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ORIGINAL ARTICLE **OPEN ACCESS**

Method Development and Validation of Stability Indicating RP-HPLC Assay Method for Estimation of Metoclopramide Injection

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ABSTRACT

A new, simple, specific, precise and robust isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for determination of metoclopramide hydrochloride from a liquid formulation. The liquid chromatographic separation was achieved Gradient employing a mobile phase of acetonitrile: water (25:75), and pH adjusted to 7.0 using ammonia. The analysis was administered using X-Bridge C18, 4.6×150 mm, 3.5µm at flow of 0.9 ml/min and therefore the PDA detection at 275nm. The tactic was validated for accuracy, precision, linearity, range, selectivity and robustness. The linearity of the proposed method was investigated within the range of 0.5–18 µg/ml (r2 = 1.000). The drug was subjected to oxidation, hydrolysis, heat and photolysis to use stress conditions. The tactic provided good peak parameters with run time of 20 min. The retention time of 9.7 min. Degradation products resulting from stress studies didn't interfere with the detection of metoclopramide hydrochloride and thus the assay can thus be considered as stability-indicating.

Keywords: *Metoclopramide hydrochloride, high-performance liquid chromatographic, validation*

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INTRODUCTION

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-N- (2-diethylamino-ethyl) benzamine, is dopamine receptor antagonist, it's also used as drugs for cancer chemotherapy induced emesis at higher doses. Metoclopramide has been approved by the FDA specifically to treat nausea and vomiting in patients with gastro oesophageal reflux disease or diabetic gastro paresis by increasing gastric motility. It is also wont to control nausea and vomiting in chemotherapy patients.[1,2] It also exerts effects on the area postrema of the brain, preventing and relieving the symptoms of nausea and vomiting. Metoclopramide is rapidly absorbed in the gastrointestinal tract with an absorption rate of about 84%. The bioavailability of the 40.7%, but can range from 30-100% in oral preparation.[3]

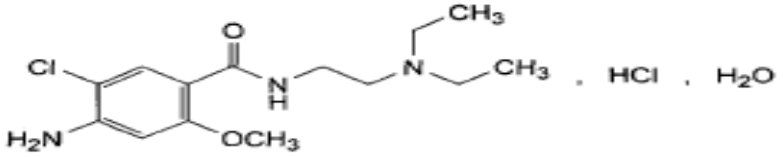

Fig. No 1 Chemical structure of metoclopramide hydrochloride

The analytical methods reported for the determination of Metoclopramide in dosage forms and in biological fluids are various chromatographic procedures. However actually because of consumption of more organic solvents, having end of the day time, utilize solid phase extraction which is time consuming and can cause a lower drug recovery once they're used. This study describes a specific, sensitive and price effective rapid assay with short run time of 5 minutes for determination of metoclopramide hydrochloride in its formulations. [4,5]

MATERIAL AND METHODS

All other ingredients utilized in formulation were obtained from Emparta grade. Acetonitrile HPLC used was obtained from Merk (Darmstadt, Germany). Water was deionized then doubly distilled. Ammonium

Acetate was Emparta grade. Orthophosphoric acid is Emparta grade. Other chemical like Ammonia, acid, sodium hydroxide pellets, peroxide (50%) are Emparta grade from Merk.

EQUIPMENTS

Analytical balance (Digital Analytical balance).The Model of XS205 .it Make of Mettler Toledo. The Capacity of Max.80g, Min. 20mg.Liquid Chromatograph (LC10AT, Shimadzu, Japan), Communications Bus Module (CBM-102, Shimadzu, Japan), Waters C18 3.9×300mm µBondapak (RP) Column, pH meter (370 pH meter, Jenway, Europe), Syringe (Hamilton Company, Reno, Nevada), Swinney filter (stainless steel, 13 mm filter, Millipore Corporation, Billerica, USA), Membrane Disc Filters (MILLIPORE, 0.45µm pore size, Millipore Corporation, Billerica, USA), Sonicator (LC20H), Vortex (FSA Lab., England)

