Modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction combined with spectrofluorimetry for trace determination of ofloxacin in pharmaceutical and biological samples

Zeeb M., *Ganjali M.R., Norouzi P.

Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, Iran.

Received 8 Oct 2011; Revised 6 Dec 2011; Accepted 7 Dec 2011

ABSTRACT

Background and the purpose of the study: Ofloxacin is a quinolone synthetic antibiotic, which acts against resistant mutants of bacteria by inhibiting DNA gyrase. This antibacterial agent is widely used in the treatment of respiratory tract, urinary tract and tissue-based infections, which are caused by Gram-positive and Gram-negative bacteria. In this work, an efficient modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction (M-IL-CIA-DLLME) was combined with spectrofluorimetry for trace determination of ofloxacin in real samples.

Methods: In this microextraction method, hydrophobic 1-hexyl-3-methylimidazolium hexafluorophosphate ([Hmim] [PF_6]) ionic liquid (IL) as a microextraction solvent was dispersed into a heated sample solution containing sodium hexafluorophosphate ($NaPF_6$) (as a common ion) and the analyte of interest. Afterwards, the resultant solution was cooled in an ice-water bath and a cloudy condition was formed due to a considerable decrease of IL solubility. After centrifuging, the enriched phase was introduced to the spectrofluorimeter for the determination of ofloxacin.

Results and major conclusion: In this technique, the performance of the microextraction method was not influenced by variations in the ionic strength of the sample solution (up to 30% w/v). Furthermore, [Hmim][PF_6] IL was chosen as a green microextraction phase and an alternative to traditional toxic organic solvents. Different parameters affecting the analytical performance were studied and optimized. At optimum conditions, a relatively broad linear dynamic range of 0.15-125 µg l^{-1} and a limit of detection (LOD) of 0.029 µg l^{-1} were obtained. The relative standard deviation (R.S.D.) obtained for the determination of five replicates of the 10 ml solution containing 50 µg l^{-1} ofloxacin was 2.7%. Finally, the combined methodology was successfully applied to ofloxacin determination in actual pharmaceutical formulations and biological samples.

Keywords: 1-Hexyl-3-methylimidazolium hexafluorophosphate, Common ion, Real samples.

INTRODUCTION

Ofloxacin (OFL), 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-ppieraziny)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid is a member of the third generation fluoroquinolones (FQ) with antibacterial activity. This antibacterial agent is widely used in the treatment of respiratory tract, urinary tract and tissue-based infections, which are caused by Gram-positive and Gram-negative bacteria (1, 2). Bactericidal action of this drug results from interference with DNA gyrase which is required for the synthesis of bacterial DNA (3, 4). To adjust the drug dosage and to study drug-drug interactions, it is critical to monitor OFL concentrations in real samples. To date, several analytical methods such as spectrophotometry (5-7), spectrofluorimetry (8, 9), high performance liquid chromatography (10-15) and capillary electrophoresis (16) have been developed for OFL determination in pharmaceutical formulations and biological samples. However, some of these methods are tedious, time-consuming, expensive, low sensitive or environmentally unfriendly. Thus, there is an increasing need to develop simple and reliable analytical methods to overcome the aforementioned problems.

Sample preparation is a critical step to isolate the analytes of interest from the sample matrix, as well as to increase the concentration of analytes prior to their determinations, especially when their levels are lower than the detection limit of analytical techniques.To reduce the amount of toxic

Correspondence: ganjali@khayam.ut.ac.ir

organic solvents in sample preparation procedures, several liquid-liquid microextraction methods have been developed and dispersive liquid-liquid microextraction (DLLME) is one the practical methods. DLLME offers several advantages such as high recovery, simplicity of operation, low cost and rapidity. It has been widely applied to preconcentrate and separate organic and inorganic compounds in different samples (17-23). In this method, however, the use of toxic solvents such as chloroform, carbon tetrachloride, etc., as the extraction phase is a common practice. Ionic liquids (ILs) have a variety of chemical and physical properties such as low vapor pressure, excellent thermal stability, adjustable miscibility, moderate solubility of organic compounds and metal ions, etc., which make them highly attractive in microextraction methods (24). To completely remove hazardous organic solvents in microextraction methods, ILs have been successfully applied in sample pretreatment methods as green solvents such as ionic liquid-based liquid-liquid microextraction (IL-LLME) (25, 26), cold induced aggregation microextraction (CIAME) (27), ionic liquid-based single-drop microextraction (IL-SDME) (28, 29), ionic liquid-based dispersive liquid-liquid microextraction (IL-DLLME) (30-32), temperature-controlled ionic liquid dispersive liquid phase microextraction (TIL-DLME) (33), etc. Recently, CIAME and IL-DLLME have received much attention and they have been applied to preconcentrate a wide range of compounds. In CIAME, IL-phase is dispersed into the sample under driving high temperature, and then aggregated at low temperature. In IL-DLLME, IL-phase is dispersed throughout the sample using a disperser solvent. In comparison with CIAME, IL-DLLME is demonstrated to be more efficient for dispersing the IL-phase into the sample solution thus significantly improves the extraction time and recovery. However, because the solubility of ILs in aqueous media depends on temperature changes, CIAME needs less IL to complete the extraction process.

