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Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 671–677

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Determination of losartan and its degradates in COZAAR® tablets by reversed-phase high-performance thin-layer chromatography

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Received 26 June 1997; received in revised form 21 October 1997; accepted 21 October 1997

Abstract

Losartan potassium is an angiotensin II receptor blocker. It has been formulated and marketed as a tablet dosage form (COZAAR®). A reversed-phase high-performance thin-layer chromatography method has been developed for the determination of losartan and its low level dimeric degradates (E and F). The method has been validated and shown to be sensitive, efficient, and reliable, and can be used as an excellent alternative to the HPLC stability testing of losartan potassium in COZAAR® tablets. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: HPTLC; Losartan potassium; Quantitation; Stability

1. Introduction

Losartan (DuP 753, MK-954), is a potassium salt of 2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole (Fig. 1) [1–3]. It has been shown to be an orally active, highly specific non-peptide angiotensin II receptor blocker. Losartan effectively reduces hypertension by suppressing the effects of angiotensin II at its receptors, thereby blocking the renin-angiotensin system. Losartan has been formulated and marketed as COZAAR® in a tablet dosage form. During the stability study of

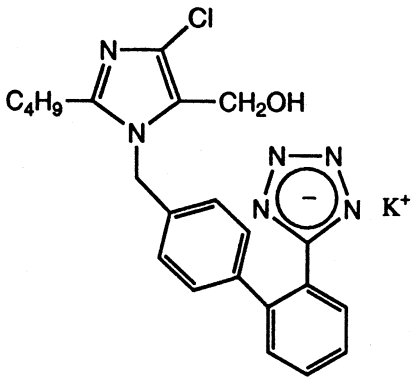

Fig. 1. Structure of losartan potassium.

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PII S0731-7085(97)00251-3

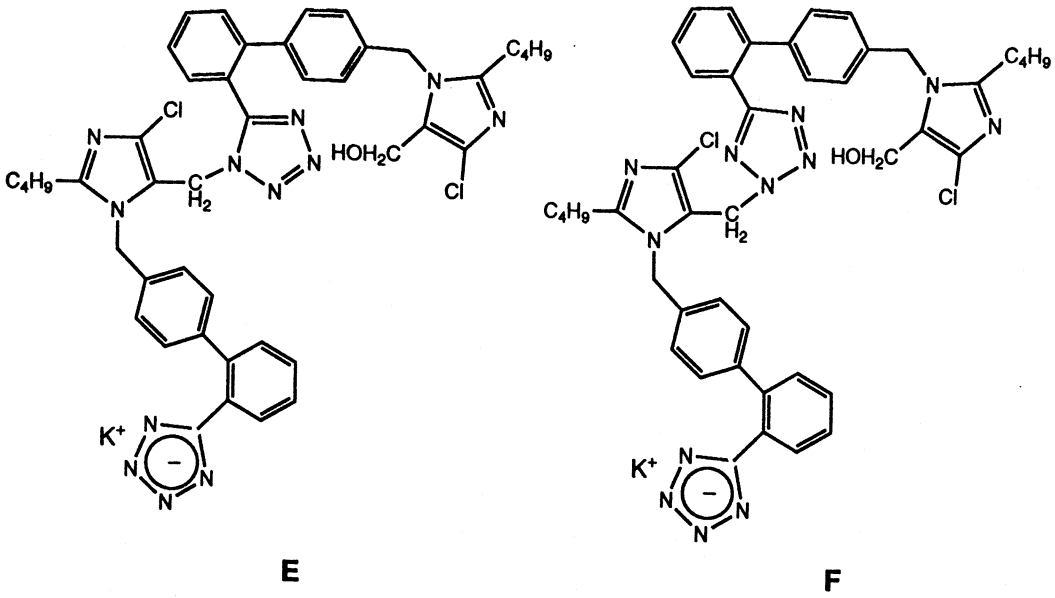

Fig. 2. Dimeric degradates of losartan potassium in COZAAR® tablet dosage fomm.