Instrumentation and chromatographic conditions –

High performance liquid chromatographic system (LC10A VP, Shimadzu, Japan), consisting of pump (LC-10A vp, Shimadzu, Japan), Communications Bus Module (CBM-102, Shimadzu, Japan), UV–VIS detector (Thermo, Shimadzu, Japan), computer (Pentium 4) and printer (HP LaserJet 2015). For the separation, X-Bridge C18, 4.6×150 mm, 3.5 µm (RP) Column was used and the chromatograms were recorded in the Lab solution, Empower software. Mobile phase was prepared from acetonitrile and buffer (Potassium Dihydrogen Phosphate, pH adjusted to three with orthophosphoric acid) within the ratio of 40:60, filtered through 0.45µm and degassed. The flow rate was kept at 0.9 ml/ minute. The Injection volume of 20µl was injected and detected at 275nm and the separation was carried out at ambient temperature.

Preparation of solutions–

Mobile Phase A: Weigh 5g of ammonium acetate and dissolve in 1000ml of water, adjusted pH 7.0 ± 0.05 with ammonia.

Mobile Phase B: Acetonitrile

Preparation of 0.01 M Phosphoric acid: Mix 0.7 mL of 85% phosphoric acid into 1000 mL of water and mix well.

Diluent: Use 0.01M Phosphoric acid as diluent

Preparation of Blank: Use Diluent as Blank.

Preparation of Standard solution: Weigh accurately 45 mg of Metoclopramide HCl Standard and transfer into 100 mL volumetric flask, dissolve and dilute to volume with diluent. Further 5.0 mL of this solution transfer into 50 mL flask dilute up to the mark with diluent and mix well. **(Concentration of Standard solution: 45ppm)**

Note: Prepare Standard solution in duplicate as standard solution 1 and standard solution 2.

Preparation of formulations of metoclopramide hydrochloride: The Tablet was prepared by compressing sustained release pellets with sugar pellets. The pellets were formulated using palletizations method incorporating ingredients like Avicel, psyllium husk, polyvinyl pyrollidone, sodium bicarbonate and ethyl cellulose.

Preparation of Sample solution: Determine the wt. /mL of sample. Accurately weigh the sample equivalent to 15 mg of metoclopramide in to 20 mL volumetric flask and dilute up to the volume with diluent. Further 3.0 mL of this solution transfer into 50 mL flask, dilute up to the mark with diluent and mix well. **(Concentration of Sample solution: 45ppm)**

Method development-

A variety of mobile phase were investigated in the development of an HPLC method suitable for analysis of metoclopramide hydrochloride in the bulk drug and in the formulation. Buss et al, in 1990 reported HPLC Method for the estimation of Metoclopramide Hydrochloride in blood (Buss et al., 1990).This reported method was modified and validated for the determination of drug in Liquid dosage formulations.[6,7]

Method Validation –

A. ***Force Degradation:***

1) **Photolytic Degradation -**Taken sample in vial (one open and another wrap with aluminium foil) exposed it under UV and white light for 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt/square for 3 days After Degradation accurately weighed the sample equivalent to 15 mg of metoclopramide in to 20 mL volumetric flask with diluent. Further 3.0 mL of this solution transferred into 50 mL flask, diluted up to the mark with diluent and mixed well.

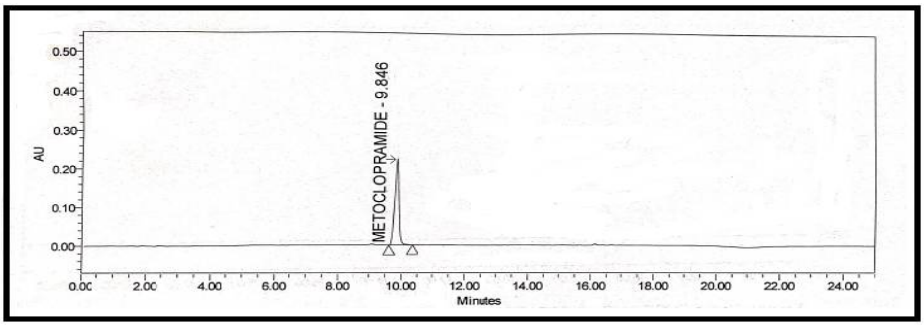

Fig. 2 Chromatogram of Photolytic Degradation

2) Heat degradation: Exposed the sample at 105°C in oven for 24 hours. After Exposure, allowed it to cool to room temperature. After degradation accurately weighed the sample equivalent to 15 mg of metoclopramide 20 mL volumetric flask and diluted with diluent. Further 3.0 mL of this solution transferred into 50 mL flask, diluted up to the mark with diluent and mixed well.

