Research Article

Novel Rp-Hplc-Pda Method for the Simultaneous Estimation of Metoprolol Succinate and Chlorthalidone in Bulk and Pharmaceutical Dosage Forms

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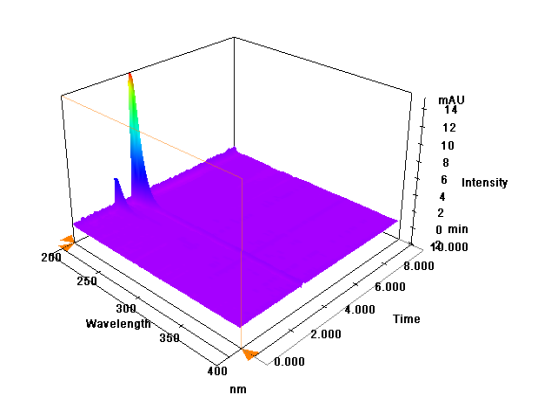
Abstract

The main objective of the study was to develop and validate a simple, precise, economical LC-MS compatible analytical method for the simultaneous estimation of Metoprolol succinate (MET) and Chlorthalidone (CT) by RP-HPLC-PDA. The method was carried out on Inertsil ODS column using mixture of 10mM ammonium acetate: acetonitrile in the ratio of 70:30%v/v as mobile phase. The flow rate was 1mL/min with PDA detection at 220nm.

The retention times of MET and CT were 5.6min and 7.5min respectively. MET and CT showed a good linearity in the concentration range of 5-25 µg/mL and 2-6 µg/mL with a correlation coefficients (R) of 0.996 and 0.995 respectively. The percentage recoveries were 99.17 and 99.75 for MET and CT respectively. The method developed was validated in accordance with ICH guidelines. All validation parameters were in compliance with the acceptance criteria. The method was successfully used for the routine analysis of MET and CT simultaneously in bulk and in combined formulations.



Keywords: PDA Detection, Metoprolol succinate, Chlorthalidone, RP-HPLC, Validation

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Introduction

Metoprolol succinate (MET), chemically is (±) 1(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanolsuccinate (2:1) (salt) used in the treatment of hypertension [1], Chlorthalidone (CT) [2], a monosulfamyl diuretic chemically is 2-chloro-5-(1-hydroxy-3-oxo-1isoindolinyl) benzene sulfonamide. The combination of MET and CT as tablet dosage form was recently approved for the treatment of patients with mild to moderate essential hypertension and cardio vascular disorders [3].

Various analytical methods have been reported in the literature for the analysis of MET and CT individually or in combination with other drugs like Amlodipine, Aspirin, Olmesartan, Clonidine, Azilsartan medoximil, Telmisartan, Hydrochlorothiazide by LC-MS [4-5], stability indicating HPLC [6-12], HPTLC [13] and spectrophotometry [14-16]. However, no HPLC methods were reported so far for the simultaneous estimation of MET and CT in bulk and in tablets. Hence, the aim of present investigation was to develop a validated RP-HPLC-PDA method for the simultaneous estimation of MET and CT in pure form and tablet dosage form which is LC-MS compatible and economical.

Material and Methods

Chemicals & reagents

MET and CT samples were obtained from Dr. Reddy's Laboratories Ltd, India. Ammonium acetate, water and methanol were purchased from E. Merck, India. All the reagents and solvents were of HPLC grade. VINICOR-D ® (manufactured by IPCA laboratories Ltd, Mumbai) tablets containing MET 23.75 mg (equivalent to Metoprolol tartrate 25 mg) and CT 6.25 mg were procured from the local market.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, SPD-M20A PDA detector and Inertsil ODS 3 aqueous column (250 x 4.6 mm, 5 µ) was used. Data acquisition was carried out using LC solutions software.

Chromatographic Conditions

Mobile phase consisting of 10mM Ammonium acetate: acetonitrile (70:30 % v/v) was filtered through nylon disc filter of 0.45 µm (Millipore), sonicated for 3 min and used in isocratic mode at a flow rate of 1 mL/min and the injection volume is 10 µL. PDA detection was performed at 220 nm and the separation was achieved at ambient temperature.

