

SIMPLE AND SELECTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF MOSAPRIDE CITRATE BY DIAZO COUPLING REACTION IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Three simple and selective spectrophotometric methods have been developed for determination of mosapride citrate (MDC) in pharmaceutical formulations. The proposed methods are based on the diazotization of MDC with sodium nitrite and hydrochloric acid, followed by coupling with resorcinol, 1-benzoylacetone and 8-hydroxyquinoline in alkaline medium for methods A, B and C respectively. The colored azo dyes were quantitated photometrically at 520, 428, and 535 nm for methods A, B and C, respectively. Different variables affecting the reactions are optimized. Beer's law is obeyed over the concentration ranges of 1.0-24.0, 1.0-28.0 and 1.0-20.0 $\mu\text{g}\cdot\text{mL}^{-1}$ for methods A, B, and C, respectively. The results of the proposed methods are validated statistically. The proposed methods were utilized for estimation of MDC in pharmaceutical dosage forms with good accuracy and precision. The results obtained by the proposed methods were compared favorably with those of the reference method.

Keywords: Mosapride citrate, Spectrophotometry, Diazo coupling reaction, Pharmaceutical formulations

INTRODUCTION

Mosapride citrate is chemically as 4-amino-5-chloro-2-ethoxy-[N-[4-(p-fluorobenzyl)-2-morpholinyl] methyl]-benzamide citrate dehydrate (Figure. 1). It is a potent gastroprokinetic drug and it is used in gastrointestinal symptoms associated with chronic gastritis. The therapeutic importance of MDC requires the development of a sensitive and rapid method for industrial quality control and clinical monitoring. Literature survey revealed that some analytical methods have been reported for determination of MSP in pure and formulations, including HPLC¹⁻³, HPTLC⁴ spectrofluorometric⁵ UV spectrophotometric⁶⁻⁷ and Visible spectrophotometric methods⁸⁻¹¹. Critical literature survey revealed that no attempt has been made to determination of MDC by diazo coupling with resorcinol, 1-benzoylacetone and 8-hydroxyquinoline in alkaline medium. In view of this, we have developed simple and sensitive spectrophotometric methods for the assay of MDC in pharmaceutical formulations. The methods are based on the aromatic amino group present in MDC was diazotized with nitrous acid at 0-5 °C temperature and the diazonium salt thus formed was coupled with resorcinol,

1-benzoylacetone and 8-hydroxyquinoline in sodium hydroxide medium for method A, B and C, respectively. The colored chromogens formed were measured at 520, 428, and 535 nm for method A, B and C, respectively. In spite of the fact that diazo coupling reactions have been extensively employed in the development of spectrophotometric analytical methodologies applied to several drugs¹²⁻¹⁴. The developed methods have the advantages of speed, sensitivity, simplicity, and cost effectiveness. The results obtained were closely comparable to those of a reported method and recovery tests were also found to be satisfactory.

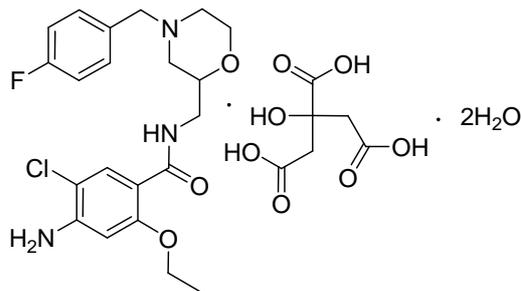


Fig. 1: Chemical structure of mosapride citrate

MATERIALS AND METHODS

Apparatus

All absorption spectra were recorded using double beam UV-Vis-NIR spectrophotometer (Shimadzu 1601, Japan) equipped with 1 cm matched quartz cells by using a personal computer loaded with the UV-PC 3.9 software package. An electronic micro balance (Sartorius MC 5, Germany) was used for weighing the solid materials.

Materials and Reagents

All solvents and reagents used were of analytical grade. Double-distilled water was used throughout the investigation. 0.2 (w/v) % sodium nitrite (S.D. Fine Chem., Mumbai, India), 2% (w/v) sulfamic acid (BDH, Mumbai, India) and 20 % (w/v) sodium hydroxide (S.D. Fine Chem., Mumbai, India) were freshly prepared in double distilled water. 1M hydrochloric acid (S.D. Fine Chem., Mumbai, India) was prepared with double distilled water. 0.5% (w/v) resorcinol (S.D. Fine Chem., Mumbai, India) was freshly prepared in double distilled water. 0.5 % (w/v) 1- benzoylacetone (Sisco research chemicals, Mumbai, India) and 0.5 % (w/v) 8-hydroxyquinoline (E-Merck, Mumbai, India) were prepared in methanol (Sisco research chemicals Ltd, Mumbai, India).