In 2010, a robust and practical sample preparation method termed cold-induced aggregation dispersive liquid-liquid microextraction (IL-CIA-DLLME) was developed based on the combination of CIAME with IL-DLLME to reduce the extraction time and required amount of IL (34). However, the performance of this microextraction method is significantly decreased by variations in the ionic strength of the sample solution. It is well established that the solubility of ILs increases as the salt content of aqueous solution increases. Consequently, the volume of the settled phase depends strongly on the ionic strength of the samples. In this study, by introducing a common ion of the ionic liquid into the sample solution, the solubility of ILs was significantly decreased. As a result, the volume of the settled phase was not affected by variations in the salt content of the sample. The aim of this work was to study the applicability of modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction (M-IL-CIA-DLLME) followed by spectrofluorimetry to determine OFL as a test compound. Spectrofluorimetric method was applied due to ease, proper selectivity, sensitivity, wide dynamic range and low cost of operation. To the best of our knowledge, until now, no method based on the microextraction with ILs and spectrofluorimetric detection has been proposed for preconcentration, extraction and determination of drugs in their trace levels. The proposed combined methodology was successfully applied to determine OFL in pharmaceutical formulations and biological samples.

MATERIAL AND METHODS

Instrumentation

Fluorescence measurements were performed by Perkin-Elmer LS 50 spectrofluorimeter equipped with xenon discharge lamp, and quartz micro-cell with a volume of 100 µl. A centrifuge from Hettich (Tuttlingen, Germany) was utilized to accelerate the phase separation process. An adjustable sampler (10-100 µl) was purchased from Eppendorf (Hamburg, Germany).

Reagents and materials

All chemicals were of analytical reagent. 1-Hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF_6], acetone, acetonitrile, methanol, ethanol, sodium hexafluorophosphate ($NaPF_6$) were obtained from Merck (Darmstadt, Germany). Pure powder of OFL was obtained from DarouPakhsh Company (Tehran, Iran). Stock solution of OFL at concentration of 1000 mg l^{-1} was prepared by dissolving the required amount of pure drug in ultra pure water. A solution of 250 mg ml^{-1} of $NaPF_6$ was prepared by dissolving appropriate amount of $NaPF_6$ in water. Ofloxacin tablets, labeled to contain 200 and 300 mg OFL per tablet, were obtained from a local pharmacy.

General analytical procedure

Aliquots of 10.0 ml sample (pH = 4.5) containing OFL in the range of 0.15-125 µg l^{-1} were placed into a screw-glass test tube with conic bottom and 0.7 ml of $NaPF_6$ (250 mg ml^{-1}) was added. Then, the resultant solution was kept in a thermo stated bath at 40 °C for 4 min. 500 µl of ethanol (as disperser solvent) containing 45 mg of [Hmim][PF_6] (as microextraction solvent) was injected rapidly into the sample solution by a 1.00 ml syringe. Then, the obtained solution was cooled in ice-water bath for 4 min and a cloudy condition was formed. In order to accelerate phase separation, the resultant solution was centrifuged for 7 min at 4000 rpm. The upper

aqueous solution was removed by a pipette. Finally, the settled phase in the glass test tube was dissolved in 200 µl of ethanol. Then 100 µl of the diluted settled phase was removed by a sampler and transferred to the micro-cell of the fluorimeter. The fluorescence intensity was measured at 501 nm with the excitation wavelength set at 305 nm. A reagent blank was prepared using a similar procedure but without OFL.

Analysis of tablets

An accurately weighed quantity of the mixed contents of 5 powdered tablets, labeled as containing 200 mg OFL each, equivalent to 0.05 g of OFL was dissolved in ultra pure water by sonication. The above mentioned solution was filtered into a 1000 ml volumetric flask by Whatman No. 42 filter paper, and made up to the mark with ultra pure water. Serial dilutions were performed and the analysis was followed up as indicated in the general analytical procedure.

