Development and Validation of a Ultra-High Performance Liquid Chromatography (UPLC) Method for the Simultaneous Estimation of Hydrochlorthaizide and Metoprolol Succinate in Tablet Formulation and Bulk Product

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ABSTRACT

A new, accurate, precise, and durable UPLC method with sensitive features has been developed for the simultaneous assessment of Hydrochlorthiazide (HCT) and Metoprolol Succinate (MET) in both bulk and tablet format. An Agilent C_{18} column with a size of 100 mm × 2.1 mm, 3 µm was used to estimate the solutes. HCT and MET were eluted in a 15-minute gradient trial at a flow rate of 1 ml/min with an ambient column temperature of 25°C and monitored at a wavelength of 226 nm using water: acetonitrile in a 95:5 v/v ratio. The retention times of HCT and MET were found to be 4.64 minutes and 5.36 minutes, respectively. The Q2A and Q2B validations of the analytical method demonstrated good linearity throughout the concentration ranges of 0-60 µg/mL for HCT and 0-60 µg/mL for MET, with r^2 of 0.994 and 0.987. High accuracy, excellent precision (inter-day and intra-day), and remarkable resilience values were also shown by the technique. The suggested analytical method proved precise, accurate, and robust for frequent analysis of the drug combination in bulk and tablet forms.

Keywords: *Hydrochlorthaizide, Metoprolol Succinate, UPLC, Simultaneous, Estimation, Validation.*

INTRODUCTION

Hydrochlorothiazide (HCT) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide (**Figure 1A**) and metoprolol succinate (MET) is 1,[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl)amino]-2-propan-ol tartrate (**Figure 1B**). The molecular mass of HCT and MET is 297.74 g/mol and 684.81 g/ mol, respectively. Few methods for simultaneous estimation of hydrochlorothiazide and metoprolol tartrate by reverse phase chromatography have been reported [1,2]. There are also some methods used for estimating individual HCT and MET [3-5]. Some pharmacopoeia methods are also available for estimating individual HCT [6] and MET [7]. The UPLC methods using the most commonly available columns and detectors like the UV detectors are preferred [8,9]. The present study describes the determination of hydrochlorothiazide and metoprolol tartrate by using reverse phase chromatography, a C18 column with a UV detector. The use of HPLC is very much preferred now-a-days for routine analysis. It is important that well validated HPLC methods are to be developed for simultaneously estimating HCT and MET. The aim of this study is development of a simple, precise, rapid and accurate reverse phase UPLC method for the simultaneous estimation of HCT and MET in pharmaceutical tablet dosage form.

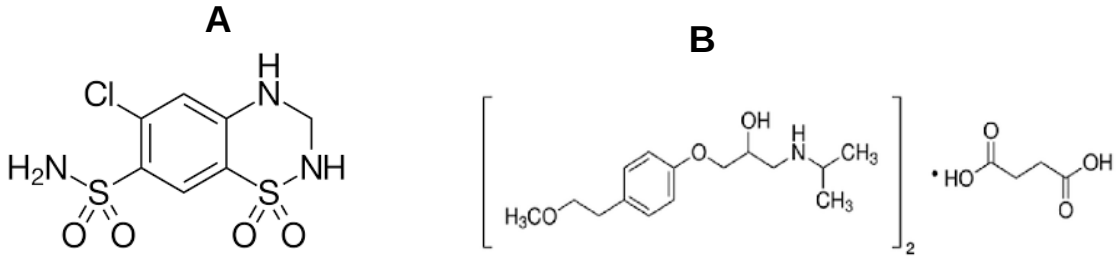


Figure 1. Structure of drugs (A) Hydrochlorthaizide (B) Metoprolol Succinate.

