Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 809 (2004) 159–165
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

An HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection

Olcay Sagirli*, Lale Ersoy

Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Beyazit, 34116 Istanbul, Turkey

Received 7 May 2004; received in revised form 7 June 2004; accepted 11 June 2004

Abstract

A selective, sensitive and precise HPLC method with fluorimetric detection has been developed for the assay of lisinopril in human plasma and urine. The clean up of the sample was carried out by solid-phase extraction, firstly with C_{18}-cartridge and secondly with a silica-cartridge. After a pre-column derivatization with fluorescamine, the reaction mixture was chromatographed on C_{18}-column with gradient elution, using methanol and 0.02 M phosphate buffer (pH = 3.2). The fluorescamine–lisinopril derivative was detected fluorimetrically by monitoring the emission at 477 nm, with excitation at 383 nm. Linear quantitative response curve was generated over a concentration range of 5–200 ng/ml and 25–1000 ng/ml for plasma and urine samples, respectively. The mean recovery of lisinopril from plasma and urine was 63.41 and 74.08%, respectively. Intra-day and inter-day R.S.D. and R.M.E. values at three different concentrations were assessed. The method was applied for pharmacokinetic study in a healthy volunteer after a single oral dose of 20 mg of the drug.

Keywords: Lisinopril; Fluorescamine

1. Introduction

Lisinopril, (S)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-prolinedihydrate, is an orally active angiotensin-converting enzyme (ACE) inhibitor which is effective in lowering blood pressure, at dosages of 20–80 mg once, daily. Successful clinical trials in essential hypertension, renovascular hypertension and congestive heart failure have been conducted with lisinopril. The drug is a lysine analog of enalaprilat, the active ACE inhibitor metabolite of enalapril [1].

Lisinopril is not significantly metabolized in humans; the absorbed drug is primarily excreted unchanged in urine. Peak serum concentrations of lisinopril are reached in about 6 h after administration. Mean maximum serum concentrations of lisinopril are about 80–140 ng/ml after a single oral dose of 20 mg [2].

Numerous analytical methods have been developed for the determination of lisinopril in tablets. These include titrimetric [3], spectrophotometric [4–9], spectrofluorimetric [4,6,10], high performance liquid chromatographic (HPLC) followed by spectrophotometric detection [4,11,12] and gas chromatographic (GC) [13] techniques. Although some of these methods are sensitive, not all of them can be directly applied to the assay of lisinopril in biological samples, due to the interferences of endogenous substances.

Pharmacological and pharmacokinetic studies that need high sensitivity have been carried out using some other techniques such as fluoroimmunoassay [14], radioimmunoassay [15] and GC with mass spectrometric detector [16]. Although HPLC is a commonly used method in bioanalytical laboratories, it is difficult to develop an HPLC method for lisinopril pharmacokinetic application, due to its low plasma concentration and endogenous interference. In addition the amphoteric nature of lisinopril makes it difficult to perform simple liquid-phase extraction in the sample preparation. The sensitivity (0.5 μg/ml) of the existing HPLC method with UV-detection for urine samples [17] is not adequate for plasma samples analysis. Another HPLC method for the determination of lisinopril in dosage forms has been extended to the in vitro analyses in spiked human plasma samples, but the application to the in vivo analyses is also not possible because of its low (0.05 μg/ml) sensitivity.

* Corresponding author. Tel.: +90 212 4400000/13587.
E-mail address: olcaysagirli@yahoo.com (O. Sagirli).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.06.014

The aim of the present study is to develop an HPLC method for the assay of lisinopril in human plasma and urine. The method is based on two solid-phase extraction steps, separation on a reversed phase column after derivatization with fluorescamine reagent and detection using a fluorescence detector. The applicability of the developed assay for pharmacokinetic studies was demonstrated.

2. Experimental

2.1. Material and chemicals

Lisinopril dihydrate was kindly supplied by Sanovel Pharmaceutical Product Ind. and Inc. (Istanbul, Turkey). Fluorescamine was obtained from Sigma (MO, USA). HPLC grade methanol and all other chemicals and solvents were from Merck (Darmstadt, Germany). Water was purified by aquaMAX^{TM}-ultra, Young lin instrument (Korea) ultrawater purification system. C_{18}-cartridges (500 mg, 3.0 ml) and silica-cartridges (100 mg, 1.0 ml) were purchased from Alltech (IL, USA). Blood plasma was obtained from human volunteers and collected into tubes treated with sodium citrate as anticoagulant. Plasma and urine samples were stored at approximately −20 °C until they were analysed.

