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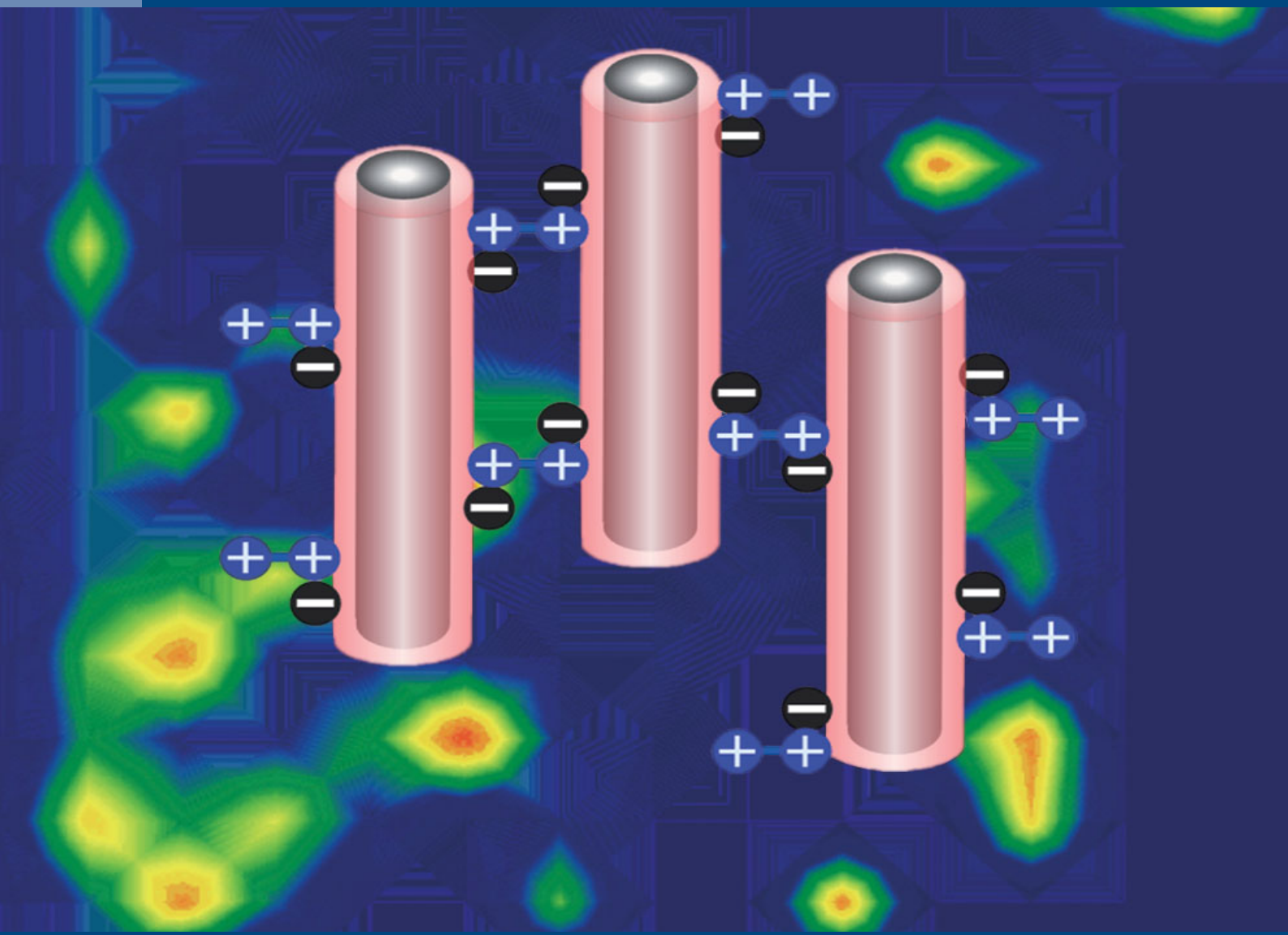

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Research Article

Determination of atenolol in human plasma using ionic-liquid-based ultrasound-assisted in situ solvent formation microextraction followed by high-performance liquid chromatography

An efficient analytical method called ionic-liquid-based ultrasound-assisted in situ solvent formation microextraction followed by high-performance liquid chromatography was developed for the determination of atenolol in human plasma. A hydrophobic ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) was formed by the addition of a hydrophilic ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate) to a sample solution containing an ion-pairing agent during microextraction. The analyte was extracted into the ionic liquid phase while the microextraction solvent was dispersed throughout the sample by utilizing ultrasound. The sample was then centrifuged, and the extracting phase retracted into the microsyringe and injected to liquid chromatography. After optimization, the calibration curve showed linearity in the range of 2–750 ng/mL with the regression coefficient corresponding to 0.998. The limits of detection (S/N = 3) and quantification (S/N = 10) were 0.5 and 2 ng/mL, respectively. A reasonable relative recovery range of 90–96.7% and satisfactory intra-assay (4.8–5.1%, $n = 6$) and interassay (5.0–5.6%, $n = 9$) precision along with a substantial sample clean-up demonstrated good performance of the procedure. It was applied for the determination of atenolol in human plasma after oral administration and some pharmacokinetic data were obtained.

