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Research Article

Development of a Stability-Indicating UPLC Method for the Determination of Metoclopramide Hydrochloride in Tablet Dosage Form

Ravikumar Vejendla*[,1], Vasavi kudidhi[1], S.Sravanthi[1], G.Taruni[1], MD.Musthafa[2]

[1]Sri Indu Institute of Pharmacy, Ibrahimpatnam, Hyderabad, A.P., India.
[2]Nizam Institute of Pharmacy, Deshmukh, Nalgonda, A.P., India.

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Abstract

A simple, sensitive, and reproducible ultra-performance liquid chromatography (UPLC) coupled with a photodiode array detector method was developed for the quantitative determination of Metoclopramide hydrochloride (MTH) tablet dosage forms. Chromatographic separation was achieved on Acquity UPLC BEH C18 50 mm, 2.1 mm, and 1.7 µm column and a mobile phase consists of 0.1% ortho-phosphoric acid: Acetonitrile (86**:**14 V/V) within a short run time of 1.2 min.. The flow rate was 0.5 mL/min, temperature of the column was maintained at 27^{0}C, injection volume was 0.5 µL and detection was made at 213 nm. The developed method was linear for Metoclopramide hydrochloride 10% to 150% of the targeted concentration and the linear regression obtained was 0.9999. Precision, evaluated by intra- and inter-day assay had relative standard deviation (R.S.D) values within 1.31 %. The drug was subjected to the International Conference on Harmonization (ICH)-prescribed hydrolytic, oxidative, photolytic, and thermal stress conditions. The performance of the method was validated according to the present ICH guidelines for specificity, linearity, accuracy, precision and robustness.

1. INTRODUCTION

Metoclopramide hydrochloride is frequently used as an antiemetic and for treatment of gut mobility disorders. The drug has a short biological half-life and is usually administered in a dose of 10–15 mg four times daily in order to maintain effective concentrations throughout the day. However, in patients with significant degrees of renal impairment, therapy should be performed at reduced dosage [1]. In long-term therapy, fluctuation in the plasma concentrations, with high concentration peaks are common for such drugs with rapid absorption and elimination [2]. The secondary effects of Metoclopramide on the central nervous system in the form of extra pyramidal symptoms, will surface, if plasma levels markedly exceed therapeutic levels [3]. Such characteristics make Metoclopramide hydrochloride a suitable candidate for controlled release delivery Metoclopramide hydrochloride (MCH) is a white crystalline, odourless substance, freely soluble in water. Chemically, it is 4-amino-5- chloro-N-[2-(diethyl amino) ethyl]-2-methoxy benzamide monohydrochloride monohydrate.

A through literature survey has revealed the following reported methods for the estimation of the metoclopramide in bulk, formulation and biological samples [4-18]. To the best of our knowledge, a complete validated RP-UPLC method for the estimation of MCH either in bulk drugs or pharmaceuticals was not reported. Therefore, it was thought worthwhile to develop a simple, precise, accurate RP-UPLC method for the estimation of MCH in tablet dosage form.

Corresponding Author:
Ravikumar Vejendla,
Sri Indu Institute of Pharmacy,
Ibrahimpatnam, Hyderabad,
A.P., India.
Mobile: +91-9246806227
E-mail:ravikumar.vejandla@gmail.com

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Milli-Q-water was used throughout the experiment. Acetonitrile (S.D. Fine Chem., Mumbai, India) and ortho-phosphoric acid (Merck, Mumbai, India) were used. MCH standard was obtained from Lee pharma Limited, Hyderabad and tablet dosage forms were purchased from local pharmacy.

2.2 Instrumentation

The UPLC system consisted of high pressure pump, Photo diode array detector and 10 µL capacity injector loop. The column used was Acquity UPLC ™ BEH C18, 2.1 x 50 mm, 1.7µm column. The output signal was monitored and processed using Empower software.

2.3 Chromatographic Conditions

The mobile phase was filtered through 0.45 µm PVDF membrane filter and degassed using vacuum before delivered into the system. The chromatographic conditions used for the analysis were given below:

Mobile phase	: 0.1% Orthophosphoric acid: Acetonitrile (86:14% v/v)
Stationary phase	: Acquity UPLC ™ BEH C18, 2.1 x 50 mm, 1.7µm
Wavelength	: 213 nm
Injection volume	: 0.5 µl
Flow rate	: 0.5 mL min^{-1}
Column temperature	: 40^{0}C
Run time	: 2 min

2.4 Preparation of Solution

2.4.1 Preparation of diluent solution
Water, acetonitrile and Ortho-phosphoric acid were mixed in a ratio of 80:20:0.1% v/v.