2.2. HPLC system

A Shimadzu (Kyoto, Japan) LC 10 liquid chromatograph, consisting of a model LC 10 AT solvent delivery system, a Rheodyn injection system with a loop of 20 μl and a model CTO 10A column oven was used. RF 10 AXL fluorescence detector was set an excitation wavelength of 383 nm and an emission wavelength of 477 nm. Detector gain was set at 1 and the sensitivity was at high. Separation was performed on a Phenomenex C_{18}-column (5 μm) (250 mm × 4.6 mm i.d.) with a guard column (4 mm × 3 mm i.d.) packed with the same material.

Gradient elution was carried out with 0.02 M phosphate buffer solution (pH = 3.2) (eluent A) and methanol (eluent B). The gradient started at 50% of B and was increased linearly to reach 70% of B in 15 min. This condition was maintained for 10 min. The flow rate of the mobile phase was 1.0 ml/min, the temperature of the column was held at 55 °C. The data were collected and analysed via the Class-LC 10, version 1.61-system software.

2.3. Solutions

Stock solution of lisinopril was prepared by dissolving 10 mg of drug in 50 ml of water. To prepare standard lisinopril working solutions, aliquot of 0.5 ml of the stock solution was diluted to 10.0 ml in a volumetric flask and 0.05–2.0 ml of this solution was further diluted to 10.0 ml with water.

The fluorescamine solution was freshly prepared at 0.7 mg/ml in acetone. Aqueous borate buffer (0.1 M, pH = 8.5) and phosphate buffer (0.02 M, pH = 3.2) solutions were prepared according to the British Pharmacopoeia [3] using boric acid–potassium chloride and potassium dihydrogene phosphate, respectively.

2.4. Solid phase extraction

1.0 ml of plasma or 0.2 ml of urine (diluted to 1.0 ml with water) was acidified with 0.2 ml of 4.0 M HCl solution and mixed thoroughly on a vortex mixer. Acidified plasma and urine samples were applied to the C_{18}-cartridge, which was preconditioned with methanol (10 ml) followed by water (10 ml) and then 0.5 M HCl (3 ml). After loading the sample, the cartridge was washed with 0.5 M HCl (5 ml) and then dichloromethane (3 ml). After the cartridge was dried by applying vacuum for 5 min, lisinopril was eluted with methanol (3 × 1 ml) and under nitrogen, solvent of the eluate was evaporated to dryness at 60 °C on a block heater. The residue was dissolved with chloroform–methanol–25% aqueous ammonium hydroxide (5:1:0.1, v/v) mixture and applied to a silica cartridge. The drug was eluated with 3 × 1 ml of chloroform–methanol–25% aqueous ammonium hydroxide (1:5:0.5, v/v) after firstly 2 ml of chloroform–methanol–25% aqueous ammonium hydroxide (5:1:0.1, v/v) and then 1 ml of chloroform–methanol–25% aqueous ammonium hydroxide (5:5:1, v/v) mixtures were passed through the cartridge. Prior to use, the cartridges were activated by washing with 4 ml of methanol and then 2 ml of chloroform. C_{18}- and silica-cartridges were connected to a vacuum manifold and washing or elution solvents were passed through the cartridges at a rate of 1.5 ml/min, by applying vacuum.

2.5. Preparation of calibration graph

Plasma (1.0 ml) or urine (0.2 ml, diluted to 1.0 ml with water) was each spiked with 0.1 ml of standard lisinopril solution containing 0.05–2.0 μg/ml lisinopril. The mixtures were applied to the C_{18}- and silica-cartridges as described above. After the extraction on silica cartridge, the solvent mixture of eluate was evaporated on a block heater at 60 °C under nitrogen. Then the residue was treated with 0.5 ml of borate buffer solution, 0.65 ml of acetone and 0.05 ml of fluorescamine solution. The content of the tubes was vigorously mixed on a vortex mixer for 1 min. A 20 μl of the reaction mixture was injected into the HPLC system, after waiting for 5 min in the dark. A calibration graph was prepared by plotting the peak areas against the concentrations of the drug.