Keywords: Atenolol / High-performance liquid chromatography / Human plasma / Ionic liquids / Microextraction
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1 Introduction

Atenolol (ATL) is an antihypertensive, antianginal, and antiarrhythmic drug; chemically 4-(2-hydroxy-3-isopropylaminopropoxy)-phenyl acetamide [1]. Most of all, it inhibits the release of rennin and angiotensin-II and the production of aldosterone [2]. The determination of biological concentration of ATL, important to investigate the pharmacokinetics of the drug, can now be used in the assessment of patients' adherence to a prescribed regimen [3].

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Abbriviations: ATL, atenolol; **IL-UA-ISFME**, ionic liquid-based ultrasound-assisted in situ solvent formation microextraction; **IL**, ionic liquid; **[Bmim][BF_4]**, 1-butyl-3-methylimidazolium tetrafluoroborate; **[Bmim][PF_6]**, 1-butyl-3-methylimidazolium hexafluorophosphate

According to literature survey, there have been several reports for the determination of ATL in biological samples. Existing analytical methods for the drug included HPLC [4–7], GC [8, 9], and UV spectrophotometry [10]. In addition, also extraction and preconcentration techniques, such as LLE [11, 12] and SPE [13], are commonly applied to monitor the drug. However, these techniques are labor intensive, time consuming, and require a large amount of hazardous organic solvents. Additionally, for trace analysis, a large volume of sample is usually needed, which may be impractical [14, 15].

LPME techniques have been used recently as an eco-friendly and very efficient alternative in the domain of sample preparation, especially for chromatography and electrophoresis [16, 17]. LPME has attracted increasing attention because it requires minimal exposure to toxic organic solvents, which makes it a simple, quick, inexpensive, and virtually solvent-free sample preparation method [18, 19]. Applications of LPME in biological and environmental analysis have been described in several papers [20, 21].

Ionic liquids (ILs) are ionic, nonmolecular solvents with low melting point, negligible vapor pressure, and high thermal stability [22, 23]. Their unique solvation properties provide unique selectivity and diverse separation mechanism, coupled to the fact that they can be structurally tailored for specific applications [24, 25]. Their miscibility in water and organic solvents can be controlled by selecting the cation/anion combination or by incorporating certain functional groups in the IL molecule [26, 27]. There has been an increased interest in exploiting the unique physicochemical properties of ILs in different microextraction schemes in recent years [28, 29].

In 2014, Zeeb et al. have developed a novel microextraction technique with high-performance and powerful preconcentration capability termed ionic-liquid-based ultrasound-assisted in situ solvent formation microextraction (IL-UA-ISFME) [30]. Its approach is based on dissolution of a hydrophilic IL in an aqueous solution containing the desired analytes, followed by the addition of an ion-exchange reagent, which undergoes an in situ metathesis reaction forming an insoluble IL. Thus, analytes are extracted and preconcentrated once the IL becomes insoluble. There is no interface between the aqueous media and the extraction phase. So, mass transfer from aqueous media into IL (which is speeded up by ultrasound wave) has no significant effect on the performance of the microextraction method. Due to the presence of common ion in the aqueous solution, solubility of IL decreases. As a result, volume of the extracting phase is not affected by variations in the ionic strength.

The aim of the present study was to investigate the applicability of IL-UA-ISFME method for determination of ATL in human plasma. The factors affecting the microextraction efficiency were studied in detail and the optimal conditions were determined. The analytical protocol was validated for quantitative purposes, and then applied to real sample analysis in combination with HPLC–UV detection.

2 Materials and methods

2.1 Instrumentation

A HPLC system (Shimadzu, Kyoto, Japan) including a pump, an automatic injector equipped with 20 μL sample loop and a UV detector (set at 225 nm) was applied for the analysis of ATL. The analytical column chosen for the separation was an RP-C_{18} (LiChrospher, Merck Millipore, Darmstadt, Germany) with 5 μm particle size and dimensions of 150 mm × 4.6 mm id, at the temperature of 30 ± 0.5°C. Isocratic mobile phase consisting of acetonitrile-methanol-phosphate buffer (0.02 moL/L, pH = 5) at a flow rate of 1.0 mL/min was run through the column. A GS-6 centrifuge (Beckman, CA, USA) was utilized for acceleration of the phase separation. The hydrophobic ionic liquid (as the extraction solvent) was dispersed throughout the aqueous media by Sonorex ultrasonic baths (Bandelin, Berlin, Germany). The mobile phase was filtered using a 0.2 μm membrane filter (Millipore, Bedford, MA, USA) and it was continuously degassed using an online degasser.

