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DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ANALYSIS OF METOCLOPRAMIDE IN PHARMACEUTICAL DOSAGE FORM AND PLASMA

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Metoclopramide in tablet dosage form. An Zodiac C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Acetonitrile:1%TEA 50:50 (/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 250nm. The retention time for Metoclopramide was 2.565 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.03ppm and 0.099ppm respectively and recovery of Metoclopramide from tablet formulation was found to be 8.73%. The proposed method was successfully applied for the quantitative determination of Metoclopramide in tablet formulation.

Key words: Metoclopramide, HPLC, Linearity, Validation.

INTRODUCTION

Metoclopramide is an antiemetic and gastroprokinetic agent described by Dr. Louis Justin-Besançon and C. Laville in 1964[1]. Thus it is primarily used to treat nausea, vomiting and to facilitate gastric emptying in patients with gastroparesis[2,3]. It is also a primary treatment for migraine headaches. Metoclopramide is the most common cause of drug-induced movement disorders. Metoclopramide is commonly used to treat nausea and vomiting (emesis) associated with conditions including: emetogenic drugs, uremia, radiation sickness, malignancy, labor and infection. It is considered ineffective in post operative nausea and vomiting (PONV) at standard doses and ineffective for motion sickness. In nausea and vomiting associated with cancer chemotherapy, it is been superseded by the more effective 5-HT_3 antagonists (e.g. Ondansetron). However, it is still a drug of choice for prophylaxis in low emetic risk chemotherapy regimens. It is also used for the prevention of nausea and vomiting when the patient is given an opiate, such as morphine.Metoclopramide has long been used in all stages of pregnancy with no evidence of harm to the mother or unborn baby [4]. The anti-emetic action of Metoclopramide is due to its antagonist activity at D_2 receptors in the chemoreceptor trigger zone (CTZ) in the central nervous system (CNS), this action prevents nausea and vomiting triggered by most stimuli [5]. At higher doses, 5-HT_3 antagonist activity may also contribute to the anti-emetic effect. The pro kinetic activity of Metoclopramide is mediated by muscarinic activity, D_2 receptor antagonist activity and 5-HT_4 receptor agonist activity [6][7]. The pro kinetic effect itself may also contribute to the anti-emetic effect.

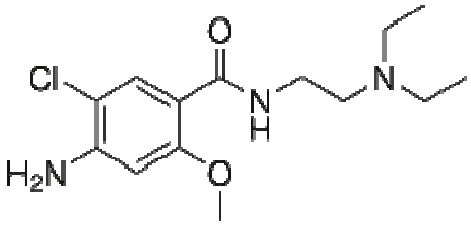

Figure.1 Structure Of Metoclopramide

IUPAC Name is 4-amino-5-chloro-*N*-(2-(diethylamino) ethyl) - 2 methoxybenzamide.Formula **is** $C_{14}H_{22}ClN_3O_2$. Molecular weight is 299.80 g/mol

REAGENTS AND EXPERIMENTS

Instrumentation: To develop a Liquid chromatographic method for quantitative estimation of Metoclopramide, an isocratic PEAK HPLC instrument and a Chromosil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Data was

analyzed by using PEAK software. Elico SL159 UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Denwar balance was used for weighing of the materials.

Chemicals and Solvents: The reference sample of Metoclopramide (API) was obtained from Lupin, Ahmedabad. The Formulation was procured from the local market. Acetonitrile, Methanol and Triethylamine used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

The buffer solution: About 10.0 mL of Triethylamine was diluted to 1000 mL with water. This solution was mixed and filtered through 0.45µ nylon filter.

The Mobile phase: A mixture of Acetonitrile and 1% Triethylamine in the ratio of 50:50%, v/v was prepared and used as mobile phase.

Standard solution of the drug: For analysis a 100 ppm standard solution was prepared in mobile phase, and further required concentrations were obtained from 100 ppm solution by proper dilution.

Sample (tablet) solution: The formulation tablets of Metoclopramide (PERINORM - 10 mg) were crushed to give finely powdered material. From the powder prepared 2.0ppm solution with Mobile phase and then filtered through Ultipor membrane sample filter paper.