A standard gift MDC was obtained from Symed Labs, Hyderabad, India. Different pharmaceutical formulations were purchased from the local market. Standard stock solution of 200 $\mu\text{g}\cdot\text{mL}^{-1}$ MDC was prepared by dissolving accurately weighted amount of pure drug equivalent to 10 mg of MDC in methanol and diluted to 50 mL of calibrated flask. The solution was further diluted to get 100 $\mu\text{g}\cdot\text{mL}^{-1}$ with methanol.

General Procedure

Method A

Aliquots of the standard MDC (100 $\mu\text{g}\cdot\text{mL}^{-1}$) solution ranging from 0.1 – 2.4 mL are transferred into a series of 10 mL volumetric flask. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1 mL of 1 M hydrochloric acid were added. The resultant solution in each flask is well shaken and allowed to stand for 5 min at 0-5 °C temperature for diazotization to complete. After that, 0.5 mL of 2.0 % sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% resorcinol (RCL) and 1.5 mL of 20 % sodium hydroxide solutions were added. The contents were diluted to the mark with double distilled water and mixed well. The absorbance of the red colored azo dye was measured at 520 nm against the reagent blank.

Method B

Different aliquots of stock reference MDC solution ($100 \mu\text{g. mL}^{-1}$) ranging from 0.1 – 2.8 mL are transferred into a series of 10 mL volumetric flask. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1 mL of 1M hydrochloric acid were added. The resultant solution in each flask is well shaken and allowed to stand for 5 min at 0-5 °C temperature for diazotization to complete. After that, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% 1- benzoylacetone (BAC) and 1.0 mL of 20 % sodium hydroxide solutions were added. The contents were diluted to the mark with double distilled water and mixed well. The absorbance of yellow colored azo dye was measured at 428 nm against the reagent blank.

Method C

Varying aliquots of stock reference MDC solution ($100 \mu\text{g.mL}^{-1}$) in the range from 0.1 – 2.0 mL are transferred into a series of 10 mL volumetric flask. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1 mL of 1 M hydrochloric acid were added. The resultant solution in each flask is well shaken and allowed to stand for 5 min at 0-5 °C temperature for diazotization to complete. After that, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% 8-hydroxyquinoline (8-HQ) and 1.0 mL of 20 % sodium hydroxide solutions were added. The contents were diluted to the mark with methanol and mixed well. The absorbance of the wine red colored azo dye was measured at 535nm against the reagent blank.

In three methods, the calibration graph was constructed by plotting the absorbance versus concentration of the MDC in $\mu\text{g.mL}^{-1}$. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Application of pharmaceutical formulations

MDC containing ten tablets were grounded into a fine powder. An amount of the powder equivalent to 10 mg of MDC was weighed into a 50-mL volumetric flask; to this 30 mL methanol was added and shaken thoroughly for about 15 min to extract the complete drug. The volume was diluted to the mark with methanol and filtered using Whatmann No. 41 filter paper. The filtrate was diluted to get $100 \mu\text{g. mL}^{-1}$ with methanol. A suitable aliquot was then subjected to analysis.

RESULTS AND DISCUSSION

An aromatic amino group present in MDC led to diazotization with nitrous acid at 0-5 °C temperature. The coupling of the resulting diazonium salt with RCL (Method A) formed a red colored chromogen, with BAC (Method B) formed yellow colored chromogen, with 8-HQ (Method C) formed wine red chromogen in presence of sodium hydroxide medium. The proposed chemical reactions are shown in Scheme 1. The resulting colored azo dye shows maximum absorption at 520, 428 and 535 nm for methods A, B and C, respectively (Figure 2)