COZAAR® tablets, two diastereoisomeric degradates of losartan have been observed, dimers E and F (Fig. 2). Various analytical methods have been developed for the quantitative determination of losartan in COZAAR® tablets using analytical techniques such as high-performance liquid chromatography (HPLC) [4], capillary electrophoresis [5], super-fluid chromatography [6], etc. HPLC is the most widely used technique for the routine stability testing of losartan in COZAAR® tablets.

Thin-layer chromatography (TLC), which is one of the oldest chromatographic methods, is commonly used in medical-biochemical analysis, food analysis, and environmental pollutant analysis [7]. By comparison with HPLC, high-performance thin-layer chromatography (HPTLC) still preserves its advantage as a rapid, reliable and economical analytical method. The main application of TLC in the pharmaceutical industry is intermediate quality control during the development and production of pharmaceutically active substances and the testing of optically pure substances [8–12]. Although TLC is mainly used as a drug screening and confirmation tool [13], quantitative pharmaceutical analysis by TLC has recently attracted considerable interest due to the improved technologies with HPTLC. In recent years, the HPTLC technique has been improved to incorporate the following features: HPTLC-grade stationary phase, automated sample application devices, controlled development environment, automated developing chamber or force-flow techniques, computer-controlled densitometry and quantitation, and fully validated procedures. These features result in methods that are not only convenient, fast, robust, and cost efficient, but also reproducible, accurate and reliable. Applications of HPTLC to the quantitative analysis of drug substances in biological and formulation matrices have been documented [14]. For example, HPTLC methods with densitometric detection were developed for the quality control of lidocaine hydrochloride bulk drug and injection solution, and the results compared well with HPLC [15].

HPTLC methods with stability-indicating features have been utilized to assess the impurity and degradation profile of bulk drug substances and drug formulations [16–20]. By selecting an appropriate developing solvent and TLC plates, satisfactory separation between active drugs and degradation products can be achieved. However,

the sensitivity and accuracy of the methods with regard to low level degradates (0.1% of active drug) present in the dosage formulation are rarely addressed. This study describes the stability indicating HPTLC method development and validation for the simultaneous determination of losartan and its low level degradates in COZAAR® tablet dosage forms.

2. Experimental

2.1. Materials

Losartan potassium and its degradates (dimers E and F) were obtained from Du Pont Merck Pharmaceutical Co. (Wilmington, DE, USA). HPLC grade acetonitrile, water, glacial acetic acid, and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPTLC plates (20 cm × 10 cm reversed-phase plates, C18 silica gel on glass) were obtained from Sigma-Aldrich (Milwaukee, WI, USA).

2.2. Instrumentation and chromatographic conditions

The HPTLC system consisted of an automatic TLC sampling device, an automatic developing chamber with programmable solvent flow, heating and cooling control, and a densiometer with fixed slit scanner with wavelength range from 200 nm to 800 nm, all from CAMAG Scientific (Wilmington, NC, USA). Data collection and data analysis were conducted using an on-line computer with Cats 3 software (CAMAG Scientific).

For optimal sensitivity, solutions of the testing samples were applied to the TLC plates as bands rather than spots. Bands were 6 mm long, and 12 μl of sample was applied to each band. The TLC plates were then developed with acetonitrile-methanol-0.1% acetic acid (35:25:40, v/v/v) as mobile phase. After developing over a distance of 30 mm, the TLC plates were dried with hot air for 2 min and with cold air for 4 min in the automatic developing chamber. The developed plates were scanned by linear scanning at 4 mm/s with a Scanner II equipped with a PC/AT and Cats 3 software. The scan length and width were adjusted to cover the entire band. The analyses were detected with UV at 254 nm.

2.3. Standard solution preparation

2.3.1. Losartan potassium standard solution

A 250.0 mg amount of losartan potassium was accurately weighed, dissolved in acetonitrile-water (1:1, v/v) and diluted to volume in a 100 ml volumetric flask. Standard solutions were obtained by diluting the above stock solution with acetonitrile-water (1:1, v/v) to give 50%, 75%, 100%, and 125% levels of the method concentration of 1.0 mg ml^{-1}. Each solution was then transferred into an HPTLC vial for analysis.