2.2 Reagents

Analytical-grade reagents, 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]), sodium hexafluorophosphate ($NaPF_6$), HCl, and NaOH, were obtained from Merck (Darmstadt, Germany). 1-Hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF_4]) was purchased from Fluka (Steinheim, Switzerland). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). ATL and metoprolol (as internal standard, IS) were obtained from Daroupakhsh (Tehran, Iran). Ultrapure water (Millipore, Bedford, MA, USA) was used in all the experiments. ATL tablets (50 mg) were purchased from commercial sources.

2.3 Preparation of stock solutions, calibration standards, and QC samples

A stock solution of ATL and metoprolol at a concentration of 100 μg/mL was prepared in methanol. Working standard solutions of ATL were prepared by diluting the stock solution with methanol to required concentration. The human plasma calibration standards were obtained by spiking working standard solutions into human plasma. The QC samples (5, 100, and 500 ng/mL) were prepared by spiking appropriate amounts of working standard solutions into human plasma. A 1.0 mg/μL solution of [Bmim][BF_4] as a hydrophilic IL was prepared in methanol. A solution of 180 mg/mL of $NaPF_6$ as an ion-exchange reagent was obtained by dissolving required amount of this salt in ultra pure water. All the stock and working solutions were stored at –20°C.

2.4 Preparation of spiked human plasma

For preparation of spiked human plasma samples, different concentrations of ATL (standard solutions) were added to 1 mL of human plasma. Afterward, the spiked real samples were deproteinized by using 0.9 mL of 10% w/v zinc sulfate/acetonitrile (60:40 v/v) and vortexed for 1 min. The samples were centrifuged for 12 min at 4000 rpm and then, the clear upper phase was transferred into new test tubes and stored frozen until analysis. Finally, the obtained samples were diluted with ultra pure water and 5 mL of the resultant samples were applied for the determination of ATL.

2.5 Ionic liquid-based ultrasound-assisted in situ solvent formation microextraction

In this microextraction procedure, 5.0 mL of the spiked human plasma was placed into a centrifuge tube (with a conical

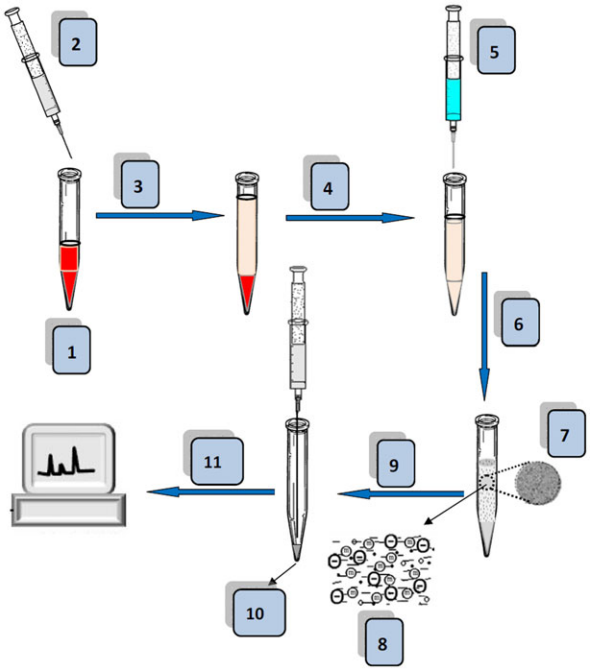

Figure 1. Schematic diagram of IL-UA-ISFME–RP-HPLC for the determination of ATL: (1) human plasma spiked with ATL; (2) addition of zinc sulfate/acetonitrile; (3) vortex and centrifugation; (4) transfer of clear phase (5) addition of $NaPF_6$ and [Bmim][BF_4]; (6) sonication; (7) fine drops of hydrophobic IL (formation of [Bmim][PF_6]); (8) interaction between ATL and IL; (9) centrifugation and decantation; (10) [Bmim][PF_6]; (11) dilution of IL and injection into RP-HPLC.

bottom). The pH of the sample solution was adjusted at 9 using NaOH (10^{-2} mol/L, by a micropipette) and 50 μL of IS was added to the sample solution. Then, 0.5 mL of $NaPF_6$ (180 mg/mL) as an ion-exchange reagent was dissolved into the resultant solution. To form water-immiscible [Bmim][PF_6] IL, 50 mg of water-miscible [Bmim][BF_4] IL was added to the aqueous sample solution. To disperse the hydrophobic IL throughout the sample, the test tube was transferred into an ultrasonic bath and sonicated for 4.5 min. Under the mentioned conditions, the in situ formed water-immiscible [Bmim][PF_6] IL was entirely dispersed into the aqueous media and thus, ATL was immediately extracted into the fine drops of [Bmim][PF_6] IL. To accelerate phase separation and to direct IL to the bottom of the test tube, the sample solution was centrifuged for 5 min at 5000 rpm. The upper phase was decanted off and the enriched phase (~12 μL) was diluted with acetonitrile to a final volume of 50 μL. Then, the diluted enriched phase was sonicated for 3 min and 20 μL was used for HPLC analysis. A scheme of the presented microextraction is shown in Fig. 1.

