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SPECTROFLUOROMETRIC DETERMINATION OF LISINOPRIL IN TABLETS

LİSİNOPRİLİN TABLETLERDE SPEKTROFLUOROMETRİC MİKTAR TAYİNİ

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Powdered tablets equivalent to 20 mg of lisinopril (I) was dissolved in 50 ml of H_2O, the mixture was sonicated for 20 minutes, diluted to 100 ml with H_2O, mixed and filtered. The first portion (20 ml) of the filtrate was discarded and 10 ml of the filtrate was diluted to 100 ml with H_2O. 10 ml of this solution was diluted to 50 ml with H_2O again. 2 ml of borax buffer (pH 9.5) was mixed with 1 ml of this solution, then 2 ml of fluorescamine (in 100 fold molar excess over I) in acetone was added and the contents were mixed again. After standing for 20 minutes, protected from light, the fluorescence intensity was measured at excitation and emission wavelengths of 365 and 488 nm respectively, against a blank prepared similarly. The fluorescence intensity was linearly related to the concentration over the 50-250 ng.ml^{-1} and 250-1250 ng.ml^{-1} concentration ranges of the I. Results obtained by applying the proposed fluorescence procedure to commercially available lisinopril dosage forms were compared with the UV spectrophotometric method. On applying the F-test and t-test at the 95% confidence level, no significant difference was found between the mean recoveries obtained with the suggested and UV spectrophotometric method.

Keywords: *Lisinopril; Fluorescence; Spectrofluorometric determination*

Lisinopril, angiotensin dönüştürücü enzim (ACE) inhibitörü olup, antihipertansif etki gösteren bir ilaçtır. Bu çalışmada lisinoprilin fluoreskamin ile sulu ortamda fluoresans gösteren türev oluşturmasına dayanan spektrofluorometrik bir yöntem geliştirildi. Optimum reaksiyon şartları araştırıldığında; reaksiyon pH 9.5'ta, fluoreskamin/amin mol oranının 100 olması halinde ve oda temperatüründe 20 dak. içerisinde tamamlanmaktadır. Fluoresans ölçmeleri 488 nm de, 365 nm eksitasyon filtresi kullanılarak yapıldı. 50-250 ng.ml^{-1} ve 250-1250 ng.ml^{-1} konsantrasyon aralıklarında doğrusal bir ilişki olduğu saptandı. Bu yöntem lisinoprilin tabletlerde miktar tayinine uygulandı. Farmasötik preparatlar ayrıca kıyas yöntemine göre analiz edildi. Her iki yöntemle elde edilen sonuçlar altışar deneme üzerinden %95 olasılık düzeyinde t ve F testi uygulanarak istatistik olarak karşılaştırıldı.

Anahtar kelimeler: *Lisinopril; Floresans; Spektrofluorometrik tayin*

Introduction

Lisinopril is the lysine analog of enalaprilat-the active inhibitor of the angiotensine converting enzyme derived from the prodrug enalapril. It has been shown to be effective in the treatment of all grades of essential hypertension (1,2). Many analytical techniques have been applied for the assay of this drug in body fluids such as RIA (3), CIBA (4), and FEA (5). HPLC analyses (6-8) of this drug was also reported. Fluorescamine is a fluorogenic reagent for the assay of primary amines in the picomole range (9-11). Its reactions with amines is almost instantaneous at room temperature in aqueous media. The proposed method depends on the reaction between lisinopril and fluorescamine in an aqueous solution.

Materials

Instruments: A Zeiss PMQ-II spectrophotometer equipped with ZFM 4 fluorescence attachment and St 41 mercury vapour lamp was used. Zeiss 365 nm filter served for the UV excitation. The emission monochromator was set at 488 nm. The slit width was varied between 0.5-0.15 mm. Glass cells (10x10x45 mm) were used as sample cells.

The responce of the spectrofluorometer was calibrated daily using appropriate concentrations of quinine sulphate solutions in 0.1 N sulphuric acid.

