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Determination of Metoclopramide Hydrochloride in Pharmaceuticals and Spiked Human Urine through Diazotization Reaction

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ABSTRACT

Three simple , rapid, sensitive and cost-effective titrimetric and spectrophotometric methods were described for the determination of metoclopramide (MCP) in its pharmaceutical dosage forms and spiked human urine. The titrimetric methods were based on the diazotization reaction involving the primary amine group of MCP and $NaNO_2$ in acid medium with visual (method A) and potentiometric (method B) end point detection. The spectrophotometric method (method C) depends upon the diazotization of MCP in acid medium followed by coupling with diphenylamine to give a red colored chromogen with a wavelength of maximum absorption at 530 nm. The experimental conditions were optimized. Both the titrimetric methods were applicable over the concentration range of 1.0 - 20.0 mg and the calculations were based on a 1 : 1 (MCP: $NaNO_2$) reaction stoichiometry. In the spectrophotometric method, Beer's law was obeyed over the concentration range of 0.3 - 7.5 μg/mL with a molar absorptivity of 4.73×10^4 L/mol cm and Sandell's sensitivity being 0.007 μg/cm^2. The limit of detection and the limit of quantification were calculated to be 0.22 and 0.67 μg/mL, respectively. The proposed methods were applied to the determination of MCP in tablets, injection and also in spiked human urine.

Key words: metoclopramide, titrimetry, spectrophotometry, diazotization, diphenylamine

INTRODUCTION

Metoclopramide hydrochloride (MCP), chemically known as 4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxybenzamide hydrochloride, is an antiemetic and gastroprokinetic agent. Thus it is primarily used to treat nausea and vomiting, to facilitate gastric emptying in patients with gastroparesis. It is also used for the prevention of cancer chemotherapy-induced emesis at much higher doses[1].

The great therapeutic importance of MCP in both clinical and experimental medicine has resulted in extensive literature on its determination in dosage forms and biological fluids. Both British Pharmacopoeia[2] and United State Pharmacopeia[3] describe acid-base titration with potentiometric end point detection. Several methods have been reported for the determination of MCP in pharmaceuticals, biological fluids or mixtures with other drugs; by HPLC[4-11], ^{1}H-NMR spectrometry[12], differential scanning calorimetry[13], X-ray powder diffractometry[13], voltammetry[14,15], potentiometry[16,17], flow-injection chemiluminescence spectrometry[18-21], fluorimetry[22], UV-spectrophotometry[23] or flow-injection spectrophotometry[24,25]. Some of the reported procedures are not simple for routine analysis and required expensive or sophisticated instruments. Literature survey revealed that no titrimetric assay of MCP has ever been reported except the official methods[2,3].

Visible spectrophotometry is perhaps the most widely used technique reported for the determination of MCP in pharmaceuticals[26-45] but most of them suffer from one or other disadvantages like poor sensitivity[26,27,29-31,33,34,41-43], narrow range of determination[30,34,35,44], need of heating[26,27,29,31,39] or extraction step[32,33], use of expensive chemicals[42] and non-aqueous systems[30,32,38-40], strict pH control[32,38] etc.

This paper describes three simple and sensitive titrimetric and spectrophotometric methods for the determination of MCP in its dosage forms. The titrimetric methods is based on the well-known diazotization reaction with $NaNO_2$ in acid medium and the spectrophotometric method on the well-characterized coupling with diphenylamine following the diazotization step.

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MATERIALS AND METHODS

I. *Apparatus*

Potentiometric titration was performed with a DI 101 digital potentiometer (Hyderabad, India) equipped with platinum-calomel electrode system. A Systronics model 106 digital spectrophotometer (Ahmedabad, India) equipped with 1-cm matched quartz cells was used to measure the absorbance.

II. *Reagents and Materials*

All chemicals and solvents were of analytical-reagent grade. Distilled water was used throughout the experiment.

(I) *Diphenylamine (0.5%)*

Five hundred milligrams of the chemical (Merck, Mumbai, India) was dissolved in glacial acetic acid (Merck, Mumbai, India) and made up to 100 mL with the same solvent.

(II) *Sodium Nitrite (6 mM for methods A and B, and 25 µg/mL for method C)*

The solutions were prepared by dissolving the chemical (Merck, Mumbai, India) in distilled water.

(III) *Sulphuric Acid (5M) and Hydrochloric Acid (5M)*

Concentrated sulphuric acid (Merck, Mumbai, India, sp. gr. 1.84) and concentrated HCl (Merck, Mumbai, India, sp. gr. 1.18) was appropriately diluted with water to get the required concentrations.