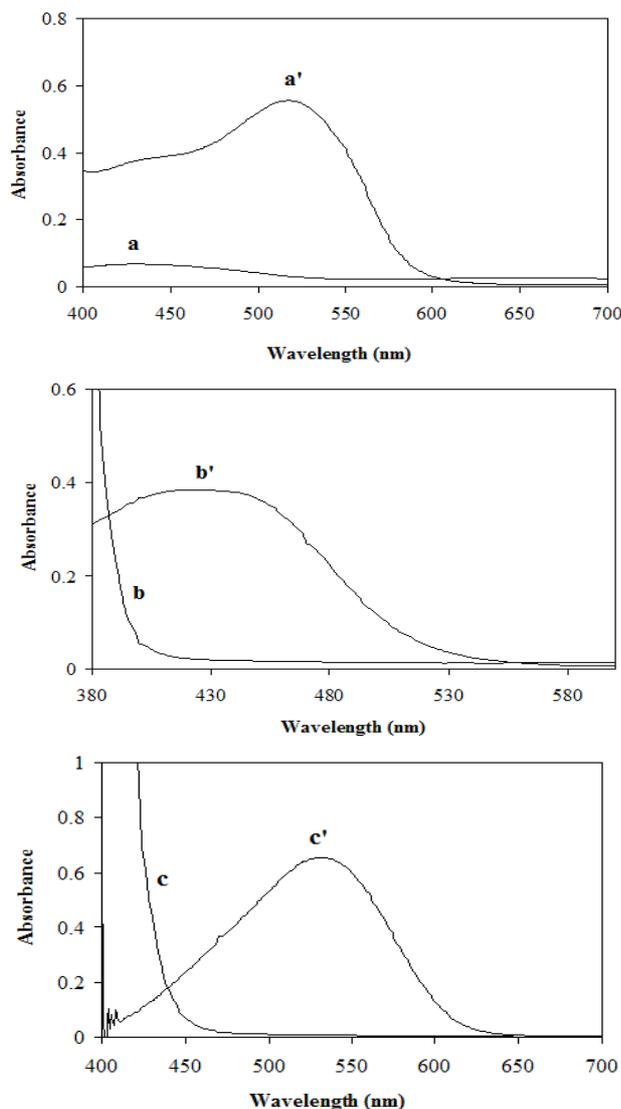


Fig. 2: a', b', c' are absorption spectra of reaction products of $10 \mu\text{g.mL}^{-1}$ MDC against reagent blanks for methods A, B and C, respectively. a, b, c are absorption spectra of reagent blanks against water for methods A, B and C, respectively.

Optimum Reaction Conditions

Effect of acid on diazotization

The diazotization reaction occurred in acidic condition. The concentration of hydrochloric acid was investigated for maximum diazotization. It was found that the absorbance was increased with increasing acid volume from 0.2-1.0 mL (Figure 3). Above this range a decrease in absorbance was observed. Thus, 1 mL of 1M hydrochloric acid was used in all methods.

Effect of Sodium Nitrite Concentration

For the maximum formation of azo dye, the influence of sodium nitrite concentration was investigated (Figure 4). The results demonstrate that the increase in absorbance tends to have a constant value for the sodium nitrite solution concentration equal to or higher than 0.8 mL, so 1.0 mL volume was selected in order to ensure an excess of reagent in the flask to guarantee the reaction development.

The interference of excess of nitrous acid was removed by adding 0.5 mL of 2 % sulfamic acid before the coupling reaction. The maximum time required for diazotization was found to be 5 min.