Analysis of spiked human plasma

Human plasma samples (1.0 ml) were spiked with OFL solutions, deproteinized by addition of 4 ml acetonitrile, and centrifuged at 4000 rpm for 15 min. Then, 2.0 ml of the clear supernatant was diluted to 50 ml and subjected to the proposed method.

Analysis of spiked human urine

Human urine samples (10 ml) were transferred into graduated centrifuge tubes. These solutions were centrifuged for 5 min at 4000 rpm. Then, aliquots of 2 ml from clear supernatant were put in new centrifuge tubes and spiked with different amounts of OFL (2 to 100 µg l^{-1}); the general analytical procedure was followed.

RESULTS AND DISCUSSION

In this study, a new simple and rapid modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction (M-IL-CIA-DLLME) was applied to preconcentrate trace levels of OFL as a prior step to its determination by spectrofluorimetry. In order to obtain high enrichment factor, effect of different variables such as amounts of IL and disperser solvent, amount of common ion, pH, ionic strength and extraction temperature were evaluated and optimized.

Type of IL and disperser solvent

The IL applied in M-IL-CIA-DLLME must be liquid in experimental conditions and hydrophobe. In addition, the density of IL has to be high enough to allow the fine particles of IL settle to the bottom of the test tube. Imidazolium-ILs containing PF_6^- as anion have the aforementioned properties, so they are suitable for liquid-liquid microextraction. By consideration of these points and the cost of ILs, [Hmim][PF_6] were selected as an extraction solvent. For M-IL-CIA-DLLME, disperser solvent should be miscible with water and microextraction solvent. In order to obtain the best extraction condition, several solvents such as acetone, methanol and ethanol were evaluated as disperser solvents. A series of sample solutions were tested using 500 µl of each disperser solvent and 45 mg of [Hmim][PF_6]. The obtained results revealed that the type of disperser solvent had no significant effect on the analytical signals. Due to the lower toxicity of ethanol, it was chosen as disperser solvent in all experiments.

Influence of diluting agent

In order to reduce the viscosity of the IL-phase, it was diluted with organic solvent prior to analysis. Different diluting agents such as methanol, ethanol, acetone and acetonitrile were investigated in order to select an organic solvent which can dissolve the enriched phase completely, to provide the best analytical signal and have less toxicity. According to these criteria ethanol was selected as a diluting agent for the rest of the work.

Influence of IL and disperser solvent

Effect of the amount of [Hmim][PF_6] on the extraction efficiency was evaluated within the range of 20-125 mg. Figuer 1 shows variation of fluorescence intensity versus [Hmim][PF_6] amount. As it can be seen, the analytical response increases as the amount of [Hmim][PF_6] increases and then starts to decrease. Therefore, in order to obtain sensitive analytical signals, 45 mg of IL was chosen for all experiments.

The influence of the volume of ethanol on the microextraction performance was also studied in the range of 300 µl to 1.0 ml (Fig. 2). At low volume of disperser solvent the cloudy condition was not formed entirely. At high volume of ethanol, the solubility of analyte in water increased and the sensitivity of the proposed method decreased. Therefore, a volume of 500 µl of ethanol was utilized in the subsequent experiments.

Influence of common ion

Effect of $NaPF_6$ amount as a common ion source on the analytical response was studied within the range of 0-250 mg (Fig. 3). Because of the common ion effect, an increase in the amount of $NaPF_6$ causes a decrease in the solubility of IL and as a result the microextraction efficiency and the analytical signal increase. As it can be found from figure 3, the fluorescence intensity increases by increase in the amount of $NaPF_6$ up to 120 mg and then remains nearly stable. However, 175 mg of $NaPF_6$ was chosen for the rest of the work.

Influence of the ionic strength

In traditional ionic-liquid microextraction methods, the volume of the settled phase depends strongly on