(IV) *Standard Drug Solution*

MCP was obtained as gift from IPCA Laboratories Ltd., Mumbai, India. A stock standard solution of pure drug containing 2 mg/mL MCP was prepared in water and used in methods A and B. For method C, the stock solution of 100 µg/mL MCP was prepared in 10 M acetic acid and the working standard solution of 15 µg/mL MCP was prepared by dilution with the same acid.

Two brands of tablets containing MCP, Perinorm-10 (IPCA Laboratories Ltd., Mumbai, India) and Reglan-10 (Cosme farma laboratories Ltd., Karnataka, India); and one brand of injection containing MCP (5 mg/mL), perinorm (IPCA Laboratories Ltd., Mumbai, India) used in the investigation were purchased from local commercial sources.

III. *Methods*

(I) *Visual Titrimetry (method A)*

Varying aliquots (1, 2, 3, 4…..10 mL) of 2 mg/mL standard MCP solution were accurately measured and transferred into a series of 100 mL titration flasks, and the total volume was made up to 10 mL with water. Five millilitres of 5 M HCl were added and the solution was titrated at room temperature (28 ± 2°C) with 6 mM $NaNO_2$ until the titration mixture produced distinct blue color on a small piece of starch iodide paper, which marked the end point. A blank titration was performed and the volume of $NaNO_2$ consumed by blank was subtracted from the volume required for sample titration to calculate the amount of $NaNO_2$ which has been reacted with drug.

(II) *Potentiometry (method B)*

Different volumes (1, 2, 3, 4……10 mL) of standard solution containing 2 mg/mL MCP were taken into a series of 100 mL beakers and the total volume was adjusted to 10 mL with water. Five millilitres of 5 M HCl were added and platinum-calomel electrodes were immersed in the titration liquid. The contents were stirred magnetically and the titrant (6 mM $NaNO_2$) was added from a microburette. Near the equivalence point, titrant was added in 0.05 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant. The equivalence point was determined by applying the graphical method. A blank titration was also performed.

In either the titrimetric method, the amount of MCP in the aliquot was computed from the following formula:

$$\text{Amount (mg)} = \frac{V \times M_{wt} \times C}{n}$$

where V = mL of $NaNO_2$ consumed
M_w = relative molecular mass of drug
C = concentration of $NaNO_2$, moles/L
n = number of moles of $NaNO_2$ reacting with per mole of MCP.

(III) *Spectrophotometry (method C)*

Different aliquots (0.2, 1.0, 2.0, 3.0, 4.0 and 5.0 mL) of 15 µg/mL standard MCP solution were accurately measured and transferred into a series of 10 mL calibrated flasks using micro burette and the total volume was adjusted to 5.0 mL by adding 10 M acetic acid. Two millilitres of 5 M H_2SO_4 and 1 mL of 25 µg/mL $NaNO_2$ were added; the content was mixed and kept aside for 5 min. Then, 1 mL of 0.5% diphenylamine was added and the volume was made up to the mark with water. After 15 min, the absorbance of each solution was measured at 530 nm against a reagent blank.

Calibration graph was prepared by plotting the increasing absorbance values *versus* concentrations of MCP. The concentration of the unknown was read from the calibration graph or deduced from the regression equation derived using the Beer's law data.

IV. *Procedure for Spiked Human Urine*

To 50 mL of MCP-free human urine taken in a 125 mL separating funnel was added with 2.5 mg MCP. Ten millilitres of 1 M NaOH were added, mixed and kept aside for 3 min. Then, 25 mL chloroform was added, shaken well for about 15 min. The lower organic layer was collected in a beaker containing anhydrous sodium sulphate. The water-free organic layer was transferred into a dry beaker and the solvent evaporated on a hot water bath. The dry residue was dissolved in 10 M acetic acid and transferred into a 50 mL calibrated flask, and diluted to the mark with the same acid. The resulting solution equivalent to 50 µg/mL MCP was diluted with the same acid to get 15 µg/mL solution and assayed using the spectrophotometric procedure (method C) described above.

