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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF ATENOLOL AND LOSARTAN POTASSIUM IN BULK AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate and precise densitometric method for the simultaneous estimation of Atenololand Losartan Potassium in Bulk and Pharmaceutical Dosage form has been developed and validated. Separation of drugs was carried out using Methanol:Ethyl acetate:Toluene: Triethylamine (4:3.9: 2: 0.1 v/v/v/v) as mobile phase on precoated Silica Gel 60 F254 plates. The densitometric evaluation of spots was carried out at 226 nm. The RFvalue for Atenolol and Losartan Potassium were found to be 0.37 ± 0.02 and 0.72 ± 0.02 respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonization (ICH) guidelines.The drug response with respect to peak area was linear over the concentration range 400-1400 ng/spot (n=6) for Atenolol and Losartan Potassium both.The limit of detection and limit of quantitation were found to be 22.4551 ng/spot and 68.0456 ng/spot for Atenolol and 11.0233 ng/spot and 33.4038 ng/spot for Losartan Potassium. The percentage recovery of Atenolol and Losartan Potassium was found to be 100.101 and100.072 respectively. The %R.S.D. values for intra-day precision study and inter-day study were ≤ 2.0%, confirming that the method was sufficiently precise.The method can be successfully employed for the simultaneous determination of Atenolol and Losartan Potassium in pharmaceutical formulation.

Keywords: Losartan Potassium, Atenolol, HPTLC, Simultaneous determination, Validation.

INTRODUCTION

Atenolol (ATN) is chemically (*RS*)-4-(2-hydroxy-3-Isopropylaminopropoxy)phenylacetamide(Fig. 1) used as antianginal, antihypertensivedrug[1-5]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia(BP) and United state Pharmacopoeia(USP). It is estimated by potentiometric titration and chromatographic method as per IP,BP & USP[6-8]. Literature review reveals thatHPLC[10-14]and UV[14-22]spectrophotometric have been reported for estimation of ATN inpharmaceutical dosage forms. Losartan potassium (LSK) is chemically Monopotassium salt of 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4yl]methyl]-1*H*-imidazole-5-methanol(Fig.2) used asantihypertensive drug by inhibiting Angiotensin II receptor[1-5]. LSK and its tablet dosage form is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United state Pharmacopoeia (USP). It is estimated by potentiometric titration and chromatographic method as per IP, BP &USP [6-8].Literature review also reveals thatHPLC [23-25], HPTLC[26]and UV[27-38]spectrophotometric methods has been reported for the estimation of LSK in pharmaceutical dosage forms.Literature survey does reveals only UV Spectrophotometric[39]and HPLC [39]methods have been developed and reported, But does not any HPTLC method for simultaneous determination of ATN and LSK in Pharmaceutical dosage form. The present developed method is simple, precise and accurate for simultaneous determination of both drugs in their Pharmaceutical Dosage form as per International Conference on Harmonization (ICH) guidelines [9].

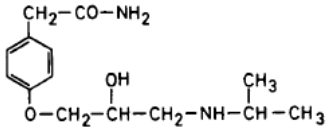

Fig. 1: Structure of Atenolol (ATN)

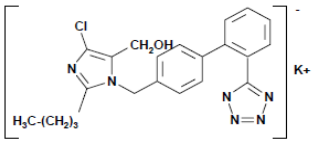

Fig. 2: Structure of Losartan potassium (LSK)

MATERIALS AND METHODS

Chemicals and reagents

Pure drug samples of Atenolol & Losartan potassium were provided as a gift sample by OlcareLaboratories,Surendranagar, Gujarat, India.Commercial pharmaceutical tablets **LOSAR*-BETA** (Unichem Laboratories, India) was procured from local pharmacy. Methanol, Ethyl acetate and Glacial acetic acid of AR Grade and all other chemicals were obtained from Allied Chemical Corporation, Vadodara, Gujarat, India.