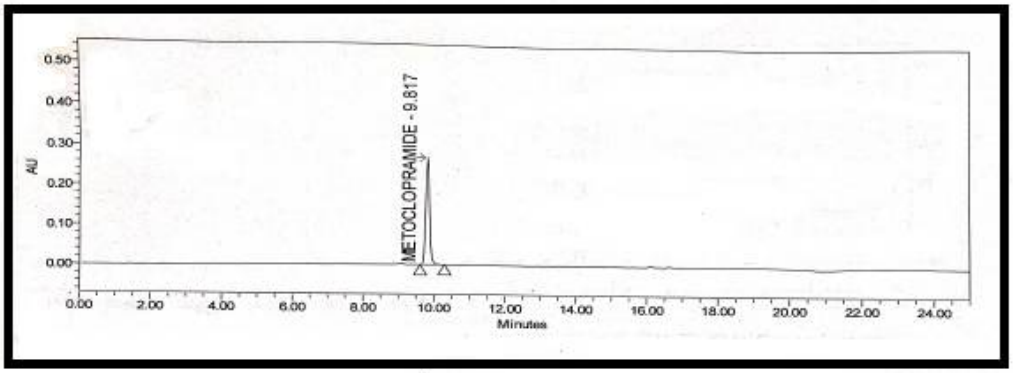

Fig. 3 Chromatogram of Heat degradation

3) Acid degradation: Accurately weighed the sample equivalent to 15 mg of metoclopramide in to 20 mL volumetric flask, add 2 mL of 5 M Hydrochloric acid solution and kept for 24 hour the water bath at 80ºC, neutralized this solution before dilution with 2 mL of 5 M Sodium hydroxide and made up the volume with diluent, and mixed well. After Degradation, 3.0 mL of this solution transferred into 50 mL flask, diluted upto the mark with diluent and mixed well.

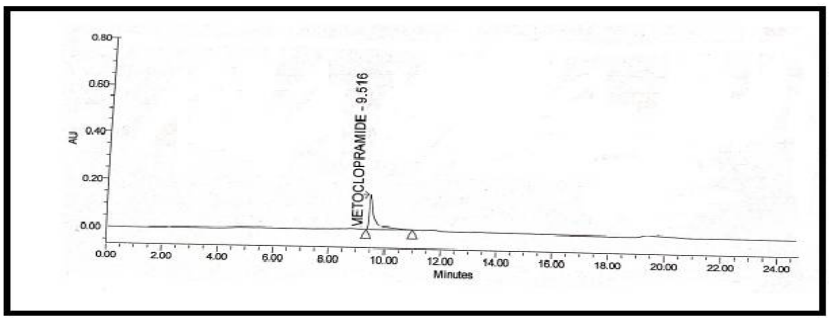

Fig. 4 Chromatogram of Acid degradation

4) Base degradation: Accurately weighed the sample equivalent to 15 mg of metoclopramide in to 20 mL volumetric flask, add 2 mL of 5 M Sodium hydroxide solution and kept for 24 hours on the water bath at 80ºC, Neutralized this solution before dilution with 2 mL of 5 M Hydrochloric acid solution and made up the volume with diluent, and mixed well. After Degradation, 3.0 mL of this solution transferred into 50 mL flask, diluted upto the mark with diluent and mixed well.

