lower than those reported in the literature. In addition, due to the simplicity

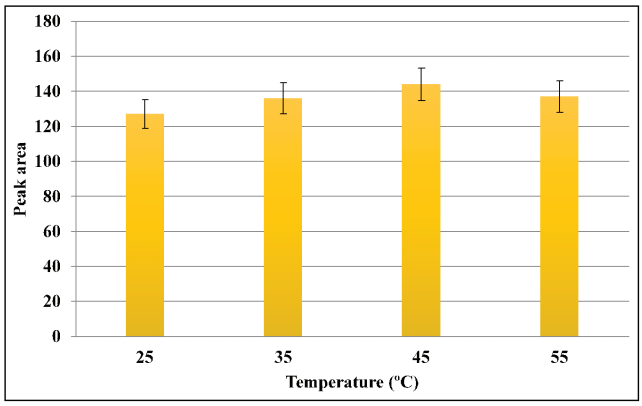

Figure 5: Time of extraction

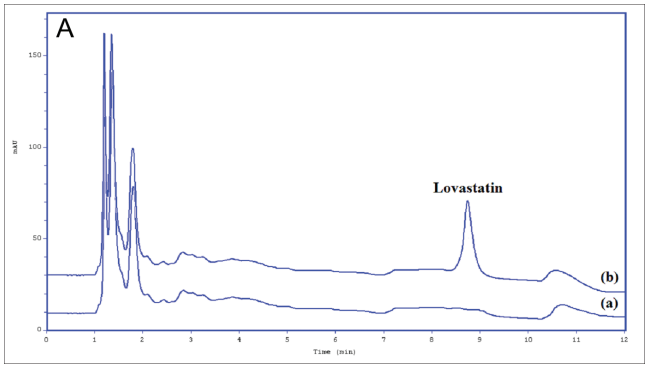

Figure 6: Representative chromatograms of urine sample spiked with 10 ng/mL of Lovastatin under the optimum conditions. (A) Control urine sample. (B) Urine sample

Table 1: Values of the corresponding regression equation and the analytical characteristics of the proposed method

Sample	Regression equation	LDR	r^2	LOQ	LOQ	PF	RSD (%)[a]	RSD (%)[b]	ER (%)
Water	$Y = 0.198X^c + 1.776$	5.0–1000	0.999	1.4	5.0	199	5.4	7.2	26.5
Urine	$Y = 0.180X + 2.267$	7.5–1000	0.998	2.0	7.5	185	7.5	9.3	24.6
Plasma	$Y = 0.165X + 1.093$	7.5–1000	0.996	2.5	7.5	170	8.1	10.5	22.7

ER = extraction recovery, LDR = linear dynamic range (µg/L), LOQ = limit of quantification, PF = preconcentration factor is calculated at 100 µg/L concentration of the analyte, RSD = relative standard division

[a]Within-day RSD (n = 5 replicates)

[b]Between-day RSD (n = 3 days)

[c]Lovastatin concentration (µg/L)

and low cost of the extraction device, the hollow fiber, it can be discarded after each extraction to avoid carryover and cross-contamination.

This work introduced a three-phase HF-LPME method combined with HPLC-UV for the extraction, preconcentration, and determination of LV in water, urine, and plasma samples. The LPME technique proved to have several advantages over the other extraction methods such as liquid–liquid extraction and solid phase extraction.[18-21] The advantages of this method are as follows:

1. The method needs very simple and inexpensive equipment.
2. Extraction recovery obtained is high (>85.5%), thus no further concentration of the extract was required before the final analysis.
3. The HF can be discarded after each extraction because of the simplicity and the low cost of the extraction device. It is good to eliminate possible carryover problems and cross-contamination. This maintains high reproducibility and repeatability for the method.
4. The organic phase volume is very low in comparison with other methods, resulting in an extremely low consumption of organic solvent per extraction. Thus, the present HF-LPME method may, therefore, be used as a green chemistry approach to reduce the consumption of hazardous organic solvents in chemical laboratories.
5. The extraction time was relatively long (45 min), but by using a multistirrer, many samples could be extracted simultaneously.

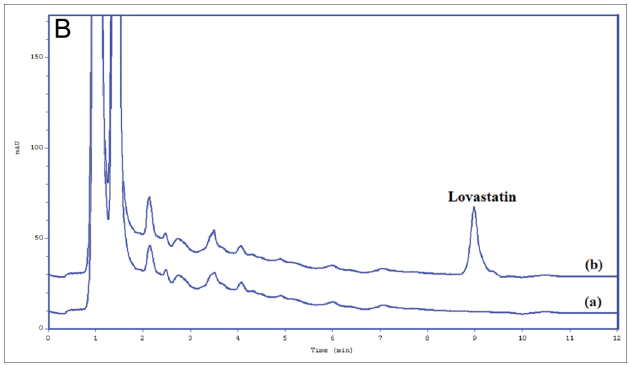

Figure 7: Representative chromatograms of plasma sample spiked with 10 ng/mL of Lovastatin under the optimum conditions. (A) Control plasma sample. (B) Plasma sample

In conclusion, a simple, fast, and inexpensive HF-LPME–HPLC-UV with high accuracy and good sensitivity for the extraction and determination of LV in biological samples was developed and validated.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Table 2: Values of the corresponding regression equation and the analytical characteristics of the proposed method

Sample	C_{added} (µg/L)	C_{found} (µg/L)	RSD% (n = 3)	Error (%)	RR (%)
Urine	–	ND	–	–	–
	25	22.1	8.9	−11.6	88.4
	50	45.0	7.0	−10.0	90.0
	200	193	5.4	−3.5	96.5
Plasma	–	ND	–	–	–
	25	21.3	9.2	−14.8	85.2
	50	44.0	7.6	−12.0	88.0
	200	194	6.6	−3.0	97.0

ND = not detected, RR = relative recovery, RSD = relative standard division

Table 3: Comparison between the present method and other methods

Ref.	PF	RSD%	LOD	LDR	Instrument	Extraction method
Wang *et al*. [15]	<7.0	5.5	0.17	1–100	HPLC-UV	DLLME
Zareh *et al*. [16]	–	6.0	–	100–5000	HPLC-UV	SPE
This study	170–199	<8.1	1.4–2.5	5–1000	HPLC-UV	SBME

DLLME = dispersive liquid–liquid micro extraction, LDR = linear dynamic range, HPLC-UV = high-performance liquid chromatography-ultraviolet detection, LOD = limit of detection, PF = preconcentration factor, RSD = relative standard division, SBME = solvent bar microextraction, SPE = solid phase extraction
All concentrations are based on micrograms per liter

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