V. *Procedure for Tablets*

Twenty tablets containing MCP were weighed and ground into fine powder. For methods A and B, an accurately weighed amount of the powdered tablet equivalent to 200 mg of MCP was transferred to a 100 mL calibrated flask and shaken with 60 mL of water for about 20 min, then made up to the mark with water, mixed and filtered using a Whatman No. 42 filter paper. A convenient aliquot (say 5 mL) was taken and assayed according to the procedures described above. For spectrophotometry (method C), an amount of powder equivalent to 5 mg of MCP was weighed into a 50 mL calibrated flask, 20 mL of 10 M acetic acid was added and the mixture was shaken for 20 min; then the volume was made up to the mark with the same acid, mixed well and filtered using Whatman No. 42 filter paper. The resulting tablet extract containing 100 µg/mL MCP was diluted appropriately with the same acid to get a working concentration of 15 µg/mL and subjected to analysis by following the procedure described above.

VI. *Procedure for Injection*

The contents of ten ampoules were pooled in a dry beaker and mixed. The aliquots containing 100 mg of MCP (method A and B) or 5 mg of MCP (method C) were measured accurately and transferred to two separate 50 mL calibrated flasks and diluted with the respective solvents. The resulting solution (100 µg/mL in MCP) in method C was further diluted to get 15 µg/mL MCP; and the general procedures were applied for assay in injection solutions.

VII. *Placebo Blank Analysis*

A placebo blank of the composition: talc (200 mg), starch (165 mg), acacia (150 mg), methyl cellulose (200 mg), sodium citrate (125 mg), magnesium stearate (145 mg) and sodium alginate (250 mg) was made and its solution was prepared in 50 mL calibration flask as described under "Procedure for tablets", and then subjected to analysis using the procedures described above.

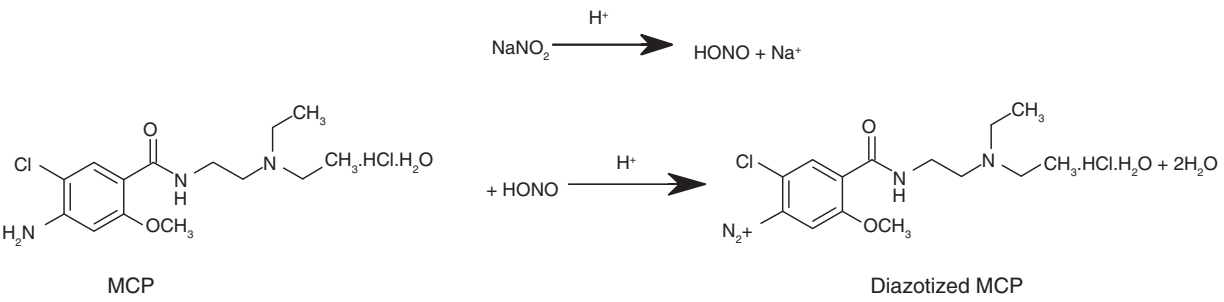
VIII. *Synthetic Mixture Analysis*

To the placebo blank of the composition described above, 100 mg of MCP (methods A and B) or 5 mg of MCP (method C) was added separately and homogenized, transferred to two separate 50 mL calibrated flasks and the solution was prepared as described under "Procedure for tablets", and then subjected to analysis using the procedures described above.

RESULTS AND DISCUSSION

Diazotization reaction has been widely used for the determination of aromatic primary amino group containing compounds of pharmaceutical importance[46-49]. $NaNO_2$ generated *in situ* nitrous acid (HONO) in acid medium and the nitrous acid reacts with amines to yield diazo compounds. The synthetic utility of such a reaction is to render the amino group labile for nucleophilic substitution, as the N_2 group is a better leaving group. Since MCP contains aromatic primary amine group, the present work employs the same reaction for its assay and the probable reaction scheme is given in Scheme 1.

In visual titrimetric method, when the reaction between the aromatic primary amine group present in MCP with HONO (generated *in situ* from $NaNO_2$ under acid medium) was completed, the excess HONO was detected using starch iodide paper. Action of starch iodide paper with reaction mixture liberates iodine from iodide in HCl medium. The liberated iodine reacts with starch to give blue color. Many internal indicators like metanil yellow, tropaeolin 00,



Scheme 1. Diazotization of MCP with HONO.