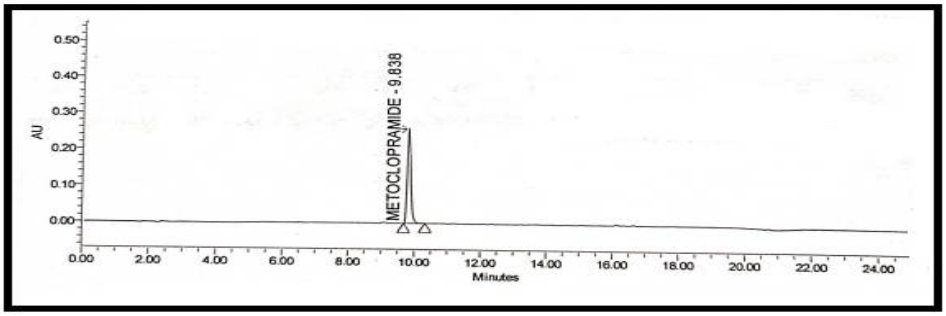

Fig. 5 Chromatogram of Base degradation

5) Peroxide degradation: Accurately weighed the sample equivalent to 15 mg of metoclopramide in to 20 mL volumetric flask, added 2 mL of 50 % hydrogen peroxide solution and heated the solution on the water bath for 80 ºC for 24 hours, allowed it to cool to room temperature and made up to volume with diluent and mixed well. Further 3.0 mL of this solution transferred into 50 mL flask, diluted upto the mark with diluent and mixed well.

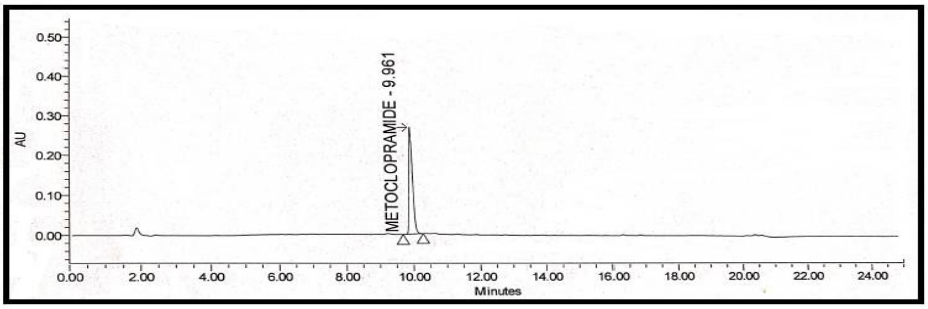

Fig. 6 Chromatogram of Peroxide degradation

6) Humidity degradation: Taken sample in a vial and exposed it at 25°C and 90% RH chamber for 2 days. After exposure, allowed it to cool to room temperature. Accurately weighed the sample equivalent to 15 mg of metoclopramide in 20 mL flask and diluted with diluent and mixed well. Further 3.0 mL of this solution transferred into 50 mL flask, diluted up to the mark with diluent and mixed well.

Parameters studied for analytical assay validation were linearity, selectivity, accuracy, precision, system suitability, solution stability, LOD, and LOQ under the guidelines of ICH Q2B (International Conference on the Harmonization, 1996).[8,9]

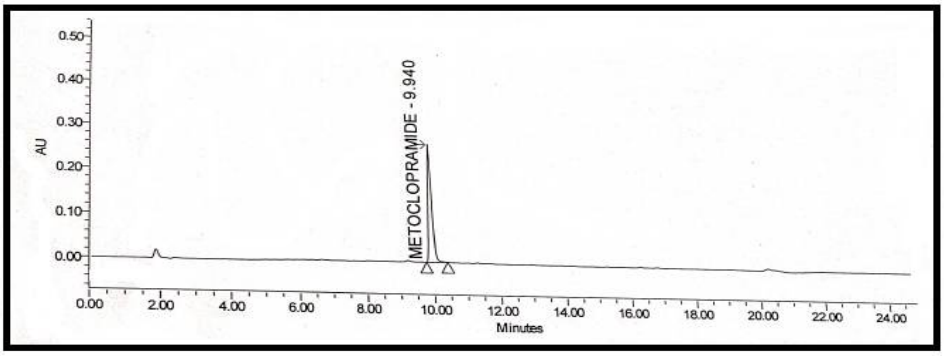

Fig. 7 Chromatogram of Humidity degradation

B. Linearity and Range:

Linearity was evaluated at 50% to 150% of the working concentration level. The working concentration level of Metoclopramide is about 45 ppm.