METHOD DEVELOPMENT

For developing the method [13-19], a systematic study on the effect of various factors was carried out by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate detection wave length and stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength: The UV absorption spectrum of diluted solutions of the Metoclopramide (at 0.5 and 3.5ppm) in methanol was recorded separately on a UV spectrophotometer. Both the spectra of Metoclopramide showed maximum absorbance at 270 nm which is selected as detection wavelength for further studies.

Choice of stationary phase: Preliminary development trials have performed with octadecyl columns of different types and configurations from different manufacturers. Finally the expected separation and good peak shapes were succeeded on Chromosil C18 column (250 mm x 4.6 mm, 5µm).

Selection of the Mobile phase: In order to get sharp peak and base line separation of the components, carried out a number of experiments by varying the composition of various solvents and its flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like Methanol, Water

and Acetonitrile with or without different buffers indifferent combinations were tested as mobile phase. A mixture of Acetonitrile and 1% Triethylamine in the ratio of 50:50%, v/v was proved to be the most suitable out of all combinations, as the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Flow rate: Flow rate of the mobile phase was changed from 0.5 – 1.5 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions: Chromatographic conditions as optimized above were shown in Table.1 These optimized conditions were followed for determination of Metoclopramide in bulk samples and in its tablet formulations. The chromatograms of standard, blank, tablet sample and plasma sample were shown in Figures 1, 2, 3 and 4 respectively.

Table.1 Optimized chromatographic conditions for estimation Metoclopramide

Mobile phase	Acetonitrile : 1% Triethylamine 50:50%, v/v
Pump mode	Isocratic
Mobile phase pH	6.8
Diluent	Mobile phase
Column	Chromosil C18 column (250 mm x 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	270 nm
Injection Volume	20 µL
Flow rate	1.0 mL/min
Run time	6 min
Retention Time	2.56 min