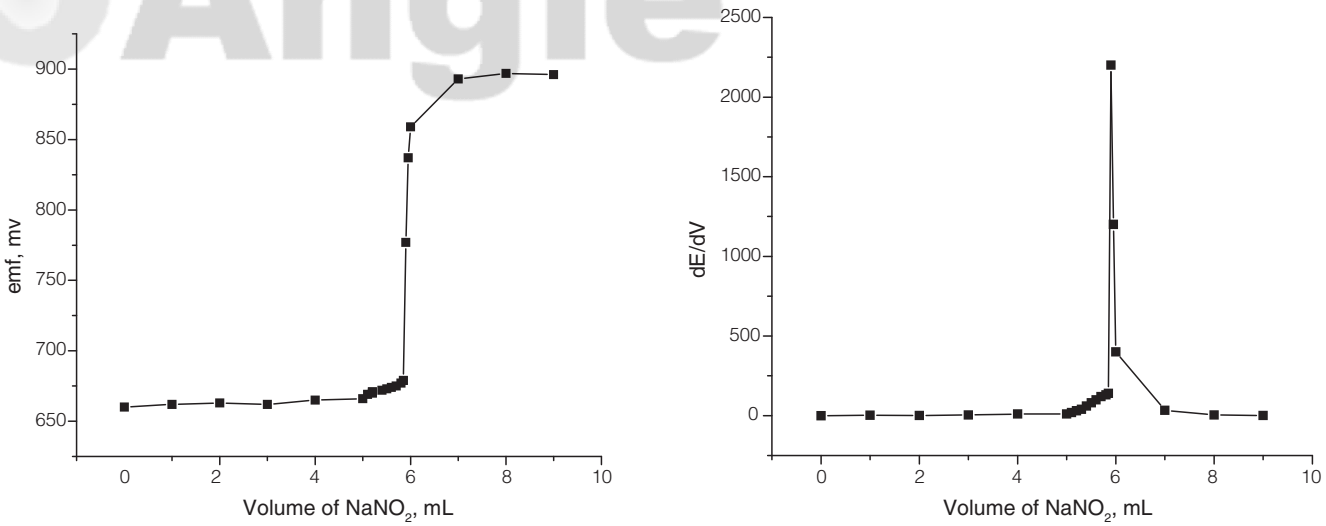
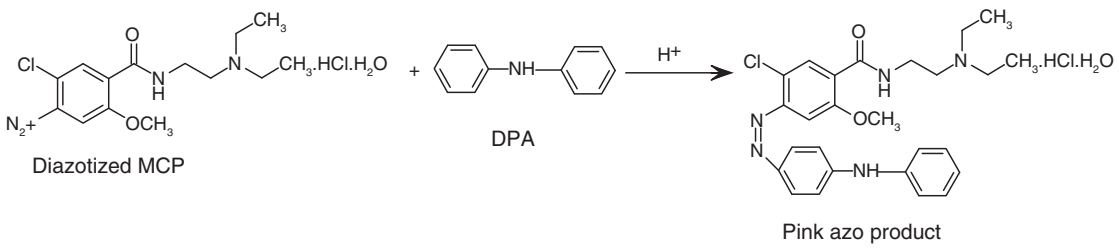


Figure 1. Typical potentiometric titration curve with a derivative curve for 12 mg MCP *Vs* 6 mM $NaNO_2$.



Scheme 2. Coupling of diazotized MCP with diphenylamine (DPA) to produce pink azo product.

methylene blue and neutral red were tried, but none of them was found satisfactory for this titration. Interestingly unlike in many reported methods, diazotization was achieved at ambient temperature (28 ± 2°C) and cooling to 0 - 5°C was not required.

The potentiometric titration used a platinum-calomel electrode system and the change in electrode potential (EMF) upon the addition of titrant was noted against the volume of the $NaNO_2$ added. Liberation of excess nitrous acid at the end point depolarizes the electrode and the end point was detected when the EMF increased rapidly (Figure 1). The stoichiometry of the reaction was assessed and found to be 1 : 1 (MCP : $NaNO_2$).

In the literature, various coupling agents have been used for the spectrophotometric assay of MCP[46-55], but diphenylamine was found to excel most of them by offering better linear dynamic range, sensitivity, non-rigid optimal experimental conditions and cost-effectiveness. Hence, the present spectrophotometric method employed diphenylamine as coupling agent. The work involves coupling of diazotized MCP with diphenylamine in acidic medium to give a pink colored azo product. Due to the fact that diazonium cations are poor electrophiles and relatively bulky species, mainly para substitution takes place in diazonium coupling[50]. In the case of *para* substitution, steric hindrance is at its weakest, while the positive charge's stabilization is at its largest, in the σ complex. If the *para* position is already occupied by another substituent, ortho substitution occurs[50]. In diphenylamine, since the *para* position is unoccupied, coupling of diazotized MCP occurs at *para* position and the reaction mechanism is represented in Scheme 2.

Even though diphenylamine and azo product are insoluble in water, they were found to be dissolved easily in acetic acid medium.

