

**SIMULTANEOUS ANALYSIS OF LOSARTAN
POTASSIUM, ATENOLOL,
AND HYDROCHLOROTHIAZIDE
IN BULK AND IN TABLETS BY HIGH-PERFORMANCE
THIN-LAYER CHROMATOGRAPHY
WITH UV ABSORPTION DENSITOMETRY**

*S. R. Sathe and S. B. Bari**

Department of Pharmaceutical Chemistry, R.C. Patel College of Pharmacy, Karwand
Naka, Shirpur 425 405, Dist-Dhule, (M.S.), India

SUMMARY

A simple high-performance thin-layer chromatographic (HPTLC) method for separation and quantitative analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in pharmaceutical formulations has been established and validated. After extraction with methanol, sample and standard solutions were applied to prewashed silica gel plates and developed with toluene–methanol–triethylamine 6.5:4:0.5 (v/v) as mobile phase. Zones were scanned densitometrically at 274 nm. The R_F values of losartan potassium, atenolol, and hydrochlorothiazide were 0.60, 0.43, and 0.29 respectively. Calibration plots were linear in the ranges 1000–5000 ng per band for losartan potassium and atenolol and 250–1250 ng per band for hydrochlorothiazide; the correlation coefficients, r , were 0.9994, 0.9993, and 0.9994, respectively. The suitability of this method for quantitative determination of these compounds was proved by validation in accordance with the requirements of pharmaceutical regulatory standards. The method was used for routine analysis of these drugs in bulk and in a formulation.

INTRODUCTION

Losartan (LOK), 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2'-(1*H*-tetrazol-5-yl)(biphenyl-4-yl)methyl]imidazole, potassium salt, is a strong non-peptide antihypertensive agent which exerts its action by specific blocking of angiotensin II receptors. It has a gradual, long-lasting effect as an antihypertensive. Atenolol (ATL), (*RS*)-4-(2hydroxy-3-isopropylaminopro-

poxy)phenylacetamide, is a cardioselective β -blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilising properties. This drug is used to treat numerous cardiovascular disorders, for example hypertension, angina pectoris, cardiac arrhythmias, and myocardial infarction. The drug is official in USP, IP, and BP [1–3]. Hydrochlorothiazide (HCTZ), 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance.

Literature survey reveals that a variety of spectrophotometric and chromatographic methods, including UV derivative, the simultaneous equation method, colorimetric determination, HPLC, ratio derivative and compensation technique, and a stability-indicating HPLC method, have been reported for determination of LOK in pharmaceutical dosage forms in combination with other drugs [4–9]. Spectrophotometric and chromatographic methods (for example HPLC and HPTLC) have been reported for determination of ATL, in combination with other drugs, in bulk and pharmaceutical dosage forms [10–13]. A variety of methods have been used for determination of HCTZ [14–20]. No method has been reported for simultaneous estimation of LOK, ATL, and HCZT in the combined dosage form.

In recent years TLC has been improved to incorporate HPTLC grade stationary phases, automated sample-application devices, a controlled development environment, automated development, forced-flow techniques, computer-controlled densitometry, quantitation, and fully validated procedures. These features result in methods which are not only convenient, rapid, robust, and cost effective but also reproducible, accurate, and reliable. The objective of this investigation was, therefore, to establish an HPTLC method for simultaneous estimation of LOK, ATL, and HCZT in bulk and in tablets.

EXPERIMENTAL

Materials and Reagents

Analytically pure samples of losartan potassium and atenolol were procured from Torrent Pharmaceuticals, India, and hydrochlorothiazide from Macleods Pharmaceuticals, India, as gifts, and were used as working standards. Toluene and methanol of HPLC grade from Merck (Darmstadt, Germany) and triethylamine of analytical reagent grade from S.D. Fine

Chemicals were used, without further purification, to prepare the mobile phase.

A solution containing 1 mg mL^{-1} losartan potassium and atenolol and 0.25 mg mL^{-1} hydrochlorothiazide, prepared by dissolving 100 mg LOK and ATL standards and 25 mg HCTZ standard in 100 mL ethanol, was used as working standard solution.

Sample Preparation

Twenty Repalol* H tablets, manufactured by Atoz Life Sciences, containing 50 mg losartan potassium, 50 mg atenolol, and 12.5 mg hydrochlorothiazide were weighed and powdered. An amount of powder equivalent to 50 mg LOK, 50 mg ATL, and 12.5 mg hydrochlorothiazide was transferred to a 50-mL calibrated volumetric flask. After addition of 40 mL methanol and sonication (30–45 min) the solution was diluted to volume with the same solvent and filtered through a $0.45\text{-}\mu\text{m}$ filter (Millipore, Milford, MA, USA). This solution ($2 \mu\text{L}$, containing 2000 ng losartan potassium, 2000 ng atenolol, and 500 ng hydrochlorothiazide) was used for assay of losartan potassium, atenolol, and hydrochlorothiazide in the tablets.