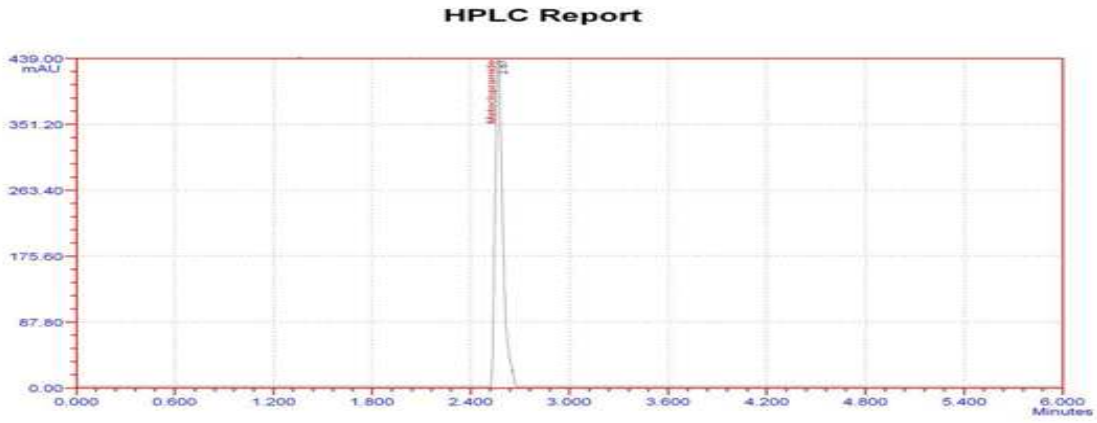

Figure.2 Chromatogram of standard solution

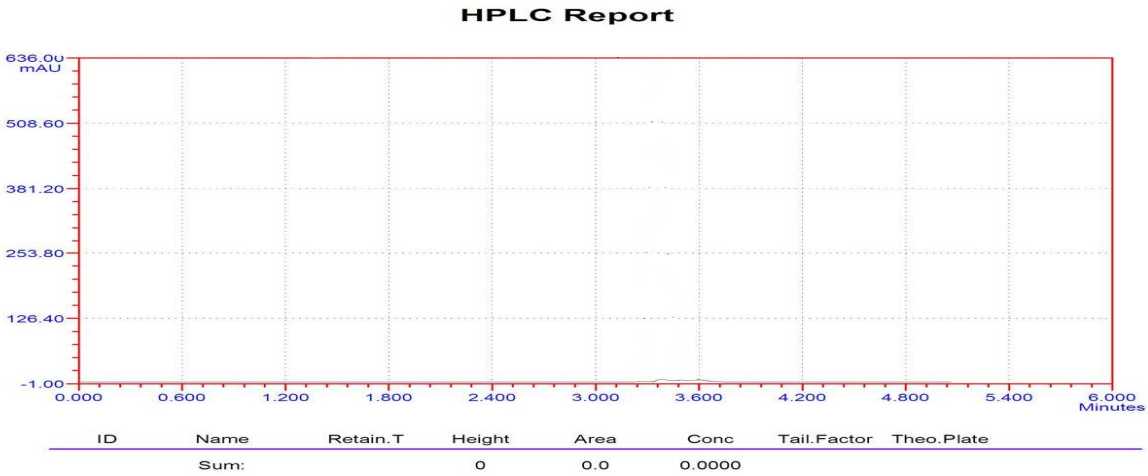

Figure.3 Chromatogram of Blank solution

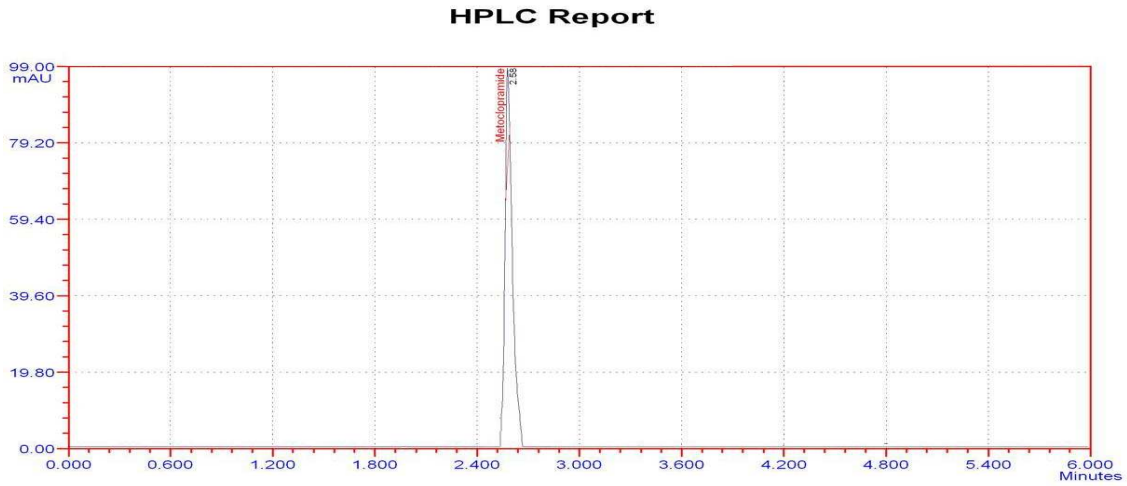

Figure.4 Chromatogram of formulation

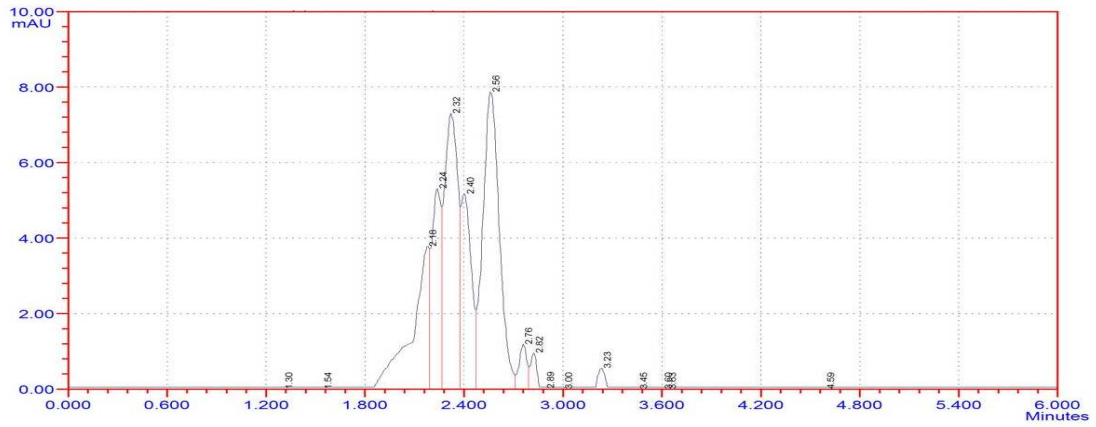

Figure.5 Chromatogram plasma sample

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated [20-28] as per ICH guidelines [20]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity: The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference resulting from excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in Table.2

Table 2: Specificity study

Name of the solution	Retention Time in Min
Blank	No peak
Standard	2.565

Linearity: Linearity was performed by preparing standard solutions of Metoclopramide at different concentration levels including working concentration mentioned in experimental condition i.e. 2.0 ppm. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 270 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table.3 and calibration plot was shown in Figure.6

Table .3 Linearity Results

Level	Concentration of Metoclopramide In ppm	Mean peak area
Level -1	0.5	78000
Level -2	1.0	137155
Level -3	1.5	203690
Level -4	2.0	261471
Level -5	2.5	312874
Level -6	3.0	377123
Level-7	3.5	435145
Range: 0.5 ppm to 3.5 ppm	Slope Intercept Correlation coefficient	118611 20700 0.9993

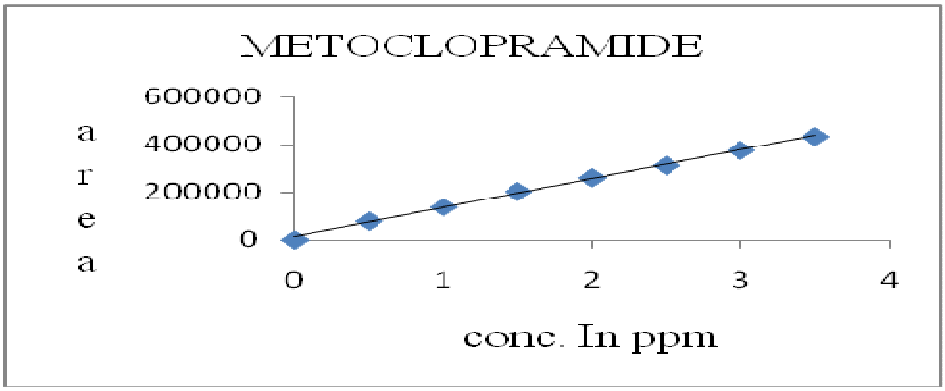

Figure.6 Calibration Plot for Metoclopramide
on X axis concentration of sample, on Y axis peak area respons