I. *Spectral Characteristics*

The absorption spectra of the pink colored azo product with λ_{max} at 530 nm and the reagent blank are shown in Figure 2. The reagent blank has negligible absorption at this wavelength.

II. *Optimization of Experimental Variables*

Various experimental variables were optimized to achieve the maximum sensitivity.

III. *Titrimetry*

(I) *Sodium Nitrite*

In both the titrimetric methods, 6 mM $NaNO_2$ was fixed

as the optimum concentration, since at this concentration the volume of $NaNO_2$ required to diazotized completely 10 mL of 2 mg/mL MCP was below 10 mL.

(II) *Hydrochloric Acid.*

The quantitative results were obtained in HCl medium and the reaction stoichiometry of 1:1 (MCP : $NaNO_2$) was unaffected in the concentration range of 0.333 - 5.0 M HCl. Hence, 5 mL of 5 M HCl in a total volume of 10 mL (i. e. overall concentration of 2.5 M HCl) was fixed as optimum in both methods.

IV. *Spectrophotometry*

(I) *Sodium Nitrite*

The amount of $NaNO_2$ required for complete diazotization was found to be 1 mL of 25 µg/mL. There was no change in the absorbance at higher concentrations.

(II) *Sulphamic Acid*

No change was observed in absorbance in the presence or absence of sulphamic acid, therefore, the use of sulphamic acid to destroy excess $NaNO_2$ was avoided.

(III) *Diphenylamine.*

The effect of diphenylamine concentration on the absorbance of the system was studied by using 0.5% diphenylamine and the results showed that there was no change in the absorbance from 0.5 - 2.0 mL of 0.5% diphenylamine (Figure 3). Hence, 1 mL of 0.5% diphenylamine used throughout the study.

(IV) *Sulphuric Acid.*

Various acids were tested and H_2SO_4 was found as the ideal one in method C, since the color intensity of the azo dye was found maximum in this medium. H_2SO_4 concentration was found to be critical. Only 1.5 - 2.5 mL of 5 M H_2SO_4 gave maximum and constant absorbance readings; below and above these concentrations decrease in the absorbance readings was observed (Figure 4). Hence, 2.0 mL of 5 M H_2SO_4 was fixed in method C.

(V) *Reaction Time and Stability of the Reaction Product*

Effect of time on both reaction steps (diazotization and coupling steps) was investigated. Diazotization reaction was found to be complete at 5 min and the coupling step took 15 min for completion and the azo dye formed remained stable for more than 24 h.

V. *Method Validation*

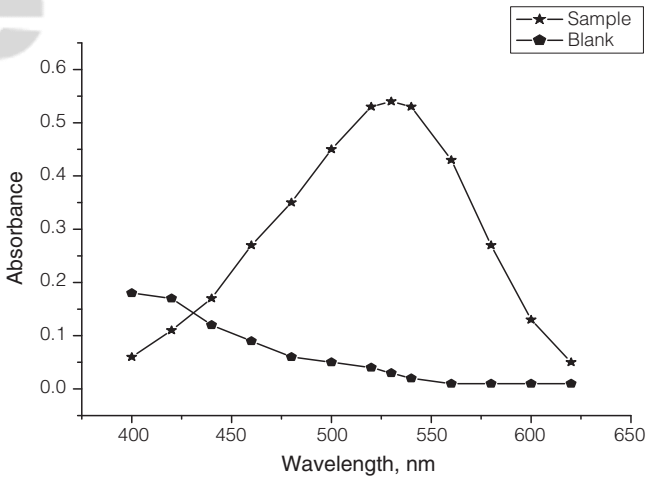


Figure 2. Absorption spectra of pink color azo product (3 mL of 15 µg/mL MCP).

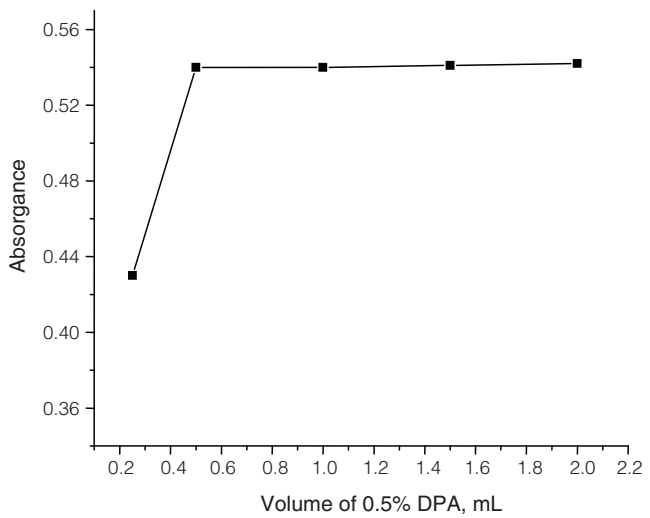


Figure 3. Effect of diphenylamine on the absorbance of pink color azo product (3 mL of 15 µg/mL MCP).