Chemicals: Lisinopril and its tablets were obtained from İlsan İlac Hammaddeleri San. A.Ş. and İltaş İlaç San. A.Ş., İstanbul. Fluorescamine was purchased from Hoffmann-La Roche, Basel, Switzerland and quinine sulphate, sulphuric acid, acetone, acetonitrile, dimethylformamide, dioxane from E. Merck., Darmstadt, Germany. All solvents used were analytical grade.

Stock solution: An accurately weighed amount of lisinopril dihydrate equivalent to 62.5 mg of lisinopril was dissolved and diluted to 100 ml with water.

Standard solutions: Aqueous solutions of lisinopril (1.25-6.25 mcg.ml^{-1} ; 0.25-1.25 mcg.ml^{-1}) were prepared by diluting the stock solution.

Reagent solutions : 0.021% and 0.004% (w/v) solutions of fluorescamine in acetone were prepared the day before usage and stored at room temperature.

Buffer solutions were prepared according to the general procedures (12, 13).

Method

2 ml of borax buffer (pH 9.5) was transferred to a 10 ml test tube. 1 ml of standard drug solution was added and the contents were mixed, then 2 ml of the fluorescamine solution was added and contents were mixed again. After standing for 20 minutes, protected from light, the fluorescence intensity was measured at excitation and emission wavelenghts of 365 and 488 nm respectively, against a blank prepared similarly.

Calibration curves were obtained by least squares linear regression of the relative fluorescence intensity values versus concentrations of lisinopril.

Sample preparation

20 lisinopril tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 20 mg of lisinopril was transferred into a 100 ml calibrated flask. Then 50 ml of water was added, and the mixture was sonicated for 20 minutes, diluted with water, mixed and filtered. The first portion (20 ml) of the filtrate was discarded and 10 ml of the filtrate was transferred into a 100 ml volumetric flask and diluted to volume with water. 10 ml of this solution was pipetted into a 50 ml volumetrik flask and diluted to volume with water again. Then , 1 ml of this solution was pipetted into a test tube and processed as described above.

Results and Discussion

The proposed method has been used for the determination of lisinopril and its dosage forms. The yields of lisinopril-fluorescamin derivative were investigated as a function of pH, reaction time, reagent excess and reagent solvent.

Fluorescence intensities were measured at the concentration of 500 ng.ml^{-1} of lisinopril. Instrument settings: excitation filter 365 nm, emission maxima 488 nm, sensitivity 1x9 and slit width 0.4 mm.

Wavelength and effect of reagent solvent

The fluorescence intensity and the position of the maxima depend on the nature of the solvent used for reagent solution. Table 1 shows that acetone gave the highest response.

Table 1. Fluorescence intensities of fluorophore formed with fluorescamine in different solvents

Solvents	Relative fluorescence arbitrary units
Acetone	44.5
Dioxane	1.5
Acetonitrile	32
Dimethylformamide	25.5

Effect of pH

The fluorophore formation is dependent on the pH of the medium. The results shown in Table 2 indicated that maximum fluorescence intensity was obtained at pH 9.5.

Table 2. Effect of pH on the reaction of lisinopril with fluorescamine

pH	7	8	9	9.5	10	11
Relative fluorescence arbitrary units	5	13	31	37	32	26.5

Reaction time

The reaction was completed within 20 min. at room temperature (Table 3).

Table 3. Effect of time on fluorescence intensity of fluorophore

Time (min.)	10	15	20	25	30	40
Relative fluorescence arbitrary units	47.7	48.5	49.2	49	48.5	48

Amount of the reagent

It was found that 100 fold excess of fluorescamine was necessary for maximum fluorescence intensity (Table 4).

Table 4. Effect of reagent concentration on the reaction of lisinopril with fluorescamine

Mole ratio of fluorescamine/lisinopril	40	80	90	100	110	120
Relative fluorescence arbitrary units	24.5	45.3	47.5	50.8	50.2	49.5

Table 5. Comparison of the results obtained by the proposed and UV spectrophotometric methods for the assay of lisinopril in tablets (A:5 mg/tablet, B:20 mg/tablet)

