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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Metoclopramide Hydrochloride by using RP-HPLC

Meghnath Chaudhary*, Dr. C. Sreedhar, T. Sreenivasarao, Katikala Dharmendra

Department of Pharmaceutical analysis, Karnataka College of Pharmacy, 33/2, Thirumenahalli, Hegde Nagar Main Road, Jakkur Post, Bengaluru, Karnataka 560064

A B S T R A C T

A simple, rapid, and sensitive high-performance liquid chromatographic method was developed and validated for determination of Metoclopramide Hydrochloride. Separation of Metoclopramide Hydrochloride was achieved within a single chromatographic run on an XBridge^{TM}column 5µm 4.6x250mm with UV detection at 274nm, under isocratic conditions, using Acetonitrile and Ammonium acetate buffer (P^{H}–3.7) in 55:45 ratio. Validation parameters were performed to demonstrate linearity, accuracy, precision, LOD & LOQ in accordance to ICH guidelines. The current method demonstrates good linearity over the range of 5-30µg mL^{-1}. The correlation coefficient (r^2) is 0.9918.Relative standard deviation for precision and accuracy was less than 2. Limit of detection (LOD) was found to be 0.1601 µg ml^{-1} and limit of quantification (LOQ) was found to be 0.5337µg ml^{-1}.

Keywords: Metoclopramide Hydrochloride, reverse phase, high performance liquid chromatography, validation

A R T I C L E I N F O

Corresponding Author
Meghnath Chaudhary
Department of Pharmaceutical Analysis,
Karnataka College of pharmacy,
#33/2, Thirumenahalli, Hegde Nagar main road,
Bangalore– 560064, KA. India.
MS-ID: JPBMAL3660

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C O N T E N T S

1. Introduction

Metoclopramide Hydrochloride (MCP) is used as Dopamine receptor antagonist; antiemetic. Chemically it is 4-amino-5-chloro- N-[2-(diethyl amino) ethyl]-2-methoxybenzamide hydrochloride (Fig1). MCP is used as

Dopamine receptor antagonist; antiemetic. It is available as white or almost white, crystalline powder or crystals which is very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride. This antiemetic, chemically related to the procainamide, acts predominantly as a dopamine antagonist[1,2] Chromatographic methods have been described for the quantitative determination of metoclopramide in formulations as well as biological fluids. These include gas chromatography [7, 8] and high performance liquid chromatography [9- 10]. These previous published methods comprise of complicated mobile systems and are not directly applicable for this novel type of dosage form which is prepared and need more investigation for method development and validation. Therefore, the main aim of this work was to develop and validate a stability indicating RP HPLC method for estimation of metoclopramide hydrochloride from a novel orally disintegrating tablet containing pellet formulation.

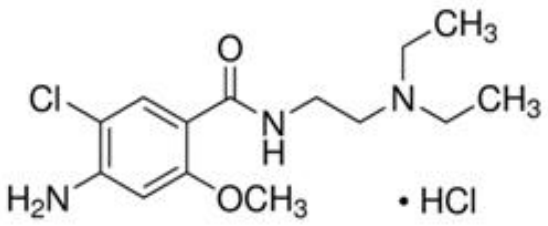

Figure 1: Chemical structure of metoclopramide

2. Materials and Methods

Drug Reagents and chemicals

Perinorm is a tablet dosage form each contains 10mg of metoclopramide hydrochloride. HPLC grade Acetonitrile (Merck), Analytical grade Ammonium Acetate was used as the solvents throughout the experiment. Pharmaceutical formulation Perinorm tablet (label claim contain 10mg) was used in HPLC analysis. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

Instrumentation

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on aAgilent Eclipse X- Bridge C18 column (5µm 4.6x250mm), column length is 15 cm with UV detection at 274nm. Analytical weighing balance. (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON230VAC, 50Hz), vaccum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam uv-visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data.

Chromatographic condition

After several trials with the different combination and ratio of solvents, the mobile phase ammonium acetate (buffer): acetonitrile (45:55v/v) at flow rate 1ml/min P^H 3.7 .Retention time (R_t) 3.16 min for metoclopramide hydrochloride. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component show reasonably good response and maximum peak at 274nm.

Standard solutions for HPLC estimation of metoclopramide hydrochloride: A tablet is powdered which contain 10 mg of active ingredient is transferred into 10 ml of volumetric flask and is dissolved in mixture of acetonitrile and the buffer(50:50) volume were made up to the mark with same solvent This gave the concentration of 1000 µg ml^{-1} of metoclopramide hydrochloride (Stock-1) . From stock solution 1, 6 dilution was prepared between 5-30µg ml^{-1} which is working concentration.

Method development and validation

A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of metoclopramide hydrochloride in the bulk drug and in the formulation. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The method was validated according to ICH [3-4] and USP guidelines [5]. The validation parameters addressed were specificity, precision, accuracy, linearity and robustness [6].

3. Results and Discussion

Method Development

Of several solvents and solvent mixtures investigated the mobile phase consisting of, using Acetonitrile and Ammonium acetate buffer (P^H – 3.7) in 55:45 ratio. Validation parameters were performed to demonstrate linearity, accuracy, precision, LOD & LOQ in accordance to ICH guideline. The retention time for the drug was 3.2 min. A typical representative chromatogram is shown in Figure 2.