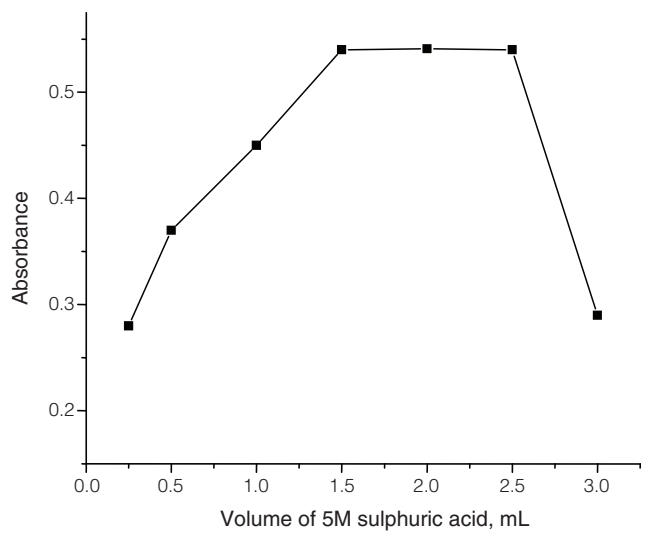


Figure 4. Effect of sulphuric acid on the absorbance of pink color azo product (3 mL of 15 µg/mL MCP).

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(I) *Analytical Parameters of Spectrophotometric Method*

A linear relation was found to exist between absorbance and the concentration of MCP in the range of 0.3 - 7.5 μg/mL (Figure 5).

The calibration graph is described by the equation:

$$Y = a + b X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in μg/mL) obtained by the method of least squares. The values of correlation coefficient, intercept and slope for the calibration data are found to be 0.999, 0.003 and 0.122, respectively. Sensitivity parameters such as apparent molar absorptivity, Sandell's sensitivity, the limits of detection (LOD) and quantification (LOQ) calculated as per the current ICH guidelines[51] are calculated and the respective values are 4.73×10^4 L/mol cm, 0.007 μg/cm^2, 0.22 μg/mL and 0.67 μg/mL. The LOD and LOQ were calculated according to the same guidelines using the formulae:

$$LOD = 3.3\ \sigma/s \text{ and } LOQ = 10\ \sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve. The standard deviation of intercept (S_a) and slope (S_b) were also calculated and found to be 0.025 for S_a and 0.004 for S_b, respectively.

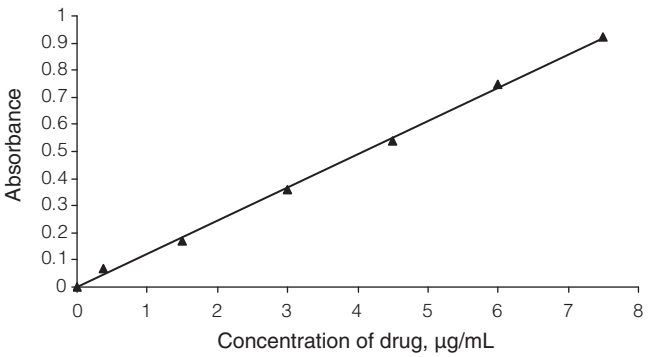

Figure 5. Calibration graph for determination of MCP in spectrophotometric method.

(II) *Accuracy and Precision*

The accuracy and precision of the methods were evaluated by performing seven replicate analyses on pure drug solution at three different amount/concentration levels (within the working ranges). The relative error (%), an indicator of accuracy was within 3.0 and within day precision, also called the repeatability, expressed as relative standard deviation (RSD %) was less than 2.0 indicating high accuracy and repeatability of the methods. The results of the study are given in Table 1. The reproducibility of the methods also known as the day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three levels over a period of five days, preparing all solutions afresh. The day-to-day RSD values were less than 2% reflecting the usefulness of the methods in routine analysis.

(III) *Robustness and Ruggedness*

Method robustness was tested by making small incremental change in HCl concentration in methods A and B; and H_2SO_4 concentration and reaction time were altered in method C. To check the ruggedness, analysis was performed by four different analysts; and on three different burettes (methods A and B) and spectrophotometers (method C) by the same analyst. The robustness and the ruggedness were checked at three different drug levels. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in Table 2.

