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Short communication

A new spectrofluorimetric method for the determination of lisinopril in tablets

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Abstract

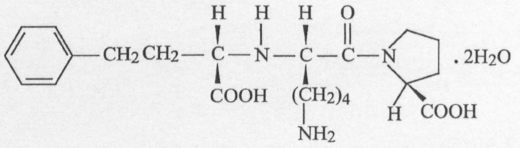
An accurate and precise spectrofluorimetric method is presented for the determination of lisinopril based on the formation of a derivative formed with 7-chloro-4-nitrobenzofurazan. The derivatization reaction proceeds quantitatively at pH 8.5–9.0 and 60 °C in 70 min when the molar ratio of reagent to the drug is 170. After the extraction with ethyl acetate the fluorescence intensity of the derivative was measured at 528 nm with excitation at 465 nm. Calibration graph is rectilinear over the range of 50–1000 ng/ml with detection and determination limits of 20 and 50 ng/ml, respectively. The regression equation is $I_f = 0.198C - 0.299$ ($r = 0.9999$). The method was applied to the commercially available tablets and the results were statistically compared with those obtained by official HPLC method.

Keywords: Lisinopril; Spectrofluorimetry; NBD-Cl

1. Introduction

Lisinopril {(*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-proline dihydrate}is a lysine analog of enalaprilat, the active metabolite of enalapril. It is a long-acting, nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor that is used for the treatment of hypertension and congestive heart failure in daily dosages of 10–80 mg [1]. Pharmacological activity of lisinopril has been proved in previous experimental and clinical studies [2,3].

The analytical methods reported for the determination of lisinopril in tablets are generally based on spectrophotometric measurements [4–8]. Other techniques such as liquid chromatographic [6,9,10] gas chromatographic [11] and spectrofluorimetric [6,7] have also been used for the same purpose. The official methods for the assay of lisinopril in the bulk form and tablets are potentiometric acid–base titration [12] and high performance liquid chromatographic (HPLC) [13] methods. On the other hand more sensitive fluoroimmunoassay [14], radioimmunoassay [15] and GC with mass detection [16] methods have been proposed for the pharmacological and pharmacokinetic studies of the drug. Plasma level of lisinopril can be estimated by these methods. Furthermore an HPLC method with UV detection has been developed for the determination of the drug in urine samples [17].



In this study a new, very sensitive, and reliable spectrofluorimetric method has been developed for the assay of lisinopril in tablets. The method is based on a derivatization with 7-chloro-4-nitrobenzofurazan (NBD-Cl) reagent that is widely used to produce fluorescence derivatives of the compounds with primary and secondary amine groups [18,19]. Developed method has been applied to the analysis of lisinopril in tablets.

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2. Experimental

2.1. Apparatus

A Shimadzu (Kyoto, Japan) spectrofluorimeter Model RF-1501 with xenon lamp and 1 cm glass cells were used. Excitation and emission wavelengths were set at 465 and 528 nm, respectively. Measuring system of the instrument was calibrated using fluorescein sodium as reference standard solution.

2.2. Material and chemicals

Lisinopril and its tablets containing 20 mg of the drug per tablet were kindly supplied by Sanovel Pharmaceutical Product Ind. and Inc. Istanbul, Turkey. NBD-Cl and other chemicals were obtained from Merck Darmstadt, Germany. Methanol and ethyl acetate were of analytical reagent grade and also purchased from Merck Darmstadt. Water was bidistilled.

2.3. Solutions

Stock solution of lisinopril was prepared by dissolving 5 mg of accurately weighed drug in 50 ml of water. Standard lisinopril solutions were prepared from the stock solution by appropriate dilutions with water.

For the preparation of sample solution twenty tablets were weighed and powdered then a portion of tablet powder equivalent to 50 mg of lisinopril was weighed and the drug was extracted with about 50 ml of water shaking mechanically for 15 min in a 100 ml volumetric flask. The solution was filtered after the volume was adjusted with water and 10 ml of the filtrate was diluted to 100 ml with the same solvent in a volumetric flask.

NBD-Cl solution was freshly prepared in methanol at 4.2 mg/ml concentration. Buffer solution was prepared adjusting the pH of 0.025 M NaH_2PO_4 solution to 9 with 0.1 M NaOH solution. Reference standard solution was prepared by diluting a stock solution of fluorescein sodium containing 1 μg/ml in 0.1 M NaOH.