Acceptance criteria for Linearity: Residual sum of square, Slope of regression line,% limit of y-intercept to be reported. Response should be linear. Co-relation coefficient (R) shouldn't be but 0.999. % Limit of Y-intercept should be within ± 2.0 % of the corresponding Y-co-ordinate of the working.

Table No.1 Linearity

Component	Metoclopramide
Tailing Factor	**1.24**
Theoretical plates	**88799**
S. No.	**Area**
1	2380380
2	2378328
3	2377306
4	2379731
5	2381226
6	2380465
Mean	**2379572.667**
SD	**1476.0487**
%RSD	**0.06**
% RSD of Bracketing Standard Solution	**0.13**
The Correlation between 1st standard solution and 2nd standard solution	**99.1**

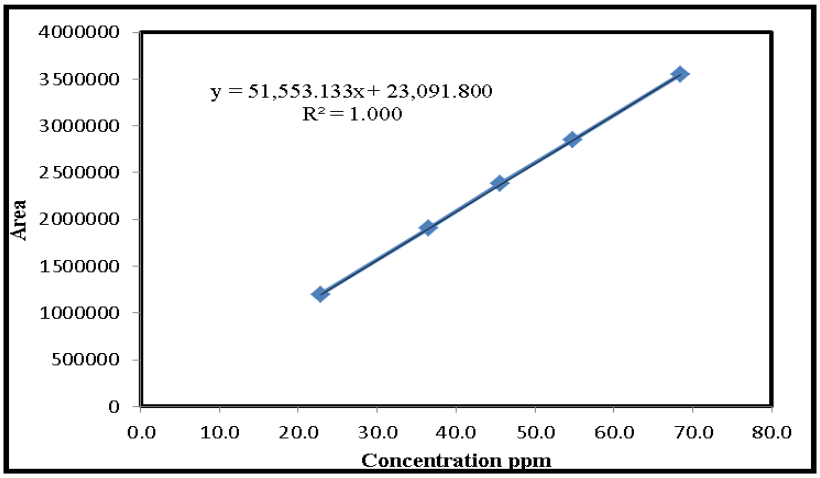

Fig no.8 Chromatogram of Linearity

C. Accuracy (Recovery):

The accuracy was evaluated at three levels 50%, 100% and 150% of the corresponding working concentration level for Metoclopramide. Working concentration level of Metoclopramide is 45 ppm. From the amount added, amount found and % recovery was calculated.

Acceptance criteria for Accuracy:

Mean recovery for 50% to 150% should be in the range of 98.0%-102.0% and individual recovery for 50% to 150% should be in the range of 97.0%-103.0%.[10]

Table No.2 Accuracy

Component	Metoclopramide
Tailing Factor	**1.23**
Theoretical plates	**98392**
S. No.	**Area**
1	2388543
2	2382523
3	2383821
4	2383224
5	2383208
6	2383650
Mean	**23841615**
SD	**2193.0739**
%RSD	**0.09**
% RSD of Bracketing Standard Solution	**0.32**
The Correlation between 1st standard solution and 2nd standard solution	**98.7**

D. Precision:

1. System Precision: Single injection of Blank (Diluent) and six replicate injections of standard solution were injected on the system.

Acceptance criteria for System precision -The Relative standard deviation for peak area of Metoclopramide in six replicate injections should not be more than 2.0%.