MATERIAL AND METHODS

Materials

Ankaleshwar, Gujarat-based Purechem Pvt. Ltd. provided a sample of HCT and MET as a present. The METPURE-H® Tablet (containing MET (25mg) + HCT (12.5mg)) was supplied by Torrent Pharmaceuticals Ltd., Mumbai. HiMedia Ltd., Mumbai provided analytical quality chemicals and UPLC grade solvents for the study.

Instruments

A Shimadzu® AUW220D balance was used for the weighing (Kyoto, Japan). The pH was measured using a VSI® VSI-1B digital pH meter (Mohali, India). The sonication was done using a Transonic Digital S sonicator (Mumbai, India). The method was developed using a reverse-phase Hypersil Gold C_{18} column with a particle size of 3 μm and a dimension of 100 mm × 2.1 mm, which was connected to an Agilent® 6200 Gradient HPLC system TOF-6500 Series with a DAD detector and a manual rheodyne injector (20 μL loop), all of which were controlled by Chemstation v.2 software.

Selection of the mobile phase

The mobile phase must be carefully selected for the elution of the solutes. The mobile phase was selected based on theoretical plates, peak purity index, and peak symmetry. The experiment started with buffer systems and an eluant like methanol, acetonitrile, or other solvents. Low-intensity peaks with a lot of tailing were produced by elution with an equal combination of buffer KH_2PO_4 and methanol. Although this was an improvement over the previous experiment, the combination of KH_2PO_4 buffer (pH 4.8) with acetonitrile resulted in the formation of a broad peak with tailing. When employed in an equal ratio with methanol, the peak symmetry improved considerably and tailing was reduced when the buffer was replaced with orthophosphoric acid (OPA) (0.05%), but it was still inadequate to elute the solutes. Water was combined with ACN to produce a crisp peak with a good Gaussian peak. The 95:5 v/v ratio generated the most theoretical plates as well as the greatest peak purity index. The mobile phase was degassed under vacuum before being filtered using a 0.45 μm membrane filter. Allowing the mobile phase to equilibrate until it achieved a stable baseline was permitted.

Chromatographic conditions

HCT and MET were eluted at a flow rate of 1 ml/min with an ambient column temperature of 25°C in a 10-minute gradient trial and monitored at a wavelength of 226 nm using a 95:5 v/v Water: ACN.

Preparation of analytical solutions

Preparation of mobile phase

Water was thoroughly mixed with ACN in a 95:5 v/v ratio. After that, the solution was degassed for 5 minutes with sonication before being filtered under vacuum through a 0.45 μm membrane filter.

Standard preparation

In a 10 mL dry volumetric flask, a precise quantity of 10 mg HCT and 10 mg MET were introduced. Sufficient amount of mobile phase was added to dissolve the drug to get a standard stock solution of 100 μg/mL and 100 μg/mL concentrations. The aforementioned content was sonicated for 10 minutes and the volume was made up to 10 mL.

Sample preparation

The average weight of 20 tablets was determined after they were properly weighed. A weight equal to a tablet was transferred to a 100 mL volumetric flask and half-filled with the diluent. The contents were sonicated for 20 minutes and then filtered to produce 10 mg/mL of MET.

Method validation

The technique was verified using the Q2A and Q2B guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), as well as guidance from the USFDA.

Linearity and Range

The linearity of the technique was tested using five different solute concentrations, ranging from 0 to 60 μg/mL for HCT and 0 to 60 μg/mL for MET. The solutions were prepared with the diluent and an equal quantity was injected into the HPLC equipment to determine the peak area. On a linearity graph, the concentration and average area of each solute were plotted. The r^2 value of the regression coefficient was computed as well [10].

Accuracy

The accuracy of the HPLC system was tested by spiking the reference drug solutions at concentrations of 80%, 100%, and 120% (recovery). The experiment was repeated three times, with the results given as % recovery % relative error based on the concentrations used [11].

Precision

The precision of the suggested method was tested in terms of inter-day and intra-day variability by spiking concentrations of 40%, 60%, and 80% six times in a single day (intra-day) and on three different days (inter-day). % relative error precision was used to describe the data [12].

