Original Article

Sensitive and accurate estimation of losartan potassium formulation by high-performance thin-layer chromatography

Abstract

Aim and Objectives: To develop a simple, sensitive, cost-effective and reproducible UV-spectrophotometric method and validate for the estimation of disodium edetate in topical gel formulations. **Materials and Methods:** Solution of disodium edetate reacts with ferric chloride to form complex in 0.1 N HCl giving λ_{max} at 270 nm. Beer's law was obeyed in the concentration range of 5–50 μg/mL ($r^2 = 0.9997$). **Results:** The limit of detection and limit of quantitation were found to be 1.190 and 3.608 μg/mL, respectively. The results show that the procedure is accurate, precise, and reproducible (relative standard deviation < 1%), while being simple and less time consuming. **Conclusions:** The study concluded that the UV-spectrophotometric method could be used for the quantification of disodium edetate in pure form as well as in pharmaceutical formulations.

Key words: Estimation, high-performance thin-layer chromatographic, losartan potassium, tablets

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INTRODUCTION

Losartan potassium, a potassium salt of 2-Butyl-4-chloro-1-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, represents the first of a new class of orally active non-peptide angiotensin II (Type AT_1) receptor antagonists employed in the management of essential hypertension.[1] Several methods have been developed using HPLC, LC-MS-MS, metabolite in biological fluids by HPLC and LC-MS-MS and capillary electrophoresis.[2-14] The aim of the present work is to develop and validate a new, accurate, specific and reproducible HPTLC method for determination of losartan potassium as in solid-based tablet formulation and also in marketed oral solid dosage formulation (COZAAR Tablets). The proposed method was validated as per ICH guidelines[15] and its updated international convention.[16]

MATERIALS AND METHODS

Drug and chemicals

Losartan potassium was obtained as kind gift sample from IPCA laboratories, Mumbai, India, and used without further purification, certified to contain 99.97% (w/w). LOSACAR tablets were procured from market (manufactured by Zydus Medica Ltd). Analytical grade acetonitrile, methanol, and acetic acid were all obtained from Qualigens Fine Chemicals, Mumbai, India.

HPTLC instrumentation and chromatographic condition

Chromatography was performed on aluminum-backed silica gel $60F_{254}$ HPTLC plates (10 × 10 cm) prewashed with methanol; plates were developed with acetonitrile-methanol-0.1% acetic acid (3.5:2.6:3.9, v/v) in a Camag twin–trough chamber (10 × 20). Standard solutions of losartan potassium were transferred to

different 10 ml volumetric flask and diluted to volume with the methanol such that the final concentrations of losartan potassium were 5.0-30.0 mcg/ml. Both standards and samples (0.3 µl) were applied to the plates as 6 mm bands by means of a Camag Linomat IV sample applicator. After development of about 7 cm and drying of the plates, evaluation of both drugs was performed by scanning densitometry at λ=235 nm (drug λ max) by means of a camag TLC scanner III controlled by CATS.V.4.06 software (Camag). Peak areas were recorded for all the peaks. The amount of losartan potassium was computed from the peak area by use of the formula: Amount of losartan potassium = (Rspl x C x D x average weight)/ (Rstd x W), where Rspl is the area of the losartan potassium sample peak, Rstd is the area of the losartan potassium standard peak, C is the concentration of standard solution [mg/ml], D is the dilution factor, and W is the weight of tablet (mg).

Calibration curves of losartan potassium

Calibration solutions of losartan potassium in methanol containing concentrations of losartan Potassium from 5.0 to 30.0 ng/ml were prepared by individual weighing. Five microliters from each solution was spotted on the HPTLC plate to obtain final concentration range of 5.0–30.0 ng per spot. Each concentration was spotted two times on the HPTLC plate. The data of peak area versus drug concentration were treated by linear least-square regression analysis.

Method validation

The HPTLC method developed was validated for following parameters.

Recovery studies

Recovery of losartan potassium was determined by spiking losartan potassium in drug to obtain three different concentrations covering the low, medium, and higher ranges of the calibration curve. The recovery was calculated by comparing the resultant peak areas with those obtained from pure standards in methanol at the same concentrations.

Precision and accuracy

Different amount of losartan potassium covering low, medium, and higher ranges of the calibration curve were spotted on the HPTLC plate. These spots were analyzed by using the above-described HPTLC method. Precision was expressed as the percent relative standard deviation (% C.V.) and accuracy was expressed as a percentage (observed concentration × 100/theoretical concentration).