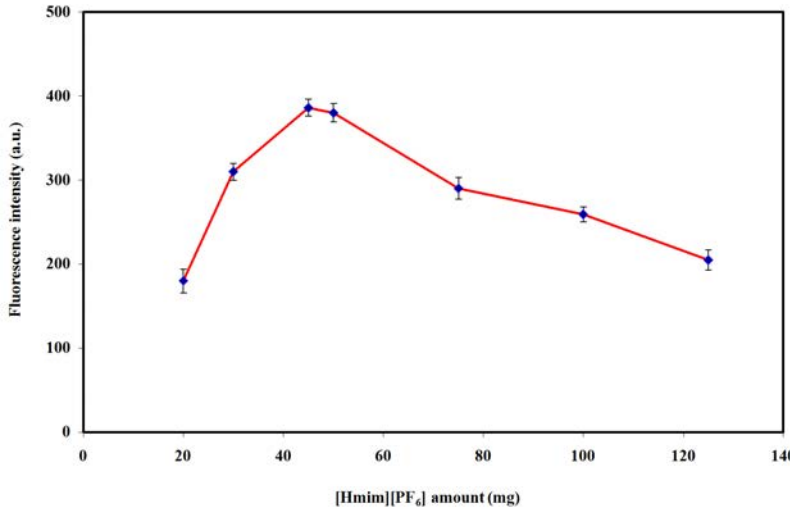

Figure 1. Effect of the amount of [Hmim][PF_6] on the fluorescence signal. Experimental conditions: OFL concentration 50 µg l^{-1}; $NaPF_6$ 175 mg; volume of disperser solvent 500 µl; pH 4.5; extraction temperature 40 °C.

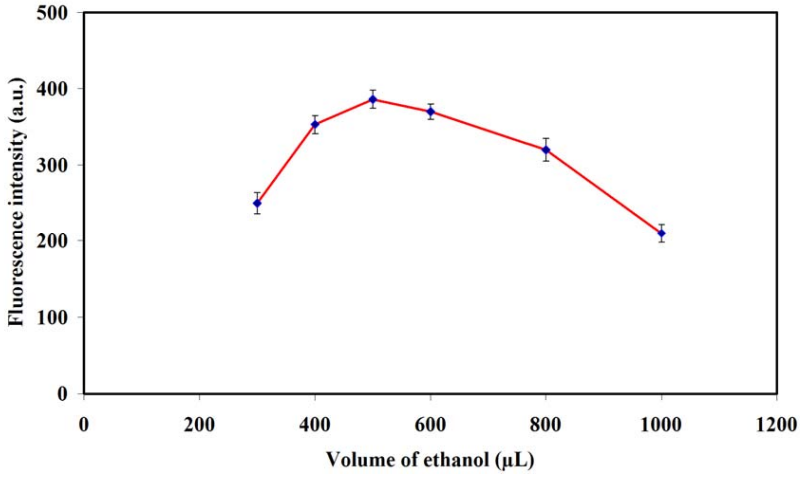

Figure 2. Effect of the volume of disperser solvent on the analytical sensitivity. Experimental conditions: OFL concentration 50 µg l^{-1}; [Hmim][PF_6] 45 mg; $NaPF_6$ 175 mg; pH 4.5; extraction temperature 40°C.

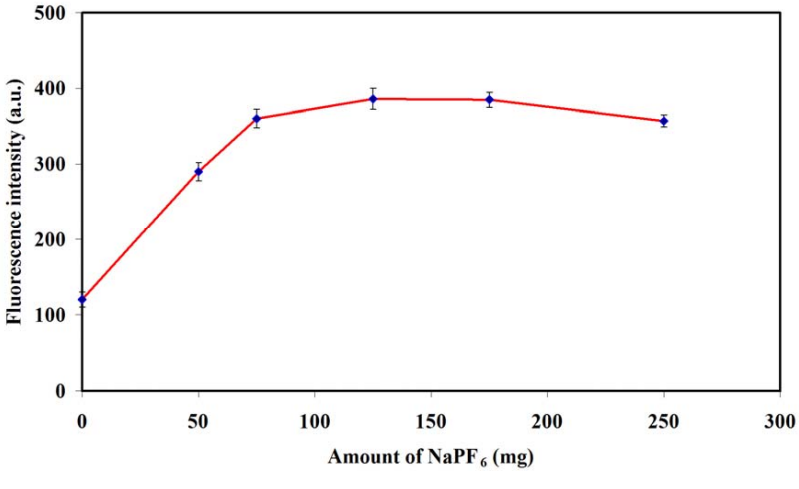

Figure 3. Influence of $NaPF_6$ amount on the analytical signal. Experimental conditions: OFL concentration 50 µg l^{-1}; [Hmim][PF_6] 45 mg; volume of disperser solvent 500 µl; pH 4.5; extraction temperature 40 ºC.