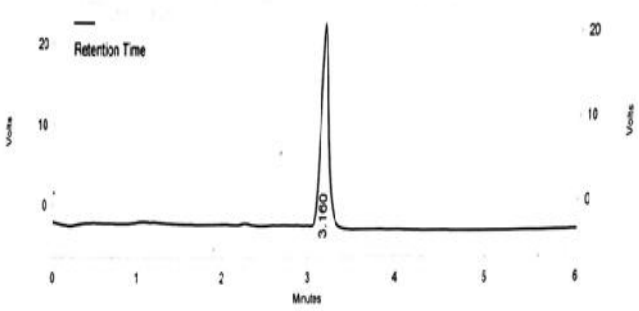

Figure 2: A typical chromatogram for metoclopramide hydrochloride

Linearity and range

Calibration curves that were constructed for metoclopramide hydrochloride were linear over the concentration range of 5 to 30µg/ml. The peak area was plotted versus the concentration. The equation for the resultant calibration curve was y = 538029x + 152662 with a linear regression coefficient of 0.9918.

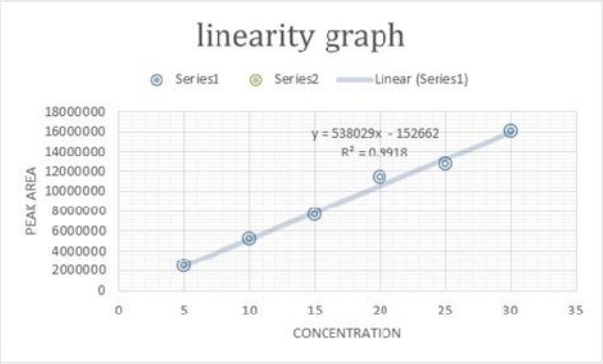

Figure 3: Linearity graph of metoclopramide hydrochloride

Table 1: Linearity data for metoclopramide hydrochloride

Concentration µgml^{-1}	**Area**
5	2500971
10	5218585
15	7615822
20	11431030
25	12776416
30	16034244

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogenous sample. The inter day (between 2 days) and intraday (at the same days: morning and evening) precision were carried out. The variations of results were calculated and %RSD was determined.

4. Conclusion

This developed RP-HPLC method for estimation of metoclopramide hydrochloride from pellets is accurate, precise, robust, specific, and stability-indicating. The method has been found to be better than previously reported methods, because of its less retention time, use of readily available mobile phase, UV detection and better resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing metoclopramide hydrochloride. All these factors make this method suitable for quantification of metoclopramide hydrochloride in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

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Table 2: Intraday precision of metoclopramide hydrochloride (morning)

Morning				
Injection (15µg/ml)	**Areas**	**Average**	**Sd**	**Rsd**
1	7784037	7690594	46094.57	0.59
2	7668002			
3	7670245			
4	7665378			
5	7680400			
6	7675504			

Table 3: Intraday precision of metoclopramide hydrochloride (afternoon)

Afternoon				
Injection (15µg/ml)	**Areas**	**Average**	**Sd**	**Rsd**
1	7760697	7721837	46998.14	0.6
2	7791690			
3	7670772			
4	7727592			
5	7691525			
6	7688746			

Table 4: Intraday precision of metoclopramide hydrochloride (day 1)

Day1				
Injection (15µg/ml)	**Areas**	**Average**	**Sd**	**Rsd**
1	7754104	7685104		
2	7798518			

3	7717862		84655.31	1.1
4	7592003			
5	7601515			
6	7646622			

Table 5: Intraday metoclopramide hydrochloride (day 2) precision

Day2				
Injection (15µg/ml)	**Areas**	**Average**	**Sd**	**Rsd**
1	7683557	7664471	37500.65	0.48
2	7639220			
3	7601398			
4	7688907			
5	7701884			
6	7671857			

Accuracy: The accuracy for estimation of metoclopramide using acetonitrile was determined by adding known amount of the analyte. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

Table 6: Accuracy for metoclopramide hydrochloride

S.N	**Level of percentage recovery**	**Amount present (mg/tablet)**	**Amount of standard drug added**	**Area response**	**Mean**	**SD**	**RSD**	**Total amount recovery**	**% recovery**
1	80%	10	8	7572240	7635249	56582.27	0.74	10.02	100.2
				7681718					
				7651790					
2	100%	10	10	11404069	11432140	64029.56	0.56	10	100
				11505411					
				11386941					
3	120%	10	12	13844734	13893282.33	86104.30	0.61	10.88	100.88
				13842415					
				13992698					

Robustness: Robustness was determined by varying the mobile phase flow rate to ± 0.1 ml/min (i.e. 0.9, 1 and 1.1 ml/ min) and the absorption maxima of the drugs by ±5 (i.e. 269, 274 and 279). The deliberate changes in the flow rate, and the change in absorption maxima did not affect the recovery of the drug which indicated the robustness of the method.

Table 7: Robustness of metoclopramide hydrochloride

S.N	**Parameter**	**Optimized**	**Used**	**Retention time (min)**
1	Flow rate	1 ml/min	0.9 ml/min	3.527
			1.1ml/min	2.883
2	Detection wavelength	274 nm	269nm	3.173
			279 nm	3.177

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