2.6. Recovery

The absolute recovery of the drug from plasma was measured by analysing 1 ml of drug free plasma samples spiked with lisinopril at 25, 75 and 150 ng/ml concentrations. The recovery of the drug from urine was determined by studying 0.2 ml of urine samples spiked with lisinopril at 250, 500

and 750 ng/ml concentrations. After solid-phase extraction and derivatization and chromatography processes, the peak areas were compared with those of standard aqueous solutions of lisinopril at the same concentrations.

2.7. Validation of the method

Repeatability (within-day precision) was evaluated by replicate analysis of spiked plasma and urine samples containing lisinopril at the same concentrations with those of the recovery study. The analyses were repeated six different times during one day. Reproducibility (between-day precision) was defined by analysing the same plasma and urine samples spiked as above on six different days.

2.8. Stability

The stability of lisinopril-fluorescamine derivative was estimated in the reaction mixture, prior to injection into the HPLC system. The freeze-thaw stability was also assessed by analysing the drug in plasma and urine samples both freshly prepared and after storage at −20 °C for 24 h and for 2 months. Stability experiments were also carried out to determine if lisinopril was decomposed during the evaporation process at 60 °C in methanol or chloroform–methanol–25% aqueous ammonium hydroxide solvent mixtures at various ratios.

2.9. Pharmacokinetic study

Venous blood samples of 2–3 ml were withdrawn into the citrated tubes at predetermined intervals for a total period of 72 h, after oral administration of a single 20 mg of lisinopril to a 33-year-old male volunteer. Urine samples were also collected at varying intervals, up to 24 h. The plasma samples obtained after centrifugation of the blood specimens at 4500 g for 10 min, and urine samples were stored at −20 °C until analysis. Urine samples were diluted, if necessary, between 1:1 and 1:5 depending on their concentration.

3. Results

The derivatization reaction between lisinopril and fluorescamine proceeded at room temperature in 1–2 min. The maximum peak area was obtained when the reaction was carried out under pH 8.5 (Fig. 1) using the borate buffer system, 250-fold molar excess reagent (Fig. 2), and a ratio of acetone to water 1.0:1.4 (v/v).

For the sample preparation, it was determined that a two-step solid-phase extraction using C_{18}- and then silica-cartridges was necessary. In this case, lisinopril was adequately separated from closely eluting fluorescamine derivatives of other amino acids in the samples, and a clear chromatogram was obtained. The samples were applied to the C_{18}-cartridges, the cartridges were washed, firstly with 0.5 M HCl, then dichloromethane, and the drug was eluted with methanol. The mixtures of chloroform, methanol and 25% aqueous ammonium hydroxide at various ratios, were used for the second solid phase extraction on silica-cartridges. The ratios were 5:1:0.1 (v/v) and 5:5:1 (v/v) for washing; and 1:5:0.5 (v/v) for elution of the drug.

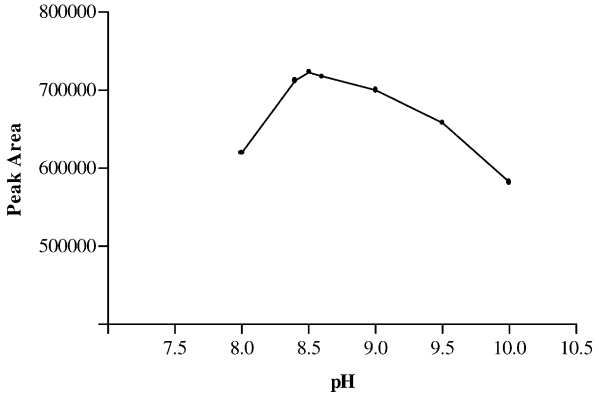

Fig. 1. Effect of pH on the reaction of lisinopril with fluorescamine.

When methanol–0.02 M phosphate buffer (pH = 3.2) solvents were used with gradient system, at 55 °C sharper and symmetrical peak was obtained for fluorescamine–lisinopril derivative in aqueous solution and sensitivity increased. Chromatography started at 50% of methanol in the buffer solution, and then methanol concentration was increased linearly to 70% in 15 min. Retention time of lisinopril derivative in this chromatographic system was 11.5 min. Representative chromatograms of (A) drug-free plasma, (B) lisinopril added to water (50 ng/ml), (C) the plasma spiked with lisinopril (50 ng/ml), (D) drug-free urine and (E) the urine spiked with lisinopril (500 ng/ml) are given in Fig. 3. No interference was observed in the drug elution region of the plasma or urine control chromatogram.