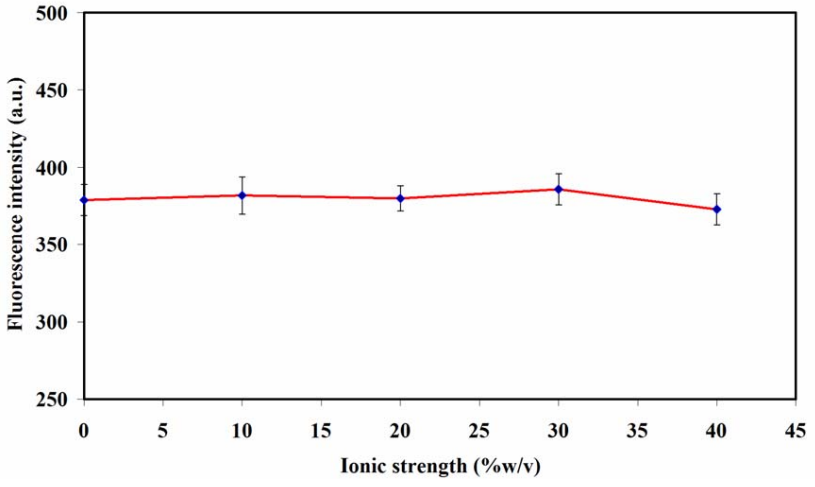

Figure 4. Influence of ionic strength on the fluorescence intensity. Experimental conditions: OFL concentration 50 µg l^{-1}; [Hmim][PF_6] 45 mg; $NaPF_6$ 175 mg; volume of disperser solvent 500 µl; pH 4.5; extraction temperature 40 ºC.

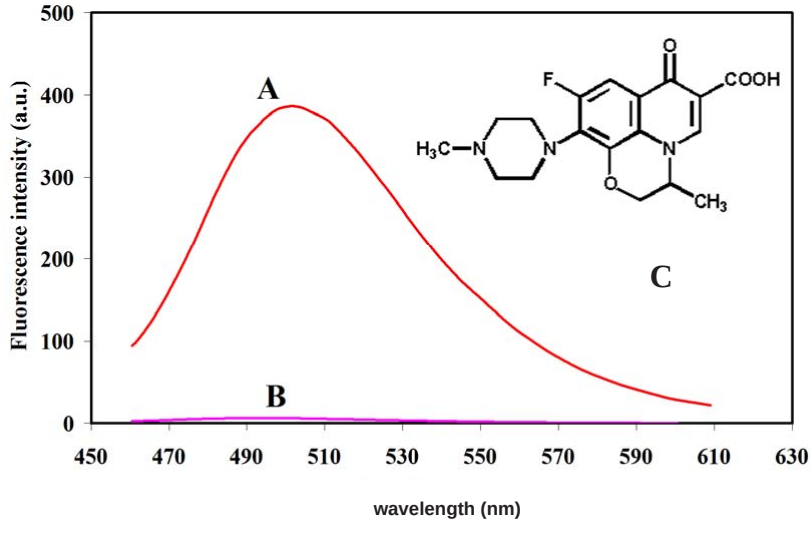

Figure 5. Emission spectrum (A) obtained for standard solution of Ofloxacin (C, 50 µg l^{-1}) treated the same as described in general analytical procedure. (B) Emission spectrum of reagent blank in [Hmim][PF_6] ionic liquid. experimental conditions: [Hmim][PF_6] 45 mg; $NaPF_6$ 175 mg; volume of disperser solvent 500 µl; pH 4.5; extraction temperature 40 °C.

the salt concentration of the sample solution which is due to the dependence of ILs solubility upon the ionic strength. In order to solve this problem, a common ion of IL was dissolved in aqueous solution. In this work, $NaNO_3$ was selected to study the effect of ionic strength within the range of 0-40% (w/v) (Fig. 4). Results revealed that phase separation occurred up to 30% of $NaNO_3$. Consequently, it was reasoned that due to the presence of common ion, the performance of the proposed microextraction method is not influenced by variations in the ionic strength of sample solutions.

Influence of pH

Effect of pH on the M-IL-CIA-DLLME was investigated in the range of 1.0-14.0 using HCl and NaOH. The results demonstrated that the maximum fluorescence intensity can be obtained at pH 4.5. However, the analytical signal decreased by increase in the pH further. Thus, pH 4.5 was selected to obtain the best sensitivity.

Influence of temperature

In this study, effect of temperature was studied within the range of 30-50 ºC. ILs can be dissolved more easily at the temperatures above 30 ºC. These results showed that the best extraction efficiency was obtained at 40 °C. Thus, this value was selected for the rest of the work. After dispersing IL, solutions were cooled to the temperature range of 0-25 °C. When the temperature decreased, the fluorescence intensity increased due to a decrease in IL solubility.

Table 1. Analytical characteristics of modified IL-CIA-DLLME for the determination of OFL.