2.4. Calibration

Aliquots of 0.005–0.1 ml of standard solution at 50 μg/ml concentration were mixed with 0.1 ml of buffer solution at pH 9 in a glass and stoppered tubes. After addition of 0.1 ml of NBD-Cl solution, the mixture was heated at 60 °C for 70 min in a block heater. The derivative NBD-lisinopril produced was extracted two times with 2 ml of ethyl acetate on a vortex mixer. After centrifugation, the organic phases were combined and volume of the mixture was adjusted to 5 ml with ethyl acetate.

The fluorescence intensity was measured against a blank solution at maximum emission wavelength, 528 while exiting at 465 nm. The fluorescence intensity of reference standard, sodium fluoressein, solution was also measured at the same wavelength combination. Relative fluorescence intensity of the NBD-lisinopril was determined by dividing the value of standard solution to that of the reference standard solution. Calibration graph was prepared by plotting the relative fluorescence intensity against the concentration of drug.

2.5. Assay procedure

An aliquot of 0.1 ml sample solution was reacted with NBD-Cl as described above at Section 2.4 part. The amount of lisinopril was calculated using the regression equation of the calibration graph.

3. Results and discussion

The reaction between lisinopril and NBD-Cl in alkaline medium at pH 9 produced a fluorophore with excitation and emission maxima of 465 and 528 nm, respectively.

Optimum conditions of the reaction with respect of the pH, temperature, heating time and amount of the reagent were investigated. The effect of the pH of the medium on the reaction was examined by changing the pH values between 7 and 10 using the buffer solutions with different pH values. The relative fluorescence intensity of the mixtures were measured after acidification and extraction with ethyl acetate as described Section 2.4 part and it was found that the fluorescence intensity is maximum at pH 8.5–9.0 (Fig. 1). Phosphate and borate buffer systems were tested and higher fluorescence intensities were obtained when the reaction was carried out using phosphate buffer system.

The derivatization reaction was carried out at different temperatures since preliminary experiments indicated that the rate of the reaction was too slow at room temperature. To determine the optimum temperature the fluorescence intensity of the ethyl acetate solution were read at 10 min intervals for 80 min. According to these experiments it was established that the reaction was completed after heating at 60 °C for 70 min. Lower temperature, 50 °C is not sufficient to complete the reaction. As it can be seen in Fig. 2, the reagent is probably degradeted since the fluorescence intensities of the final solutions are too low when the reaction proceeds at 80 °C. Although the fluorescence intensities are high by heating at 70 °C, the values measured were not stable even if for a short period. Therefore, 60 °C and 70 min were chosen as optimum temperature and time period Fig. 3.

Optimum reagent amount was determined by varying the molar ratio of NBD-Cl to the drug from 75 to 300 and a 170-fold molar excess was found sufficient.

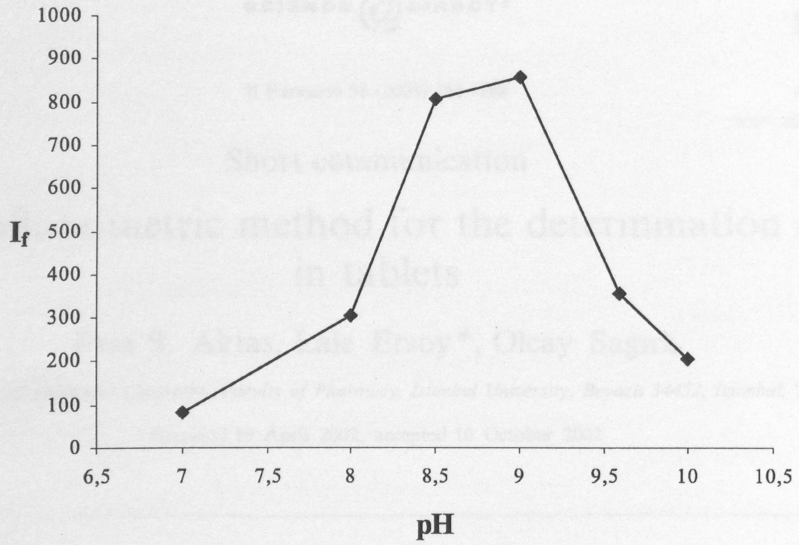

Fig. 1. Effect of the pH on the reaction of lisinopril with NBD-Cl.