3 Results and discussion

A univariate approach was employed to optimize the influential factors within this study. A fixed concentration of ATL (250.0 ng/mL) was used in the optimization process. To improve precision and accuracy of the procedure in all of the experiments, metoprolol with the concentration of 500 ng/mL was used as the IS and added into the sample solution. Quantifications were performed by calculating peak areas relative to the IS from the average of three replicate measurements. Blanks were run periodically to confirm the absence of contaminations.

3.1 Selection of hydrophilic ionic liquid and ion-exchange reagent

In IL-UA-ISFME, a water-immiscible IL is formed by addition of a water-miscible IL to a sample solution containing an ion-exchange reagent. To select a desired water-miscible IL, significant points should be considered: (i) hydrophilic properties of IL, (ii) density of the in situ formed hydrophobic IL (iii), cost of IL, (iv) extraction capability of the analyte of interest, and (v) chromatographic behavior. Regarding hydrophilic properties, ILs containing Cl^-, BF_4^-, and $CF_3SO_3^-$ are water-miscible and ILs containing PF_6^- and $(CF_3SO_2)_2N^-$ are water-immiscible. It is well known that ionic compounds containing $(CF_3SO_2)_2N^-$ are relatively expensive and those containing BF_4^- are relatively inexpensive. Therefore, 1-butyl-3-methylimidazolium tetrafluoroborate [Bmim][BF_4] and 1-hexyl-3-methylimidazolium tetrafluoroborate [Hmim][BF_4] ILs, which meet the mentioned requirements, were selected for the optimization process. These ILs show acceptable hydrophilic properties, which is in accordance with the principles of ISFME, but in case of [Hmim][BF_4] IL, the extraction recovery was lower in comparison with [Bmim][BF_4] IL. For this reason, [Hmim][BF_4] IL was omitted in the evaluation. In addition, the density of the in situ formed hydrophobic IL must be higher than water, to be collected at the bottom of the test tube. Finally, to achieve a compromise between these points, 1-butyl-3-methylimidazolium tetrafluoroborate [Bmim][BF_4] IL was selected as the optimal hydrophilic IL. By dissolving this IL in an aqueous media containing $NaPF_6$ (ion-exchange reagent), it is possible to obtain hydrophobic 1-butyl-3-methylimidazolium hexafluorophosphate [Bmim][PF_6] IL, which acts as the extraction solvent.

3.2 Effect of hydrophilic ionic liquid amount

The effect of [Bmim][BF_4] IL amount on the method performance was studied. This parameter was investigated in the range of 20–90 mg. As shown in Fig. 2, reproducible and sensitive signals were obtained using 50 mg of [Bmim][BF_4] IL. It is obvious that by increasing the amount of [Bmim][BF_4] IL, the volume of the hydrophobic IL formed under these conditions significantly increases, which causes a decrease in analytical response. As a result, a value of 50 mg was used for the rest of the work.

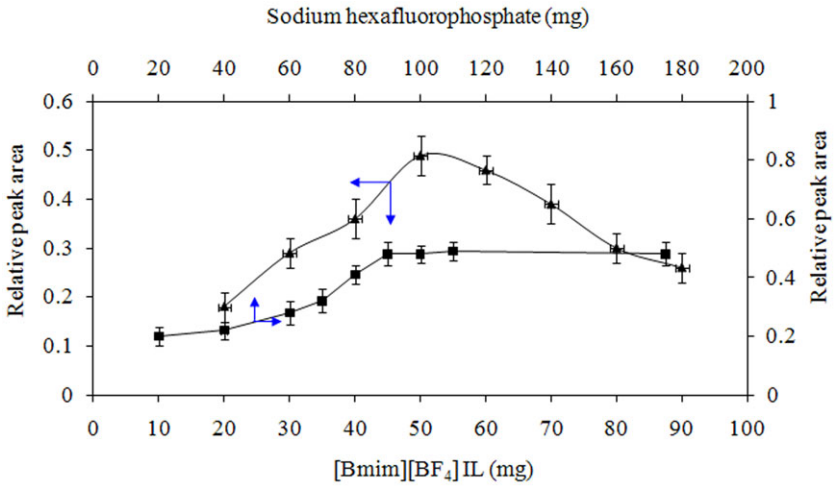

Figure 2. Effect of [Bmim][BF_4] (▲) and $NaPF_6$ (■) amounts on the extraction efficiency. Experimental conditions: ATL concentration: 250 ng/mL; pH: 9; sonication time: 4.5 min; sample volume 5 mL; centrifugation rate: 5000 rpm; centrifugation time: 5 min.

3.3 Effect of $NaPF_6$ and salt addition

To produce water-immiscible [Bmim][PF_6] IL, an excess amount of $NaPF_6$ was dissolved in an aqueous media. The influence of $NaPF_6$ amount was tested in the range of 20–175 mg. As it can be seen in Fig. 2, by increasing the amount of this reagent, the relative area increases and after 90 mg, analytical signals reach stable conditions. Finally, to obtain a balance between sensitivity and reproducibility, 90 mg of this reagent was selected as the optimal value. It is reported in the literature that a little change in the ionic strength of the sample causes a considerable change in the solubility of the IL [31, 32]. As a result, performance of the sample pretreatment method is significantly affected by variation of salt concentration of the sample [33]. To fix this problem, a common ion of the IL was dissolved in an aqueous media [34]. The effect of salt concentration on the analytical signals was tested within the range of 0–20% w/v using NaCl. Based on the results obtained in this study, this parameter has no effect on analytical responses.