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

Intraday precision: To study the Intraday precision, six replicates of standard solution of Metoclopramide (at 2ppm) were prepared and injected using the proposed method conditions. The percent relative standard deviation (% RSD) for peak responses was calculated and was found to be 0.7%, which is well within the acceptance criteria of not more than 2.0%. Results of Intraday system precision studies were shown in Table.4

Table.4 Intraday Precision Results for Metoclopramide

SAMPLE	CONC (PPM)	INJECTION NO	PEAKS AREA	RSD (Acceptance criteria ≤ 2.0%)
Metoclopramide	2	1	262549	0.7
		2	266384	
		3	260994	
		4	262856	
		5	264189	
		6	263005	

Inter Day precision: To study the Inter day precision, six replicate standard solutions of Metoclopramide at 2ppm concentration was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.9%, which is well within the acceptance criteria of not more than 2.0%. Results of Inter day system precision studies are shown in Table.5

Table.5 Inter Day Precision Results for Metoclopramide

SAMPLE	CONC (PPM)	INJECTION NO	PEAKS AREA	RSD (Acceptance criteria ≤ 2.0%)
Metoclopramide	2	1	263618	0.9
		2	265472	
		3	269085	
		4	267634	
		5	266057	
		6	262517	

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level of 2ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table.6 . Satisfactory recoveries ranging from 98.0 to 101.0 were obtained for the proposed method with mean RSD of 1.0% for %recoveries, across the three levels. This indicates that the proposed method was accurate.