Chromatography

Chromatography was performed on $10 \text{ cm} \times 20 \text{ cm}$ aluminium HPTLC plates coated with 0.2-mm layers of silica gel 60 F₂₅₄ (Merck). Before use the plates were washed with methanol then dried in an oven at 50°C for 5 min. Samples were applied as 6-mm bands by means of a Camag (Muttens Switzerland) Linomat V automatic sample applicator equipped with a $100\text{-}\mu\text{L}$ syringe (Hamilton, Reno, Nevada, USA); the distance between the bands was 13.0 mm and the spraying rate was $15 \text{ s } \mu\text{L}^{-1}$. Ascending development of the plate, migration distance 70 mm, was performed at $25 \pm 2^\circ\text{C}$, with toluene–methanol–triethylamine 6.5:4:0.5 (v/v) as mobile phase, in a Camag twin-trough chamber previously saturated with mobile phase vapour for 30 min. The average development time was 20 min. Densitometric scanning at 274 nm was then performed with a Camag TLC scanner 3 equipped with Camag Wincats software version 1.3.0 using the deuterium light source; the slit dimensions were $6.00 \text{ mm} \times 0.45 \text{ mm}$.

RESULTS AND DISCUSSION

Validation of the Method

The method was validated in accordance with ICH guidelines [21].

Linearity

Amounts of standard solution equivalent to 1000–5000 ng LOK and ATL per band and 250–1250 ng HCTZ per band were applied to the prewashed HPTLC plates and the plates were developed, dried, and scanned as described above. Calibration plots were constructed by plotting peak areas against the corresponding amounts of the drugs (ng per band). For LOK and ATL response was a linear function of amount in the range 1000–5000 ng per band; for HCZT the linear range was 250–1250 ng per band. The correlation coefficients, r , were 0.9998 for LOK, 0.9987 for ATL, and 0.9981 for HCZT. The average linear regression equations were $Y = 1.7928X + 1241.71$ for LOK, $Y = 1.8833X + 2003.36$ for ATL, and $Y = 3.2510X + 1063.57$ for HCZT.

Sensitivity

The sensitivity of measurement of losartan potassium, atenolol, and hydrochlorothiazide was estimated in terms of the limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by use of the equations $LOD = 3 \times N/B$ and $LOQ = 10 \times N/B$, where N is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and B is the slope of the corresponding calibration plot. LOQ and LOD for losartan potassium were 570.95 and 188.41 ng, respectively. For atenolol they were 560.24 and 184.82 ng, respectively, and for hydrochlorothiazide they were 137.55 and 45.39 ng, respectively.

Evaluation of Precision for Assay of the Pharmaceutical Preparation

The amounts of losartan potassium, atenolol, and hydrochlorothiazide in the pharmaceutical preparation were determined by replicate ($n = 5$) analysis. The results are reported in Table I.

Precision was determined by analysis of standard solutions containing concentrations of LOK, ATL, and HCTZ covering the entire calibration range. The precision of the method, as intra-day variation (CV , %) was determined by analysis of these solutions three times on the same day. Inter-

Table I

Results from assay of losartan potassium, atenolol, and hydrochlorothiazide in Repalol* H tablets

Component	Label claim (mg)	Amount found (mg \pm <i>SD</i> , <i>n</i> = 5)	Percentage of label claim (\pm <i>SD</i>)
Losartan potassium	50	49.66 \pm 0.58	99.33 \pm 1.17
Atenolol	50	49.87 \pm 0.13	99.72 \pm 0.28
Hydrochlorothiazide	12.5	12.44 \pm 0.08	99.60 \pm 0.71

n is the number of replicates

day precision (*CV*, %) was assessed by analysis of these solutions on three different days over a period of one week. The results of the precision studies are as shown in Table II.

Table II

Results from evaluation of precision

Drug	Concentration (ng per band)	Intra-day precision (<i>CV</i> , %, <i>n</i> = 3)	Interday precision (<i>CV</i> , %, <i>n</i> = 3)
Losartan potassium	1000	1.135	1.138
	2000	0.879	0.890
	3000	0.535	0.519
Atenolol	1000	0.385	0.318
	2000	0.192	0.252
	3000	0.356	0.487
Hydrochlorothiazide	250	1.495	1.532
	500	0.889	0.806
	750	1.219	1.370

Accuracy

The accuracy of the method was determined by analysis of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution obtained from the tablet formulation (2000 ng mL⁻¹ losartan potassium and atenolol and 500 ng mL⁻¹ hydrochlorothiazide) was spiked with amounts equivalent to 80, 100, and 120% of amounts of drugs in the original solution. When these solutions were analysed the recoveries were found to be within acceptable limits (Table III).