Robustness

The method's robustness was evaluated by varying the mobile phase composition by 1% v/v (i.e. 71:29 % v/v and 69.31 % v/v), flow rate by 0.1 mL/min (i.e. 0.6 mL/min and 0.7 mL/min), and wavelength by 1 nm (i.e. 256 nm and 258 nm), while keeping all the other chromatographic parameters fixed [13].

Systems suitability parameters

The analytical method's repeatability profile was determined by injecting five times the standard solution and monitoring data such as retention length, peak area, theoretical plates, and tailing factor [14].

Limit of detection

Although it is not necessary to define the exact amount, the limit of detection (LOD) is the lowest concentration that any analytical method can detect [15].

The limit of detection (LOD) was determined by the formula:

$$\mathbf{LOD = 3.3\ (\sigma / S)}$$

Where, **σ ═** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The limit of quantification is the smallest amount that can be measured with a given degree of accuracy and precision using any analytical method (LOQ) [16].

The limit of quantification (LOQ) is determined by the formula:

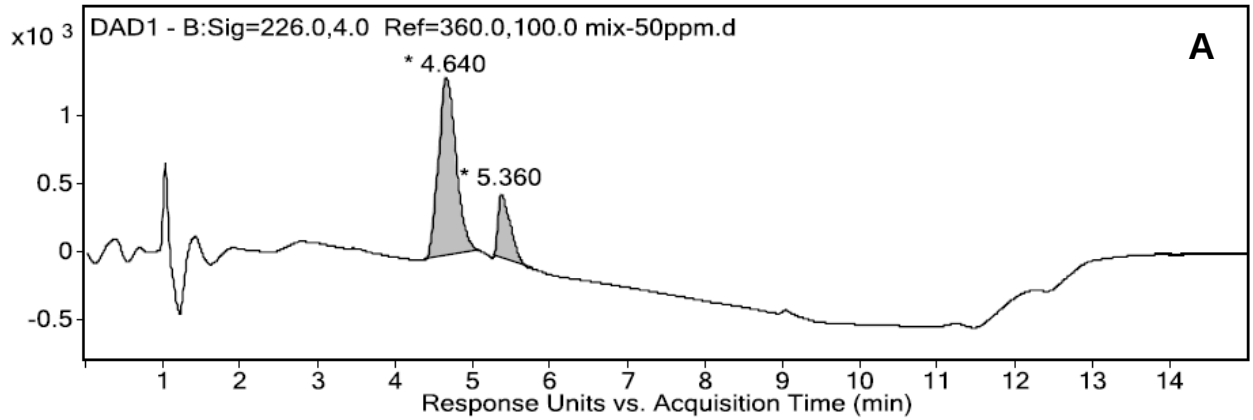
$$\mathbf{LOQ = 10\ (\sigma / S)}$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Because there were no previous similar methods, the new methodology was entirely based on trial and error. However, considerable influence was drawn from earlier reports when deciding on the stationary phase. The reverse phase C_{18} stationary phase from Agilent® was utilized, with a particle size of 3 µm and a diameter of 100 mm × 2.1 mm i.d. The mobile phase Water: ACN in the ratio 95:5 v/v was utilized for the elution after several continuous trials. Peak tailing was minimized and the analytical method's robustness was significantly enhanced by keeping the mobile phase at a low pH. The use of acidic pH was justified to a greater extent because high basic pH caused dissolution in silica-based reverse-phase columns. The pH of the mobile phase and the pKa of the solute were also found to be in close agreement, enabling them to remain in the unionized state. As a consequence, the pH value was chosen based on two units. The elution was place on an Agilent® C_{18} column in isocractic mode for 8 minutes with a mobile phase of 95:5 v/v Water: ACN. The flow rate was maintained at 1 mL/min, the column temperature at 25°C, and the detection wavelength at 226 nm. The retention times for HCT and MET were 4.64 minutes and 5.36 minutes (**Figure 2A**), respectively. In the tablet sample solution, HCT had a retention time of 4.34 minutes while MET had a retention time of 5.24 minutes (**Figure 2B**). This clearly showed that the suggested analytical method for routine medicine combination analysis in bulk and tablet forms was exact, accurate, and robust.



