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Development and validation of RP-HPLC method for the Simultaneous Estimation of Eprosartan mesylate and chlorthalidone in Tablet Dosage Form

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Abstract: A simple, rapid, accurate and precise RP-HPLC method was developed and validated for the determination of Eprosartan mesylate (EPM) and chlorthalidone (CHL) in pharmaceutical formulation. Chromatographic separation was performed on a Phenomenox, Gemini C18 (250×4.6 mm, 5µm) column from thermo isocratic mode with mobile phase 55:45 water: acetonitrile with pH adjusted to 3.4 with ortho phosphoric acid at flow rate 1 ml/min. Peak intensity of both the drugs was monitored at 250 nm with UV detection. The retention time (RT) of EPM and CHL were found to be 2.14 and 3.80 min, respectively. The linearity of EPM and CHL were found in the range of 10-400 µg/ml for EPM and 0.5-12.5 µg/ml for CHL. The proposed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantification. Furthermore, no interference was observed with extra pharmacopoeial excipients in tablet suggesting its utility for routine quality control analysis of EPM and CHL in pharmaceutical formulations.
Keywords: Eprosartan mesylate, Chlorthalidone, RP-HPLC.

Introduction

Eprosartan mesylate (EPM) is a 4-[[2-butyl-5-(2-carboxy-3-thiophen-2-yl-prop-1-enyl)-imidazol-1-yl] methyl] benzoic acid mesylate[1-2] (Figure. 1A). It is an AT_1 subtype angiotensin II receptor antagonist used in the treatment of hypertension. Chlorthalidone (CHL) is an oral diuretic used along with oral antihypertensive agent. Chemically it is (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide[3](Figure. 1B). Many analytical methods are reported in the literature for the determination of EPM by UV-Spectrophotometry[4], RP-HPLC[5-7]. Several methods have been described for determination of CHL by UV-Spectrophotometry[8], RP-HPLC.[9-11] Although there are several chromatographic methods reported for determination of both these drugs. However, to the best of our knowledge, there is no LC analytical method reported for simultaneous determination of EPM and CHL in combined dosage form. In the present investigation an attempt has been made to develop accurate and precise RP-HPLC method with UV-detection for the quantification of these drugs in pharmaceutical formulation.

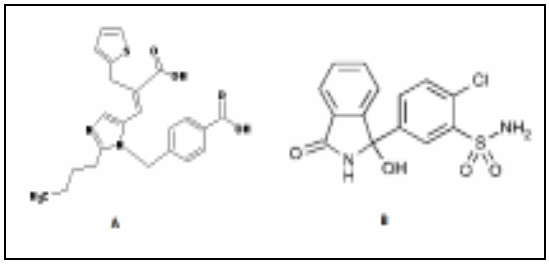

Figure 1: Chemical structures of analytes A. Eprosartan mesylate, B. Chlorthalidone

Material and Methods

Material and reagent

Eprosartan mesylate was a kind gift of Mylan Pharma Ltd. (Nashik, India), Chlorthalidone was provided by Ipca laboratories (Mumbai, India). Pharmaceutical formulation of tablets Eprozar® containing Eproesartan mesylate 400mg and CTD® containing chlorthalidone 12.5 mg was purchased from local market of Nagpur. HPLC grade acetonitrile and phosphoric acid was procured from Merck, Mumbai. Double distilled de-ionized water was used throughout the study. All solutions were filtered through a Millipore vacuum filter system (0.22 µm) and degassed by sonicator.

Instrument

A HPLC system consisted of LC solution data handling system (Shimadzu LC-20AD), a SPD-20A Shimadzu UV visible detector, Rheodyne injector with 20µL sample loop. A 25 µL Hamilton syringe was used for injecting the samples. Data acquisition was performed by using LC 2010 solution software. Ultrasonic bath sonicator was used for degassing of the mobile phase. Weighing of the materials were carried on a Shimadzu balance with high accuracy.

Chromatographic conditions

The mobile phase consisted of water and acetonitrile (55:45 v/v), pH of which is maintained at 3.4 using orthophosphoric acid (85%). The mobile phase was always freshly prepared and filtered through a Millipore vacuum filter system equipped with 0.22µm filter and degassed by sonicator. Chromatography was performed at ambient temperature by pumping the mobile phase at a flow rate of 1.0 ml/min. The column effluent was monitored at 250 nm.