Parameter	Analytical feature
Linear range (µg l^{-1})	0.15-125
Correlation coefficient (R^2)	0.9991
Limit of detection (µg l^{-1})	0.029
Repeatability (R.S.D.[a], %) (n = 5)	2.7
Preconcentration factor (PF)	50
Sample volume (ml)	10
Extraction time (min)	<5

[a] R.S.D. was obtained for the determination of five replicates of 50 µg l^{-1} OFL.

Table 2. Determination of OFL in tablets by the proposed methodology and by a reference method (14).

Claimed (mg/tablet)	Proposed methodology (mg)[a]	Reference method (mg)[a]	Error (%) [b]	Error (%) [c]
200	194.3 ± 6.2	201.8 ± 4.0	-2.8	-3.7
300	304.6 ± 9.5	307.0 ± 5.9	+1.5	-0.8

[a] Standard deviations are based on four replicates.
[b] Error against the declared value.
[c] Error against the reported method.

Thus, a temperature of 0 ºC was used for the rest of the work by transferring the test tubes in the ice-water bath.

Influence of microextraction time
Microextraction time is one of the important factors which can affect the microextraction efficiency. In M-CIA-DLLME method, the microextraction time is defined as an interval between transferring the sample solution containing the dissolved IL into the ice-water bath and starting centrifugation. The dependence of microextraction efficiency upon extraction time was studied within the range of 2-8 min. Based on the results obtained in this experiment no significant improvement was obtained after 4 min. Therefore, 4 min was selected as an optimum value.

Selectivity of the method
Under the above optimized conditions, the influence of some foreign substances on the assay of OFL at 50 µg l^{-1} level was studied. It was found 150-fold Na^+, NH_4^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}, SO_4^{2-}, starch, glucose, lactose, fructose, sucrose, ascorbic acid, citric acid, uric acid, oxalic acid, lactic acid and tartaric acid have almost no effect on the analytical signal of OFL (analytical signal change below 5%). All results indicated the proposed method had good selectivity for determination of OFL in pharmaceutical and biological samples.

Spectral characteristics of OFL and reagent blank
The emission spectrum of OFL was recorded as described in the general analytical procedure (Fig. 5). As it can be ascertained, the emission peak of OFL is at 501 nm, while its excitation peak is at 305 nm. Fluorescence spectrum of the reagent blank was recorded. Figuer 5 shows that the emission of reagent blank has no notable effect on the determination of OFL. Thus, the wavelengths mentioned above were selected for quantitative analysis of OFL.

APPLICATION

Figures of merits
Under optimized conditions, calibration graph was achieved using 10 ml of standard solutions of OFL. Results revealed that the calibration curve was linear from 0.15 to 125 µg l^{-1} OFL. Analytical characteristics of the proposed methodology are shown in Table 1. The limit of detection (LOD), calculated as three times the standard deviation of the measurement of blanks divided by the slope of the calibration curve, was found to be 0.029 µg l^{-1}.

Precision and accuracy
For repeatability monitoring, 5 replicate standard samples of 0.15, 5, 25, 50 and 100 µg/l were measured. The mean concentrations by the proposed method were 0.140±0.005, 4.6±0.1, 24.5±1.1, 52.0±1.4 and 104.1±2.0 µg/l with respective relative standard deviation (R.S.D.) values of 3.6, 2.2, 4.5, 2.7 and 1.9%.

Analysis of OFL in pharmaceutical formulations
In order to show the validity of the proposed method, it was applied for OFL determination in commercial tablets. Three replicate determinations were performed, and satisfactory results were achieved. Table 2 shows the results obtained by applying the

Table 3. Determination of OFL in spiked human urine and spiked human plasma by present work.

Drug	Spiked urine				Spiked plasma			
	Amount added (µg l^{-1})	Amount found (µg l^{-1}) ± S.D.[a]	R.S.D. (%)	Recovery (%)	Amount added (µg l^{-1})	Amount found (µg l^{-1}) ± S.D.[a]	R.S.D. (%)	Recovery (%)
OFL	2	1.83 ± 0.09	4.9	91.5	2	1.79 ± 0.11	6.1	89.5
	5	4.92 ± 0.18	3.6	98.4	5	4.72 ± 0.16	3.4	94.4
	10	9.49 ± 0.31	3.3	94.9	10	9.71 ± 0.46	4.7	97.1
	100	106.91 ± 4.01	3.7	106.9	100	93.02 ± 5.11	5.5	93.0

[a] Average of four independent measurements.

present method and those obtained by a reference method (14). The results show the applicability of the proposed methodology and its accuracy for quantitative analysis of OFL in this type of samples.

