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Simultaneous estimation of metoprolol tartrate and chlorthalidone by using RP-HPLC and method development as per ICH guidelines

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ABSTRACT

A simple, rapid and accurate reverse phase-high performance liquid chromatographic method for the simultaneous determination of Metoprolol Tartrate and Chlorthalidone in tablet dosage form is developed and validated. The chromatographic analysis was performed on a C 18column grace smart RP18 (250×4.6 mm, 5 µm) in isocratic mode, the mobile phase consisted of methanol, acetonitrile and 0.05 M phosphate buffer (adjusted topH 4.5 with ortho-phosphoric acid) at a ratio of 60:20:20 v/v/v, and a flow rate of 1.0 mL/min and ASPD detector is used. The eluents were monitored at 254 nm. The retention time of lamivudine and stavudine were found to be 2.50 min and 4.25 min, respectively. The linear ranges were found to be 10-602 2 µg/mL (r =0.9992) for lamivudine and 10-60 µg/mL (r =0.999) for stavudine . The proposed method is also found to be accurate, precise androbust. The method could be applied to routine quality control of pharmaceutical formulations containing Metoprolol Tartrate and Chlorthalidone.

Keywords: Metoprolol Tartrate, Chlorthalidone, RP-HPLC.

INTRODUCTION

The HPLC system consisted of LC-10AT VP Shimadzu liquid chromatograph (Japan) equipped with diode array detector (ASPD), connected to Class-VP 5.032 Software. Chromatographic separations were performed on C 18column grace smart RP18 (250×4.6 mm, 5 m). Samples were 18 injected by means of a Rheodyne injector fitted with a 20 L loop. A Bandelin sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustments. In addition, electronic balance, micropipette and micro pore filtration assembly were used in this study.[1,2].

MATERIALS AND METHODS

2.1 Preparation of standared stock solution

The standard stock solutions were prepared by transferring 100 mg of 3TC and 100 mg stavudine working standards into 100 mL volumetric flasks. To that about 50 mL methanol was added, and the solution wassonicated to dissolve and the volume made up to mark with methanol[4]. The standard solutions were filtered through a 0.45µm membrane filter. Aliquots of these solutions were transferred using A-grade bulb pipettes into100 mL volumetric

flasks and volume make up to the mark with mobile phase to give the final concentration100µg/mL of each analyte [5].

2.2 Preparation of internal standard solution

Accurately weighed 50 mg of internal standard (IS) was transferred into 50 mL volumetric flask containing 30 mL of methanol (HPLC grade) and sonicated for about 20 min. The volume was made up to the mark with methanol. The stock solution was further diluted with the mobile phase to concentration 10 µg/mL [6]. To development the method, a variety of mobile phases were investigated for the development of a suitable assay method for simultaneous analysis of Stavudine and 3TC in tablets. These included methanol-water (80:20 v/v), acetonitrile-water (80:20 v/v), methanol-0.05M phosphate buffer (80:20 v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid), methanol acetonitrile-0.05M phosphate buffer (80:10:10 v/v/v, pH 3.5- 6.5 adjusted with ortho-phosphoric acid) and methanolacetonitrile- 0.05M phosphate buffer (60:20:20 v/v/v, pH 3.5- 6.5 adjusted with ortho-phosphoric acid). The suitability of the mobile phase was decided on the basis of the good resolution, suitability for stability studies, time required for the analysis, ease of preparation and use of a readily available cost-effective solvents

Method Validation

To determine linearity, aliquots of primary standard 3TC and chlorthalidone stock solutions were taken into 100 mL volumetric flask and diluted up to the mark with the mobile phase such that the final concentration of 3TC and chlorthalidone standard solution was added to get a final concentration 10µg/mL in all solutions. The solutions (20µL) were injected three times in to the column according to the optimized chromatographic conditions, and the peak areas and retention times were recorded. The calibration curve was constructed by plotting the analyte to internal standard peak area ratio (Responsefactor) against the concentration (µg/mL).The accuracy was carried out byrecovery studies using standard addition method; known amounts of standard drugs were added to pre-analyzedsample of 3TC and chlorthalidone in according to 0, 50, 100 and 150% of labeled claim, and then subjected to the proposed HPLC method[7]. The experiment was performed in triplicate. The percentage recovery, RSD (%) and standard error mean (SEM) were calculated for each concentration level. Precision was determined as repeatability, intermediate precision and reproducibility in accordance with ICH recommendations19.Repeatability was determined as intraday variation and intermediate precision was determined by measurement of inter day variation[3]. The reproducibility was checked by measuring the precision of the method in same laboratory on a same instrumentation with analysis being performed by another person. For both intra-day and inter-day variation, standard solutions of 3TC and Stavudine at three different concentrations (30, 40 and 50µg/mL) were determined in triplicate. Limit of detection (LOD) and Limit of quantification (LOQ) were calculated based on the ICH guidelines. Robustness was done by deliberately changing the chromatographic conditions like ± 0.2 in pH of the buffer and ±0.1mL in flow rate [8]. To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like asymmetry factor, theoretical plate number(N), resolution (Rs) and retention time (t) were analyzed by R using 20µL of the working standard solution containing 3TC (50µg/mL) and EFV (50 µg/mL) injecting five times into HPLC system. For analysis of marketed samples, twenty tablets of metoprolol tartrate each containing 3TC (300 mg) and chlorthalidone (600mg) were weighed and finely powdered. A quantity of the powder equivalent to one tablet content was accurately weighed, transferred into 100 mL volumetric flask containing 70 mL of methanol, sonicated for about 15 min and the volume make up to the mark with methanol. This solution was filtered through a 0.45µm membrane filter paper and filtrate was again diluted to get a final concentration of 50µg/mL of each drug with mobile phase. Suitable aliquot of internal standard solution was added to get a final concentration of 10µg/mL in all solutions[10]. The standard and sample solutions (20µL) were separately injected into HPLC system. The possibility of interference from the excipients in the analysis was studied[9,11].