(IV) *Effect of Co-formulated Substances*

The effect of co-formulated substances was tested by

Table 1. Evaluation of intra-day and inter-day accuracy and precision

Methods[a]	MCP taken	Intra-day accuracy and precision			Inter-day accuracy and precision		
		MCP found	%RE	%RSD	MCP found	%RE	%RSD
Visual titrimetry	6.0	6.14	2.3	0.7	6.16	2.7	1.0
(method A)	12.0	12.21	1.8	0.8	12.27	2.3	1.0
	18.0	18.24	1.3	0.7	18.30	1.7	1.0
Potentiometry	6.0	6.11	1.8	0.7	6.17	2.8	1.0
(method B)	12.0	12.19	1.6	0.5	12.25	2.1	1.0
	18.0	18.25	1.4	0.6	18.32	1.8	0.7
Spectrophotmetry	1.5	1.48	1.3	0.8	1.54	2.7	0.8
(method C)	4.5	4.60	2.2	0.7	4.63	2.9	0.9
	7.5	7.68	2.4	0.8	7.71	2.8	0.7

RE: Relative error and RSD: Relative standard deviation.
[a]In titrimetry methods, MCP taken/found are in mg and they are μg/mL in spectrophotometry.

Table 2. Robustness and ruggedness expressed as intermediate precision (%RSD)

Method	MCP Taken[a]	Method robustness: Parameter altered			Method ruggedness	
		HCl[b] (5M) mL RSD, % (n = 3)	H_2SO_4[c] (5 M) mL RSD, % (n = 3)	Reaction time[d] RSD % (n = 3)	Inter-analysts' RSD, % (n = 4)	Inter-instruments' RSD, % (n = 3)
Visual titrimetry	6.0	0.7	-	-	0.9	1.8
(method A)	12.0	0.8	-	-	1.1	0.9
	18.0	0.8	-	-	0.9	0.9
Potentiometry	6.0	0.9	-	-	0.7	1.2
(method B)	12.0	0.7	-	-	0.7	0.8
	18.0	0.8	-	-	0.7	0.9
Spectrophotometry	1.5	-	0.8	1.0	0.8	1.0
(method C)	4.5	-	0.9	0.8	0.9	1.1
	7.5	-	0.9	0.8	0.8	1.0

[a] mg in titrimetry and μg/mL in spectrophotometry.
[b]HCl volumes used were 4.8, 5.0 and 5.2 ml in both visual titrimetry and potentiometry.
In spectrophotometric method, [c]H_2SO_4 volumes of 1.8, 2.0 and 2.2 ml and [d]reaction time of 13, 15 and 17 min were employed.

placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution was subjected to analysis according to the recommended procedures. In all cases, there was no interference by the inactive ingredients as indicated by the near blank absorbance in spectrophotometry and near blank titre volume in titrimetry.

The analysis of synthetic mixture solution yielded percent recoveries which ranged between 96.83 and 104.1 with standard deviation of 1.24 - 1.42 in all cases. The results of this study are presented in Table 3 indicating that the inactive ingredients did not interfere in the assay.

(V) *Application of the Proposed Methods to Spiked Human Urine and Dosage Forms*

The proposed spectrophotometric method was successfully applied to the spiked human urine sample. The analysis of human urine sample spiked with MCP yielded percent recoveries in the range of 105.7 - 108.2 (n = 5) with standard deviation of 1.28 - 1.41. In order to evaluate the analytical applicability of the proposed methods to the quantification of MCP in commercial tablets and injection, the results obtained by the proposed methods were compared to those of the reference method[(2)] by applying Student's *t*-test for accuracy and *F*-test for precision. In reference method, 0.2500 g of MCP was dissolved in 5.0 mL of 0.01 M HCl and 50 mL of alcohol; and the resulting solution was titrated with 0.1 M NaOH to potentiometric end point detection. The results (Table 4) show that the Student's *t*- and *F*-values at 95% confidence level are less than the theoretical values, which confirmed a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table 3. Recovery of the drug from synthetic mixture

Method	MCP in synthetic mixture taken[a]	MCP recovered[b] (Percent ± SD)
Visual titrimetry	6.0	103.6 ± 1.94
(method A)	12.0	104.1 ± 2.61
	18.0	103.4 ± 1.75
Potentiometry	6.0	97.01 ± 1.66
(method B)	12.0	96.83 ± 2.72
	18.0	98.78 ± 2.53
Spectrophotometry	1.5	97.39 ± 2.88
(method C)	4.5	97.68 ± 3.71
	7.5	98.82 ± 2.59

[a] mg in titrimetry and μg/mL in spectrophotometry.
[b]Mean value of five determinations.