Analysis of OFL in spiked biological samples

The accuracy of the present method was evaluated by determination of OFL in spiked human urine and spiked human plasma. The recovery of the drug under study was investigated at four concentration levels. The obtained results are shown in table 3. As can be seen, calculated amounts of recoveries varied between 91.5-106.9% and 89.5-97.1% for human urine and human plasma, respectively, indicating both accuracy and precision.

CONCLUSION

For the first time, an efficient modified ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction (M-IL-CIA-DLLME) followed by spectrofluorimetry was applied to determine OFL as a test compound in pharmaceutical formulations and biological samples. [Hmim][PF_6] was selected as an environmentally friendly extraction solvent which put very few dangers to environment. On the other hand, ILs have no measurable vapor pressure, and hence can emit no volatile organic compounds (VOCs). In the present work, by dissolving one of the IL's ions as a common ion into the sample solution, the robustness of the microextraction method against the variations of the ionic strength was significantly increased. The combined methodology was demonstrated to be rapid, practical, inexpensive and green for determination of analytes in trace levels. In addition, the present method revealed to be a practical tool for routine quality control of OFL in pharmaceutical and biological samples with low operation cost and simplicity of instrumentation. In order to assess the possible analytical applications of the proposed method, the effect of concomitant species on the determination of OFL in real samples was studied. The study was focused on some common compounds abundant in pharmaceutical and biological samples. The obtained results revealed the recommended method had good selectivity to the determination of OFL in real samples.

REFERENCES

1. Ichihara N, Tachizawa H, Tsumura M, Une T, Sato K. Phase I study on DL-8280, Chemotherapy, 1984; 32:118-149.
2. Tanwar YS, Patel D, Sisodia SS. In vitro and in vivo evaluation of ocular inserts of ofloxacin. DARU, 2007; 15:139-145.
3. Popa D, Oprean R, Curea E, Preda N. TLC-UV densitometric and GC-MSD methods for simultaneous quantification of morphine and codeine in poppy capsules. J. Pharm. Biomed. Anal. 1998; 18:645-650.
4. Moriya F, Chan K, Hashimoto Y. Concentrations of morphine and codeine in urine of heroin abusers. Legal Med. 1999; 1:140-144.
5. Prasad AB, Reddy N, Reddy YVB, Chakravathy IE. Simultaneous spectrophotometric determination of ofloxacin and tinidazole from combined dosage forms from pharmaceutical formulations. Asian J. Chem. 2004; 16:1905-1910.
6. Mostafa S, El-Sadek M, Alla EA. Spectrophotometric determination of enrofloxacin and pefloxacin through ion-pair complex formation. J. Pharm. Biomed. Anal. 2002; 28:173-180.
7. Kapetanovic V, Milovanovic L, Erceg M. Spectrophotometric and polarographic investigation of the ofloxacin-Cu(II) complexes. Talanta, 1996; 43:2123-2130.
8. Du LM, Yang YQ, Wang QM. Spectrofluorometric determination of certain quinolone through charge transfer complex formation. Anal. Chim. Acta, 2004; 516:237-243.
9. Wu H, Zhao G, Du L. Determination of ofloxacin and gatifloxacin by mixed micelle-mediated cloud point extraction-fluorimetry combined methodology. Spectrochim. Acta Part A, 2010; 75:1624-1628.
10. Fabre D, Bressolle F, Kinowski JM, Bouvet O, Paganin F, Galtier M. A reproducible, simple and sensitive