Instrumentation and chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E.Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were pre-washed with methanol and activated at 80^0C for 5 min prior to sample application. The standard and formulation samples of ATN and LSK in mixture were spotted on Pre-coated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nl/s. The mobile phase consists ofMethanol: Ethyl acetate: Toluene: Triethylamine (4: 3.9: 2: 0.1 v/v/v/v). Linear ascending development was carried out in twin trough chamber (10 x 10 cm). The optimized chamber saturation time for mobile phase was 15 min, at ambient temperature; the length of chromatogram run was 7 cm. Densitometric scanning was performed on CAMAG TLC scanner 3 in Absorbance/Reflectance mode, operated by winCATS 1.3.4planar chromatography software. The spots were analyzed at a wavelength of 226 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. The parameters were selected as recommended by the CAMAG TLC scanner 3 manual. Evaluation was performed using linear regression analysis of peak areas.

Preparation of standard stock solutions and calibration curves

Accurately weighed Atenolol(10 mg) was transferred to 10 mL volumetric flask, dissolved in and diluted with methanol up to the mark (1000 µg/ml). For preparation of LSK stock solution,accurately weighed Losartan potassium(10 mg) was transferred to 10 mL volumetric flask,

dissolved in and diluted with methanol up to the mark (1000 µg/ml). For preparation of working standard solution, 2 ml of stock solution of ATN (1000µg/ml) and LSK (1000µg/ml) were transferred to 10 ml volumetric flask and diluted with methanol upto the mark to obtain final concentration containing 200µg/ml of ATN and LSK. Calibration was done by applying mixture of standard solutions ranging from 2.0 – 7.0 µl by Hamilton syringe with the help of Linomat V autosprayer on TLC plate that gave concentration 400-1400 ng/spot for ATN and LSK Both. Each concentration was spotted six times on TLC plates. From the developed plates calibration curve was plotted as peak areas versus corresponding concentrations (Fig. 5 and 6).

Analysis of ATN and LSK in marketed Tablet Formulation

To determine the content of ATN and LSK simultaneously in conventional tablets (LOSAR*-BETA, label claim 50 mg ATN and LSK Both); twenty tablets were accurately weighed, average weight determined and grounded to fine powder. A quantity of powder equivalent to 50 mg (ATN) and 50 mg (LSK) was transferred into 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to mark with same solvent to obtain 0.5 mg/ml of ATN and LSK. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). From the above solution 4 mL was transferred into 10 ml volumetric flask and diluted to mark with same solvent. So, Resultant solution was found to contain 200µg/ml (200 ng/µl) Atenolol and Losartan Potassium. 5µl of this solution applied on TLC plate followed by development and scanning at 226 nm. The analysis was repeated for three times.

Method Validation

Linearity

For the linearity study the 2-7 µl from the working standard solution containing 200 ng/spot of ATN and LSK was injected. So, linearity responses for ATN and LSKboth were assessed in the concentration range 400-1400 ng/spot, of working standard solutions.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (ATN and LSK) was spiked at three different levels (80%, 100% and 120%). These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of ATN and LSK by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

LOD= 3.3 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOQ = 10 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOD and LOQ were determined from the standard deviations of theresponses for six replicate determinations.

Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Fig. 7. The spot for ATNand LSK was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 226 nm for detecting peak purity of ATN and LSK was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Repeatability

Repeatability of sample application was assessed by spotting 3µl (600 ng/spot of ATN and LSK) of drug solution six times on a TLC, followed by development of plate and recording the peak area for six spots.

RESULTS AND DISCUSSION

Method development

The TLC procedure was optimized for simultaneous determination of ATN and LSK. The mobile phase Methanol: Ethyl acetate: Toluene: Triethylamine (4: 3.9: 2: 0.1 v/v/v/v) resulted in good resolution with sharp and symmetrical peaks of Rf 0.37 ± 0.02 for ATN and 0.72 ± 0.02for LSK. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 15 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both the drugs(Fig. 3).