Calibration curve was linear over a range of 5–200 ng/ml for plasma and 25–1000 ng/ml for urine. The limits of

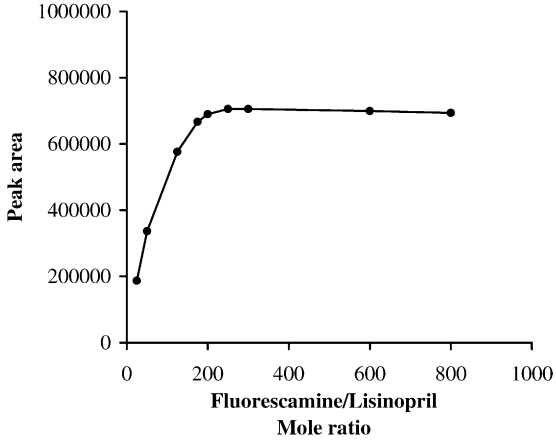

Fig. 2. Effect of fluorescamine amount on derivatization reaction.

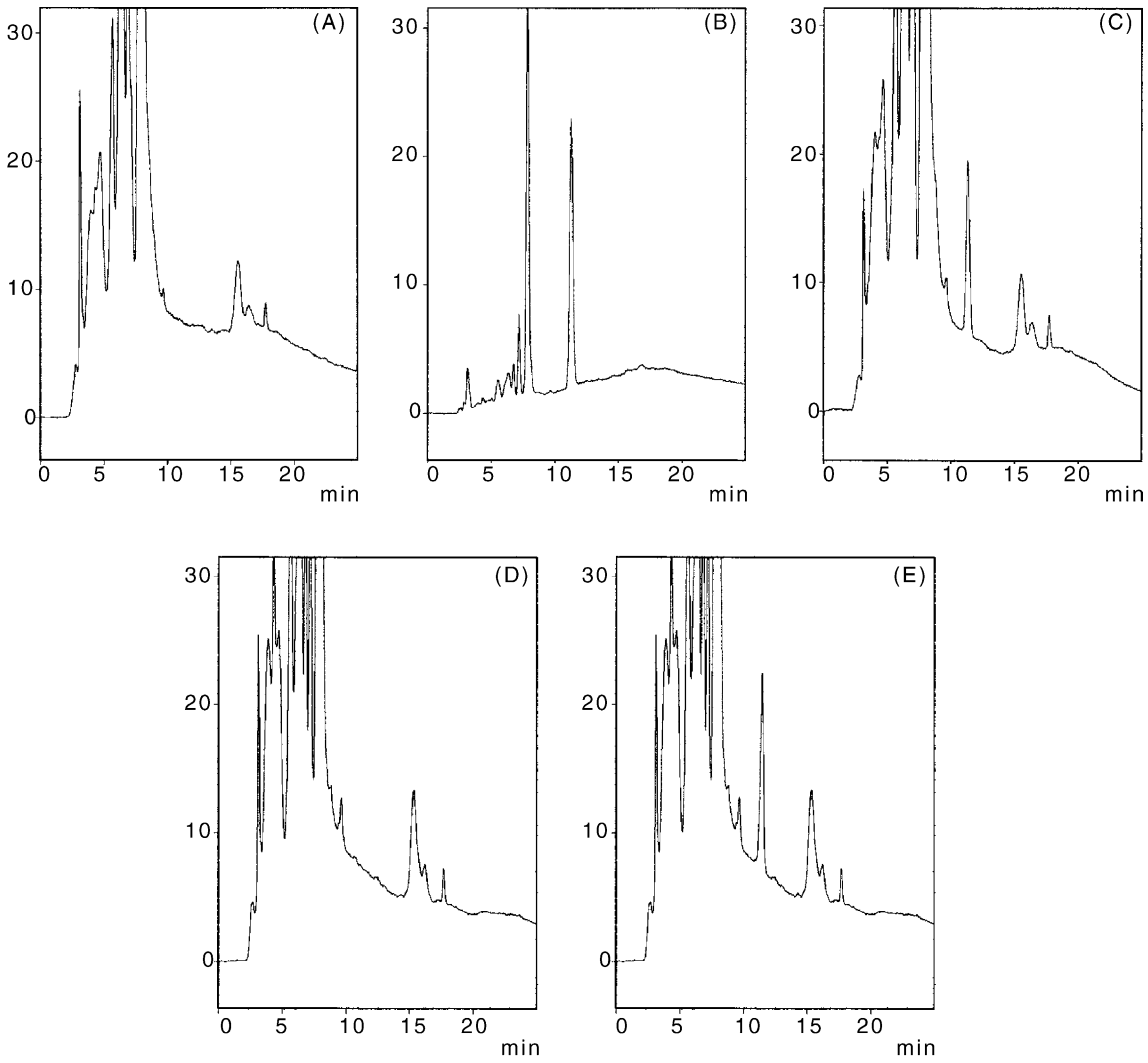

Fig. 3. Representative chromatograms of (A) drug-free plasma, (B) lisinopril added to water (50 ng/ml), (C) the plasma spiked with lisinopril (50 ng/ml), (D) drug-free urine and (E) the urine spiked with lisinopril (250 ng/ml).

quantitation (LOQ) were 5 ng/ml and 25 ng/ml (the lowest concentration on the calibration curves) for plasma and urine, respectively. Limit of detection (LOD) was 2 ng/ml for plasma and 10 ng/ml for urine, at a signal to noise ratio of 3.

The average absolute recovery of lisinopril decreased to 63.41% in plasma samples although a great loss of lisinopril was not observed in urine samples with 74.08% recovery (Table 1). The recovery was determined as 75% when the same procedures were applied to the aqueous lisinopril solution.

Intra-day and inter-day relative standard deviation (R.S.D.) values were found to be within 1.12 and 3.81% for plasma and 1.61 and 4.14% for urine, respectively. The results were determined by analysing the samples spiked with lisinopril at three different concentrations. Accuracy of the method expressed as relative mean error (R.M.E.) was below 5.07%. The statistical parameters are given in Table 2.