Table.6 Accuracy Results

Level	Amount of Metoclopramide (in ppm)		% Recovery	%RSD
	Spiked	Recovered		
50 %	1	0.99	99.0	**1.5**
	1	0.98	98.0	
	1	1.01	101.0	
100%	2	2.02	101.0	**1.0**
	2	1.99	99.5	
	2	1.98	99.0	
150%	3	2.99	99.7	**0.5**
	3	3.02	100.7	
	3	3.01	100.3	
Mean			**99.8**	**1.0**

Robustness: The robustness study was performed by slight modification in mobile phase flow rate, pH and its composition. Metoclopramide at 2 ppm concentration was analyzed under these changed experimental conditions. Three replicate injections were performed with each of the altered chromatographic condition and the mean peak area was compared against the mean peak area obtained with the unaltered conditions. It was observed that there were no marked changes in chromatography and the %assay when compared with unaltered conditions was with in ± 2%, demonstrating that the developed method was robust in nature. The results of robustness study are shown in Table.7

Table .7 Robustness of Metoclopramide

Condition	Mean area	% assay	% Difference
Unaltered	260514	100.0	-
Flow rate at 0.8 mL/min Flow rate at 1.2mL/min	263687 259143	101.2 99.5	1.2 -0.5
Mobile phase: ACN : 1% TEA 48% 52% 52% 48%	 259036 258317	 99.4 99.2	 -0.6 -0.8
pH of mobile phase at 7.0	259680	99.7	-0.3
pH of mobile phase at 6.6	261327	99.2	0.3

System suitability: System suitability was studied under each validation parameter by injecting six replicates of the standard solutions at 2ppm concentration. For the method following limits were considered as acceptance criteria, Tailing factor ≤ 2, Theoretical plates > 2000 and %RSD for peak area ≤ 2%. The system suitability results are given in Table.8

Table: 8 Stability Results for Metoclopramide

Parameter	Tailing factor	Theoretical plates	% RSD for peak response
Specificity study	1.61	14025.6	1.2
Linearity study	1.81	13751.6	0.7
Precision study	1.88	13406.2	0.7

Stability test: To perform the Stability test, three replicates of standard and sample solutions at 2ppm (stability samples) were prepared and stored separately at ambient temperature (25±10˚C) for two days. After the intended storage period, both the standard and sample stability solutions were compared against a freshly prepared standard solution (comparison sample) using the proposed method. It is noticed that the % stability of Metoclopramide was more than 98%, demonstrating insignificant degradation in both standard and formulation samples. The results of stability test were shown in Table.9

Table.9 System Suitability Results for Metoclopramide

S. No	Concentration (ppm)	Solution	Mean Peak Area	% Stability
1	2	Fresh standard Solution (Comparison sample)	265534	-
2	2	Stored Standard Solution (Stability sample)	264284	99.5
3	2	Stored Sample Solution (Stability sample)	264437	99.6

Limit of detection and Limit of quantification: To determine the Limit of Detection (LOD) sample was dissolved in mobile phase and injected until peak was disappeared. After 0.01ppm dilution, peak was not clearly observed. So it confirms that 0.01ppm is Limit of Detection and Limit of Quantification was found to be 0.05ppm. For establishing LOQ, six replicates of standard at 0.05ppm were prepared and quantified with a relative standard deviation of 1.9%. The LOD and LOQ of Metoclopramide are given in Table.10

Table.10 Limit of Detection and Limit of Quantification for Metoclopramide

parameter	Measured volume
Limit of Quantification	0.05ppm
Limit of Detection	0.01ppm