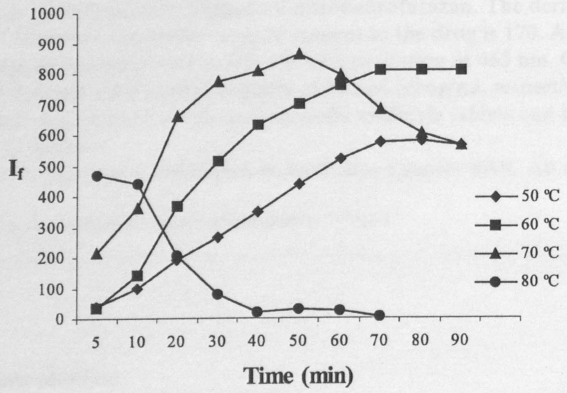

Fig. 2. Effect of the temperature and heating time on the reaction of lisinopril with NBD-Cl.

The derivatization reaction between lisinopril and NBD-Cl proceeds quantitatively under the conditions described above at pH 8.5–9, 60 °C in 70 min when the mole ratio of reagent to the drug is 170. A linear correlation was obtained between relative fluorescence intensities (I_f) and lisinopril concentration over the range 50–1000 ng/ml in the final solution. The regression equation is $I_f = 0.198C - 0.299$ ($r = 0.9999$).

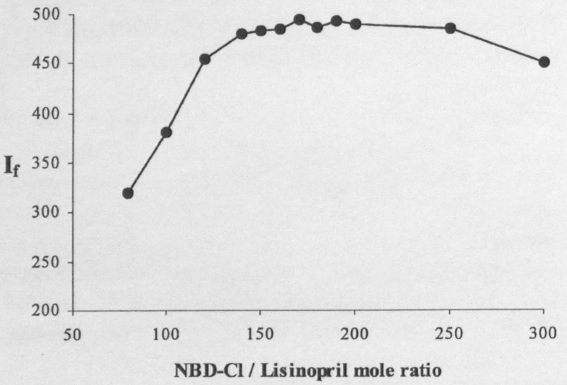

Fig. 3. Effect of the reagent amount on the reaction between lisinopril and NBD-Cl.

The NBD derivative of lisinopril is stable in the dark and at 4 °C in ethyl acetate for at least 1 day. The fluorescence intensity of the solution does not change on exposure to UV light for 10 min.

The reproducibility of the proposed method was determined by analyzing ten replicate samples of standard lisinopril solution containing 500 ng/ml of the drug. The coefficient of variation was calculated as 0.5% from intraday assays (Table 1).

The proposed method was applied to the determination of lisinopril in tablets and the results were compared with those of the official HPLC method [13] using t- and F-tests. There is no significant difference between two methods in terms of the mean values and the standard deviations since the calculated t- and F-values are lower than the theoretical ones (Table 1).

Table 1
Statistical evaluations of the results obtained by proposed and comparison method for the assay of lisinopril in tablets containing 20 mg of the drug ($n = 6$)

Statistical value	Proposed method	Comparison method
Mean	20.07	20.19
Drug found of declared (%)	100.33	100.98
RSD (%)	0.7	0.4
Confidence limits	20.07±0.13	20.19±0.07
t-Test of significance [a]	$t = 1.84$	
F-test of significance [a]	$F = 3.06$	

[a] $t_{teor} = 2.23$, $F_{teor} = 5.05$ ($P = 0.05$).

4. Conclusion

The proposed spectrofluorimetric method provides very sensitive, accurate and reproducible quantitative determination for lisinopril in tablets. It is suitable for routine analyses of the drug in its formulation form since there is no interference from calcium phosphate, mannitol, starch, magnesium stearate and iron oxide, which present in tablets as inactive ingredients. It is also possible to apply the method to the assay of the drug in binary mixtures with hydrochlorothiazide that does not react with the reagent.

If the methods are compared in terms of sensitivity and reproducibility, developed method is sensitive and the results are reproducible as previously reported spectrofluorimetric methods based on the derivatization reaction of lisinopril with *o*-phtalaldehyde (OPA) [6] and acetyl acetone–formaldehyde [7]. Determination limits and RSD values of these reported methods are 20, 30 ng/ml and 0.6, 0.7%, respectively whereas those of developed method are 50 ng/ml and 0.7%. Major advantage of OPA-derivatization method is the instability of the derivatization product. Although NBD-lisinopril derivative is stable, limitation of the proposed method is relatively long heating time.