Preparation of standard solutions

Stock solutions were prepared individually by dissolving 9.5 mg of Metaprolol succinate (equivalent to 10 mg of Metaprolol tartrate) and 10 mg of CT in 10 mL volumetric flasks containing 5 mL of methanol and the volume was made up to mark with methanol. Aliquots of the individual stock solutions were further diluted with ammonium acetate to get the required standard concentrations in the range of 5-25 µg/mL and 2-6 µg/mL for MET and CT respectively.

Validation of the HPLC method

The method developed was validated in accordance with ICH guidelines.

Linearity

A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of MET and CT were prepared over a concentration range of 5-25 µg/mL (5, 10, 15, 20, 25 µg/mL) and 2-6 µg/mL (2, 3, 4, 5, 6 µg/mL) respectively from stock solution and injected in triplicate. Linearity was estimated by a plot of peak areas as a function of analyte concentration and the test results were evaluated by calculating appropriate statistical parameters such as slope, intercept, correlation coefficients (R) and regression (R^2)

Precision

Precision is the measure of concordance between repeatedly obtained experimental values. Repeatability was assessed by using a minimum of six determinations at 100 % of the test concentration. This study was carried out by injecting six replicates of the standard and sample at a concentration of 15 µg/mL and 4 µg/mL of MET and CT respectively. The standard deviation and the RSD were computed for precision.

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the diluent, placebo and sample solution. The retention times of drug substances and the drug products were observed. Absence of interference of excipients in the tablet proves the specificity of the proposed method.

Accuracy

Accuracy is the measure of closeness the experimental value to it's the true value. It should be established across the specified range of the analytical procedure. The accuracy of the proposed method was evaluated by performing recovery studies were performed by the standard addition method. The % recovery and the % RSD were calculated at each level of addition.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (Where,σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

Robustness is a measure of the method's capability to remain unaltered by small, but calculated variations. The method parameters like no. of theoretical plates, capacity factor, tailing factor and % assay were noted. It can be partly assured by good system suitability specifications. To study the effect of flow rate, ± 20 % change was made in flow rate.

The effect of wavelength was studied by changing wavelength by ±1nm and effect of mobile phase by ± 2 %. The data was given in **Table 2**.

System suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50 µL. The test parameters were noted and % RSD was calculated.

Assay

Twenty tablets were weighed and finely powdered, the powder was accurately weighed which contains 10 mg of MET and 2.63 mg of CT and transferred into a 10 mL volumetric flask containing methanol and vortexed for 5 min and volume was adjusted up to the mark with methanol. This solution was centrifuged and then filtered using disposable syringe filter (13 mm, 0.4 µm). An aliquot of filtrate was diluted with ammonium acetate and analyzed in triplicate. The amount of drug present in the each tablet was quantified by comparing the area of standard MET and CT with that of the sample.

Results and Discussion

From literature review it is clearly evident that there were no HPLC methods so far reported on the simultaneous estimation of MET and CT in combined dosage form. Hence, the present investigation was aimed to develop a simple, precise, economical RP-HPLC-PDA method for the simultaneous estimation of MET and CT in bulk and pharmaceutical dosage forms.

Method Development

In this investigation efforts were made to develop a validated RP-HPLPDA method for the simultaneous analysis of MET and CT in bulk and pharmaceutical dosage forms. Initial trail was carried out with Phenomenex C_{18} column (250 x 4.6 mm, 5 µ) using a mobile phase of water and methanol (50:50 % v/v) at a flow rate of 1 mL/min with the detector set at 220 nm. Under these conditions MET was eluted at 5.2 min and CT was not eluted within 15 min run