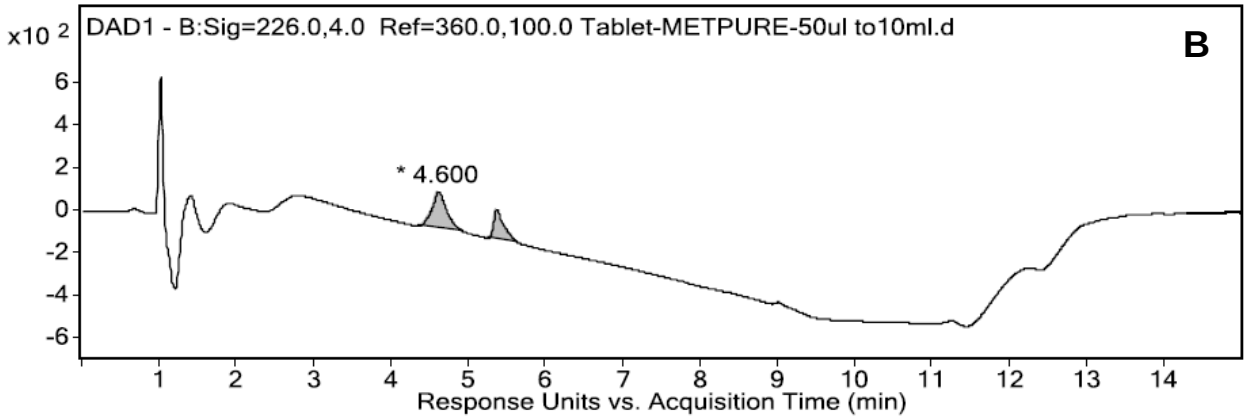


Figure 2. Chromatogram of Hydrochlorthaizide and Metoprolol Succinate (A) after method optimization and (B) in tablet sample.

Method validation

Linearity and range

Throughout the dose and peak area ranges of 0-60 µg/mL for HCT and 0-60 µg/mL for MET, there was very high linearity, with linear regression equations of y = *435.7x* and y = *103.1x*, respectively. The regression coefficient values were 0.994 and 0.987, respectively, suggesting that there was a high level of linearity (**Figure 4**).