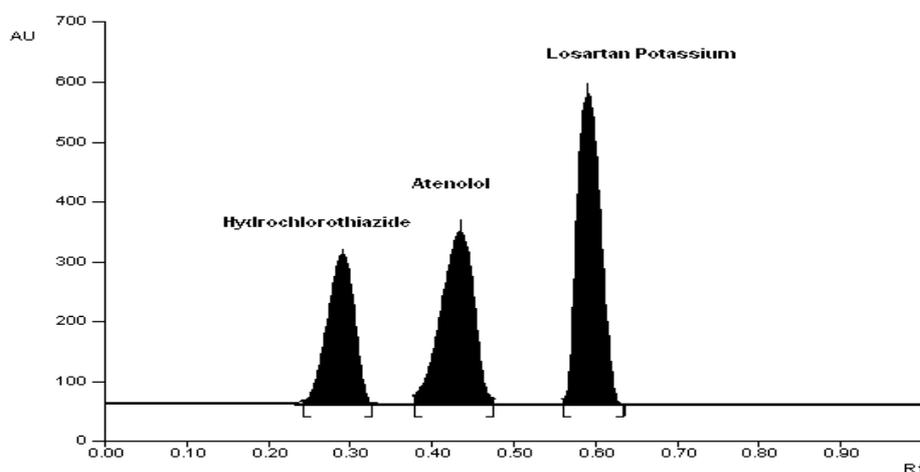
Table III

Results from recovery studies

Brand name	Drug	Recovery level (%)	Initial amount (ng)	Amount added (ng)	Recovery (%)	CV (%)
Repalol* H	Losartan potassium (50 mg)	80	2000	1600	99.33	0.421
		100	2000	2000	100.22	0.300
		120	2000	2400	99.62	0.322
	Atenolol (50 mg)	80	2000	1600	100.30	0.230
		100	2000	2000	100.08	0.078
		120	2000	2400	99.81	0.383
	Hydrochlorothiazide (12.5 mg)	80	500	400	99.35	0.443
		100	500	500	99.18	0.939
		120	500	600	100.33	0.200

Specificity

The mobile phase used resolved the drugs very efficiently (Fig. 1). The R_F values of losartan potassium, atenolol, and hydrochlorothiazide were 0.60, 0.43, and 0.29, respectively. Typical absorption spectra of LOK,

**Fig. 1**

Typical chromatogram obtained from losartan potassium (R_F 0.60), atenolol (R_F 0.43), and hydrochlorothiazide (R_F 0.29). Detection was at 274 nm and the mobile phase was toluene–methanol–triethylamine 6.5:4:0.5 (v/v)

ATL, and HCTZ are shown in Fig. 2. Peak purity for the drugs was tested by acquiring spectra at the peak start (S), peak apex (A), and peak end (E) positions. Results from correlation of the spectra were: for losartan potassium $r(S,M) = 0.9999$ and $r(M,E) = 0.9998$; for atenolol $r(S,M) = 0.9994$ and $r(M,E) = 0.9994$; and for hydrochlorothiazide $r(S,M) = 0.9999$ and $r(M,E) = 0.9994$. It can thus be concluded that no impurities or degradation products were eluting with the peaks obtained from the standard drug solution.

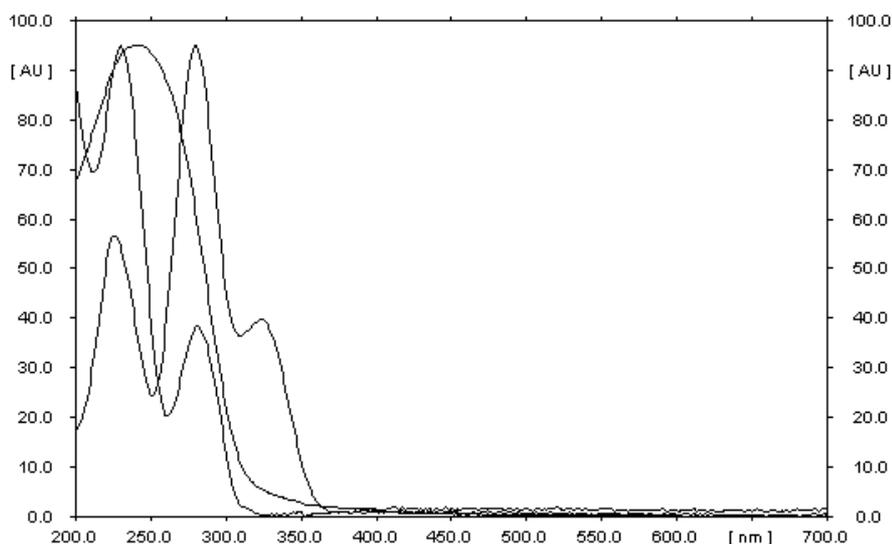


Fig. 2

Typical absorption spectra of losartan potassium, atenolol, and hydrochlorothiazide