	A				B			
	Proposed Method		UV Spectrophotometric Method		Proposed Method		UV Spectrophotometric Method	
n	mg/tablet	%	mg/tablet	%	mg/tablet	%	mg/tablet	%
1	4.92	98.42	4.97	99.47	19.82	99.12	19.71	98.54
2	4.95	98.89	4.87	97.33	19.68	98.42	19.83	99.13
3	4.90	97.95	4.93	98.67	19.92	99.60	19.94	99.71
4	4.91	98.19	4.89	97.87	19.78	98.89	19.77	98.83
5	4.91	98.19	4.91	98.13	19.97	99.83	19.83	99.13
6	4.95	98.89	4.93	98.67	19.97	99.83	19.88	99.42
Mean	4.92	98.42	4.91	98.36	19.86	99.28	19.83	99.13
Standard Deviation	0.02		0.03		0.11		0.07	
R.S.D.	0.41		0.67		0.54		0.37	
Confidence Limits	4.90-4.94		4.88-4.94		19.77-19.95		19.77-19.89	
t-test of significance	t=0.35	p=0.05	t=2.23		t=0.31	p=0.05	t=2.23	
F- test of significance	F=2.25	p=0.05	F=5.05		F=2.04	p=0.05	F=5.05	

Stability of the derivative in aqueous medium was investigated by performing repeated readings of the same sample at different times. I_f values were found to be stable for at least three days at +4°C in dark.

Under the specified experimental conditions, the fluorescence intensity was linearly related to the concentration over the 50-250 ng.ml^{-1} and 250-1250 ng.ml^{-1} concentration ranges of the amine. Using the method of least squares, the linear regression equations obtained were I_f=0.232 C-0.680 (0.9999) and I_f=0.177C+3.970 (0.9999) for 50-250 ng.ml^{-1} and 250-1250 ng.ml^{-1} concentration ranges, respectively.

Results obtained by applying the proposed fluorescence procedure to commercially available lisinopril dosage forms are presented in Table 5. The results obtained were compared with the UV spectrophotometric method.

On applying the F-test and t-test at the 95% confidence level, no significant difference was found between the mean recoveries obtained with the suggested and UV spectrophotometric method.

The proposed method is simple and rapid and shows good precision, accuracy and selectivity.

References

1. Garavaglia, G.E., Messerli, F.H., Nunez, B.D., Schmieder, R.E., Frohlich, E.D.: Am.J. Cardiol. 62, 912 (1988)
2. Donahoe, J.F., Laher, M., Dotle, G.D., Cooper, W.D.: J. Cardiovasc. Pharmacol. 9 (Suppl. 3) 66 (1987)
3. Worland, P.J., Jarrot, B.: J.Pharm. Sci. 75, 512 (1986)
4. Fyhrquist, F., Grönhagen-Riska, C., Tikkanen, I., Junggren, I.L.: J. Cardiovasc. Pharm. 9 (Suppl. 3) 61 (1987)
5. Shepley, K., Rocci, M. I., Patrick, H., Mojaverian, P.: J.Pharm. Biomed. Anal. 6(3) 241 (1988)- Ref: Chem. Abstr. 109, 31461z (1988)
6. Brittain, H.G. (Ed.) Analytical Profiles of Drug Substances, 21, p. 262, Academic Press Inc., San Diego, California 1992
7. The United States Pharmacopoeia (USP XXII) 3rd Supplement, p. 2355 United States Pharmacopoeial

Convention Inc. Rockville 1990
8. The United States Pharmacopoeia (USP XXIII) p. 895 United States Pharmacopoeial Convention Inc. Rockville 1995
9. Weigele, M., De Bernardo, S. L., Tengi, J. P., Leimgruber, W.: J. Am. Chem. Soc. 94, 5927 (1972)
10. Udenfriend, S., Stein, S., Böhlen, P., Daiman, W., Leimgruber, W., Weigele, M.: Science 178, 871 (1972)
11. Böhlen, P., Stein, S., Dairman, W., Udenfriend, S.: Arch. Biochem. Biophys. 155, 213 (1973)
12. British Pharmacopoeia (BP) II Appendix ID, A76, HMSO, London 1993
13. Meites, L. (Ed.): Handbook of Analytical Chemistry 11-7 McGraw-Hill Book Company Inc. New York 1963

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