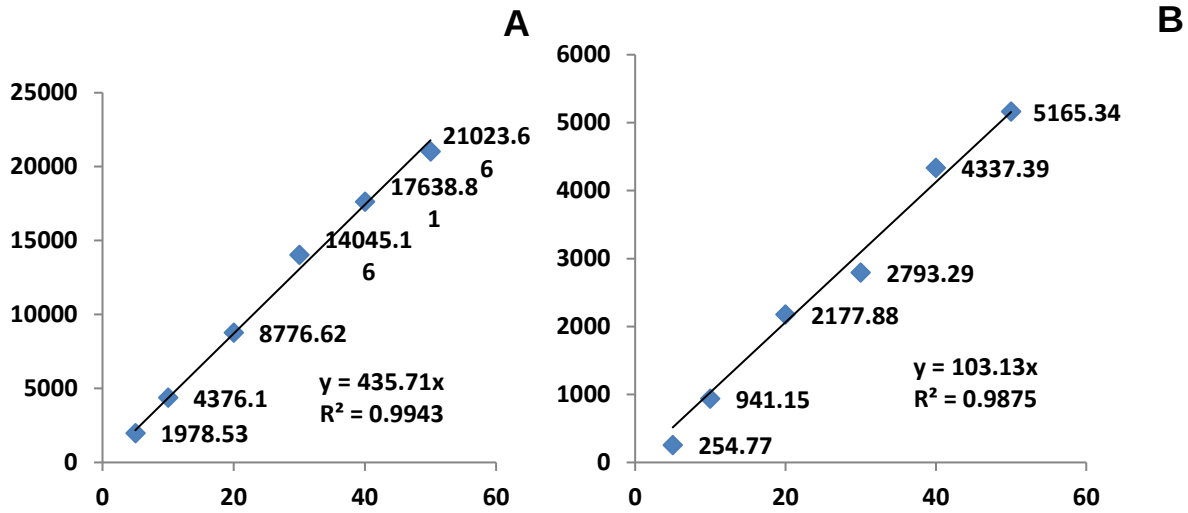


Figure 4. Linearity plot of (A) Hydrochlorthaizide and (B) Metoprolol Succinate.

Accuracy

The % recovery characteristic of the proposed method for simultaneous estimation by utilizing the calibration curve was determined in part by the Y-intercept and slope of the graph. HCT's % RSD values were 0.83, 0.39, and 0.66, respectively, while MET's were 0.67, 0.42, and 0.78, all of which were less than the US Pharmacopeia's acceptance threshold of 2% (**Table 1**). Overall, the method revealed that the data retrieved was correct.

Table 1. Recovery for accuracy studies for the combination.

Spiked level %	Conc. of drug added (µg/mL)	Conc. of drug found (µg/mL)	Recovery %	Mean %	% RSD
HYDROCHLORTHAIZIDE					
80	16	16.09386	101.52	101.68	0.83
	16	16.18407	101.84		
100	20	20.24659	101.23	100.80	0.39
	20	20.0717	100.36		
120	24	24.3347	101.39	101.35	0.66
	24	24.31274	101.30		
METOPROLOL SUCCINATE					
80	8	8.117429	100.76	100.77	0.67
	8	8.208364	100.78		
100	10	10.960449	99.59	99.80	0.42
	10	10.979085	100.01		
120	12	11.972432	101.74	101.76	0.78
	12	11.937307	101.77		

Conc., Concentration; RSD, relative standard deviation

Precision

In both intra-day and inter-day variability testing for precision data, the method was proven to be highly accurate across the tested ranges of 20-100 µg/mL for HCT and MET. The peak area of the sample solution matched that of the standard solution in both cases, with a % RSD of less than 2%. HCT and MET had % RSDs of 0.19 % - 0.34 % and 0.23 % - 0.61 % in intra-day studies (**Table 2**), respectively, whereas HCT and MET had % RSDs of 0.24 % - 0.56 % and 0.26 % - 0.87 % in inter-day studies, indicating high precision and minimal variation (**Table 3**).

Table 2. Precision data of intra-day variability.

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
HCT	20	1486.364	1486.83	100.53	0.19
	30	2225.869	2226.15	100.71	0.34
	40	2905.418	2905.97	98.77	0.29
MET	40	622.0565	621.86	98.70	0.33
	60	931.2762	931.50	100.00	0.23
	80	1240.078	1244.28	100.91	0.61

Conc., Concentration; RSD, relative standard deviation

Table 3. Precision data of inter-day variability.

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
HCT	20	2266.545	2265.54	153.76	0.56
	30	3333.542	3325.25	150.81	0.35
	40	4460.325	4464.77	152.05	0.24
MET	40	2102.517	2118.54	164.59	0.87
	60	3170.365	3174.79	164.78	0.26
	80	4255.317	4260.33	166.03	0.47