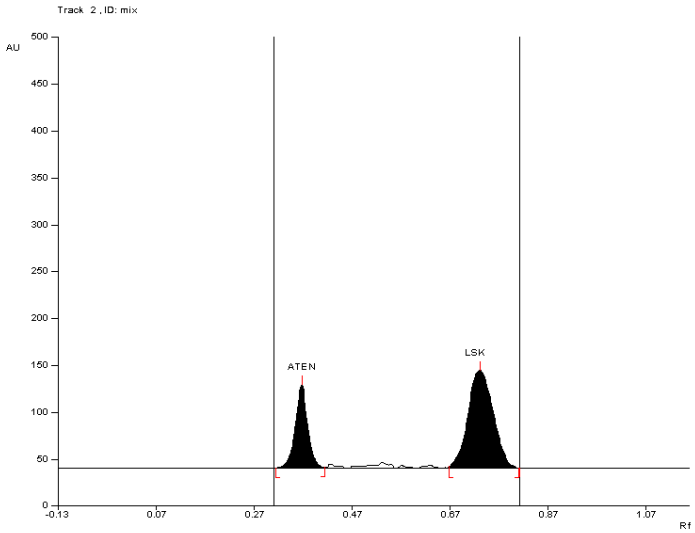

Fig. 3: HPTLC Chromatogram of Standard ATN and LSK in mixture

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 400-1400 ng/spot for ATN and LSK. The linear equations for the calibration plots were y = 1.5413x + 889.08 and y = 5.7403x + 353.64 with Regression (r^2) being 0.9978 and 0.9969 for ATN and LSK, respectively. (Fig. 4, 5, 6) (Table 1, 2 and 3).

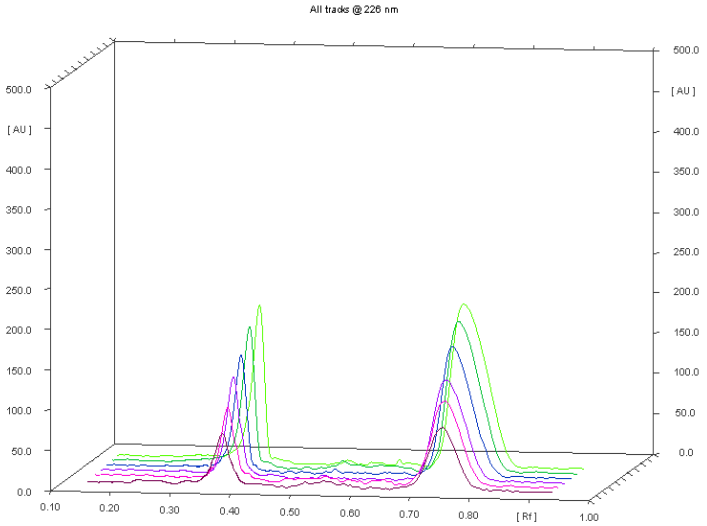

Fig. 4: 3D Representation of Densitogram for Calibration curve of ATN and LSK

Table 1: Result of Calibration readings for ATN

Concentration (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
400	0.37	1510.883 ± 6.966	0.4610
600	0.37	1813.283 ± 6.548	0.3611
800	0.37	2128.917 ± 17.773	0.8348
1000	0.37	2364.617 ± 11.189	0.4732
1200	0.37	2757.883± 13.440	0.4873
1400	0.38	3060.317± 7.1714	0.2343

Table 2: Result of Calibration readings for LSK

Concentration (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
400	0.72	2709.750± 7.365	0.2718
600	0.72	3862.600 ± 11.241	0.2910
800	0.72	4721.500 ± 16.171	0.3425
1000	0.72	6034.520± 59.758	0.9903
1200	0.73	7312.333± 8.810	0.1205
1400	0.74	8394.500± 11.428	0.1361