Preparation of stock solution

Accurately 10 mg of standard EPM and CHL were weighed and dissolved into 100 ml mobile phase to obtain final concentration of 100 µg/ml. Appropriate dilutions were made into 10 ml volumetric flask with mobile phase to produce working solutions in the concentrations range 10-400 µg/ml and 0.5-3 µg/ml for OLM and CHL, respectively. 20µl of samples were injected into the chromatographic system and peak areas were measured.

Preparation of sample solution

Twenty tablets of Eprozar® and CTD® were weighed and crushed to get fine powder in mortar. A powder equivalent to 400 mg of eprosartan mesylate and 12.5 mg of chlorthalidone were accurately weighed and transferred in a 100 ml volumetric flask and dissolved in a 20 ml of mobile phase. The solution was subjected to intermittent sonication for 1h in order to extract drug completely. The solution is then filter through 0.22 µ Millipore membrane and volume was brought to the mark. Appropriate dilutions were made with mobile phase to produce working sample solutions in the concentrations range 10-400 µg/ml and 0.5-12.5 µg/ml for EPM and CHL, respectively. 20µlof samples were injected into the chromatographic system and peak areas were measured.

Results and Discussion

Method development

Different trials were carried out by varying the ratio of water : acetonitrile (v/v) and optimizing the chromatographic conditions. The mobile phase consisting of water : acetonitrile (55 : 45 v/v), pH 3.4 was found to have good resolution at wavelength 250 nm with 1.0 ml/min flow rate. The optimized conditions gives well resolved and sharp peak for both the drugs (Figure 2). Retention times for EPM and CHL were determined as 2.14 and 3.80 min, respectively (Figure 3).

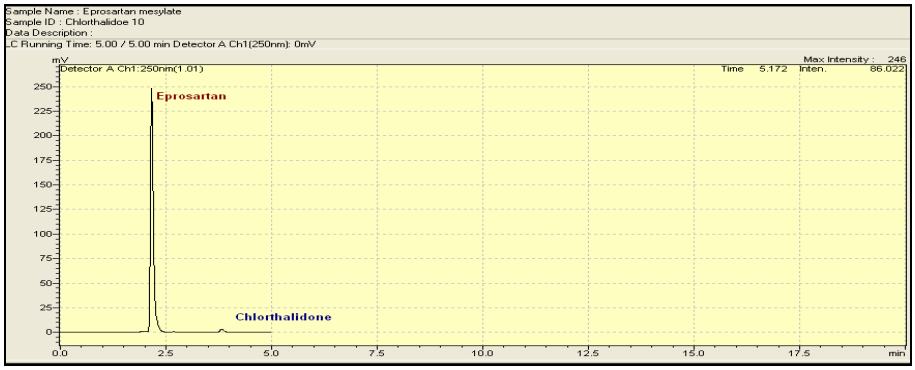

Figure 2: A typical chromatogram showing well resolved and sharp peaks of EPM and CHL

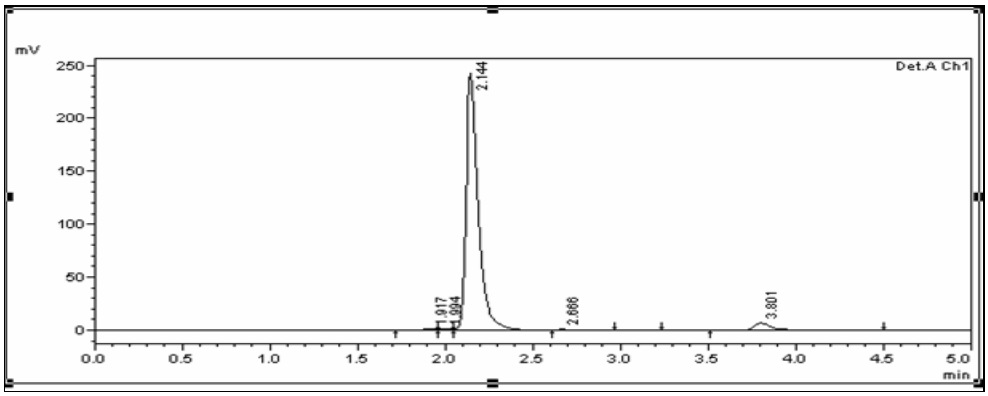

Figure 3: Representative chromatogram of EPM and CHL having RT 2.14 and 3.80 min respectively.