The fluorescamine–lisinopril derivative is stable in the dark and at 4 °C in the reaction mixture for at least 1 day. The fluorescence intensity of the solution does not change on exposure to UV light for 10 min. Lisinopril is not effected by freezing, thawing or heating processes. No significant change was observed in plasma and urine samples spiked with the drug and stored at −20 °C for a period of 2 months.

An interference of other ACE inhibitors commonly used in therapy, is not under consideration since these drugs do

Table 1
Absolute recovery of lisinopril from plasma and urine ($n = 5$)

Sample	Concentration (ng/ml)		Recovery (%)	R.S.D. (%)
	Added	Found (mean ± S.D.)		
Plasma	25.00	14.97 ± 0.54	59.88	3.61
	75.00	48.19 ± 0.59	64.25	1.22
	150.00	99.15 ± 1.32	66.10	1.33
Urine	250.00	168.28 ± 6.05	67.31	3.59
	500.00	383.47 ± 13.86	76.69	3.61
	750.00	586.88 ± 12.45	78.25	2.12

Table 2
Intra-day and inter-day precision and accuracy of lisinopril in plasma and urine ($n = 6$)

Sample	Concentration (ng/ml)		R.S.D. (%)	R.M.E. (%)
	Added	Found (mean ± S.D.)		
Plasma				
Intra-day				
	25.00	25.18 ± 0.76	3.01	0.72
	75.00	74.34 ± 0.83	1.12	−0.88
	150.00	153.50 ± 2.43	1.58	2.33
Inter-day				
	25.00	26.27 ± 1.00	3.81	5.07
	75.00	73.70 ± 1.52	2.06	−1.73
	150.00	145.50 ± 2.77	1.91	−2.99
Urine				
Intra-day				
	250.00	243.10 ± 7.70	3.17	−2.76
	500.00	504.20 ± 10.50	2.08	0.84
	750.00	741.45 ± 11.95	1.61	−1.14
Inter-day				
	250.00	240.20 ± 9.95	4.14	−3.92
	500.00	498.10 ± 13.45	2.70	−0.38
	750.00	738.95 ± 13.85	1.87	−1.47

not have a primary amine group and thus do not react with fluorescamine reagent. Hydrochlorothiazide, which is generally used in therapy with lisinopril as diuretic, does not interfere for the same reason.