Limit of detection and limit of quantification

These were calculated by use of the equations Limit of detection (LOD) =3 × N/B and limit of quantification (LOQ) = 10 × N/B where N is the standard deviation of the peak areas of the drugs (n=3), taken as a measure of the noise, and B is the slop of the corresponding calibration curve.

Analysis of marketed formulation

The developed method can be applied in determination of losartan potassium in COZAAR tablets, which is marketed oral solid dosage formulation.

To determine the contents of losartan potassium in tablets (COZAAR, label claim: 25 mg per tablet), the drug from the powder was extracted with 10 ml methanol. To ensure complete extraction of the drug, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the supernatant was suitably diluted to give desired concentration. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

RESULTS AND DISCUSSION

The mobile phase used was resolving the two drugs very efficiently, as shown in Figure 1. HPTLC offers several advantages over reported methods. It facilitates automatic application and scanning *in situ*. The composition of the mobile phase for the development of the chromatographic method was optimized by testing different solvent mixtures of varying polarity. Acetonitrile-0.2% acetate (6:4, 7:3, v/v) and methanol-0.2% acetate (8:2, 9:1, v/v) were tried. The best results were obtained using acetonitrile-

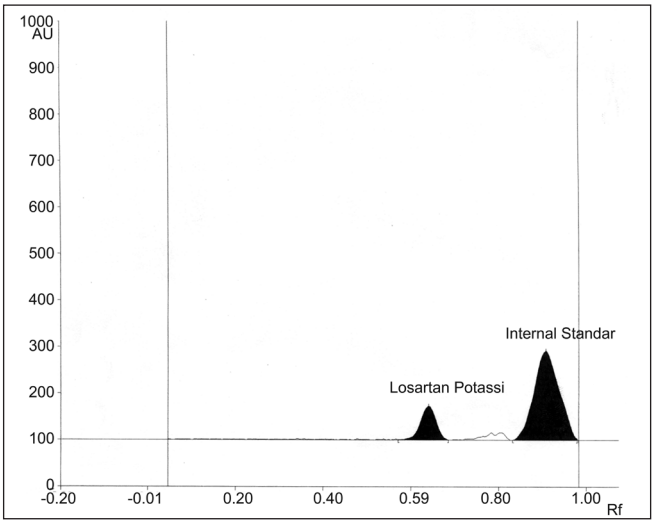

Figure 1: Typical chromatogram of losartan potassium and internal standard

methanol–0.1% acetic acid (3.5:2.6:3.9, v/v). This mobile phase showed good resolution of losartan potassium peak from other formulation components or excipients tested. Densitometric scanning of all the tracks showed compound with Rf value 0.61 (single violet spot), identified as losartan potassium. The present method is quicker as the time needed for development of plate is reduced considerably to less than half an hour for chamber saturation. The method was successfully used in the analysis of losartan potassium from the tablet dosage forms, and in case of Cozaar Tablets without interference of the formulation excipients.

Recovery study

Results showed high extraction efficiency of losartan potassium from formulation components. The recovery of losartan potassium ranged from 96.58 to 98.27%, average of 97.33%. This confirms that the proposed method can be used for determination of losartan potassium in tablet formulation.

Precision and accuracy

Five microliter aliquots of samples containing 5.0, 15.0, and 30.0 ng losartan potassium were analyzed according to the proposed method. In order to control the scanner parameters, one spot was analyzed several times. By spotting and analyzing the same amount several times the precision of the automatic spotting device and the derivatization technique, was evaluated. The coefficient variation (% C.V.) for the analysis of eight replicates indicated good precision for the proposed TLC method (% C.V. consistently less than 5) and scanning eight spots in one run is the method of choice. Results obtaining were of good accuracy and high precision. The accuracy was found to be in the range of 88.76–98.88% and % C.V. in range of 1.02-6.81. The results are presented in Tables 1-3.

Limit of detection and limit of quantification

The limit of detection was 3.0 ng/ml and the limit of quantification was 16.0 ng/ml and the solutions were stable for the 3 days.

CONCLUSIONS

The developed HPTLC technique is precise, specific, accurate, and sensitive. It proves that the method is repeatable and selective for the analysis of losartan potassium as bulk drug and in pharmaceutical formulations without any interference from the excipients.