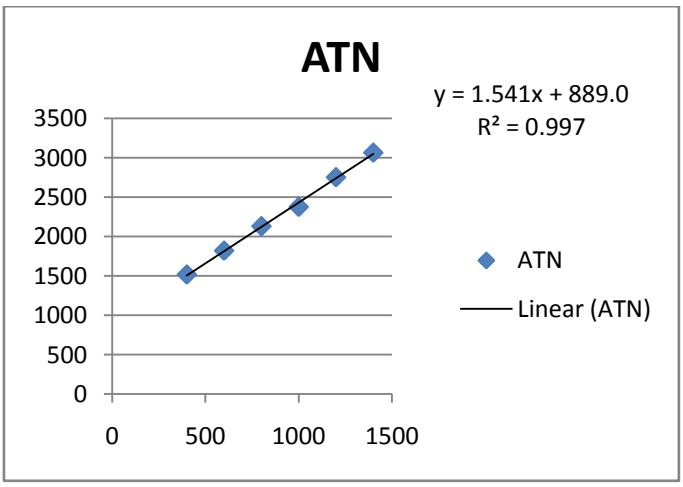

Fig. 5: Calibration curve of ATN in Methanol at 226 nm

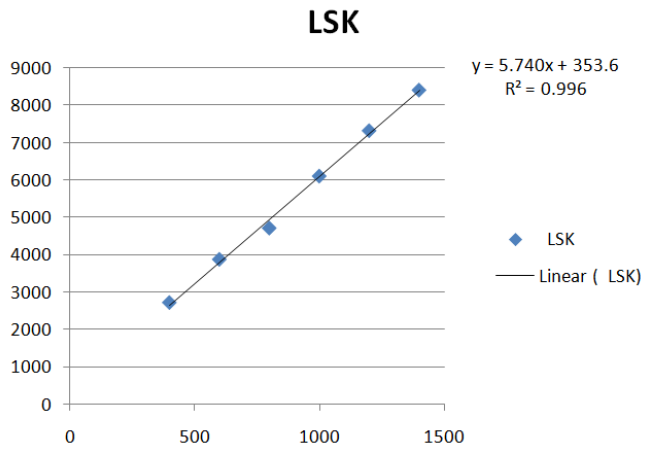

Fig. 6: Calibration curve of LSK in Methanol at 226 nm

Table 3: Statistical Data of ATN and LSK

Parameters	Results	
	ATN	LSK
Linear Range(ng/spot)	400-1400	400-1400
Slope	1.5452	5.7266
Intercept	882.003	351.957
Std. Deviation of Slope	0.0059	0.0098
Std. Deviation of Intercept	9.8713	10.0292
Limit of Detection(ng/spot)	22.4551	11.0233
Limit of Quantitication(ng/spot)	68.0456	33.4038
Regression Equation	y = 1.5413x+ 889.08	y = 5.7403x+353.64
Co-Relation Co-Efficient (r)	0.9989	0.9984
Co-Efficient of Determination (r^2)	0.9978	0.9969

Precision

The precision of the method was expressed as relative standard deviation (RSD %). The %RSD values for intra-day precision study and inter-day study listed in (Table 4 and 5) were≤2.0%, confirming that the method was sufficiently precise.

Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs from the pharmaceutical dosage form, and spiked with 80, 100, and 120% of additional pure drug, the recovery was found to be99.86- 100.25% for ATN and 99.99- 100.25% for LSK(Table 6 and 7).

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 22.4551 and 68.0456 ng/spot for ATN and 11.0233 and 33.4038 ng/spot for LSK.

Specificity

The peak purity of ATN and LSK wereassessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9994 and r (M, E) =0.9990 for ATN and r (S, M) = 0.9986 and r (M, E) = 0.9996 for LSK. Good match was obtained between standard and sample spectra of ATN and LSKrespectively (Fig. 7).