Fig. 4 shows the plasma drug levels versus time after a single oral dose of 20 mg lisinopril in a healthy volunteer. Plasma concentration was measurable up to 24 h. A maximum concentration of 87.4 ng/ml in plasma was reached at 7 h. The elimination half-life ($t_{1/2}$) and the area under the curve ($AUC_{0–72\,h}$) were calculated as 28.4 h and 1399 ng h/ml, respectively. The cumulative urinary excretion of lisinopril is shown in Fig. 5. Approximately 25% of the

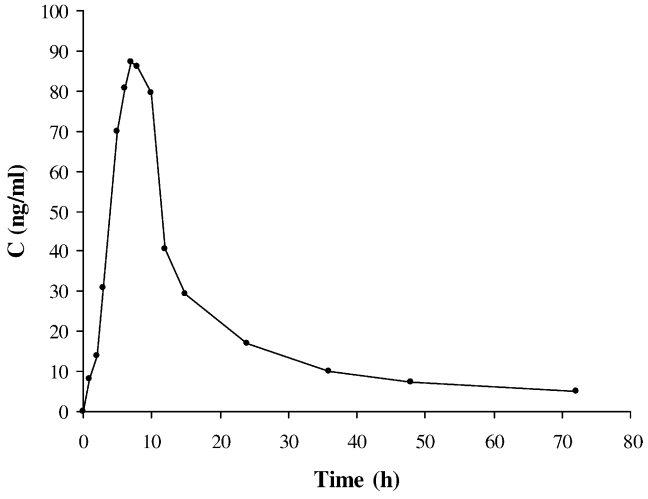

Fig. 4. A representative plasma concentrations vs. time profile of lisinopril in a healthy volunteer after a single 20 mg oral dose.

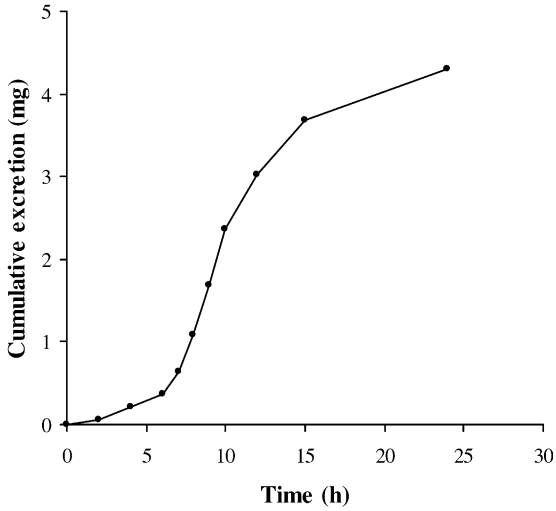

Fig. 5. Cumulative excretion of lisinopril in urine of a healthy volunteer after a single 20 mg oral dose.

administered drug was excreted unchanged, within 24 h after oral administration.

Pharmacokinetic results obtained using this method are in agreement with those of the studies reported previously [1,19].

4. Discussion

4.1. Optimisation of the reaction mixture

Lisinopril contains a primary aliphatic amine group, known to react immediately with fluorescamine, and produces a highly fluorescent product with excitation and emission maxima of 383 and 477 nm, respectively. The reaction is proposed to proceed [20] as shown in Scheme (Fig. 6).

Fluorescamine which has been used for the analyses of the substances, which contain primary amine group [21–23] was chosen as the derivatizing reagent because of its fast reactivity and formation of a fluorigenic group that offers a high sensitivity. Optimum reaction conditions between lisinopril and the reagent were investigated. The effect of pH on the reaction was examined using borate and phosphate buffer systems at the pH values between 8–10 since fluorescamine reacts with the substances containing aliphatic primary amine group under alkaline conditions [14]. The required reagent amount was determined by varying the molar ratio of fluorescamine to lisinopril from 25–800. The acetone–water ratio of the reaction mixture also affects the result, for this reason various acetone–water ratios were tested. Reaction mixture was immediately chromatographed on HPLC and also after 5, 10 and 20 min later to determine optimum time period necessary to proceed the reaction quantitatively.