3.4 Effect of sample pH

When an ionizable compound is selected for analysis, pH of the aqueous media plays a critical role in the extraction process [35, 36]. It is well known that the extraction efficiency of an ionic form of the natural analyte is not acceptable. To obtain the best yield of extraction, the uncharged form of ATL must be prevalent. The effect of sample pH was evaluated in the range of 2–12 using HCl and NaOH (10^{-2} mol/L of each, by a micropipette). As it can be seen in Fig. 3, reproducible and sensitive data were obtained at pH 9, which is compatible with the equilibrium constant of ATL ($pK_a = 9.6$) [37]. Therefore, pH value of 9 was selected for the rest of the work.

3.5 Effect of sonication time

Since the ultrasound irradiations can increase dispersion phenomenon and mass transfer [38, 39], the effect of sonication on the extraction efficiency was investigated. In the sample pretreatment method, ultrasound radiation was used as a disperser agent, which significantly improves the extraction yield and the speed of analyte migration into the tiny droplet of [Bmim][PF_6] IL. The influence of this parameter was tested in the range of 1–8 min at the power of 50 W. Based on Fig. 3, relative peak area increases as the time of ultrasound radiation increases, but after 4.5 min there is no meaningful change in the analytical signal and stable conditions are observed. So, this value was chosen for all experiments.

3.6 Analytical performance

To access practical applicability of the method, the optimized conditions were adopted in the evaluation of linear range (LR), correlation coefficient (r-squared, r^2), LOD (S/N = 3) and LOQ (S/N = 10). The LR of 2–750 ng/mL with r^2 = 0.998 is sufficient to cover possible concentrations of ATL in various real samples. Furthermore, LOD of 0.5 ng/mL and LOQ of 2 ng/mL imply the high sensitivity of the developed method. The chromatograms for blank and spiked human plasma are shown in Fig. 4 and revealed that there was no significant interference in the entire analytical procedure.

3.7 Precision and accuracy

Results of the intraday and interday precision at three different concentration levels (5, 100, and 500 ng/mL) for the QC samples are presented in Table 1. As it is illustrated, the intra-assay RSD measured at six runs a day of a sample was 4.8–5.1%. Also, the interassay precision was evaluated on a 3-day period with a total of nine runs provided RSD values in the range of 5.0–5.6%.

Calculations for enrichment factor (EF) and extraction recovery (ER) were done according to the previously reported documents [40]. ER experiments were carried out by analyzing plasma samples spiked with ATL at different concentration levels (5, 100, and 500 ng/mL). As shown in Table 2, ER% and EF were within 90.0–96.7% and 18.0–19.3, respectively.

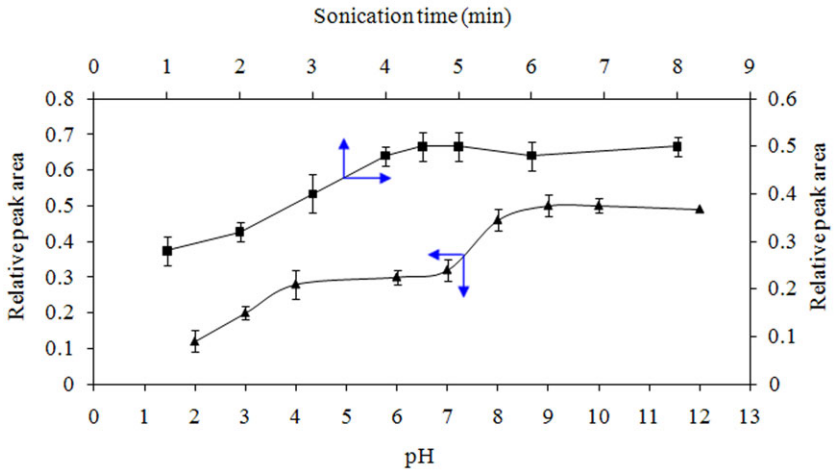

Figure 3. Effect of pH (▲) and sonication time (■) on the extraction efficiency. Experimental conditions: ATL concentration 250 ng/mL; amount of ion exchange reagent 90 mg; amount of [Bmim][BF_4] IL 50 mg; sample volume 5 mL; centrifugation rate 5000 rpm; centrifugation time 5 min.