(VI) *Recovery Studies*

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure MCP at three levels (50, 100 and 150% of that found in tablet powder) and the total was determined by the proposed methods. The percent recovery of pure MCP added was in the ranged of 97.00 - 104.3% with standard deviation of 1.19 - 1.38 (Table 5) indicating that the recovery was good and the co-formulated substance did not interfere in the determination.

CONCLUSIONS

Three simple methods for the determination of MCP

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Table 4. Results of analysis of tablets by the proposed methods

Tablet/injection Brand name	Label claim, mg/tablet	Found[a] (Percent of label claim ± SD)			
		Reference method	Visual titrimetry (method A)	Potentiometry (method B)	Spectrophotometry (method C)
Perinorm-10[b]	10	99.67 ± 1.32	100.98 ± 0.74 **t = 2.00** **F = 3.18**	101.5 ± 0.81 **t = 2.77** **F = 2.65**	98.42 ± 0.58 **t = 2.08** **F = 5.18**
Raglan -10[c]	10	100.3 ± 0.92	101.0 ± 0.81 **t = 1.28** **F = 1.29**	100.98 ± 0.70 **t = 1.33** **F = 1.73**	98.57 ± 0.71 **t = 2.69** **F = 1.46**
Injection Perinorm-10[b]	10	97.33 ± 1.18	98.97 ± 0.86 **t = 2.54** **F = 1.88**	98.35 ± 0.76 **t = 1.66** **F = 2.41**	97.85 ± 0.85 **t = 0.81** **F = 1.93**

[a]Mean value of five determinations.
[b]IPCA Laboratories Ltd., Mumbai, India; [c]Cosme farma laboratories Ltd., Karnataka, India.
The value of t (tabulated) at 95% confidence level and for four degrees of freedom is 2.77.
The value of F (tabulated) at 95% confidence level and for four degrees of freedom is 6.39.

Table 5. Accuracy assessment by recovery experiments

Methods	Tablet studied	MCP in tablet[a]	Pure MCP added[a]	Total found[a]	Pure MCP recovered[b] Percent ± SD
Visual titrimetry (method A)	Perinorm -10	6.06	3.0	8.99	97.67 ± 0.72
		6.06	6.0	11.88	97.00 ± 0.82
		6.06	9.0	14.81	97.22 ± 0.79
Potentiometry (method B)	Perinorm -10	6.1	3.0	9.23	104. 3 ± 0.65
		6.1	6.0	12.32	103.7 ± 0.67
		6.1	9.0	15.29	102.1 ± 0.81
Spectrophotometry (method C)	Perinorm -10	2.95	1.5	4.42	98.00 ± 0.62
		2.95	3.0	5.93	99.33 ± 0.78
		2.95	4.5	7.40	98.89 ± 0.82

[a]mg in titrimetry and μg/mL in spectrophotometry.
[b]Mean value of three measurements.

in tablets and in injection were developed and validated as per the ICH guidelines[51]. The methods are based on well-characterized diazotization/coupling reactions. The diazocoupling reaction involving diphenylamine has earlier been used by Abbas Afkhami *et. al*., for the determination of nitrite[46]; however, the authors have used triton X-100, a neutral surfactant, for dissolving the reaction product and ethanol to prepare diphenylamine solution. In the present work, the use of surfactant and ethanol which are not eco-friendly has been avoided, since diphenylamine and azo dye were found to be easily dissolved in acetic acid medium. This is a greener approach and the novelty of the proposed spectophotometric method. The present titrimetric methods are the simplest methods ever reported and the visual titrimetric method is the first ever reported for the determination of MCP. The titrimetric methods are applicable over wide linear dynamic ranges and were successfully applied to the tablets and injection. The high sensitivity of the spectrophotometric method enabled its application to the determination of MCP in spiked human urine besides the dosage forms. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Besides the simplicity and sensitivity of the procedures, the relative cheapness of apparatus and reagents demonstrate their advantageous characteristics. The methods are also useful due to high tolerance limit for common excipients found in pharmaceutical formulations. These merits coupled with the use of simple and inexpensive instrument and high selectivity of the methods recommend the use of the methods in routine quality control laboratories.

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