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Analytical Method Development and Validationfor the Simultaneous Quantitization of Metolazone and Losartan Potassium in Bulk Drug and in Pharmaceutical Dosage Form by RP-HPLC

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Abstract

A reverse phase liquid chromatography (RP-HPLC) method have been developed and subsequently validated for the determination of Metolazone and losartan potassium in bulk and Pharmaceutical dosage form. Metolazone belongs to diuretics and Losartan postassium belongs to anti-hypertensive drugs. In HPLC the separation was achieved with an Agilent TC-C18 4.6 x250 mm 5µm Column and the mobile consists of pH 2.85 phosphate buffer (0.02M) and methanol (35:65) v/v at a flow rate of 1.0 mL/min. The eluents were detected at 230 nm with a run time of 10 min. The described method of metolazone and losartan potassium is linear over a range of 1µg/mL to 5µg/mL and 10µg/mL to 50µg/mL respectively with correlation coefficient of 0.995and0.996. The RSD value of less than 2 % shows that the methods were precised. The method enables accurate, precise, and rapid analysis of metolazone and losartan potassium in bulk and Pharmaceutical dosage form. These developed methods were simple, rapid, and selective and can be applied for the routine analysis of Metolazone and Losartan potassium in bulk and Pharmaceutical dosage form.

Key words: RP-HPLC, Metolazone, Losartan potassium,RP-HPLC.

INTRODUCTION:

According to US-FDA SIAM, defined as validated quantitative analytical methods can detected the changes with time in the chemical,physical or microbiological properties of the drug substance and drug producs,and that are specific so that the contents of active ingredients can be accurately measured with out interference.

Developing and validating new analytical method is costly and time consuming. Before starting the arduous process, a through literature search should be conducted for existing methodologies of the intended analytes or similar compounds. This should include a computerized search of chemical abstract and other relevant sources such as compendia monograph (USP,EP), journal articles, manufacturer literature and internet.[1-4]

Metolazone [fig.1] is chemically 7-chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-o tolyl-6-quinazoline sulphonamide. Its molecular formula is $C_{16}H_{16}ClN_3O_3S$ having molecular weight 365.832g/mole. It is a quinazoline diuretic. It is marketed as tablets 2.5mg,5.0mg. The action of metolazone result from interference with the renal tubular mechanism of electrolyte reabsorption. Metolazone acts primarily to inhibit sodium reabsorption at the cortical diluting site and to a lesser extent in the proximal convoluted tubule. Sodium and chloride ions are excreted in approximately equivalent amounts. The increased delivery of sodium to the distal tubular exchange site results in increased potassium excretion. Metolazone does not inhibit c belongs to arbonic anhydrase. The antihypertensive mechanism of action metolazone is not fully understood but is presumed to be related to its saluretic and diuretic properties.[5-6]

Losartan potassium[fig.2] is chemically [2-butyl-4chloro-1-({4-[2-92H-1,2,3,4-tetrazol 5-5-yl)phenyl}methyl)-1H-imidazol-5-yl]methanol. Its molecular formula was $C_{22}H_{23}ClN_6O$ having molecular weight 422.911g/mole belongs to anti-hypertensive category. It is available in tablet dosage form. It is an angiotensin-receptor blocker (ARB) that may be used alone or with other agrnts to treat hypertension. Losartan may be used to treat hypertension , isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also used for the treatment of systolic dysfunction, myocardial infraction, coronary artery disease and heart failure.[7-9]

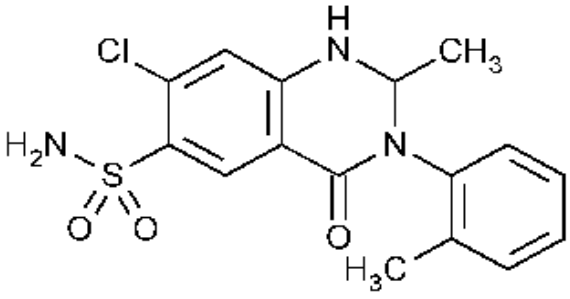

FIG:1Metolazone

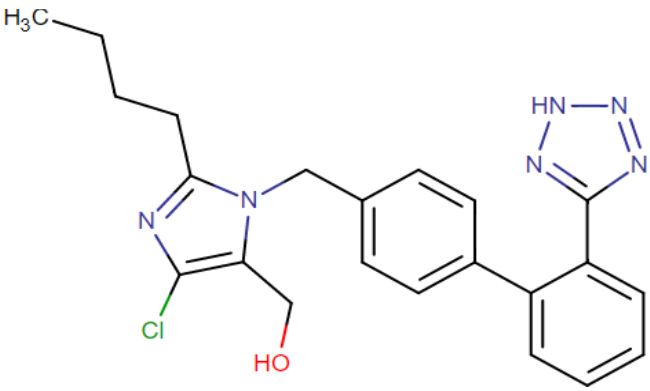

FIG:2 Losartan Potassium

MATERIALS AND METHODS

Reagents and chemicals:

Pharmacopeial grade standard of Metolazone was provided by century pharmaceutical pvt ltd. Losartan potassium was provide by Hetero drugs ltd. Analytical HPLC grade solvents Methanol, Acetonitrile, O-Phosphoric acid, potassium dihydrogen phosphate, Water were obtained from Spectrochem Pvt. Ltd.,Mumbai, India).

Instumentation:

The chromatographic system used to perform development and validation of this method was comprised of a an Agilent TC-C18 4.6 x250 mm 5µm Column,photodiode-array detector with manual injection connected to a multi instrument data acquisition and data processing system.

Chromatographic conditions:

Chromatographic analysis was performed on Agilent C18(250*4.6mm,5µm) column. The mobile phase was consisted of potassium dihydrogen ortho phosphate buffer and methanol in the ratio of 35:65 % v/v and pH of mobile phase was adjusted to 2.85 using dilute orthophosphoric acid. Mobile phase was filtered through a 0.45 µm nylon membrane filter (Millipore Pvt. Ltd Bangalore,India) and degassed in an ultrasonic bath (Spincotech Pvt.Ltd.,Mumbai). The flow rate of mobile phase was adjusted to 1.0ml and the injection volume was 20 µl. Detection was performed at 230nm.

Stock solutions:

Metolazone stock solutions:

Accurately weighed and transferred 10mg of Metolazone into 10ml volumetric flask, added about 5 mL of HPLC grade methanol shake well, diluted to the mark with HPLC grade methanol and mixed well. From this working standards will be prepared.

Losartan potassium stock solutions:

Accurately weighed and transferred 10mg of Losartan potassium into 10ml volumetric flask, added about 5 mL of HPLC grade methanol shake well, diluted to the mark with HPLC grade methanol and mixed well. From this working standards will be prepared.

Preparation of working standard solution of 1µg Metolazone and 10µg Losartan potassium:

Working standards solution of Metolazone was prepared by taking 1 ml from metolazone stock solution into a 10 ml volumetric flask and made up to 10ml with mobile phase.Again from this working standard solution 0.1ml of metolazone solution was taken into another 10ml volumetric flask and 0.1ml of losartan potassium solution was taken from the above losartan potassium stock solution into the previously added 0.1ml metolazone solution volumetric flask and make upto the mark with the mobile phase.

Solution

pH 2.85 phosphate buffer:

About 136 mg of potassium dihydrogen phosphate was transferred to a 100mL reagent bottle and made upto the mark with Milli Q water of HPLC grade water. Mixed well and sonicated in an ultrasonicator for 5 minutes. Stored the solution at room temperature (20±5 °C).Adjusted the p^H with dilute ortho phosphoric acid.

RESULTS AND DISCUSSION:

Method development:

The method has been developed by preliminary trails using different composition of mobile phases consisting various mixtures of methanol and water, methanol and phosphate buffer in different ratios which does not shows proper elution. Finally a mixture of mobile phase consisting of Methanol and Phosphate buffer (65:35v/v) at a flow rate of 1ml/min, shown better elution of peak with satisfying system suitability studies. The column used is Agilent C18 (250×4.6mm, 5µm). A detection wavelength of 230 nm was selected after scanning the standard solution over the range 190-400 nm by using photo-diode array (PDA) detector. Detection at 230 nm resulted in good response and good linearity, with a retention time of Metolazone 3.91min and Losartan potassium 6.14min. The method was carried out by standard addition method. After developing the analytical method, it was validated. The analytical method validation gave evidence that the procedure was suitable for the intended purpose; it was carried out as per guidelines of ICH. Typical chromatogram of Metolazone and Losartan potassium was shown in Figure 3.