Formulation: For assay of Metoclopramide in tablet formulation (PERINORM - 10 mg of Metoclopramide as mono hydrochloride salt), 20 tablets were weighed and calculated the average tablet weight. Accurately weighed and transferred a quantity of powder equivalent to 10mg of Metoclopramide in to a 10mL volumetric flask. 5mL of mobile phase was added and sonicated to completely dissolve the drug and final volume was made with the same diluent. Mixed well and filtered the solution through 0.45µ filter. Further pipetted out 0.1mL of the above solution into a 50mL volumetric flask and diluted up to the mark with Mobile phase to get a final concentration of 2ppm. An aliquot of the prepared solution was injected onto HPLC system and the peak area of Metoclopramide was measured. The %assay was calculated by applying the molecular weight correction for hydrochloride salt. The proposed method was able to estimate Metoclopramide in the tablet formulation with an accuracy of 98.9%

5.10 Plasma sample analysis

Blood Plasma Preparation[29]

Blood taken into vacutainer tube(s) containing 1.8 mg K2EDTA per ml blood . Be sure to draw the full volume to ensure the correct blood-toanticoagulant ratio.Invert vacutainer tubes carefully 10 times to mix blood and anticoagulant and store at room .Temperature until centrifugation.Samples should undergo centrifugation immediately. This should be carried out for a minimum of 10 minutes at 1000-2000 RCF (generally 1300 RCF) at room temperature (refer to speeds and times recommended by manufacturer). Do not use brake to stop centrifuge. This will give three layers: (from top to bottom) plasma, leucocytes, erythrocytes. Carefully aspirate the supernatant (plasma) at room temperature and pool in a centrifuge tube. Take care not to disrupt the cell layer or transfer any cells. Inspect plasma for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble

matter.Aliquot plasma into cryovials and store at –80 °C. Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood. 0.5ml of plasma sample solution was taken in a test tube and added 100µl of 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded Metaclopramide 3 ppm concentrated sample was prepared in plasma. By the using of developed method, but due to more interference between plasma and drug Metaclopramide is not estimated with this method.

DISCUSSION ON THE RESULTS

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile: 1% Triethylamine in the ratio of 50:50%, v/v at a flow rate of 1.0 mL/min. The optimum wavelength for detection was set at 270nm at which much better detector response for drug was obtained. As it was shown in Fig2. The retention time was 2.56 min for Metoclopramide and no interferences was observed in formulation samples. The number of theoretical plates was found to be > 13000, which indicates the efficient performance of column. System suitability test was performed with each of the validation parameter and has comfortably met the set acceptance criteria. The results obtained were represented in Table 8

The calibration curve was obtained with a seven standard points in the range of 0.5-3.5ppm was found to be Linear. The representative graph of calibration curve was shown in Fig. 10.C and the regression data was presented in Table 9.4. Calibration curve was found to be linear with correlation coefficient of r=0.9993, and the Intercept and Slope values were found to be 20700 and 118611 respectively. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%.

Precision was evaluated by carrying out six independent sample preparations of standard at 2ppm concentration. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correct and hence the developed analytical method is highly repetitive. RSD of intraday precision was found to 0.7%. For the

inter day precision a study carried out on consecutive days resulted in a RSD of 0.9%. This indicates good method precision and the results are shown in Table 5 and 6

Standard addition method at 50%, 100% and 150% of 2ppm, showed good recoveries ranging from 98.0 to 101.0%. The mean recovery data obtained at each level as well as on overall three levels (Table 10.7) was within 2.0%, which satisfied the acceptance criteria set for the study.

The stability studies of Metoclopramide was evaluated by comparing the results of freshly prepared standard solution (2ppm) against the standard and sample solutions (stability samples) after two successive days of storage at ambient temperature (25±10°C). Metoclopramide was found stable under the tested conditions with insignificant degradation.

The proposed method has been applied to the assay of commercial tablets (PERINORM - 10 mg) containing Metoclopramide mono hydrochloride. Three independent samples were analyzed after extracting the drug, as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. The Proposed method was able to estimate Metoclopramide with an accuracy of 98.9% in tablet formulation.

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters made us to conclude that it could be used for rapid and reliable determination of Metoclopramide in tablet formulation as well as in bulk samples. The current method can successfully applied in the quality control of tablet formulations, plasma samples with good accuracy and precise results.

CONCLUSION

Based on the above results reveals that the developed method applicable for the determination of Metoclopramide In Pharmaceutical Dosage Form And Plasma and applicable for regular analysis.

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