Table 1: Precision and accuracy data of HPTLC method performed on losartan potassium

Parameter	Values		
Actual amount of losartan potassium spotted (ng)	5.0	15.0	30.0
Amount detected[a] (ng±S.D.)	4.34 ± 0.36	14.43 ± 0.44	29.6 ± 0.38
C.V. (%)	8.18	3.01	1.29

[a] One spot is scanned eight times.

Table 2: Accuracy and precision of the assay

Amount of losartan potassium spotted (ng)	Amount detected (ng) (Mean±SD, *n*=5)	C.V. (%)	Accuracy (%)
5.0	4.44 ± 0.30	6.81	88.76
15.0	14.50 ± 0.40	2.75	96.64
30.0	29.60 ± 0.31	1.02	98.88

Table 3: Precision data of the HPTLC assay for losartan potassium

Amount of losartan potassium spotted (ng)	Amount detected (ng) (mean±SD)	C.V (%)
Inter day (n=5)		
5.0	4.68 ± 0.27	5.82
15.0	14.48 ± 0.41	2.84
30.0	29.70 ± 0.24	0.81
Intra day (n=5)		
5.0	4.72 ± 0.13	2.68
15.0	14.13 ± 0.28	1.95
30.0	29.37 ± 0.40	1.37

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REFERENCES

1. Smith RD, Chiu AT, Wong PC, Herblin WF, Timmermans PB. Pharmacology of Nonpeptide Angiotensin II Receptor Antagonists. Annu Rev Pharmacol Toxicol 1992;32:135-65.
2. Ansari M, Kazemipour M, Khosravi F, Baradaran M. A comparative study of first-derivative spectrophotometry and high-performance liquid chromatography applied to the determination of losartan potassium in tablets. Chem Pharm Bull 2004;52:1166-70.
3. Prasaja B, Sasongko L, Harahap Y, Hardiyanti, Lusthom W, Grigg M. Simultaneous quantification of losartan and active metabolite in human plasma by liquid chromatography–tandem mass spectrometry using irbesartan as internal standard. J Pharm Biomed Anal 2009;49:862-7.
4. Hertzog DL, McCafferty JF, Fang X, Tyrrell RJ, Reed RA. Development and validation of a stability-indicating HPLC

method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. J Pharm Biomed Anal 2002;30:747-60.
5. Farthing D, Sica D, Fakhry I, Pedro A, Gehr TW. Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate J Chromatogr B Biomed Sci Appl 1997;704:374-8.
6. Furtek CI, Lo MW. Simultaneous determination of a novel angiotensin II receptor blocking agent, losartan, and its metabolite in human plasma and urine by high-performance liquid chromatography. J Pharm Biomed Anal 1997;15:1021-9.
7. Carlucci G, Palumbo G, Mazzeo P, Quaglia MG. Simultaneous determination of losartan and hydrochlorothiazide in tablets by high-performance liquid chromatography. J Pharm Biomed Anal 2000;23:185-9.
8. Iwasa T, Takano T, Hara K, Kamei T. Method for the simultaneous determination of losartan and its major metabolite, EXP-3174, in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. J Chrom B Biomed Sci Appl 1999;734: 325-30.
9. Jia JY, Zhang MQ, Liu YM, Liu Y, Liu GY, Li SJ, *et al*. Pharmacokinetics and bioequivalence evaluation of two losartan potassium 50-mg tablets: A single-dose, randomized-sequence, open-label, two-way crossover study in healthy Chinese male volunteers. Clin Ther 2010;32:1387-95.
10. Lastra OC, Lemus IG, Sanchez HJ, Perez RF. Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets. J Pharm Biomed Anal 2003;33:175-80.
11. Lusina M, Cindrić T, Tomaić J, Peko M, Pozaić L, Musulin N. Stability study of losartan/hydrochlorothiazide tablets. Int J Pharm 2005;291:127-37.
12. Ritter MA, Furtek CI, Lo MW. An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Pharm Biomed Anal 1997;15:1021-9.
13. Dubey R, Bhusari VK, Dhaneshwar SR. Validated HPTLC Method for Simultaneous Estimation of Losartan potassium and Metolazone in Bulk. Drug Formulation Der Pharm Lett 2011;3:334-42.
14. Sathe SR, Bari SB. Simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in tablets by high-performance thin layer chromatography with UV absorption densitometry. Acta Chromatogr 2007;19;270-8.
15. ICH Guidance on Analytical Method Validation In: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September 2002.
16. ICH, Q2B Validation of Analytical Procedure: Methodology. In: Proceedings of the International Conference on Harmonization, Geneva, March 1996.

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