2.4.2 Preparation of standard solution
25 mg of MCH working standard was accurately weighed and transferred into a 500 mL volumetric flask. 300 ml of diluent was added to the above flask and sonicated for 10 min to completely dissolve and made up the volume with diluent.

2.4.3 Preparation of sample solution
Twenty tablets of MCH were separately weighed and grounded to fine powder. An amount equivalent to 10 mg of MCH was transferred into a 200 mL volumetric flask and about 150 mL of diluent was added, sonicated for not less than 10 min with occasional stirring and made up the volume with diluent.

2.5 Method Validation
Method validation was performed as per ICH guidelines [19]. The following validation characteristics were addressed.

2.5.1 System suitability
Standard solution was used for the system suitability check. System suitability was analysed in terms of USP tailing factor (≤2.0), theoretical plate counts (≥30000) and % R.S.D. (relative standard deviation) for five replicate injections (should be ≤ 2.0).

2.5.2 Precision
The precision of the method was evaluated by carrying out six independent assays of MCH test sample against a qualified reference standard and the % R.S.D of assay was calculated. The intermediate precision of the method was also evaluated using a different analyst, different columns of the same brand (Waters Acquity, BEH C18, 50 x 2.1 mm, particle size 1.7 µm) and instrument (UPLC system, Waters Acquity) in the same laboratory.

2.5.3 Linearity
Test solutions were prepared from MCH stock solution at seven concentration levels from 10 to 150 % of analyte concentration (5, 12.5, 25, 37.5, 50, 62.5 and 75 µg/ml). The peak area versus concentration data was treated by least squares linear regression analysis.

2.5.4 Accuracy
The accuracy of the assay method was evaluated in triplicate at seven concentration levels (between 10 and 150 %), i.e., 5, 12.5, 25, 37.5, 50, 62.5 and 75 µg/ml. Percent recoveries were calculated.

2.5.5 Robustness
To determine the robustness of the developed method, experimental conditions were deliberately altered, and system suitability parameters were checked. The flow rate of the mobile phase was 0.5 ml/min. To study the effect of flow rate, flow rate was changed by 0.05 units from 0.45 to 0.55 ml/min, the proportion of acetonitrile in the mobile phase 14 % was changed by ±1 %, and UV detection wavelength (213 nm) was changed ± 3nm. To study the effect of column temperature, it was altered by ±0.1 units. To study the effect of concentration of phosphoric acid buffer, it was altered by ±1.0%. Changes in chromatographic parameters, i.e., theoretical plates, tailing factor and % R.S.D. were evaluated for the method.

3. RESULTS AND DISCUSSION

3.1 Method development
The main aim of the developed method was to achieve separation and quantification of MCH using an isocratic mobile phase with UPLC system. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis. Detection of MCH was adequate at 213 nm. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for MCH, which eluted at around 1.0 min giving a total run time of 1.2 min as shown in Fig 1.

3.2 Method Validation

3.2.1 System suitability
The system suitability results for the proposed UPLC method are given in Table 1. The results prove that the optimized UPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision
The % R.S.D. of MCH assay during the method precision and the intermediate precision was 1.3 and 1.1, respectively, indicating good precision of the method.

3.2.3 Linearity
The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 5 - 75 µg/ml, and the correlation coefficient obtained was > 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte. The results are shown in Table 2.

3.2.4 Accuracy
Percent recovery of MCH samples ranged from 101.2 to 102.0, and % R.S.D. values were within 1.0 %, showing the good accuracy of the method. The result is shown in Table 3.

3.2.5 Robustness
In all the deliberately varied chromatographic conditions, the assay results were between 98 and 101 % and no significant changes were obtained in chromatographic parameters (Table 3). This shows the robustness of the developed method.

4. CONCLUSION
The new, isocratic RP-UPLC method proved to be simple, linear, precise, accurate, robust and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 1.01 min allows the analysis of a large number of samples in a short period of time and is therefore more cost effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of MCH in tablet formulations.

Table 1: System suitability Data

System suitability Results				
Injection No.	**RT (in min)**	**Area**	**Tailing**	**Plate count**
1	0,974	203635	1.4	6555
2	0,976	204307	1.4	6591
3	0,976	204323	1.4	6596
4	0.977	203956	1.4	6624
5	0.974	203326	1.4	6638
Mean	**0.976**	**203909**	**1.4**	**6601**
SD	**0.002**	**432.1**	**0.001**	**32.2**
%RSD	**0.1**	**0.2**	**0.08**	**0.5**

SD: Standard Deviation

Table 2: Linearity Data

Concentrations (µg/ml)	**Area**
5	20142
12.5	49795
25	100484
37.5	150928
50	201579
62.5	251412
75	302694
Regression Statistics	
Multiple R	0.999986
R^2	0.999972