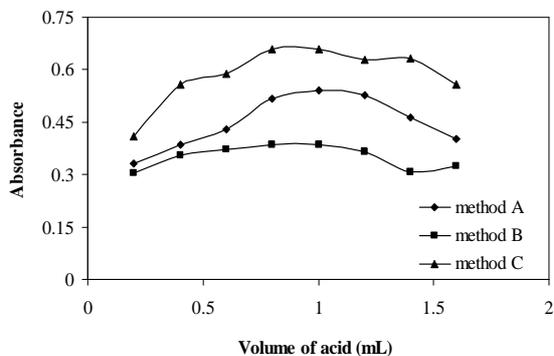
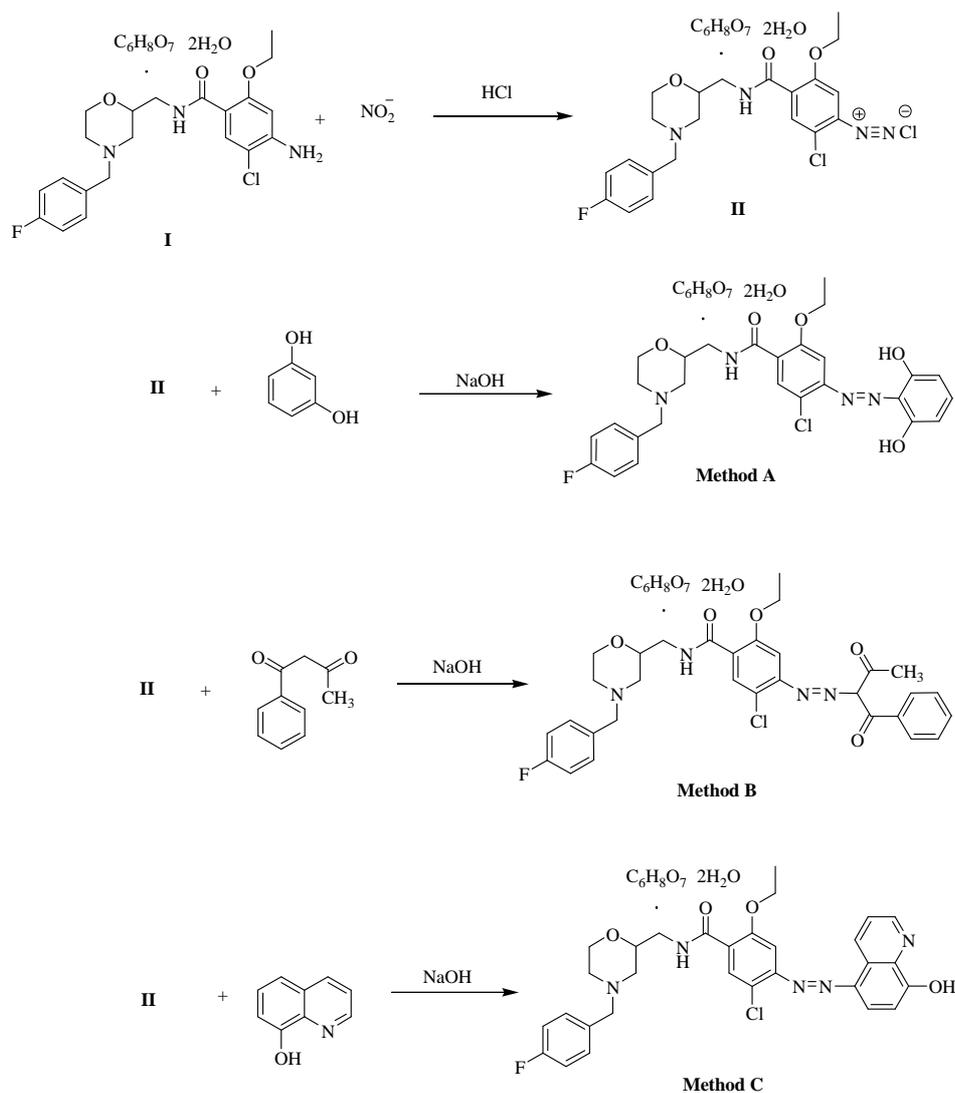


Fig. 3: Effect of hydrochloric acid on the reaction product of 10 µg mL⁻¹ MDC in methods A, B, and C.



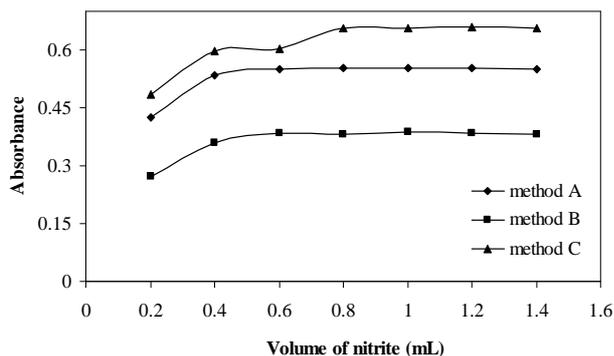


Fig. 4: Influence of sodium nitrite on the reaction product of 10 µg mL⁻¹ MDC in methods A, B, and C.

Effect of coupling reagents concentration

The influence of concentration of RCL, BAC and 8-HQ were investigated in the proposed methods by measuring the absorbance at specified wavelengths in the standard procedure for solutions containing a fixed concentration of MDC and varying amounts of coupling reagents. These results indicate that the maximum absorbance was obtained with 1 mL of reagent in all methods for quantitative determination of the investigated drug. The higher concentration of reagents does not affect the sensitivity of methods in all methods (Figure 5). This clearly indicates that all MDC had been reacted by coupling reagents and forms azo products reached a maximum as well.

Effect of sodium hydroxide

The stability and formation of azo dye depends upon the nature of reaction medium.