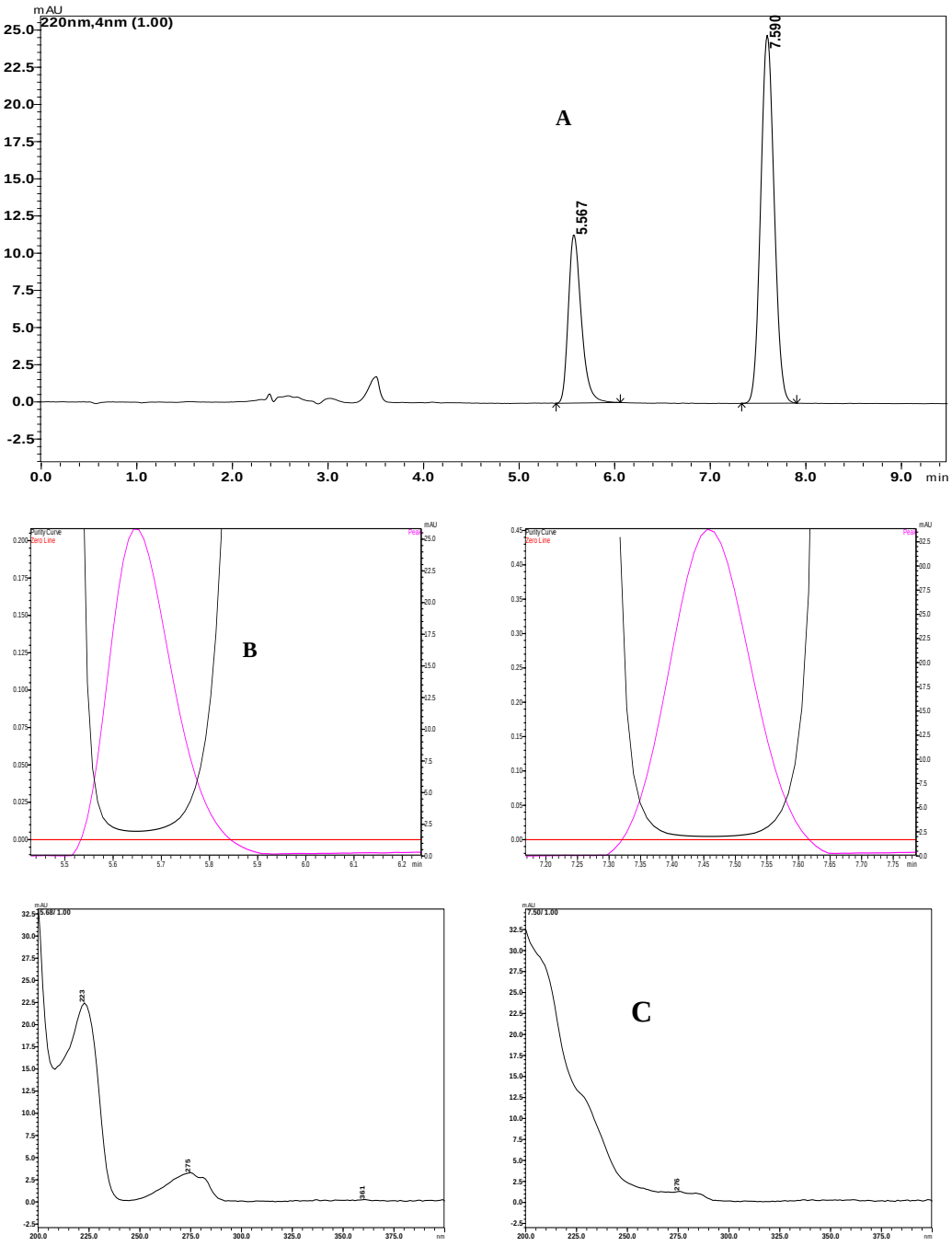

Figure 1 A Standard chromatogram of MET and CT mixture; **B** - Peak purity index of MET and CT; **C** - UV spectrum