Table 4: Intra-Day and Inter-Day study of ATN

Concentration (ng/spot)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
600	1812.40 ± 6.60	0.3639	1817.03 ± 5.61	0.3089
800	2122.10 ± 6.66	0.3136	2116.93 ± 6.33	0.2990
1000	2366.07 ± 5.55	0.2347	2358.00 ± 4.83	0.2048

Table 5: Intra-Day and Inter-Day study of LSK

Concentration (ng/spot)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
600	3866.47 ± 5.7143	0.1478	3866.07 ± 5.7657	0.1491
800	4714.43 ± 5.7588	0.1222	4724.23 ± 5.9911	0.1268
1000	6030.87 ± 63.6344	1.0551	6073.43 ± 64.4116	1.0605

Table 6: Determination of Accuracy for ATN

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
600	480	1080	1079.37	99.8681	0.0788
	600	1200	1201.50	100.2500	0.3189
	720	1320	1321.33	100.1852	0.1178
Average				100.1011	

Table 7: Determination of Accuracy for LSK

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
600	480	1080	1079.97	99.9930	0.0466
	600	1200	1199.83	99.9722	0.3610
	720	1320	1321.80	100.2500	0.1328
Average				100.0717	

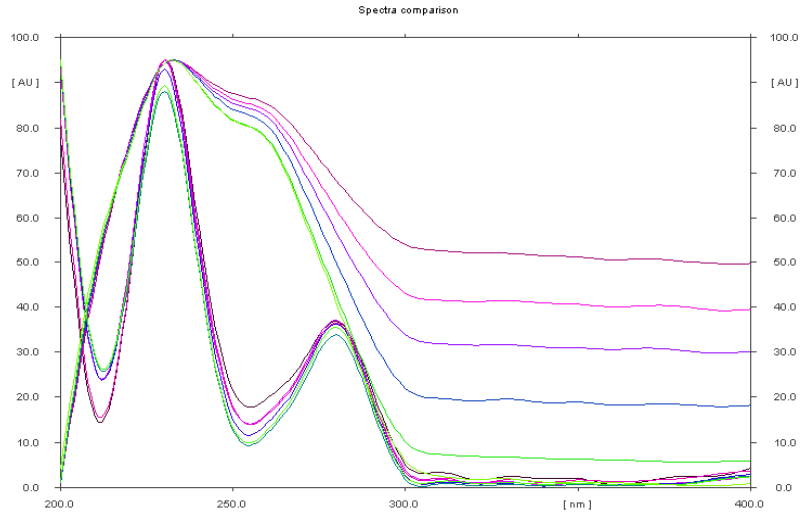

Fig. 7: UV Absorption (Reflectance Mode) of the corresponding spots for ATN and LSK

Table 8: Repeatability study of ATN and LSK

Concentration	ATN (600 ng/spot)	LSK (600 ng/spot)
Area	1818.9	3871.3
	1802.3	3850.0
	1820.3	3858.2
	1812.5	3880.5
	1810.4	3860.2
	1815.3	3855.4
Mean	1813.3	3862.6
± SD	6.5484	11.2412
%RSD	0.3611	0.2910

Table 9: Assay Result of Marketed Formulation

Parameters	LOSAR*-BETA	
	ATN	LSK
Actual Concentration (ng/spot)	1000	1000
Concentration Obtained (ng/spot)	993.518	1000.938
%Purity	99.35	100.09
%RSD	0.0003	0.0099
Limit[6]	92.5% - 107.5%	90% -110%

Table 10: Validation Parameters

Summary of Validation Parameters		
	ATN	LSK
Recovery (%)	100.10	100.07
Repeatability (%RSD)	0.3611	0.2910
Precision (CV)		
Intra-day (n=3)	0.0030	0.0044
Inter-day (n=3)	0.0027	0.0044
Specificity	Specific	Specific
Selectivity	Selective	Selective

Repeatability

The % RSD for peak area values of ATN and LSK were found to be 0.3611and 0.2910 respectively, as given in Table 8.

Analysis of ATN and LSK in marketed formulation

When the LOSAR*-BETA tablets were analyzed, ATN and LSK gave sharp and well defined peaks at R_f 0.37±0.02 and 0.72 ±0.02, respectively, when scanned at 226 nm. The results in Table 9 indicate that there was no interference from the excipients commonly present in the tablet formulation. The % purity was 99.35% for ATN and 100.09 % for LSK.

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of ATN and LSK in pharmaceutical dosage forms. The method was validated as per International Conference on Harmonization (ICH) guidelines.

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