A study was conducted to determine the most effective alkalis and the optimum alkali concentration to be used. Sodium hydroxide was found to be more suitable for coupling reaction compared to sodium carbonate or aqueous ammonia because the formed dye was stable and more intense in sodium hydroxide medium. The results of study revealed that 1.5 mL volume of 20% sodium hydroxide is optimum for method A and 1.0 mL volume of 20% sodium hydroxide is optimal for methods B and C to produce maximum absorbance.

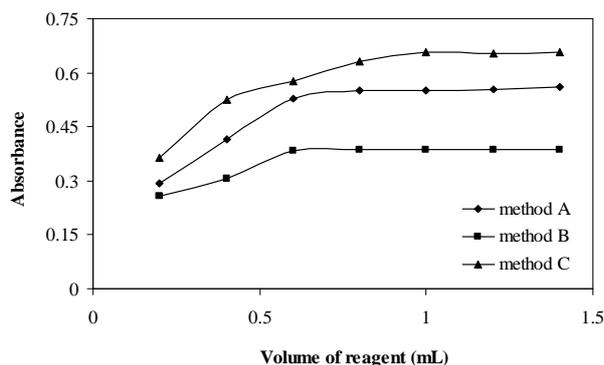


Fig. 5: Effect of concentration of coupling reagents on the reaction product of 10 µg mL⁻¹ MDC in methods A, B, and C

Effect of solvent

The choice of diluent for the reaction mixture was also studied. Water, methanol, ethanol, acetone, and isopropanol were tested as diluting solvents. Water was the best diluent for methods A and B. But, in case of method C the highest absorbance and reproducible value were obtained when methanol was used as a diluting solvent.

Effects of reaction time and temperature

The colored dyes developed rapidly after the addition of reagents and attained maximum intensity after about 5 min at room temperature (27 ± 1°C). A stability study of the chromogens was carried out by measuring the absorbance values at time intervals of 10 min. It was found that the formed azo dyes were stable for more than 2 hours in all methods.

Validation of the Proposed Methods

Calibration curves, linearity and sensitivity

Under optimum experimental conditions, a linear relation was obtained between absorbance and concentration of MDC in the

range 1.0-24.0 µg mL⁻¹ in method A, 1.0-28.0 µg mL⁻¹ in method B and 1.0-20.0 µg mL⁻¹ in method C. The regression analysis of the plot using the method of least squares was made to evaluate the intercept (a), slope (b), regression coefficient (r²) and standard deviations of slope and intercept. In all cases, Beer's law plots were linear with good correlation coefficients as shown Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines¹⁴ using the formulae: $k \cdot S.D.a/b$, where k is 3.3 for LOD and 10 for LOQ, S.D.a is the standard deviation of the intercept, and b is the slope of regression line.

Precision and accuracy of proposed methods

The accuracy and precision of the proposed methods was carried out by measuring the content of MDC in pure form at three different concentration levels (low, medium and high). The short-term (intra-day assay) and the daily precisions (inter-day assay) were performed by measuring five independent analyses at specified concentration levels within 1 d and on 5 consecutive days, respectively (Table 2). The standard deviation, relative standard deviation and relative errors obtained by proposed methods can be considered to be very satisfactory.

Table 1: Analytical and regression parameters of proposed methods

Parameter	Method A	Method B	Method C
λ_{max} (nm)	520	428	535
Beers law limit ($\mu\text{g.mL}^{-1}$)	1.0-24.0	1.0-28.0	1.0-20.0
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	3.1903×10^4	2.3583×10^4	3.8653×10^4
Sandell's sensitivity ($\mu\text{g.cm}^{-2}$)	0.019	0.026	0.016
Regression equation ($Y = a + bC$) [*]	0.052	0.0384	0.0629
Slope (b)	0.019	0.0002	0.0137
Intercept (a)	0.9998	0.9994	0.9998
Correlation coefficient (r^2)	3.35×10^{-4}	3.55×10^{-4}	3.78×10^{-4}
Standard deviation of slope (S_b)	4.52×10^{-3}	5.6×10^{-3}	4.2×10^{-3}
Standard deviation of intercept (S_a)	0.29	0.48	0.22
Detection limit LOD ($\mu\text{g.mL}^{-1}$)	0.87	1.46	0.68
Quantification limit LOQ ($\mu\text{g.mL}^{-1}$)			

^{*}Y = a + bC, where Y is absorbance and C is the concentration in $\mu\text{g.mL}^{-1}$