RESULTS AND DISCUSSION

In order to achieve simultaneous elution of the two components, initial trails were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and theirproportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions such as methanol-water (80:20 v/v), acetonitrile water (80:20 v/v), methanol-0.05M phosphate buffer (80:20 v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid) methanol-acetonitrile-0.05M phosphate buffer (80:10:10v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid) and methanol-acetonitrile-0.05M phosphate buffer (60:20:20 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid). Finally, methanol, acetonitrile and 0.05 M phosphate buffer (adjusted topH 4.5 with ortho-phosphoric acid) at a ratio of 60:20:20 v/v/v was selected as the optimum mobile phase and aflow rate of 1.0

mL/min. Under these conditions, the analyte peaks were well resolved and were free from tailing. The tailing factor was <1.5 for both the analytes. The retention times of 3TC and chlorthalidone were found to be 2.50 min and 4.25 min, respectively. The resolution (Rs) between IS and 3TC was found to be 4.12, and3TC and chlorthalidone was found to be 6.34, indicating good separation of both analytes from each other. The theoretical plate number for 3TC and chlorthalidone were found to be 6480 and 7025, respectively, thus indicating good column efficiency. A typical chromatogram was recorded at 254 nm, shown in Figure 1. The calibrationplot was constructed by plotting response factor (RF) versus concentration (µg/mL) of 3TC and chlorthalidone which were found to be linear in the range of 10-60µg/mL (r2=0.9992) and 10-60µg/mL (r2=0.999),respectively (Table 1). Limit of detection (LOD) values of 3TC and chlorthalidone were experimentally verified to be 0.16µg/mL and 0.14µg/mL, respectively. Limit of quantitation (LOQ) values of 3TC and chlorthalidone were found to be 0.49µg/mL and 0.41µg/mL, respectively, which indicated that the method can be used for analysis of 3TC and chlorthalidone over a very wide range of concentrations. The percentage recoveries of 3TC andchlorthalidone were found to be in the range of 99.37-100.57% and 99.54-100.35%, respectively. The results were shown in Table 2, which indicates that the method is accurate. The precision of ananalytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample. Results from determination of repeatability and intermediate precision, expressed as RSD (%). The low values of %RSD indicated that the method is precise. The reproducibility results were shown that, there were no significant differences between %RSD values for intra-day and inter-dayprecision, which indicated that the method, is reproducible. Robustness was done by small deliberate changes inthe chromatographic conditions. There were no significant changes in the peak areas and retention times of 3TCand chlorthalidone when the pH and flow rate of the mobile phase were changed. The results were indicating that the proposed method is robust. The proposed method was applied to the simultaneous estimation of 3TC and chlorthalidone tablets. The assay results show that the proposed method was selective for the simultaneous determination of 3TC and chlorthalidone without interference from the excipients used in the tablet dosage form.The values were shown in Table 3. The assay results and low %RSD values indicated that the developed method can be used for routine analysis of metoprtartrate and chlorthalidone pharmaceutical dosage forms.