Table 3: Accuracy Data

Amount Added		% Recovery*	% RSD*
Level (%)	Concentration (µg/ml)		
10	5	102.0	0,4
25	12.5	101.2	0.5
50	25	101.4	0.3
75	37.5	101.6	0.5
100	50	102.2	0.8
125	62.5	101.6	0.2
150	75	101.8	0.2

** Average of three determinations*.

Table 3: Summary of Robustness results

Robust Parameter	Deliverable condition		RT	Response	USP Tailing	%RSD	USP Plate count
	Column No.	Column serial No.					
Different column Lots	1	1953124215721	0.976	203909	1.4	0.2	6605
	2	1953124215702	0.937	206101	1.5	0.4	4628
	3	1873034315787	1.123	206884	1.4	1.4	5128
	4	1683912615535	1.240	204299	1.6	1.6	7332
Phosphoric acid Buffer	**Strength**						
	0.09%(pH-2.18)		0.955	206013	1.4	0.5	6494
	0.10%(pH-2.21)		0.976	203909	1.4	0.2	6605
	0.11%(pH-2.15)		0.969	205661	1.4	0.8	6692
Organic Modifier	**Ratio change (%)**						
	13		1.148	205362	1.4	0.2	7281
	14		0.976	203909	1.4	0.2	6605
	15		0.847	206119	1.4	0.5	6063
Flow rate	**in mL/min**						
	0.45		0.966	201729	1.4	0.5	6670
	0.50		0.976	203909	1.4	0.2	6605
	0.55		0.958	202857	1.4	0.3	6648
Column Temperature	**in °C**						
	39		0.992	207717	1.4	0.9	6709
	40		0.976	203909	1.4	0.2	6605
	41		0.976	207395	1.4	1.1	6746
Wavelength	**in nm**						
	210		0.976	204945	1.4	0.5	6622
	213		0.976	203909	1.4	0.2	6605
	216		0.978	208755	1.4	1.9	6622

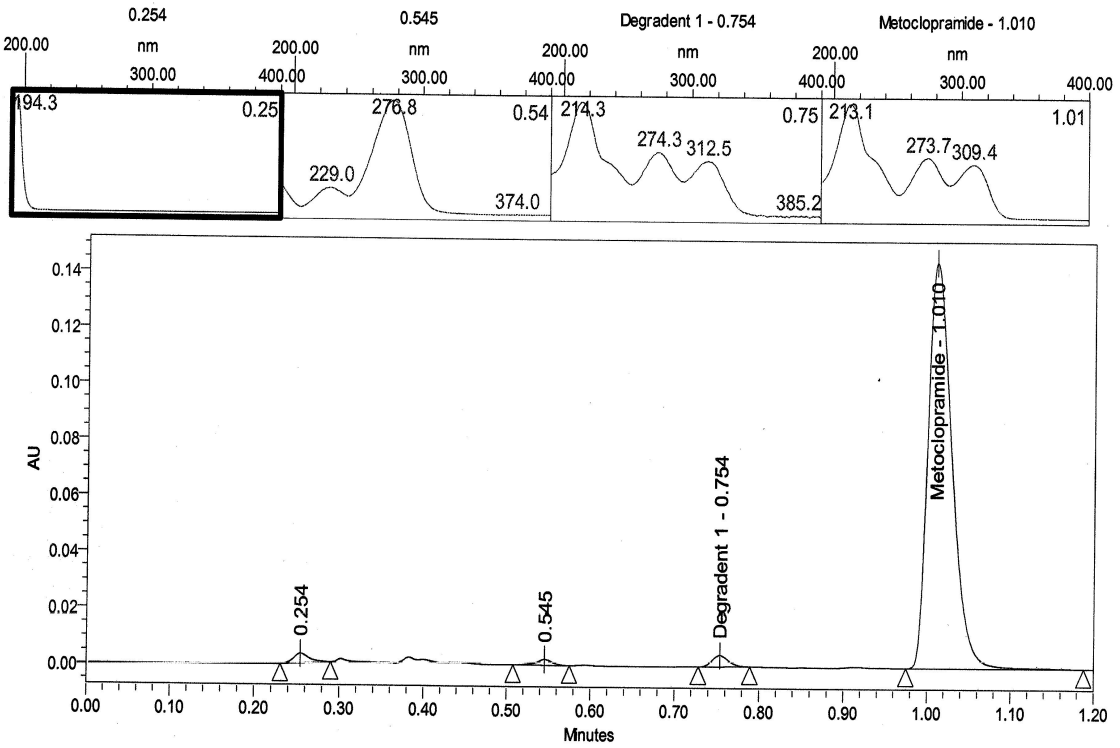

Fig.1: Chromatogram of Metoclopramide sample solution

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