Table 3. System suitability of standard solution

Component	Metoclopramide
Tailing Factor	**1.24**
Theoretical plates	**88799**
S. No.	**Area**
1	2372462
2	2381794
3	2377458
4	2378902
5	2370404
6	2374149
Mean	**2375861.5**
SD	**4272.272**
%RSD	**0.06**
% RSD of Bracketing Standard Solution	**0.19**
The Correlation between 1st standard solution and 2nd standard solution	**99.6**

2. Method Precision: Six independent sample preparations were prepared and injected in duplicate on the HPLC.

Table No.4 Precision

Component	Metoclopramide
Tailing Factor	**1.24**
Theoretical plates	**88799**
S. No.	**Area**
1	2377215
2	2381426
3	2380380
4	2375731
5	2378328
6	2380465
Mean	**2378924.161**
SD	**2201.1709**
%RSD	**0.09**
% RSD of Bracketing Standard Solution	**0.11**
The Correlation between 1st standard solution and 2nd standard solution	**99.1**

E. Ruggedness-Six independent sample preparations were prepared and injected in duplicate on the HPLC. The RSD for % assay of six independent samples should not be more than 2.0%. Absolute difference for mean % Assay obtained from method precision and intermediate precision should not more than 3.0.[11]

Solution Stability:

The Standard solution and sample solution was kept at sample temperature condition (25°C). And injected from time to time on to the HPLC the absolute difference of % assay should be within ± 3.0, when compared to the initial solution.

F. Robustness: This parameter was studied after making small, deliberate changes in the chromatographic conditions and observing the effect of these changes on the system suitability parameters and % Assay by injecting standard and sample solutions the absolute difference of % Assay value in each modified condition should be within ± 3.0 when compared to the original condition.[12,13]

Table No.4 Validation Parameters

Changes in parameters	Values	Retention time of Metoclopramide	Tailing Factor of standard	Theoretical plates of standard	% RSD of standard area
Control (Precision)	As per method	10.090	1.24	88799	0.06
Change in Wavelength (±2 nm)	273 nm	9.764	1.21	41426	0.17
	277 nm	9.764	1.21	41442	0.13
Change in Column temperature (± 5°C)	32°C	9.867	1.24	41777	0.07
	42°C	9.687	1.25	40664	0.27
Change in pH of mobile phase (± 0.2 units)	6.8	9.752	1.30	31803	0.26
	7.2	9.853	1.35	28388	0.11

RESULT AND DISCUSSION

The liquid chromatographic separation was achieved Gradient employing a mobile phase of acetonitrile: water (25:75), and pH adjusted to 7.0 using ammonia. The analysis was administered using X-Bridge C18, 4.6×150 mm, 3.5µm at flow of 0.9 ml/min and therefore the PDA detection at 275nm. The tactic was validated for accuracy, precision, linearity, range, selectivity and robustness. [14] The linearity of the proposed method was investigated within the range of 0.5–18 µg/ml (r2 = 1.000). The drug was subjected to oxidation, hydrolysis, heat and photolysis to use stress conditions. The tactic provided good peak parameters with run time of 20 min. The retention time of 9.7 min. Degradation products resulting from stress studies didn't interfere with the detection of Metoclopramide hydrochloride and thus the assay can thus be considered as stability-indicating.[15]