Fig.1: A typical chromatogram of IS, 3TC and chlorthalidone

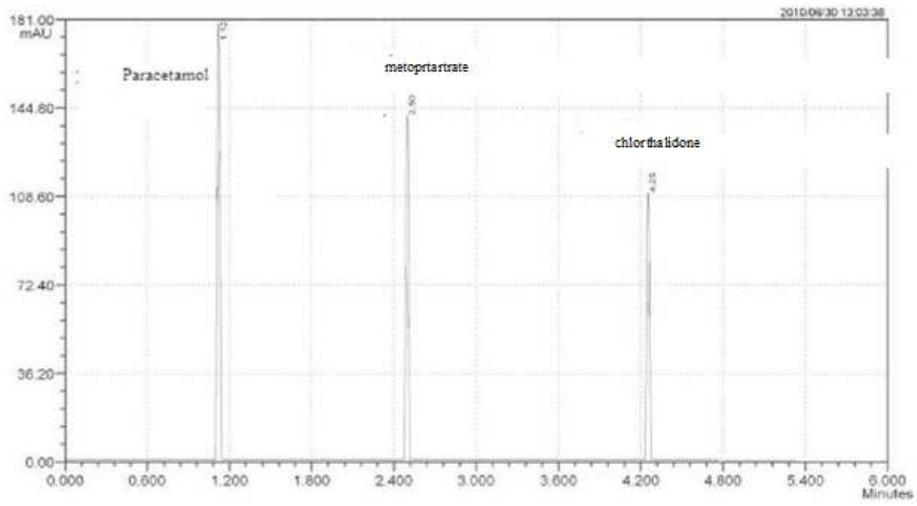

TABLE 1: Linearity data of 3TC and chlorthalidone

Analyte	Conc µg/mL	Mean RF±SD (n=3)	RSD%	SEM	Linear regression
3TC	10	2.0052±0.0066	0.331	0.0038	Y=0.0447x+0577 R^2 =0.9992
	20	2.5081±0.0327	0.508	0.0074	
	30	2.8971±0.0206	0.709	0.0019	
	40	3.3836±0.0195	0.577	0.0113	
	50	3.1981±0.0132	0.348	0.0076	
	60	4.2659±0.001	0.305	0.0075	
chlorthalidone	10	0.8763±0.001	0.113	0.0006	Y=0.0253X+0.614 R^2=0.9991
	20	1.1242±0.0016	0.146	0.0009	
	30	1.3485±0.0101	0.147	0.0052	
	40	1.6235±0.0214	1.321	0.0026	
	50	1.9002±0.0113	0.577	0.0061	
	60	2.1279±0.0116	0.544	0.0063	

SEM=Standard error mean, RSD=Relative standard deviation, RF=Response factor

Table 2: Results of recovery studies by standard addition method

Analyte	Amount(%) of drug added to analyte	Theoretical content	Conc found (µg/mL)±SD n=3	Recovery	RSD%	Bias%	SEM
3TC	0	10	9.9366±0.084	99.37	0.841	-0.634	0.0485
	50	15	14.9477±0.0825	99.65	0.555	-0.348	0.0476
	100	20	20.1136±0.1313	100.57	0.653	0.5682	0.0758
	150	25	25.1216±0.2632	100.49	1.042	0.4864	0.0152
chlorthalidone	0	10	10.0353±0.0771	100.35	0.701	0.3527	0.0445
	50	15	14.9601±0.0396	99.73	0.194	-0.266	0.0171
	100	20	19.9069±0.0915	99.54	0.463	-0.465	0.0529
	150	25	24.9816±0.1427	99.66	0.573	-0.341	0.0824

Table 3: Estimation of amount present in tablet dosage form

Brand name	Tablet formulation	Label claim/tablet mg	% label claim estimated (mean±sd) N=3	RSD%	SEM	%Drug estimated
	metoprtartrate	300	298.78±1.5211	0.509	0.878	99.54
metoprtartrate	chlorthalidone	600	601.24±0.7102	0.218	0.411	100.21

CONCLUSION

The developed RP-HPLC method was accurate, precise, reproducible and robust. The developed method has been found to be better, because of its wide range of linearity, use of a readily available mobile phase, lack of extraction procedure and low retention times. All these factors make the proposed method suitable for the quantification of 3TC and in bulk drugs and in table dosage form. The method can be successfully used for the routine analysis of chlorthalidone and metoprtartrate in pharmaceutical dosage forms without interference

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REFERENCES

[1] World Health Organization says failure to deliver AIDS medicines is a global health emergency. WHO Press Release September **2003**.
[2] Rouzes A, Berthoin K, Xuereb F, *J Chromatogram B* **2004**; 813: 209-16.
[3] International Pharmacopoeia Monograph on efavirenz, World Health Organization, Geneva-27 Switzerland, The World Health Organization **2005**; Working document QAS/05.145, 1-7.
[4]Sean C Sweetman: Martindale The Complete Drug Reference. 34th Edition. USA: ThePharmaceutical Press; 648-9.
[5]Jayakar B, Kumar M, Saravanan C, Kumudhavali MV. *J Chem Pharm Res* **2010**; 2(1): 478-81.
[6]. Schuman M, Schneider S, Omes C *Bull Soc Sci Med* **2005**; 3: 317-25.

[7]. Uslu B, Ozkan SA. *Anal Chimica Acta* **2002**; 466(1): 175-85.
[8]. Kano EK, Serra CHR, Emiko E, Koono M, Andrade SS, Porta V. *Int J Pharmaceutics* **2005**; 297(1-2): 73-9.
[9]. Gholamreza B, Shahla M, Amir K, Bahareh M. *J Chromatogr B* **2005**; 823(2): 213-7.
[10]. Ramachandram G, Kumar AKH, Swaminathan S, Venkatesan P, Kumaraswami V, Greenblatt DJ. *J Chromatogr B* **2006**; 835(1-2): 131-5.
[11]. Rao BU, Nikalje AP. *Afr J Pharm Pharmacol* **2009**; 3(12): 643.