HPLC assay for determination of ofloxacin in plasma and lung-tissue. Application in pharmacokinetic studies. J. Pharm. Biomed. Anal. 1994; 12:1463-1469.
11. Lillenberg M, Yurchenko S, Kipper K, Herodes K, Pihl V, Sepp K, Lohmus R, Nei L. Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. J. Chromatogr. A, 2009; 1216:5949-5954.
12. Al-Dgither S, Alvi SN, Hammami MM. Development and validation of an HPLC method for the determination of gatifloxacin stability in human plasma. J. Pharm. Biomed. Anal. 2006; 41:251-255.
13. Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. J. Chromatogr. A, 2005; 1092:206-215.
14. Shervington LA, Abba M, Hussain B, Donnelly J. The simultaneous separation and determination of five quinolone antibiotics using isocratic reversed-phase HPLC: Application to stability studies on an ofloxacin tablet formulation. J. Pharm. Biomed. Anal. 2005; 39:769-775.
15. Zhang H, Ren Y, Bao X. Simultaneous determination of (fluoro)quinolones antibacterials residues in bovine milk using ultra performance liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal. 2009; 49:367-374.
16. See KL, Elbashir AA, Saad B, Alia ASM, Aboul-Enein HY. Simultaneous determination of ofloxacin and ornidazole in pharmaceutical preparations by capillary zone electrophoresis. Biomed. Chromatogr. 2009; 23:1283-1290.
17. Zeeb M, Ganjali MR, Norouzi P. Dispersive liquid-liquid microextraction followed by spectrofluorimetry as a simple and accurate technique for determination of thiamine (vitamin B1). Microchim. Acta, 2010; 168:317-324.
18. Yan H, Wang H, Qin X, Liu B, Du J. Ultrasound-assisted dispersive liquid-liquid microextraction for determination of fluoroquinolones in pharmaceutical wastewater. J. Pharm. Biomed. Anal. 2011; 54:53-57.
19. Jafarvand S, Bidari A, Hemmatkhah P, Hosseini MRM, Assadi Y. Dispersive liquid-liquid microextraction of silver prior to determination by microsample introduction-flame atomic absorption spectrometry. Anal. Lett. 2009; 42:2214-2231.
20. Rezaee M, Assadi Y, Milani-Hosseini MR, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid-liquid microextraction. J. Chromatogr. A, 2006; 1116:1-9.
21. Bidari A, Ganjali MR, Assadi Y, Norouzi P. A novel methodology based on solvents less dense than water through dispersive liquid–liquid microextraction: application in quantitation of L-ascorbate in fruit juices and soft drinks by fiber optic-linear array detection spectrophotometry. Anal. Methods, 2011; 3:724-730.
22. Rezaei F, Bidari A, Birjandi AP, Hosseini MRM, Assadi Y. Development of a dispersive liquid–liquid microextraction method for the determination of polychlorinated biphenyls in water. J. Hazard. Mater. 2008; 158: 621-627.
23. Zhang SH, Li C, Song SJ, Feng T, Wang C, Wang Z. Application of dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for trace analysis of six carbamatepesticides in apples. Anal. Methods, 2010; 2:54-62.
24. Poole CF, Poole SK. Extraction of organic compounds with room temperature ionic liquids. J. Chromatogr. A, 2010; 1217:2268-2286.
25. Hirayama N, Deguchi M, Kawasumi H, Honjo T. Use of 1-alkyl-3-methylimidazolium hexafluorophosphate room temperature ionic liquids as chelate extraction solvent with 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione. Talanta, 2005; 65:255-260.
26. Berton P, Martinis EM, Martinezc LD, Wuilloud RG. Room temperature ionic liquid-based microextraction for vanadium species separation and determination in water samples by electrothermal atomic absorption spectrometry. Anal. Chim. Acta, 2009; 640:40-46.
27. Gharehbaghi M, Shemirani F, Farahani MD. Cold-induced aggregation microextraction based on ionic liquids and fiber optic-linear array detection spectrophotometry of cobalt in water samples. J. Hazard. Mater. 2009; 165:1049-1055.
28. Vidal L, Chisvert A, Canals A, Salvador A. Ionic liquid-based single-drop microextraction followed by liquid chromatography-ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples. Talanta, 2010; 81:549-555.
29. Aguilera-Herrador E, Lucena R, Cardenas S, Valcarcel M. Determination of trihalomethanes in waters by ionic liquid-based single drop microextraction/gas chromatographic/mass spectrometry. J. Chromatogr. A, 2008; 1209:76-82.
30. Gharehbaghi M, Shemirani F, Baghdadi M. Dispersive liquid–liquid microextraction based on ionic

liquid and spectrophotometric determination of mercury in water samples. Int. J. Environ. Anal. Chem. 2009; 89:21-33.
31. Berton P, Wuilloud RG. An online ionic liquid-based microextraction system coupled to electrothermal atomic absorption spectrometry for cobalt determination in environmental samples and pharmaceutical formulations. Anal. Methods, 2011; 3:664-672.
32. Abdolmohammad-Zadeh H, Sadeghi GH. Combination of ionic liquid-based dispersive liquid–liquid micro-extraction with stopped-flow spectrofluorometry for the pre-concentration and determination of aluminum in natural waters, fruit juice and food samples. Talanta, 2010; 81:778-785.
33. Zhou QX, Bai HH, Xie GH, Xiao JP. Temperature-controlled ionic liquid dispersive liquid phase micro-extraction. J. Chromatogr. A, 2008; 1177:43-49.
34. Zhang H, Cheng M, Jiang X. Determination of benzoic acid in water samples by ionic liquid cold-Induced aggregation dispersive LLME coupling with LC, Chromatographia, 2010; 72:1195-1119.