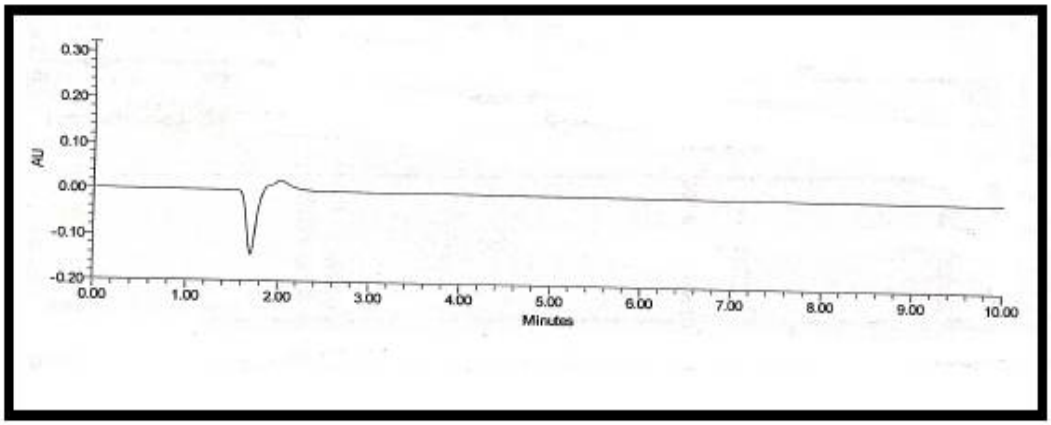

Figure 9: Typical Blank chromatogram of metoclopramide Hydrochloride

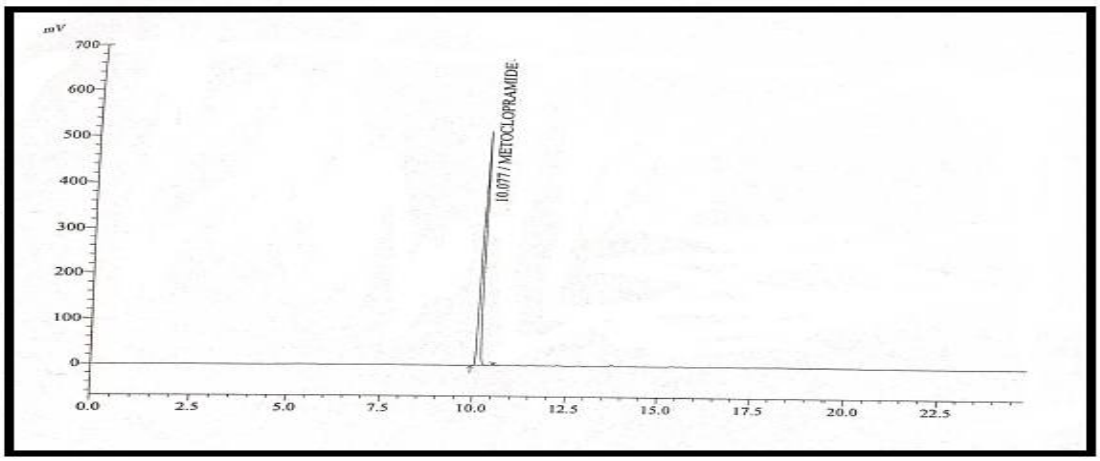

Figure 10: Typical Standard chromatogram of metoclopramide Hydrochloride

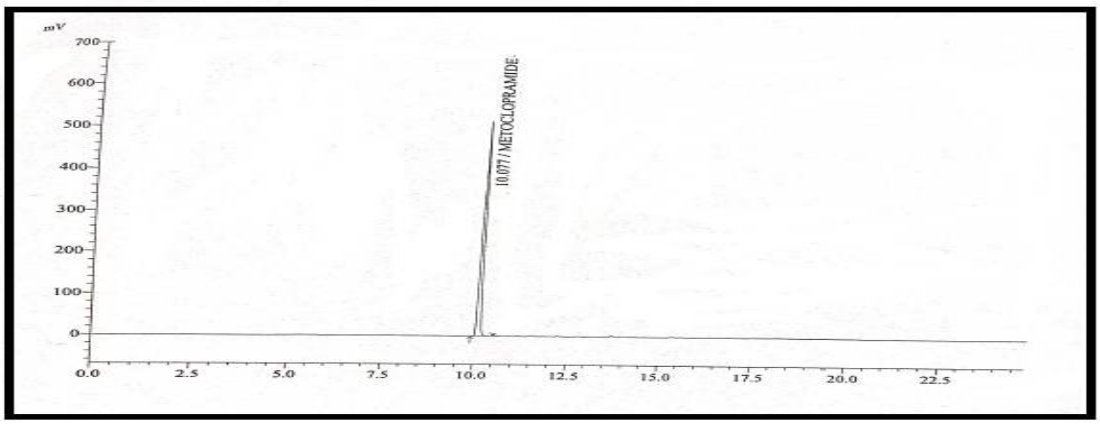

Figure 11: Typical chromatogram of sample of metoclopramide Hydrochloride

CONCLUSION

The high-performance liquid chromatographic method for measuring metoclopramide hydrochloride in pharmaceutical dosage formulations. The method has been shown to be specific for Metoclopramide HCl Injection. The method has been shown to be precise, Linear and Accurate across the range of 50% to 150% of working concentration of Metoclopramide. The method can be used in quality control laboratory for release of production batches and stability study.

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