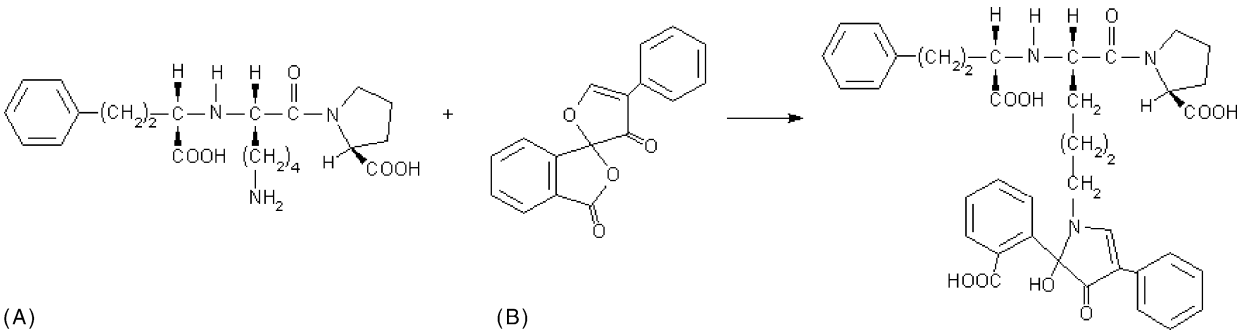

Fig. 6. The reaction proposed between lisinopril (A) and fluorescamine (B).

4.2. Optimization of the sample preparation

The sample preparation step is the main difficulty of lisinopril analyses. The amphoteric character of lisinopril, which has both amino acid and carboxylic acid groups, makes impossible liquid-phase extraction. The substance is charged at any pH value and can not be transferred into organic phase even if the aqueous phase was saturated using sodium chloride and the polarity of the organic phase was increased. Therefore solid-phase extraction was tested and the method reported by Leis at al. [16] was applied with a little modification. Acidified aqueous lisinopril solution was passed through the cartridge. To increase the recovery, 0.5 M HCl solution was used instead of 0.1 M HCl for washing. However lisinopril in aqueous solution was recovered at 95% level, a clear chromatogram could not be observed when plasma and urine samples were prepared by the same way because of the endogenous substances.

Some processes were tested to obtain a clear chromatogram. Plasma samples were deproteinized using organic solvents or acidic solutions before applying the samples to the cartridges. Acetone and methanol or aqueous trichloroacetic acid and perchloric acid solutions were used for this purpose, but a clear chromatogram could not be obtained. Aqueous cupper(II), nickel(II) or zinc(II) chloride solutions at neutral pH were also tried for deproteinization and the removal of α-amino acids in plasma. Although the inorganic salts proved to be efficient agents for plasma, the chromatograms were unsuccessful, with a very noisy baseline for the urine sample.

A second solid phase extraction using silica-cartridges was tested to wash the impurities that could not be removed. In this case, polar and nonpolar interfering substances were entired and a clear chromatogram was obtained. Solvent mixture of chloroform, methanol and 25% aqueous ammonium hydroxide at various ratios were examined to apply the sample to the cartridge, to wash the impurities and to elute the drug. This solvent mixture was chosen considering the thin layer chromatographic studies on silica plates previously reported [24]. The ratios of the solvent mixture were critical. Because either the impurities were not removed sufficiently and the baseline was very noisy or the chromatogram was clear but the recovery was very low. Optimum ratios were determined as described in experimental part, after numerous tests. Sample preparation procedure developed for plasma, which is based on solid phase extraction with $C_{18\infty}$ and silica-cartridges, was successfully applied to the urine samples.

4.3. Optimization of the mobile phase

The mixtures of methanol or acetonitrile with water at various ratios were examined as mobile phase on C_{18}-column, but the peak was asymmetric and broad since the fluorescamine–lisinopril derivative was retained by stationary phase. To prevent the tailing of the peak, acidic solutions as 5% acetic acid, 0.1 M phosphoric acid, 0.1 M HNO_3 and phosphate buffers at pH between 2.5–4.0, were tested instead of water. A different effect on chromatogram could not be observed when various acidic solutions were used eventhough the lisinopril–fluorescamine peak became sharper. The temperature of the column was changed to 30, 40, 50 and 55 °C. Although the chromatogram was better this way, a sharper and symmetrical peak was obtained and the best separation was achieved with a linear gradient programme.

As internal standard, a substance, which is able to extract using two solid-phase extraction steps, reacts with fluorescamine and gives an isolated peak on the chromatogram, could not be found. For this purpose firstly ACE inhibitors that have similar structure with lisinopril were considered. But as mentioned above, none of these substances (fosinopril, enalapril, quinapril, cilazapril, benazepril, ramipril) have a primary amine group and hence do not react with fluorescamine. Although a fluorophoric derivative was obtained with some other substances that have primary amine group, either their behaviours were not similar with lisinopril on C_{18}- or silica-cartridges (vigabatrin, aspartam, gabapentin, baclofen, amlodipin, tranylcypromine) or an isolated peak could not be obtained on the chromatogram (tranexamic acid, ampicillin, loracabef, cefaclor and fluvoxamine). On the other hand, since good reproducibility was obtained, an internal standard to overcome sample-to-sample variation was found to be unnecessary.