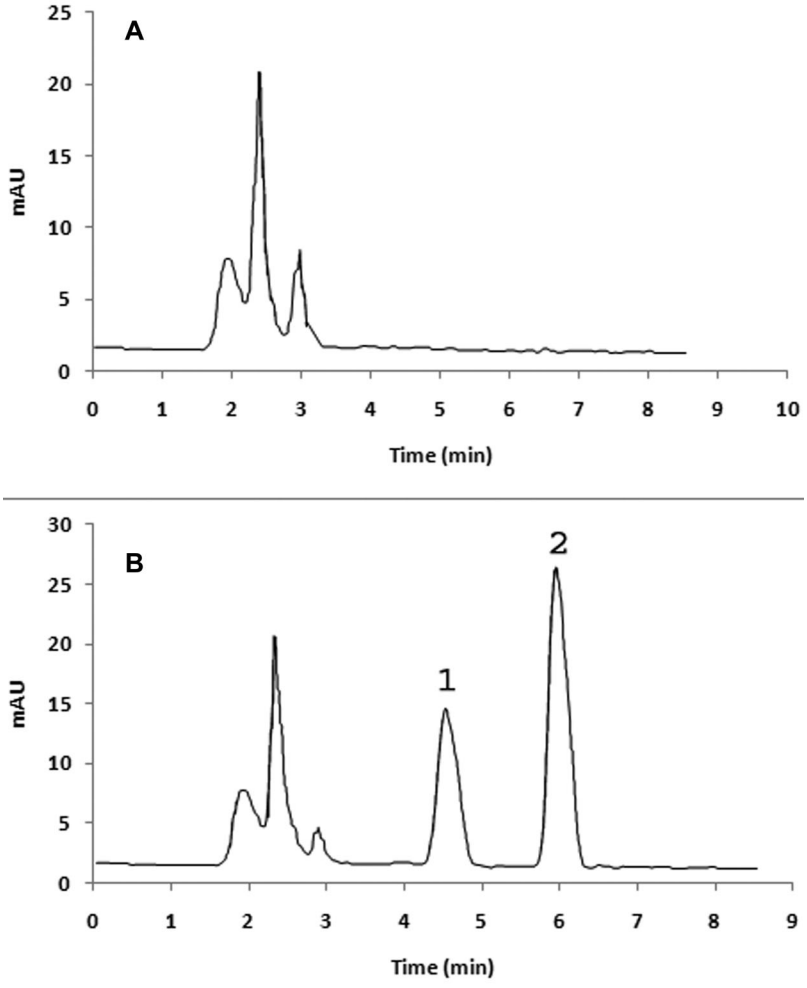

Figure 4. Typical chromatograms of ATL in human plasma; A: blank plasma; B: spiked plasma with 250 ng/mL of ATL. Peaks: 1 = ATL; 2 = metoprolol (internal standard).

3.8 Application

The developed procedure under optimal conditions was used to determine ATL concentration in human plasma. ATL was administered orally as a single dose of 50 mg to three healthy male volunteers. The real samples were collected at 0, 1, 2, 3, 4, 6, 8, 12 h after the drug administration. The main pharmacokinetic parameters including T_{max}, C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, and $T_{½}$ are summarized in Table 3. A comparison of the method with other previously reported techniques for the determination of ATL is provided in Supporting Information Table S1.

Table 1. Intraday and interday precision for the determination of ATL in spiked human plasma samples

Drug	Intraday, $n = 6$				Interday, $n = 9$		
	Concentration (ng/mL)	Found value (ng/mL)[a)]	RSD (%)	E_r (%) [b]	Found value (ng/mL)[a)]	RSD (%)	E_r (%)
ATL	5	5.2	4.8	4.0	5.5	5.1	10.0
	100	96.0	4.5	4.0	91.3	5.0	8.7
	500	486.3	5.1	2.7	470.3	5.6	5.9
IS	500	473.9	4.0	5.2	470.2	4.9	6.0

a) The average of three independent measurements.
b) Relative error.

Table 2. Extraction recovery and enrichment factor of the proposed method for the determination of ATL in human plasma

ATL concentration (ng/mL)	Plasma sample	
	ER[a)] ± SD[b)](%)	EF
5	90.0 ± 5.0	18.0
100	93.5 ± 4.9	18.7
500	96.7 ± 5.1	19.3

a) Extraction recovery.
b) Standard deviation, $n = 3$.

Table 3. Pharmacokinetic parameters of the drug in human plasma after oral administration of an ATL tablet

Pharmacokinetic parameters	ATL (50 mg)	
	Mean	SD
T_{max} (h)	3.3	0.5
C_{max} (ng/mL)	144.5	14.6
$AUC_{0–12}$ (ng h mL^{-1})	591.0	87.9
$AUC_{0-\infty}$ (ng h mL^{-1})	801.6	61.2
$T_{½}$ (h)	5.6	0.7

T_{max}: Time required to reach maximum plasma concentration.
C_{max}: Maximum plasma concentration.
$AUC_{0–12}$: Area under curve.
$AUC_{0-\infty}$: Area under curve at infinite time.
$T_{½}$ (h): Time required to reach half of the concentration.