2.3.2. Degradate standard solution

Two milligram amounts of dimers E and F authentic material were accurately weighed and transferred to a 100 ml volumetric flask. Acetonitrile-water (1:1, v/v) was added to volume and the solution was mixed thoroughly. This was the working standard of dimers E and F. Standard solutions for low level linearity and limit of detection (LOD) and limit of quantitation (LOQ) determination were obtained by diluting the working standard with acetonitrile-water (1:1, v/v) to concentrations ranging from 0.05% to 2.0% levels of the losartan method concentration of 1.0 mg ml^{-1}.

2.4. Sample solution preparation

One 25 mg COZAAR® tablet was placed into each of ten 25 ml volumetric flasks and diluted to volume with acetonitrile-water (1:1, v/v). A magnetic stirring bar was placed into each flask and stirred for 45 min followed by sonication for 10 min. An aliquot was centrifuged and the clear supernatant was transferred into HPTLC vials for analysis.

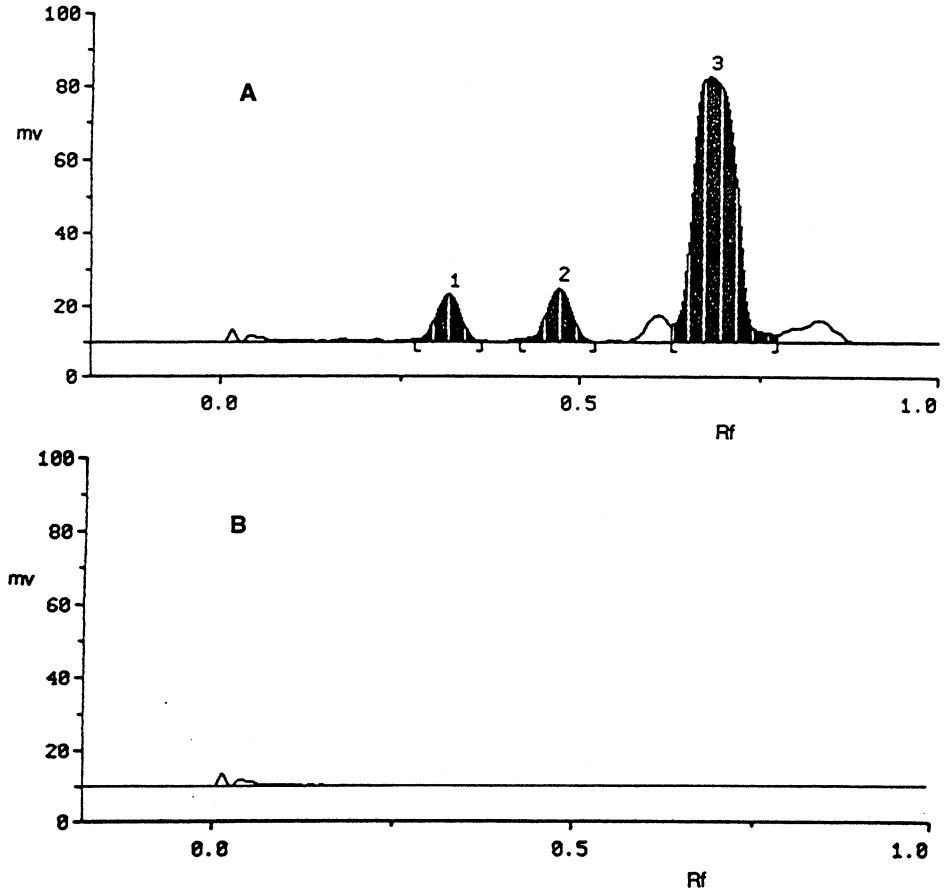

Fig. 3. Typical chromatogram of COZAAR® tablets stored at 40°C and 75% relative humidity for 3 years: 1, dimer F; 2, dimer E; 3, losartan potassium. (A) COZAAR® tablet solution; (B) placebo tablet solution.