5. Conclusion

As mentioned in the Introduction part, sensitivity of the HPLC method with UV detection [17] or fluorescence detection [18] is too low to determine the low drug plasma concentrations. Other methods developed for pharmacokinetic studies such as radio-, fluoro-immunoassay and GC-MS techniques [14–16] are very sensitive with 0.2–0.7 ng/ml LOQ values but not readily applicable for many researchers since they generally require expensive devices or reagents.

In summary, this is the most sensitive HPLC method is being reported thus far for the analysis of lisinopril in plasma. The method is selective, reproducible, accurate and can be reliably used for both pharmacokinetic study and drug monitoring.

Acknowledgements

The authors would like to thank the Research Fund, Istanbul University for the sponsorship of this study (project nos.: T-54/23072002 and UDP-132/18042003).

References

[1] S.G. Lancaster, P.A. Todd, Drugs 35 (1988) 646.
[2] B. Beermann, A.E. Till, H.J. Gomez, M. Hichens, J.A. Bolognese, I.L. Junggren, Biopharm. Drug Disposit. 10 (1989) 397.
[3] British Pharmacopoeia, Her Majesty's Stationery Office, London, 1998, pp. 799–800.
[4] A. El-Gindy, A. Ashour, L. Abdel-Fattah, M.M. Shabana, J. Pharm. Biomed. Anal. 25 (2001) 913.
[5] A. El-Gindy, A. Ashour, L. Abdel-Fattah, M.M. Shabana, J. Pharm. Biomed. Anal. 25 (2001) 923.
[6] F.A. El-Yazbi, H.H. Abdine, R.A. Shaalan, J. Pharm. Biomed. Anal. 19 (1999) 819.
[7] P.D. Panzade, L.R. Mahadik, Indian Drugs 36 (1999) 321.
[8] S. Atmaca, S. Tatar, G. Iskender, Acta Pharm. Turc. 36 (1994) 13.
[9] J.H. Kumar, R.K. Agrawal, Indian Drugs 37 (2000) 196.
[10] E.S. Aktas, L. Ersoy, O. Sagirli, Il Farmaco 58 (2003) 165.
[11] R.T. Sane, G.R. Valiyare, U.M. Desmukh, S.R. Singh, R. Sodhi, Indian Drugs 29 (1992) 558.
[12] The United States Pharmacopoeia, 24th revision, Asian ed., United States Pharmacopoeial Convention, Inc., Twinbrook Parkway, Rockville, MD, 2000, pp. 2149–2152.
[13] A.B. Avadhanulu, A.R.R. Pantulu, Indian Drugs 30 (1993) 646.
[14] A.S. Yuan, J.D. Gilbert, J. Pharm. Biomed. Anal. 14 (1996) 773.
[15] P.J. Worland, B. Jarrott, J. Pharm. Sci. 75 (1986) 512.
[16] H.J. Leis, G. Fauler, G. Raspotnig, W. Windishhofer, Rapid Commun. Mass Spectrom. 13 (1999) 650.
[17] Y. Wong, B.G. Charles, J. Chromatogr. B 673 (1995) 306.
[18] A.A. El-Emam, S.H. Hansen, M.A. Moustafa, J. Pharm. Biomed. Anal. 34 (2004) 35.
[19] V.J. Crillo, A.E. Till, H.J. Gomez, W.J. Shih, G. Theime, Clin. Pharmacol. Ther. 39 (1986) 187.
[20] S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, M. Weigele, Science 178 (1972) 871.
[21] H.J. Mascher, C. Kikuta, J. Chromatogr. A 812 (1998) 221.
[22] C.C. Hong, F. Kondo, J. Food Protect. 63 (10) (2000) 1421.
[23] T. Bantan-Polak, M. Kassai, K.B. Grant, Anal. Biochem. 297 (2001) 128.
[24] Analytical Profiles of Drug Substances and Excipients, vol. 21, 1992, pp. 234–276.