4 Concluding remarks

This paper outlined the successful development and application of IL-UA-ISFME technique combined with HPLC–UV for qualitative and quantitative determination of ATL in human plasma. The results showed that IL-UA-ISFME–HPLC is a simple, sensitive, and very effective method, with no need to use hazardous extraction and disperser solvents. The mass transfer of the analyte was speeded up by sonication, which provided high recovery and enrichment factor. The performance of this eco-friendly method in real samples was satisfactory. Putting all the benefits together, it possesses great potential to be employed in bio-analytical and pharmacokinetic studies.

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The authors have declared no conflict of interest.

5 References

[1] Vaikosen, E., Ebeshi, B., Joffa, P., Simple, Sensitive and reproducible acetous perchlorate and spectrophotometric determination of atenolol in tablet dosage form. *J. Pharm. Sci. & Res.* 2012, *4*, 1933–1938.

[2] Smith, R. D., Yokoyama, H., Averill, D. B., Schiffrin, E. L., Ferrario, C. M., Reversal of vascular hypertrophy in hypertensive patients through blockade of angiotensin II receptors. *J. Am. Soc. Hypertens.* 2008, *2*, 165–172.

[3] Brayfield, A., Martindale: The Complete Drug Reference. 38th Edition, *Pharmaceutical Press*, London 2014.

[4] Verghese, C., McLeod, A., Shand, D., Rapid high-performance liquid chromatographic method for the measurement of atenolol in plasma using UV detection. *J. Chromatogr. B: Biomed. Sci. Appl.* 1983, *275*, 367–375.

[5] Black, S., Stenhouse, A., Hansson, R., Solid–phase extraction and derivatisation methods for β–blockers in human post mortem whole blood, urine and equine urine. *J. Chromatogr. B: Biomed. Sci. Appl.* 1996, *685*, 67–80.

[6] Chiu, F., Zhang, J., Li, R., Raymond, K., Efficient assay for the determination of atenolol in human plasma and urine by high–performance liquid chromatography with fluorescence detection. *J. Chromatogr. B: Biomed. Sci. Appl.* 1997, *691*, 473–477.

[7] de Abreu, L. R. P., de Castro, S. A. C., Pedrazzoli, J., Atenolol quantification in human plasma by high–performance liquid chromatography: Application to bioequivalence study. *AAPS PharmSciTech.* 2003, *5*, 116–122.

[8] Angier, M. K., Lewis, R. J., Chaturvedi, A. K., Canfield, D. V., Gas chromatographic–mass spectrometric differentiation of atenolol, metoprolol, propranolol, and an

interfering metabolite product of metoprolol. *J. Anal. Toxicol.* 2005, *29*, 517–521.

[9] Kasture, A., Ramteke, M., Simultaneous UV–spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. *Indian J. Pharm. Sci.* 2006, *68*, 394–396.

[10] Ferraro, M. C., Castellano, P. M., Kaufman, T. S., Chemometrics–assisted simultaneous determination of atenolol and chlorthalidone in synthetic binary mixtures and pharmaceutical dosage forms. *Anal. Bioanal. Chem.* 2003, *377*, 1159–1164.

[11] Yilmaz, B., Arslan, S., Determination of atenolol in human urine by gas chromatography–mass spectrometry method. *J. Chromatogr. Sci.* 2011, *49*, 365–369.

[12] Spanakis, M., Niopas, I., Determination of atenolol in human plasma by HPLC with fluorescence detection: validation and application in a pharmacokinetic study. *J. Chromatogr. Sci.* 2013, *51*, 128–132.

[13] Basan, H., Yarımkaya, S., A novel solid–phase extraction–spectrofluorimetric method for the direct determination of atenolol in human urine. *Luminescence* 2014, *29*, 225–229.

[14] Demeestere, K., Dewulf, J., De Witte, B., Van Langenhove, H., Sample preparation for the analysis of volatile organic compounds in air and water matrices. *J. Chromatogr. A*, 2007, *1153*, 130–144.

[15] Lee, J., Lee, H. K., Rasmussen, K. E., Pedersen–Bjergaard, S., Environmental and bioanalytical applications of hollow fiber membrane liquid–phase microextraction: a review. *Anal. Chim. Acta* 2008, *624*, 253–268.

[16] Arce, L., Nozal, L., Simonet, B., Valcárcel, M., Ríos, A., Liquid–phase microextraction techniques for simplifying sample treatment in capillary electrophoresis. *TrAC, Trends Anal. Chem*. 2009, *28*, 842–853.

[17] Sarafraz-Yazdi, A., Amiri, A., Liquid–phase microextraction. *TrAC, Trends Anal. Chem.* 2010, *29*, 1–14.

[18] Abdulra'uf, L. B., Sirhan, A. Y., Huat Tan, G., Recent developments and applications of liquid phase microextraction in fruits and vegetables analysis. *J. Sep. Sci.* 2012, *35*, 3540–3553.

[19] Hu, B., He, M., Chen, B., Xia, L., Liquid phase microextraction for the analysis of trace elements and their speciation. *Spectrochim. Acta, Part B* 2013, *86*, 14–30.

[20] Halvorsen, T. G., Pedersen-Bjergaard, S., Reubsaet, J. L. E., Rasmussen, K. E., Liquid–phase microextraction combined with liquid chromatography–mass spectrometry. Extraction from small volumes of biological samples. *J. Sep. Sci*. 2003, *26*, 1520–1526.