time. In the other trail 0.05 % v/v orthophosphoric acid (pH 2.3) was used as aqueous phase and methanol as organic modifier (80:20 % v/v) at a flow rate of 1mL/min with Phenomenex C_{18} column (250 x 4.6 mm, 5 µ) and the peak retentions were not changed with this trial. In the next trail, 10mM ammonium acetate (pH 4.5) and acetonitrile (75:25 % v/v) was used using Inertsil ODS-3 column and the peaks were eluted at 5.2 min and 11min respectively for MET and CT however, peak tailing was observed with MET. To optimize the mobile phase composition the trails were carried out by using mixture of 10 mM ammonium acetate: acetonitrile (70:30 % v/v) as mobile phase and ammonium acetate as diluent at a flow rate of 1 mL/min. Under these conditions MET and CT were eluted at 5.6 min, 7.5 min respectively within 10 min of run time and no interference peaks were found. The validation of the method was performed as per ICH guidelines. The peak purity index of greater than 0.9998 indicates peak purity of the drug sample used in the analysis and shown in **Figure 1** along with UV spectra.

Method validation

Linearity

The range of reliable quantification was set at the concentrations of 5-25 µg/mL and 2-6 µg/mL of MET and CT respectively. This range was selected based on 80-120 % of the standard concentration used for accuracy and were analyzed in triplicate. Concentrations and Peak areas were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (R) was found to be 0.996 and 0.995 indicating a linear response over the range used. The calibration data was given in **Table 1.**

Table 1 Linearity, Accuracy, Precision, Assay and stability data for MET and CT

	Validation data of MET	**Validation data of CT**
Linearity (n=3)		
Range	5-25 µg/mL	2-6 µg/mL
Regression equation	y =15051x – 16095	y =36401x – 11173
Correlation coefficient	R = 0.998	R = 0.998
Regression coefficient	R^2 = 0.996	R^2 = 0.995
Accuracy (n=3)		
% Level of Addition	Mean Percent Recovery(%RSD)	
80	99.83 (1.80)	100.3 (1.64)
100	99.50 (1.57)	99.23 (1.37)
120	100.1 (1.44)	98.86 (0.67)
Precision (n=6)		
System Precision	Average peak area (%RSD) 163591.3 (0.47%)	123736.4 (0.12%)
Method Precision	129494.4 (0.10%)	154041.9 (0.58%)
Percent Assay (% RSD) (n=3)	99.86 (0.578)	100.8 (0.717)

Precision

Precision studies were carried out in terms of repeatability.

System precision:

Repeatability of standard application was carried out using six replicates of the mixed standard concentration (15 µg/mL of MET and 4 µg/mL of CT). The % RSD of the peak area was found to be less than 1 indicating an acceptable level of precision for the analytical system. The data was given in **Table 1** and the overlay of chromatograms shown in **Figure 2A**.

Method precision:

Repeatability of sample measurement was carried out in six replicates of the same sample preparations from same homogenous blend of marketed formulation(VINICOR-D ® tablets). The % RSD of the peak area was found to be less than 1.The data was given in **Table 1** and the overlay of chromatograms shown in **Figure 2B**