Conc., Concentration; RSD, relative standard deviation

Robustness

The intentional change of several critical chromatographic parameters such as mobile phase composition, flow rate, and wavelength by 1%, 0.1 mL/min, and 1 nm, respectively, resulted in a substantial shift in the chromatogram for both medicines. When the mobile phase combination was adjusted to 66:34 v/v, the % RSD was determined to be 2% (0.22 for HCT and 0.34 for MET). Similarly, the % RSD was found to be less than 2% when the composition was altered by 64:36 v/v where HCT has a value of 0.26, whereas MET has a value of 0.49. When the flow rate was raised by 0.1 ml/min, the % RSD was determined to be 2% (0.12 for HCT and 0.31 for MET). A similar reduction in flow rate, on the other hand, resulted in a % RSD of < 2 (specifically, HCT showed 0.16 while MET showed 0.38). A variation of 1 nm in wavelength resulted in a RSD value of less than 2% where HCT demonstrated 0.21 and 0.18, respectively and MET demonstrated 0.26 and 0.36, respectively. All of the tests indicated that the suggested method has robust characteristics due to the deliberate change of the parameters.

Systems suitability parameters

The system suitability features of the suggested approach demonstrated a high degree of repeatability and may be utilized for routine drug combination analyses. The suggested HCT method yielded an average retention time (Rt) of 8.162 minutes and a mean theoretical plate (TP) of 7148. The Rt and TP for MET were 10.106 minutes and 6897, respectively (Table 5). A tailing value of less than 2% showed no specific tailing in any cases. Both symmetric and asymmetric components are of similar magnitude in an ideal Gaussian peak with excellent peak symmetry (asymmetric factor = 1). Because the suggested method met the minimum requirements of US Pharmacopoeia monographs (minimum theoretical plates of 2000 and tailing factor of less than 2%), it has a high resolution, significant separation, high column effectiveness, and enhanced repeatability. The separation factor (α) and resolution factor (Rs)

were found to be significantly higher than the ICH limits and required recommendations of 1 and 1.5, respectively, indicating that the suggested analytical technique produces a greater separation of both peaks with less tailing and greater resolution. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

Table 4. Systems suitability parameters.

HYDROCHLORTHAIZIDE						METOPROLOL SUCCINATE					
Rt (min)	**Area (mV)**	**Theoretical Plates (TP)**	**Separation Factor**	**Resolution Factor**	**Tailing Factor**	**Rt (min)**	**Area (mV)**	**Theoretical Plates (TP)**	**Separation Factor**	**Resolution Factor**	**Tailing Factor**
4.253	379967	3504	1.531	1.936	1.46	6.927	2521645	5583	1.534	1.898	1.39
4.259	375014	3764	1.537	1.933	1.48	6.923	2517961	5555	1.529	1.889	1.30
4.255	378053	3881	1.539	1.921	1.59	6.924	2516517	5656	1.533	1.890	1.36
4.258	378516	3505	1.532	1.930	1.55	6.927	2517892	5893	1.532	1.896	1.31
4.251	377337	3641	1.541	1.926	1.49	6.926	2512491	5885	1.544	1.881	1.29
4.255	**377499**	**3687**	**1.539**	**1.924**	**1.513**	**6.922**	**2519297**	**5711**	**1.531**	**1.882**	**1.337**
% RSD		**0.17**							**0.46**		

Limit of detection and Limit of quantification

HCT had a LOD of 0.312867 µg/mL and a LOQ of 0.722524 µg/mL, while MET had a LOD of 0.214769 µg/mL and a LOQ of 1.8156 µg/mL, showing the method's remarkable detection capacity for the lowest possible concentration of the solute concurrently from the combination or formulation.

CONCLUSION

The suggested analytical method may be utilized to estimate HCT and MET in bulk and tablet formulations at the same time. According to the ICH validation criteria, the method exhibits linearity throughout the range, accuracy, precision, and resilience. The % RSD, theoretical plates, and tailing values all met the minimum requirements of the US Pharmacopoeia. The validated stress degradation tests under thermal, oxidative, alkali, and acid conditions showed the possibly damaged components, which chemists would find very helpful for quality control and assurance. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

CONFLICT OF INTEREST

No conflict of interest is declared.

FINANCIAL SUPPORT
None

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