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Development and validation of analytical methods for the simultaneous estimation of Losartan potassium and Metolazone in bulk and in pharmaceutical dosage form by RP-HPLC and HPTLC

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Abstract: Two new, simple, accurate and precise Reverse Phase High Performance Liquid Chromatograhic (RP-HPLC) method and High Performance Thin Layer Chromatographic (HPTLC) method has been developed for simultaneous analysis of losartan potassium and metolazone in tablet formulations. The mobile phase consisting of acetonitrile: 0.1% trifluoroacetic acid, (40:60% v/v) with flow rate of 0.8 mL/min was used for RP-HPLC with a UV detector at 236 nm. For the HPTLC method, chloroform: methanol, 10:1% v/v, as mobile phase, UV detection was performed densitometrically at 230 nm. The retention time for losartan potassium and metalazone were found to be 4.72 and 6.40 min, respectively. The R_f values were found to be 0.18 and 0.42 for losartan potassium and metalazone, respectively. The methods were validated in accordance with the requirements of ICH and FDA guidelines. These two methods can be successfully applied for the routine quality control analysis of losartan potassium and metalazone in bulk and tablet formulation.

Keywords: Losartan potassium; metalazone; RP-HPLC, HPTLC, ICH guidelines, validation

1. Introduction

Losartan Potassium (LOS) is monopotassium salt of 4-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-y] methyl]-1H-imidazole-5-methanol (**Figure 1.**) and Metolazone (MET) is 7-chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-tolyl-6-quinazoline sulphonamide (**Figure 1.**). Both drugs are antihypertensive drugs. LOS interferes with binding of angiotensin-II to AT_1-receptor by binding reversibly to the receptors. Angiotensin is a vasoconstrictor and blockage of its activity decreases systemic vascular resistance and hence reduces the pressure. MET interferes with the renal tubular mechanism and electrolyte reabsorption. It primarily inhibits sodium reabsorption. The antihypertensive activity of MET is due to its saluretic and diuretic properties.

Various methods have been reported for the estimation of LOS individually and in combination with other drugs in pharmaceutical formulations by UV Spectroscopic methods,[1-5] by HPLC method.[6-9] Similarly, various methods have been reported for the estimation of MET individully by UV Spectroscopy method[10] and HPLC method[11] and in biological fluids.[12-14] From the literature survey it was found that no method has been reported for the simultaneous estimation of LOS and MET in cobmined dosage form. The aim of this study was to develop and validate simple, rapid, selective and economical RP-HPLC and HPTLC methods for the simultaneous estimation of LOS and MET in bulk and in combined dosage form. The developed methods were validated as per the ICH Guidelines.[15, 16]

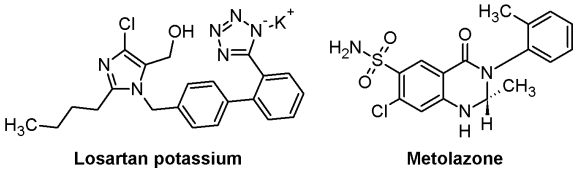

Figure 1. The Structures of Losartan potassium (LOS) and Metolazone (MET)

2. Results and discussion

2. 1. Method development

The mobile phase consisting of Acetonitrile and 0.1% trifluoroacetic acid in the ratio of 40:60% v/v was used with flow rate 0.8 mL/min. The detection is made at 236 nm. 20 µL of the sample solution was loaded into the column and the chromatogram were recorded.

The mobile phase consisting of chloroform: methanol in the ratio of 10:1% v/v selected for the analysis. The detection was made with CAMAG TLC scanner 3, at 230 nm. The ascending development mode was made in a twin trough chamber. The system suitability

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Table 1. System Suitability Parameters

Parameters	RP-HPLC		HPTLC		Limit
	LOS	MET	LOS	MET	
Retention time/ R_f	4.72	6.40	0.18	0.42	----
Tailing factor	1.37	1.44	1.12	1.85	≤ 2
Asymmetrical factor	1.75	1.78	1.08	1.71	≤ 2
Number of Theoretical plates	4149	4461	3654	4258	>2000
Capacity factor	2.13	3.10	---	---	1-10
Resolution	2.67		4.78		≥ 2

2. 2. Quantification of formulation

Analysis of the formulation was carried out and the results are expressed in the **Table 2**. The chromatogram for the analysis of formulation by RP-HPLC and HPTLC are given in **Figure 2** and **3**, respectively. The percentage label claim of LOS and MET were found to be 100.81±1.4742 and 99.14±0.7494, respectively for HPLC method. For HPTLC method, the percentage label claim was found to be 100.66±1.4046 for LOS and 98.97±0.6129 for MET parameters are shown in **Table 1**.