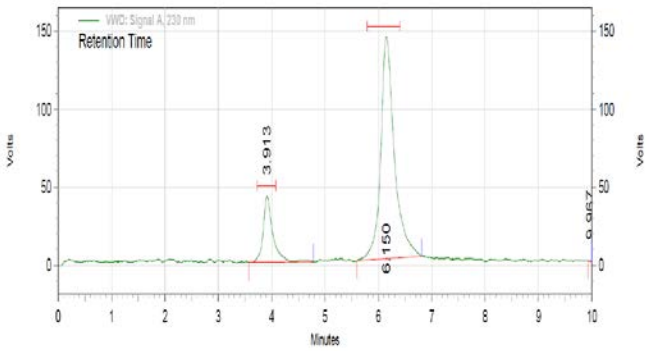

FIG:3 Chromatogram of satandard preparation of Losatan potassium & Metolazone

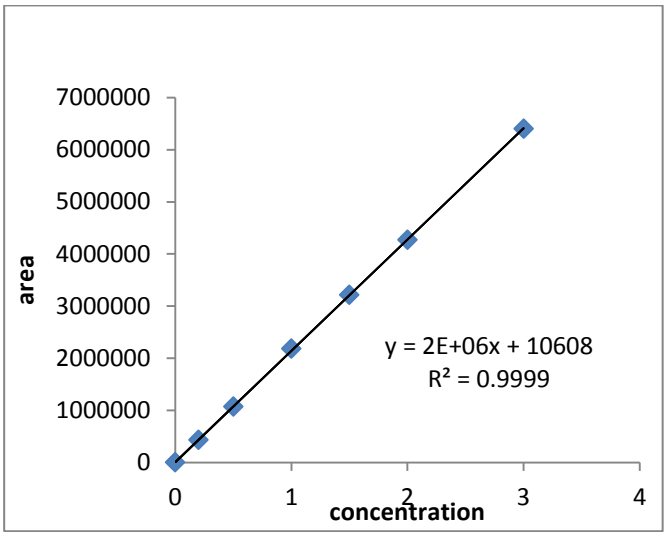

FIG:4 Calibration graph of Metolazone

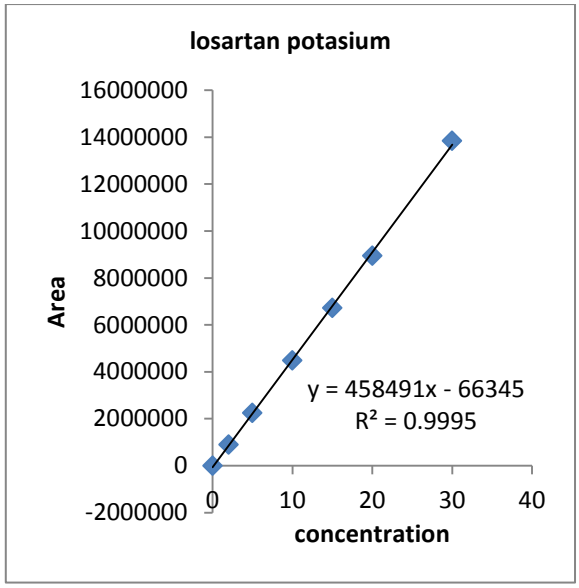

FIG:5 Calibration curve of Losartan Potassium

Validation of the method:

The develoed method has been validated as per ICH guidelines (ICH Q2B) for precision, accuracy, linearity, LOD & LOQ, ruggedness and robustness.

LINEARITY

The linearity of the method was established by spiking a series of dilutions of Metolazone and Losartan potassium. Solutions of six different concentrations 0.2-3.0 µg/ml and 2.0-30.0µg/ml of Metolazone and Losartan potassium were injected into the HPLC system. The calibration curve was constructed for the standard solutions by plotting their concentrations against their respective peak areas. Regression equation was obtained and the values of slope-a, intercept-b, and correlation coefficient (R^2) were determined as shown in Fig 4&5 and the results are tabulated in Table 1&2.

ACCURACY:

The accuracy study was performed on 50 %, 100 % and 150 % of the analytical method target concentration of Metolazone and Losartan potassium. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level were obtained. The percentage recoveries of Metolazone and Losartan potassium were calculated using standard at the same concentration at each concentration level as presented in Table 3&4.

PRECISION AND INTERMEDIATE PRECISION:

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and calculated the % Relative standard Deviation (RSD). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition. The %RSD and assay results are shown in table 5&6.

ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like mobile phase composition and wavelength which may differ but the responses were still within the specified limits of the assay. The standard solution was injected into the chromatograph at varied conditions of mobile phase composition ± 5 % v/v , pH ±0.05, Flow rate ±0.2ml/min and wavelength by ± 5 nm. The results are shown in Table 07&08.