Repeatability

The repeatability of sample application was assessed by application of 1 μ L standard drug solution seven times to an HPTLC plate, development of plate, and recording peak height and peak area for the zones. *CV* (%) of peak height and area were 0.58 and 0.59, respectively, for LOK, 0.21 and 0.22 for ATL, and 0.44 and 0.45 for HCZT. Repeatability of measurement of peak height and area were determined by application of 1 μ L standard drug solution to an HPTLC plate, developing the plate, and scanning the zone seven times without changing the position of the plate. *CV* (%) for measurement of peak height and area were 0.56 and 0.21, respectively, for LOK, 0.26 and 0.09 for ATL, and 0.40 and 0.13 for HCZT.

CONCLUSION

An HPTLC method for simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in pharmaceutical dosage forms has been established for the first time. Use of HPTLC enables analysis of several samples at the same time. The method is very simple and rapid, and provides accurate and precise results.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr S.J. Surana, Principal, R.C. Patel College of Pharmacy, Shirpur, M.S., India, for providing necessary facilities, and to Torrent Pharmaceuticals Ltd, Ahmedabad, and Macleods Pharmaceuticals Ltd, India, for the generous gifts of pure samples of losartan potassium, atenolol, and hydrochlorothiazide.

REFERENCES

- [1] US Pharmacopoeia, 28th edn, US Pharmacopoeial Convention, 2005, p. 193
- [2] Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Delhi, 1996, p. 72
- [3] British Pharmacopoeia, International edn, Vol. I, HMSO, Cambridge, 2004, p. 179
- [4] O.C. Lastra, I.G. Lemus, H.J. Sanchez, and R.F. Perez, *J. Pharm. Biomed. Anal.*, **33**, 175 (2003)
- [5] M.B. Shankar, F.A. Metha, K.K. Bhatt, R.S. Metha, and M. Geetha, *J. Pharm. Sci.*, **65**, 167 (2003)
- [6] A.H. Prabhakar and R. Giridhar, *J. Pharm. Biomed. Anal.*, **27**, 861 (2002)
- [7] N. Erk, *J. Pharm. Biomed. Anal.*, **24**, 603 (2001)
- [8] G. Carlucci, *J. Pharm. Biomed. Anal.*, **23**, 185 (2000)
- [9] D.L. Hertzog, J.F. McCafferty, X. Fang, R.J. Tyrrell, and R.A. Reed, *J. Pharm. Biomed. Anal.*, **30**, 747 (2002)
- [10] C.V.N. Prasad, C. Parihar, K. Sunil, and P. Parimoo, *J. Pharm. Biomed. Anal.*, **17**, 877 (1998)
- [11] S.M. Al-Ghannam, *J. Pharm. Biomed. Anal.*, **40**, 151 (2006)

- [12] A.P. Argekar and S.G. Powar, *J. Pharm. Biomed. Anal.*, **21**, 1137 (2000)
- [13] G. Lamprecht, T. Kraushofer, K. Stoschitzky, and W. Lindner, *J. Chromatogr. B*, **740**, 219 (2000)
- [14] H. Taomin, H. Zhang, Y. Bei, S. Luping, Z. Xiaowei, and D. Gengli, *J. Pharm. Biomed. Anal.*, **41**, 644 (2006)
- [15] O.A. Razak, *J. Pharm. Biomed. Anal.*, **34**, 433 (2004)
- [16] M. Lusina, T. Cindrić, J. Tomaić, M. Peko, L. Pozaić, and N. Musulin., *Int. J. Pharm.*, **291**, 127 (2005)
- [17] E. Sidiko, M.C. Sevil, and A. Sedef, *J. Pharm. Biomed. Anal.*, **33**, 505 (2003)
- [18] J.A. Murillo, A.A. Pulgarin, M. Alanon, and G. Prez-Olivares Nieto, *Anal. Chim. Acta*, **518**, 37 (2004)
- [19] T. Takubo, H. Okada, M. Ishii, K. Hara, and Y. Ishii, *J. Chromatogr. B*, **806**, 199 (2004)
- [20] N. Erk, *J. Chromatogr. B*, **784**, 195 (2003)
- [21] ICH Guidelines Q2B, Validation of Analytical Procedures – Methodology, 1996