[21] Huang, C., Seip, K. F., Gjelstad, A., Shen, X., Pedersen-Bjergaard, S., Combination of electromembrane extraction and liquid–phase microextraction in a single step: Simultaneous group separation of acidic and basic drugs. *Anal. Chem.* 2015, *87*, 6951–6957.

[22] Han, D., Tang, B., Ri Lee, Y., Ho Row, K., Application of ionic liquid in liquid phase microextraction technology. *J. Sep. Sci.* 2012, *35*, 2949–2961.

[23] Stanisz, E., Werner, J., Zgoła-Grześkowiak, A., Liquid–phase microextraction techniques based on ionic liquids for preconcentration and determination of metals. *TrAC, Trends Anal. Chem.* 2014, *61*, 54–66.

[24] Ge, D., Lee, H. K., Ionic liquid based dispersive liquid—liquid microextraction coupled with micro–solid phase extraction of antidepressant drugs from environmental water samples. *J. Chromatogr. A* 2013, *1317*, 217–222.

[25] Wang, S.-R., Wang, S., Ionic liquid-based hollow fiber–supported liquid–phase microextraction enhanced electrically for the determination of neutral red. *J. Food Drug Anal.* 2014, *22*, 418–424.

[26] Yu, H., Ho, T. D., Anderson, J. L., Ionic liquid and polymeric ionic liquid coatings in solid–phase microextraction. *TrAC, Trends Anal. Chem.* 2013, *45*, 219–232.

[27] Marcinkowski, Ł., Pena-Pereira, F., Kloskowski, A., Namieśnik, J., Opportunities and shortcomings of ionic liquids in single-drop microextraction. *TrAC, Trends Anal. Chem.* 2015, *72*, 153–168.

[28] Fontanals, N., Borrull, F., Marcé, R. M., Ionic liquids in solid–phase extraction. *TrAC, Trends Anal. Chem.* 2012, *41*, 15–26.

[29] Ruiz-Aceituno, L., Sanz, M. L., Ramos, L., Use of ionic liquids in analytical sample preparation of organic compounds from food and environmental samples. *TrAC, Trends Anal. Chem.* 2013, *43*, 121–145.

[30] Zeeb, M., Mirza, B., Zare-Dorabei, R., Farahani, H., Ionic liquid-based ultrasound-assisted in situ solvent formation microextraction combined with electrothermal atomic absorption spectrometry as a practical method for preconcentration and trace determination of vanadium in water and food samples. *Food Anal. Method* 2014, *7*, 1783–1790.

[31] Baghdadi, M., Shemirani, F., In situ solvent formation microextraction based on ionic liquids: a novel sample preparation technique for determination of inorganic species in saline solutions. *Anal. Chim Acta* 2009, *634*, 186–191.

[32] Yousefi, S. R., Shemirani, F., Development of a robust ionic liquid–based dispersive liquid—liquid microextraction against high concentration of salt for preconcentration of trace metals in saline aqueous samples: Application to the determination of Pb and Cd. *Anal. Chim. Acta* 2010, *669*, 25–31.

[33] Pedersen-Bjergaard, S., Rasmussen, K. E., Bioanalysis of drugs by liquid–phase microextraction coupled to separation techniques. *J. Chromatogr. B* 2005, *817*, 3–12.

[34] Zeeb, M., Ganjali, M. R., Norouzi, P., Preconcentration and trace determination of chromium using modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction: application to different water and food samples. *Food Anal. Method* 2013, *6*, 1398–1406.

[35] Martínez, V., Maguregui, M., Jimenez, R., Alonso, R., Determination of the pKa values of beta–blockers by automated potentiometric titrations. *J. Pharm. Biomed. Anal.* 2000, *23*, 459–468.

[36] Tolcha, T., Merdassa, Y., Megersa, N., Low–density extraction solvent based solvent–terminated dispersive liquid—liquid microextraction for quantitative determination of ionizable pesticides in environmental waters. *J. Sep. Sci.* 2013, *36*, 1119–1127.

[37] http://www.drugbank.ca/drugs/DB00335

[38] Yamini, Y., Saleh, A., Ultrasound–assisted emulsification microextraction combined with injection–port derivatization for the determination of some chlorophenoxyacetic acids in water samples. *J. Sep. Sci.* 2013, *36*, 2330–2338.

[39] Chen, X., You, X., Liu, F., Hou, F., Zhang, X., Ionic–liquid–based, manual–shaking– and ultrasound–assisted, surfactant–enhanced emulsification microextraction for the determination of three fungicide residues in juice samples. *J. Sep. Sci.* 2015, *38*, 93–99.

[40] Ho, T. S., Pedersen-Bjergaard, S., Rasmussen, K. E., Recovery, enrichment and selectivity in liquid-phase microextraction: comparison with conventional liquid–liquid extraction. *J. Chromatogr A* 2002, *963*, 3–17.