Method validation

Method validation was carried out for different parameters including linearity, accuracy, inter-day and intra-day precision, reproducibility. The limit of detection (LOD) and limit of quantification (LOQ), were also determined. All the parameters were studied considering the ICH guidelines. [12]

System suitability

The efficiency of column is expressed in terms of system suitability parameters, such as number of theoretical plates, resolution, tailing factor. The data are summarized[Table 1].

Table 1: System suitability parameter

Parameters	Retention time (t_r)	Theoretical plates (N)	Tailing factor (T_f)	Resolution (R_s)
EPM				
pH				
3.2	2.13	3890	1.62	0.753
3.4	2.14	4010	1.52	0.661
3.6	2.12	4265	1.66	0.686
Flow rate				
0.9	3.29	3995	1.64	0.742
1.0	2.14	4180	1.49	0.666
1.1	2.52	4165	1.69	0.688
Mobile phase				
43:57	2.85	4069	1.52	0.775
45:55	2.14	4021	1.51	0.628
47:53	3.12	4213	1.59	0.698

CHL				
pH				
2.9	3.89	4354	1.32	4.763
3.4	3.80	4251	1.85	4.652
3.1	3.88	4359	1.39	4.564
Flow rate				
0.9	4.34	4384	1.69	4.365
1.0	3.80	4299	1.75	4.651
1.1	3.47	4187	1.89	4.765
Mobile phase				
43:57	3.90	4952	1.64	4.589
45:55	3.80	4289	1.71	4.463
47:53	3.92	4649	1.82	4.330

Linearity

The linearity of the method was determined at six concentration levels ranging from 10-400 µg/ml for EPM and 0.5-12.5 µg/ml for CHL. The calibration curve was constructed by plotting peak area against concentration of drugs. The results show that an excellent correlation exits between the peak area and concentration of drugs. [Table 2], lists the linearity parameters of the calibration curves for EPM and CHL.

Table 2: Regression characteristics of pure drug

Parameters	**Eprosartan mesylate**	**Chlorthalidone**
Conc. Range (µg/ml)	10 to 400	0.5 to 12.5
Correlation coefficient (r^2)	0.996	0.995
Slope (m)	11840	2990
Intercept (b)	22449	3387
LOD (ng/ml)	30	15
LOQ (ng/ml)	80	60

Accuracy

The accuracy of analytical procedure measures the closeness of measured values to the true value. Standard solution of accuracy of 80%, 100% and 120% solutions were injected into the chromatographic system. [Table 3], represents the high percent recovery values indicating that the proposed method is accurate and reproducible.

Table 3: Result for recovery studies

Analytes	**Label claim**	**Amount added (%)**	**Total amount (mg)**	**Amount recovered * (mg)**	**Mean Recovery (%)**	**RSD (%)**
Eprosartan mesylate	400 mg/tab	80	320	318.82	99.63±1.04	1.043
		100	400	396.53	99.13±0.89	0.897
		120	480	476.50	99.27±0.21	0.271
Chlorthalidone	12.50 mg/tab	80	10	9.94	99.40±0.18	0.181
		100	12.5	12.43	99.44±0.96	0.965
		120	15	14.92	99.46±0.76	0.764

* Mean of three determinations, RSD: Relative standard deviation

Precision

The precision of the method was evaluated by inter day and Intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage of RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and the response factor of drug peaks and percentage of RSD were calculated. From the result obtained [Table 4], the proposed HPLC method was found to be precise.

Table 4: Result of precision study

Parameter	%Mean*		SD		% RSD	
	EPM	CHL	EPM	CHL	EPM	CHL
Intraday precision	99.65	99.25	0.82	1.046	0.822	1.053
Inter-day precision	99.76	99.65	0.68	1.028	0.681	1.031

* Mean of six determinations, SD: Standard deviation, RSD: Relative standard deviation

Detection and quantitation limits

The limit of detection (LOD)and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed RP-HPLC method. The LOD is the smallest concentration of analyte that gives measurable response. The LOD of EPM and CHL was found to be 30 ng/ml and 15 ng/ml, respectively. LOQ was 80 ng/ml and 60 ng/ml for EPM and CHL, respectively.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It has been observed that the proposed method is able to withstand relatively minor alterations in pH, compositions of mobile phase and flow rate [Table 1], but retention times for EPM and CHL were found to be altered.

Conclusion

The RP-HPLC method was developed and validated successfully in terms of accuracy, precision, linearity etc. The proposed method was found to be simple, rapid and accurate. Hence, this method can be used for easy and efficient routine analysis of EPM and CHL in quality control laboratory.

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