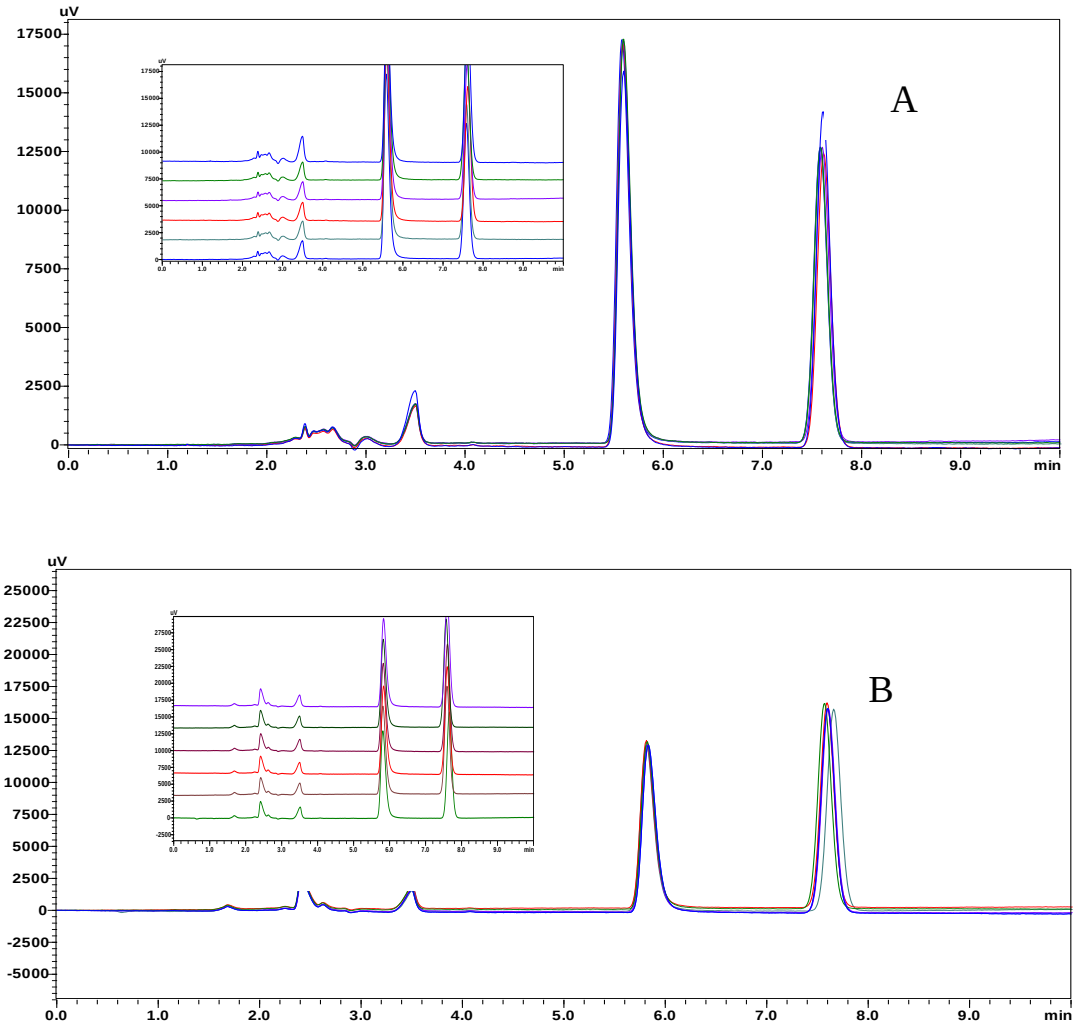

Figure 2 A-System precision data; **B**- Method precision data for MET and CT

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80 %, 100 %, 120 % to the drug product solution of 15 µg/mL and 4 ug/mL and these solutions were analyzed in triplicate in each level of addition. The % RSD and the % Recovery were in compliance with specified limits. It is clear from the results of accuracy study given in **Table 1**, that the proposed method enables accurate quantitative estimation of MET and CT.

Assay

Assay of VINICOR-D ® tablets was performed by the proposed method and the % assay of the formulation was calculated as an average of 3 determinations, which was about 99.8 ± 0.58 and 100.8 ± 0.73 for MET and CT respectively. These results indicate that the present HPLC method can be successfully used for the simultaneous analysis of MET and CT in bulk and pharmaceutical dosage forms, the results of assay given in **Table 1.**

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard, sample individually to examine any interference. The 3D plots of diluent, placebo, standard and sample are shown in **Figure 3** and overlay of chromatograms as shown in **Figure 4** and From these figures it can be inferred that there were no co-eluting peaks at the retention time of MET and CT, this shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analysis and the peak purity indices for sample and standard was found to be more than 0.999 and this depicts specificity of the method.