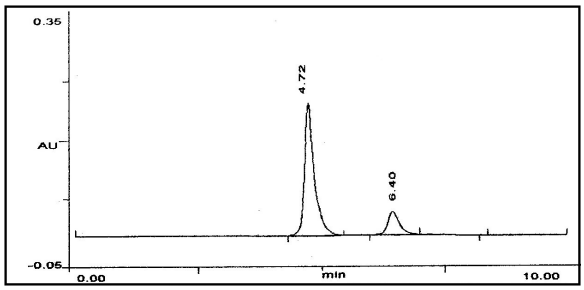

Figure 2. RP-HPLC chromatogram for formulation

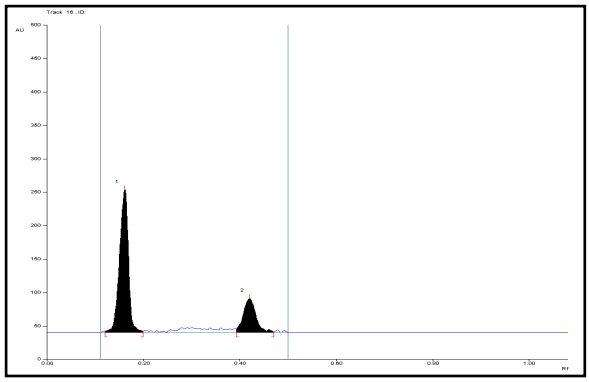

Figure 3. HPTLC Chromatogram for formulation

Table 2. Results of Analysis of Formulation

Drug	Label Claim (mg)	% Label Claim		SD (%RSD)	
		RP-HPLC	HPTLC	RP-HPLC	HPTLC
LOS	25	100.81	100.66	1.4742 (1.4623)	1.4046 (1.3954)
MET	2.5	99.14	98.97	0.7494 (0.7559)	0.6129 (0.6193)

2. 3. Validation of the Method

Linearity

RP-HPLC

LOS was found to be linear in the concentration range of 5-35 µg/mL and MET was in the range of 1-7 µg/mL. The regression equation for LOS and MET were y=768938.65x+62999.92 and y=2221068.94x–153297.11, respectively. The correlation coefficients, r, were 0.9998 and 0.9997, respectively, over the concentration ranges. The calibration graph for LOS and MET are shown in **Figures 4** and **5**, respectively.

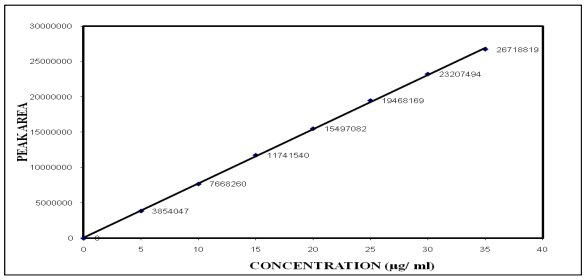

Figure 4. Calibration Curve of LOS by RP-HPLC

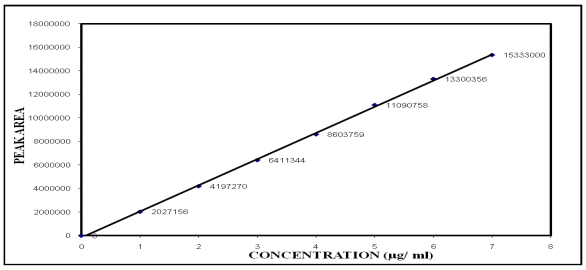

Figure 5. Calibration Curve of MET by RP-HPLC

HPTLC

LOS and MET were found to be linear in the range of 50-500 ng/spot and 10-100 ng/spot, respectively. The regression equation for LOS and MET were y= 4.699x+384.223 and y=22.195x+342.411, respectively. The correlation coefficients r, were 0.9992 for both LOS and MET. The calibration graph for LOS and MET are shown in **Figures 6** and **7**, respectively.