Determination of lisinopril in biological fluids such as urine or plasma needs sensitive analyze methods. The proposed spectrofluorimetric method developed for the determination of lisinopril in tablets can also be applied for this purpose, after chromatographic separation on HPLC and using fluorescence detector. The applicability of the method to the biological samples is now under investigation. Only the urine samples can be analyzed using spectrophotometric method reported by Wong and Charles [17] since the sensitivity is not enough to permit the analyses of plasma samples. However, the assay of the drug in biological samples at very low concentrations is possible using radio-, fluoro-immunoassay and GC MS techniques [14–16], these are not readily available for many researchers.

References

[1] S.G. Lancaster, P.A. Todd, Drugs Lisinopril. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure, Drugs 35 (1988) 646–669.

[2] J.E. Rush, D.D. Merrill, The safety and tolerability of lisinopril in clinical trials, J. Cardiovasc. Pharm. 9 (1987) 99–107.

[3] H.J. Gomez, S.G. Smith, F. Moncloa, Efficacy and safety of lisinopril in older patients with essential hypertension, Am. J. Med. 85 (1988) 35–37.

[4] A. El-Gindy, A. Ashour, L. Abdel-Fattah, M.M. Shabana, Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorothiazide in binary mixtures, J. Pharm. Biomed. Anal. 25 (2001) 923–931.

[5] J.H. Kumar, R.K. Agrawal, Spectrophotometric methods for simultaneous determination of amlodipine besylate and lisinopril in tablets, Indian Drugs 37 (2000) 196–199.

[6] A. El-Gindy, A. Ashour, L. Abdel-Fattah, M.M. Shabana, Spectrophotometric, spectrofluorimetric and LC determination of lisinopril, J. Pharm. Biomed. Anal. 25 (2001) 913–922.

[7] F.A. El-Yazbi, H.H. Abdine, R.A. Shaalan, Spectrophotometric, spectrofluorimetric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms, J. Pharm. Biomed. Anal. 19 (1999) 819–827.

[8] S. Atmaca, S. Tatar, G. Iskender, Spectrophotometric determination of lisinopril in tablets, Acta Pharm. Turc. 36 (1994) 13–16.

[9] F. Barbato, P. Morrica, F. Quaglia, Analysis of ACE inhibitor drugs by high performance liquid chromatography, Farmaco 49 (1994) 457–460.

[10] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), J. Pharm. Biomed. Anal. 16 (1997) 431–438.

[11] A.B. Avadhanulu, A.R.R. Pantulu, Gas liquid chromatographic estimation of lisinopril in its pharmaceutical dosage forms, Indian Drugs 30 (1993) 646–649.

[12] British Pharmacopoeia, Her Majesty's Stationery Office, London, 1998, pp. 799–800.

[13] The United States Pharmacopoeia, 24 revision, Asian Edition, United States Pharmacopoeial Convention, Inc., Twinbrook Parkway, Rockville, MD, 2000, pp. 2149–2152.

[14] A.S. Yuan, J.D. Gilbert, Time-resolved fluoroimmunoassay for the determination of lisinopril and enalaprilat in human serum, J. Pharm. Biomed. Anal. 14 (1996) 773–781.

[15] P.J. Worland, B. Jarrott, Radioimmunoassay for the quantitation of lisinopril and enalaprilat, J. Pharm. Sci. 75 (1986) 512–516.

[16] H.J. Leis, G. Fauler, G. Raspotnig, W. Windishhofer, An improved method for the measurement of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry, Rapid Commun. Mass Spectrom. 13 (1999) 650–653.

[17] Y. Wong, B.G. Charles, Determination of the angiotensin-converting enzyme inhibitor lisinopril in urine using solid-phase extraction and reversed-phase high-performance liquid chromatography, J. Chromatogr. B 673 (1995) 306–310.

[18] P.B. Ghosh, M.W. Whitehouse, 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole: a new fluorigenic reagent for amino acids and other amines, Biochem. J. 108 (1968) 155–156.

[19] S. Tosunoglu, L. Ersoy, Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection, Analyst 120 (1995) 373.