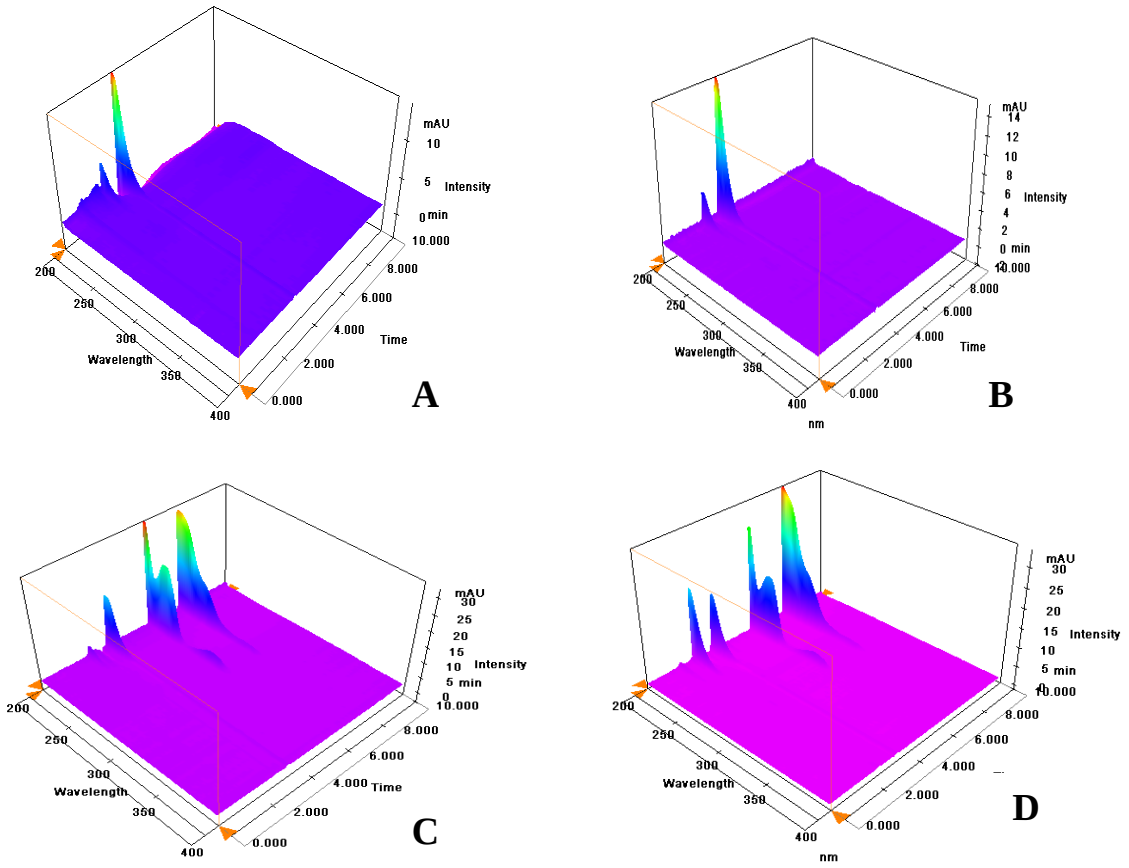

Figure 3 3D plots of the chromatograms for diluent (A); placebo (B); standard(C) and drug product (D)