Effect of variation of Wavelength

A study was conducted to determine the effect of variation in wavelength. Standard solution was prepared and injected into the HPLC system by keeping variation in wavelength ± 5 nm. The effect of variation of wavelength was evaluated.

Effect of variation in the mobile phase composition

A study was conducted to determine the effect of variation in mobile phase composition. Standard solution was prepared and injected into the HPLC system by keeping variation in mobile phase i.e., ± 5 % of organic phase. The effect of variation of mobile phase was evaluated.

Table 01: Showing results from linearity study of Metolazone

S. No	Conc (µg/ml)	Peak area (mV*min)
1.	0.2	4276195
2.	0.5	107198374
3.	1.0	21834682
4.	1.5	32112510
5.	2.0	42683346
6.	3.0	64039195
(Correlation coefficient) R^2		0.999

Table 02: Showing results from linearity study of Losartan potassium

S. No	Conc (µg/ml)	Peak area (mV*min)
1.	2.0	897492
2.	5.0	2241971
3.	10	4479720
4.	15	672182
5.	20	8963840
6.	30	13845760
(Correlation coefficient) R^2		0.999

Table 03: Showing result from accuracy study of Metolazone

%Concentration (at specification Level)	Area			Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	3456140	3448470	3511451	0.5	0.49	98.20%	**98.57%**
	3464551						
	3424719						
100 %	4251428	4216841	4268334	2.0	1.97	98.97%	
	4201251						
	4197844						
150 %	5184211	5134391	5210124	3.0	2.95	98.54%	
	5124812						
	5094152						

Table 04: Showing result from accuracy study of Losartan potassium

%Concentration (at specification Level)	Area			Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	4481920	4398749	4479720	10	9.81	98.1%	**98.80%**
	4312782						
	4401547						
100 %	8912432	8872385	8945123	20	19.83	99.15%	
	8891243						
	8813481						
150 %	13484512	13716228.6	13845760	30	29.71	99.06%	
	13864233						
	13799941						

Table 05: Intraday **precision for proposed method**

Concentration (µg/ml)	Injection (n)	Peak areas of Metolazone(mV*min)
2 µg/ml standard solution	1	4268334
	2	4212423
	3	4127845
	4	4199920
	5	4215463
	6	4241256
Statistical analysis	Mean	421074
	SD	47459.4
	% RSD	1.12

Table 06: Intraday precision for proposed method

Concentration (µg/ml)	Injection (n)	Peak areas of Losartan potassium(mV*min)
20 µg/ml standard solution	1	8953740
	2	8841246
	3	8945613
	4	8874124
	5	8912453
	6	8712431
Statistical analysis	Mean	8873268
	SD	89598.76
	% RSD	1.00

Table 07: Robustness of the proposed method for Metolazone

S. No	Parameter	Metolazone		
		Retention time (min)	Peak area (mV*min)	Tailing factor
1.	Standard	6.150	4269129	1.05
2.	Mobile phase (60:40 % v/v)	6.153	4152173	1.11
3.	Mobile phase (70:30 % v/v)	6.097	4217909	1.20
4.	Wavelength (235 nm)	6.207	4197921	1.54
5.	Wavelength(225 nm)	6.197	4217775	1.32
6.	pH(2.35)	6.147	4125143	1.24
7.	pH(3.35)	6.124	4214576	1.23

Table 08: Robustness of the proposed method of losartan potassium

S. No	Parameter	Losartan potassium		
		Retention time (min)	Peak area (mV*min)	Tailing factor
1.	Standard	3.913	8963840	1.05
2.	Mobile phase (60:40 % v/v)	3.153	8841253	1.11
3.	Mobile phase (70:30 % v/v)	3.097	8712453	1.20
4.	Wavelength (235 nm)	3.207	8789431	1.54
5.	Wavelength (225 nm)	3.197	8841275	1.32
6.	pH(2.35)	3.148	8864713	1.23
7.	pH(3.35)	3.142	8741356	1.14

CONCLUSION:

The RP-HPLC method for determination of Metolazone and Losartan potassium in bulk drug and pharmaceutical dosage form was successfully developed and validated for is intended purpose. Sample recoveries using the developed method were in good agreement with their theoretical drug content.The method shown to Specific, linear, precise, accurate and robust. Because the method separates Metolazone and Losartan potassium.This method is easily recommended for the routine quality control analysis Metolazone and Losartan potassium to quantify in pharmaceutical preparations.

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