3. Results and discussion

3.1. Method development

In this study, the selection of stationary phase was very important in order to detect low level (0.1% of losartan method concentration of 1.0 mg ml^{-1}) of dimeric degradates. Normal phase TLC plates were tried during the method development; however, peak tailing was constantly observed for losartan, and the dimeric degradates, probably due to the interaction of the tetrazol group of losartan with the silanol group of the TLC plate. This tailing phenomenon reduced the peak symmetry and the sensitivity of the method dramatically. The peak tailing was eliminated by using C18 reversed-phase HPTLC plates and the sensitivity was improved. The selection of mobile phase was also critical to the method sensitivity. Losartan potassium has pK_a values of 2.36 and 5.55. In this respect, using buffer with $pH < 2.3$ would be preferred to avoid peak splitting and peak broadening. Finally, by comparison with spots, bands render sharper peaks and hence higher sensitivity for the method.

Under the conditions described, the R_f value of losartan standard was found to be 0.79 ± 0.06 and the R_f values of dimeric degradates E and F were 0.54 ± 0.06 and 0.31 ± 0.06, respectively. Severely stressed COZAAR® tablets were used to demonstrate the method specificity. A typical chromatogram of COZAAR® tablets stressed at 40°C

and 75% relative humidity for 3 years is shown in Fig. 3. Losartan and its dimeric degradates (E and F) are totally baseline separated within 30 mm developing length. Two other peaks with unknown identity were also observed eluting close to losartan. Since they have not been observed in tablets within the shelf-life, these two peaks were probably the results of unrealistic stressing of the tablets. Due to the large difference of polarity (hydrophobicity) between losartan and its dimeric degradates, the currently used HPLC method utilized a gradient method in order to shorten the analysis time [4]. In this study, the stationary phase contains reversed-phase C18 with a large portion ($\approx 40\%$) of silanol groups which would pose stronger interaction with losartan than the dimeric degradates. Overall, the polarity difference between losartan and the dimeric degradates is compromised on the TLC plate, dramatically shortening the elution time. However, by comparison with HPLC, the measurement repeatability (precision) and method reproducibility are still a critical issue with HPTLC methods. Therefore, the instrument parameters and quality of TLC plates have to be well controlled.

3.2. Method validation

As demonstrated in Fig. 3, baseline separation of losartan and two dimeric degradates is achieved. Analysis of the sample solution by the HPLC method revealed a similar peak profile with no extra peaks. There is also no interference of either losartan and the degradates from the placebo, which further demonstrates the specificity of this method.

The measurement precision of the method was evaluated by scanning ten spots of the losartan standard solution. The relative standard deviation of the peak areas was found to be 1.2%.

The sensitivity of the method was demonstrated by analyzing dimers E and F at different concentrations. Three bands of each concentration were scanned and their peak areas were averaged. The limit of quantitation of dimers E and F was found to be 0.1% with a signal/noise ratio of 0.1. The limit of detection of dimers E and F was found to be 0.05% with a signal/noise ratio of greater than

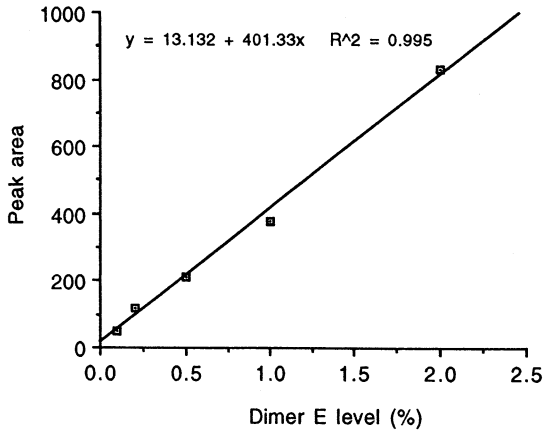

Fig. 4. Linearity of detector response versus dimer E degradate concentrations ranging fro 0.1% to 2% levels of losartan potassium method concentration (1.0 mg ml^{-1}).

5. A satisfactory linear relationship between the detector response and concentration of between 0.1% and 2.0% of losartan method concentration (1.0 mg ml^{-1}) was established for dimers E and F. For example, a correlation coefficient, R^2, of 0.995 was obtained with dimer E (Fig. 4).