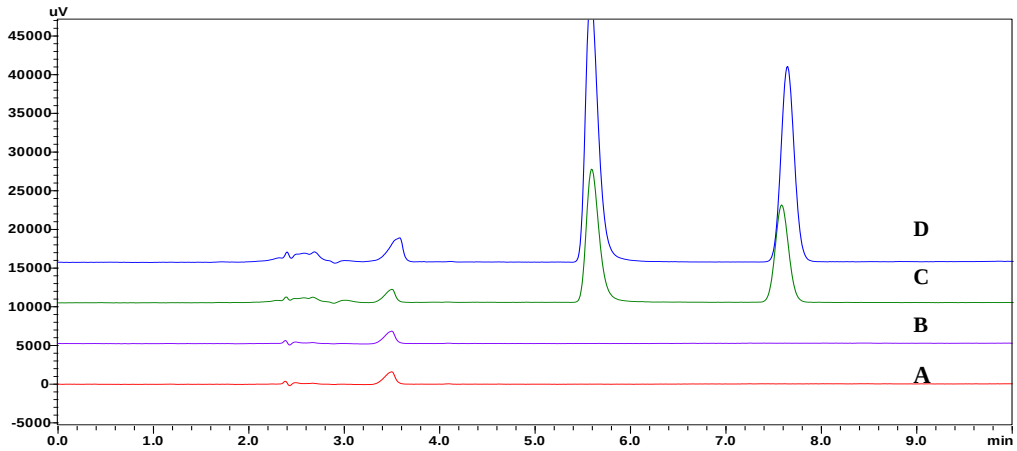

Figure 4 Specificity data for the chromatograms of diluent (A); placebo (B); standard (C) and drug product (D)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on statistical calculation from the calibration curves. The limit of detection for MET and CT was found to be 0.339 µg/mL and 1.029 µg/mL respectively, the analyte peak could be distinguished without any base line disturbances at this concentration. The limit of quantification for MET and CT was found to be 0.217 µg/mL and 0.659 µg/mL respectively.

Robustness

As part of the robustness, a deliberate change in the flow rate and wavelength was made to evaluate the impact on the method. Retention times were slightly changed with flow rate but no change in the retention time was observed. Percent assay values were also estimated under these changed conditions and the results were given in **Table 2**. These results indicated that the method is robust in terms of deliberate changes with the mobile phase ratio, flow rate and wavelength.

Table 2 Robustness data of MET and CT

Chromatographic parameters	Retention time(min)		Theoretical plate number		Tailing factor (T_f)	
	MET	CT	MET	CT	MET	CT
Wavelength(nm)						
218	5.56	7.59	7473.43	12287.22	1.41	1.12
220	5.56	7.59	7470.35	12285.70	1.41	1.12
222	5.56	7.59	7468.19	12290.10	1.41	1.12
% B of mobile phase						
32	5.27	6.71	7162.45	11950.76	1.43	1.12
30	5.81	7.65	7457.55	12801.89	1.43	1.09
28	6.52	8.78	7914.29	13902.21	1.42	1.08
Flow rate (mL/min)						
0.9	6.57	8.39	7890.63	13336.06	1.45	1.09
1.0	5.02	7.60	7457.75	12256.74	1.43	1.08
1.1	5.27	6.89	7095.22	11930.52	1.19	1.10

System suitability

System suitability testing is an integral part of the analytical procedure. The studies were carried out by injecting five times a 15 μg/mL and 4 μg/mL standard concentration of MET and CT at different injection volumes ranging from 10-50 μL. The % RSD values for system suitability test parameters like tailing factor, retention time and theoretical plate number were all less than 2% and indicating the given conditions were suitable for the simultaneous analysis of MET and CT in tablets.

Stability of the stock solution

The stability of the stock and standard solutions were determined by analyzing the samples under refrigeration (8 ± 1°C) at different time intervals up to 48 hrs. The % variation in assay values at different time intervals were found to be less than 2 % of the initial zero time interval solution, demonstrating that the solutions were stable for a period of 48 hrs when stored at 8°C.

Conclusion

A new, RP- HPLC method has been developed for the simultaneous analysis of MET and CT in tablet dosage forms. The method was fully validated as per International Conference on Harmonisation (ICH) Guidelines and found to be applicable for routine quality control analysis for the simultaneous estimation of MET and CT in tablets using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity were all within limits and proved the reliability of method. The method provides selective quantification of MET and CT without interference from diluents. Therefore, this method can be employed in quality control for the simultaneous estimation of MET and CT in bulk and in tablets.

Acknowledgements

The authors are thankful to Dr. Reddy's Laboratories Ltd, India for providing gift samples of drugs and also to the Siddhartha Academy of General and Technical Education, A.P., for providing necessary facilities to carry out the research work**.**

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Publication History
Received 30th Sep 2014
Revised 10th Oct 2014
Accepted 18th Oct 2014
Online 30th Oct 2014