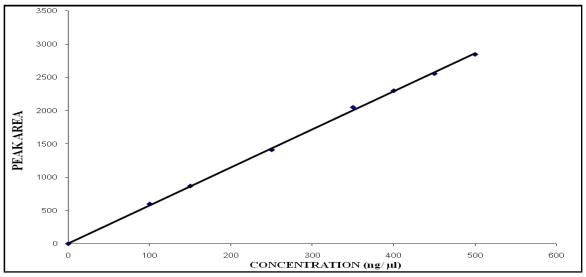

Figure 6. Calibration Curve of LOS by HPTLC

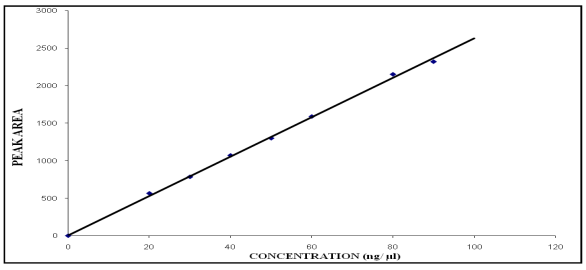

Figure 7. Calibration Curve of MET by HPTLC

Precision

The analysis of the formulation containing both LOS and MET were repeated for six times. For RP-HPLC method the % RSD value for LOS and MET were found to be 1.4623 and 0.7559, respectively. For HPTLC method it

was found to be1.3954 and 0.6193 for LOS and MET, respectively. The %RSD values were found to be less than 2. Hence the developed methods were found to be highly precise.

Accuracy

The accuracy of the method was confirmed by recovery studies. The %RSD values for recovery analysis were found to be less than 2. This indicates that there are no interferences due to the excipients used in formulation. Hence the accuracy of the method was confirmed. The results of analysis are shown in **Table 3**.

Specificity

The chromatogram of the solution of the non-spiked capsule matrix did not show any spots. On the other hand, the chromatogram of the tablet matrix spiked with drugs showed clear, compact and well-separated peaks of LOS and MET. Moreover, no other peaks eluted besides the active compounds. Therefore, the method was considered specific.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done with different analysts. The %RSD for analyst 1 and analyst 2 were found to be 0.6224 and 0.4666, 1.7359 and 1.8957, for LOS and MET, respectively.

Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) were calculated from the calibration graphs. The method based on the determination of slope of linearity plot and standard deviation of y intercept was used for the determination of LOD and LOQ and were found to be 0.3545 µg/mL and 1.0743 µg/mL for LOS and 0.01500 µg/mL and 0.0454 µg/mL for MET, for RP-HPLC method. For the HPTLC method, the LOD and LOQ were found to be 5.0135 ng/spot and 15.1924 ng/spot for LOS and 0.5218 ng/spot and 1.5811 ng/spot for MET.

3. Experimental

Materials and methods: The drug standards and the formulation METOZ L-25 were obtained as gift samples from Centaur Pharmaceuticals Pvt. Ltd., Mumbai, India. The formulation METOZ L-25 contains LOS 25 mg and MET 2.5 mg. Acetonitrile (HPLC grade) and HPLC water, analytical grade chloroform and methanol were used throughout the analysis.A Shimadzu HPLC system with LC - 10 ATvp Pump, SPD - 10 ATvp detector, rheodyne injector and 20 µL loop was used to perform the RP – HPLC analysis. The column used was C_{18} ODS column. Winchrome computer based data station was used. HPTLC was performed in CAMAG HPTLC instrument with a CAMAG Automatic sampler and CAMAG TLC Scanner 3 with WINSCAT software. Pre-coated silica gel 60 F 254 plates were used for the analysis. Twin trough glass chamber was used as the development tank. Remi Centrifuge apparatus was used for centrifugation of the samples. Sonica Ultrasonic Cleaner – Model 2200 MH was used for the sonication of the sample solutions.

3. 1. Method development

RP-HPLC

HPLC was done on a Phenomenax® Luna C_{18} column (150 × 4.6 mm ID, 5µ particle size) with a UV detector system, with an isocratic mode of operation. An ambient temperature was maintained throughout the run. The mobile phase was sonicated for 15 min to remove the dissolved gases present in the system. The mobile phase was allowed to run through the column to saturate the column before the analysis. Various mobile phases in different ratios and different compositions were tried to select a suitable mobile phase for the selected drug components.