The accuracy of the method was determined by investigating the recovery of losartan potassium at five levels ranging from 50% to 125% of the method concentration (1.0 mg ml^{-1}) from the solution spiked placebo. The results showed good recoveries ranging from 99.5% to 100.5% (Table 1). The detector responses for losartan solution in the presence of the placebo tablet were also linear over the range of 50-125% of the method concentration (1.0 mg ml^{-1}) with a correlation coefficient, R^2, of 0.994 (Fig. 5). The y-intercept for this linear regression is not zero as would be expected with HPLC methods. Unlike HPLC methods, for

Table 1
Accuracy of method determined by the recovery of losartan from placebo tablets spiked with losartan potassium solutions

Level	Amount added (mg)	Amount recovered (mg)	Recovery (%)
50%	5.01	4.98	99.4
75%	7.52	7.53	100.1
100%	10.02	10.07	100.5
125%	12.53	12.50	99.8

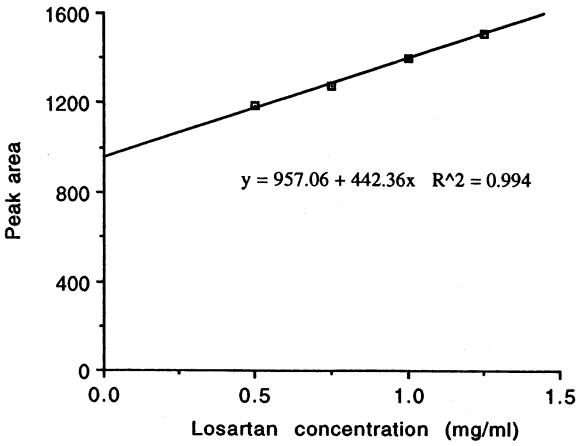

Fig. 5. Linearity of detector responses versus losartan potassium concentrations ranging from 50% to 125% of losartan potassium method concentration (1.0 mg ml^{-1}).

which linearity of detector response over a wide range of concentrations of analyses can be obtained, the calibration curve of detector response (especially for UV) versus a wide range of concentrations for HPTLC often does not follow linear regression but rather polynomial regression. With HPTLC, the analyses interact with the layer surface of the stationary phase where scattering and absorption tend to take place, especially with high concentrations of analyses. These combined processes are not adequately described by Beer-Lambert law, but the Kubelka-Munk model [21]. Therefore the relationship of detector response and concentration ranging from 0.1% to 125% does not follow linear regression, but a polynomial regression. However, linearity within a relatively narrow range of concentrations (e.g. 0.1-2% or 50-125%) can be achieved, which is sometimes useful for quantitation.

The method precision was demonstrated by analyzing ten COZAAR® tablet solutions on a single HPTLC plate. For quantitation of losartan, the sample solutions and losartan standard solutions were chromatographed on the same plate. The results obtained from single level external standards compared well with those obtained from calibration curve. Also the same tablet solutions were diluted (to give a concentration of 0.05 mg ml^{-1}) and analyzed by HPLC. The results shown in Table 2 demonstrate that data generated by HPTLC method agree well with the HPLC results.

Table 2
Assay results for ten COZAAR® tablet (25 mg) solutions by HPTLC and HPLC

Tablet no.	Potency found (mg per tablet)	
	HPTLC method	HPLC method
1	24.27	24.59
2	24.50	25.04
3	24.36	24.66
4	24.53	24.59
5	24.87	24.72
6	25.28	24.23
7	25.03	24.85
8	24.79	24.88
9	24.95	24.96
10	24.77	24.90
Average	24.74	24.64
R.S.D.	1.2%	1.5%

4. Conclusion

HPTLC has been explored for the simultaneous determination of losartan and its low level dimeric degradates in the tablet dosage form. In comparison with HPLC methods, this method appears to be equally suitable for routine testing of losartan in its formulations. Among its advantages are short run time and large sample capacity, both of which significantly reduce the duration of the analysis. This method can be an excellent alternative for the dosage uniformity and stability testing of COZAAR® tablets.

Acknowledgements

The authors thank Michael Honeycutt and Roger James of CAMAG Scientific for their valuable suggestions. The authors also thank Paul Dradransky for providing COZAAR® tablets and the authentic materials of dimers E and F.

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