HPTLC System

HPTLC was performed on HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use, the plates were washed with methanol and stored in a desiccator. Samples were applied to the plates as 8 mm bands by means of a Camag automatic TLC sampler 4 equipped with a 25 µL micro syringe. Ascending development was employed for the method development. Camag twin-trough TLC chamber was previously saturated with the mobile phase for 30 min. After development the plates were dried for 5 min in an oven at 50 °C. Densitometric scanning was then performed with a Camag TLC scanner 3 equipped with Wincats Software Version 1.3.0, using the deuterium light source.

3. 2. Standard solution and calibration graphs

RP-HPLC

Standard stock solutions of 500 µg/ mL LOS and MET were prepared in methanol. The stock solutions were further diluted with mobile phase to get a concentration of 5-35 µg/mL of LOS and 1-7 µg/mL of MET. The calibration graph was plotted by using peak area versus concentration.

HPTLC

Standard stock solutions of 1 mg/mL LOS and MET were prepared in methanol. The stock solutions were further diluted with mobile phase to get a concentration of 50-500 ng/spot of LOS and 10-100 ng/spot of MET. The calibration graph was plotted by using peak area versus concentration.

Table 3. Recovery analysis

Levels of % recovery	Amount present (µg/mL)		Amount added (µg/mL)		Amount recovered (µg/mL)		%Mean Recovery		%RSD	
	LOS	MET	LOS	MET	LOS	MET	LOS	MET	LOS	MET
RP-HPLC method										
80	15.1275	1.4853	12	1.2	11.9899	1.1821	99.92	98.51		
100	15.1275	1.4853	15	1.5	14.9920	1.4843	99.95	98.95	0.0458	0.2466
120	15.1275	1.4853	18	1.8	18.0013	1.7739	100.01	98.55		
HPTLC method										
80	201.0875	19.7705	160	16	160.5590	16.2507	100.35	101.57		
100	201.0875	19.7705	200	20	198.8650	20.5174	99.43	102.59	1.0073	0.5347
120	201.0875	19.7705	240	24	236.0432	24.5814	98.35	102.42		

3. 3. Quantification of formulations

RP-HPLC

Twenty tablets were weighed accurately and the average value of each tablet is determined. The weighed tablets were crushed into a fine powder. The tablet powder equivalent to 25 mg of LOS was weighed and transferred into a 50 mL of standard flask, dissolved in methanol and sonicated for 10 min. The solution was made up to the mark with methanol and centrifuged for 15 min at 2000 rpm. The supernatant liquid is filtered through a Whatmann filter paper No. 41. The filtered solution was further diluted with mobile phase to get a concentration of 20 µg/mL of LOS. This solution also contains 2 µg/mL of MET theoretically. Sample solutions were injected and the chromatograms were recorded.

HPTLC

The tablet powder equivalent to 10 mg of LOS was weighed accurately in to a 10 mL volumetric flask, dissolved in methanol and sonicated for 10 min. The solution was made up to the mark with methanol and centrifuged for 15 min at 2000 rpm. The supernatant liquid is filtered through a Whatmann filter paper No. 41. This was further diluted with methanol to get a concentration of 50 ng/µL of LOS and 5 ng/µL of MET, theoretically. Six spots of 4 µL each were placed on the plates and the chromatogram was developed in the Twin Trough Chamber.

3. 4. Validation of the Method

The method was validated in accordance with ICH guidelines.

Precision

The precision of both the method were assessed by replicate analysis (n=6) of formulation containing both LOS and MET.

Accuracy

The accuracy of the method was determined by recovery studies. This was performed by the method of standard additions at three different levels, i.e. by multiple level recovery studies. To the pre analysed formulations, 80%, 100% and 120% of the standard solutions were added. The amount of drug recovered was calculated.

4. Conclusion

Two simple, precise, rapid and accurate HPLC and HPTLC methods were developed for the simultaneous determination of LOS and MET in bulk and in pharmaceutical dosage form. When comparing both the methods, HPTLC method is highly sensitive but the instrument is costly and the cost of analysis is low. HPLC instrument is at low cost when compare to HPTLC but the cost analysis is more. Among its advantages are short run time and large sample capacity both of which significantly reduce the duration of the analysis. Hence the developed methods can be applied for the routine quality control analysis of LOS and MET in bulk and in pharmaceutical dosage form.

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