Table 2: Evaluation of precision and accuracy of proposed methods

Proposed method	MDC taken ($\mu\text{g.mL}^{-1}$)	Intra-day precision (n=6)*			Inter-day precision (n=6)*		
		MDC found ($\mu\text{g.mL}^{-1}$)	% RSD	% RE	MDC found ($\mu\text{g.mL}^{-1}$)	% RSD	% RE
Method A	4	4.04±0.063	1.56	1.00	4.04±0.08	1.98	1.00
	12	12.13±0.196	1.62	1.08	11.95±0.13	1.09	-0.41
	20	20.08±0.22	1.12	0.40	19.98±0.19	0.95	-0.10
Method B	4	4.02±0.038	0.95	0.50	4.03±0.07	1.73	0.75
	16	16.05±0.16	0.98	0.31	16.05±0.17	1.06	0.31
	24	23.96±0.168	0.7	-0.17	23.98±0.2	0.83	-0.08
Method C	4	3.94±0.065	1.65	-1.50	4.08±0.08	1.96	2.00
	12	12.01±0.132	1.1	0.08	12.07±0.18	1.50	0.58
	20	19.97±0.065	0.33	-0.15	20.08±0.22	1.10	0.40

*Mean value of six determinations; SD. Standard deviation; RSD. Relative standard deviation ; RE. Relative error

Accuracy and recovery

The accuracy and validity of the proposed methods was ascertained by recovery experiments through standard addition method. In this study, pre analyzed tablet powder was spiked with pure MDC at two different levels and the total amount of drug was determined by the

proposed methods. The recoveries of the pure drug added to the tablet powder were shown in Table 3. The results reveal that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually formulated with pharmaceutical preparations. The recovery values are indicating that the accuracy of method is not affected by co formulated substances.

Table 3: Results of recovery of proposed methods by standard addition method

Proposed methods (%) Recovery ^a ± SD						
Proposed methods	Formulation taken ($\mu\text{g.mL}^{-1}$)	Pure drug added ($\mu\text{g.mL}^{-1}$)	Moza plus	Mopride-5	Mosid-OD	Mosafe
Method A	8	4	99.35±1.21	102.4±1.52	101.05±2.2	98.16±1.8
	8	8	101.17±1.6	101.08±1.41	102.72±1.77	99.71±1.64
Method B	12	6	99.41±0.43	100.79±0.9	100.14±0.74	100.1±0.44
	12	12	100.68±0.71	100.7±0.27	100.05±0.61	100.64±0.21
Method C	8	4	100.34±0.92	100.52±1.38	100.65±1.41	100.28±0.82
	8	8	100.29±0.3	101.14±0.57	101.2±1.83	100.34±0.66

^a Mean value of three determinations

Application of the methods to the analysis of formulations

The developed methods were successfully applied to the analysis of MDC in various pharmaceutical formulations. The results of assay are given in Table 4 and compared favorably with the reference

method⁸. The results showed that the *t*- and *F*-values are less than the theoretical value for five degrees of freedom and for 95% confidence level, suggesting that the proposed methods are comparable to the reference method with respect to accuracy and precision. The results are given in Table 4.

Table 4: Application of proposed methods to the assay of MDC in different pharmaceutical formulations

Pharmaceutical preparations #	Amount per tablet (mg)	% Found* ± SD			
		Reference method	Method A	Method B	Method C
Moza plus ^a (Capsules)	15	101.03±1.2	100.16±0.59	99.96±0.78	99.3±0.65
			t = 1.6 F = 4.13	t = 1.83 F = 2.36	t = 1.57 F = 3.4
Mopride ^b (Tablets)	5	101.4±1.15	101.16±1.04	101.01±0.9	100.3±0.67
			t = 0.38 F = 1.22	t = 0.65 F = 1.63	t = 2.02 F = 2.94
Mosid-OD ^c (Tablets)	15	99.2±1.35	100.62±0.67	99.4±0.71	99.17±0.8
			t = 2.31 F = 4.06	t = 0.32 F = 3.61	t = 0.04 F = 2.84
Mosafe ^d (Tablets)	5	98.9±1.6	99.92±1.05	99.37±1.14	100.03±1.01
			t = 1.3 F = 2.32	t = 0.59 F = 1.96	t = 1.46 F = 2.51

#Marketed by: ^aIntas pharma, India; ^bMicro Nova, India; ^cTorrent pharma, India; ^dGlenmark, India. *Mean value of six determinations. The theoretical values of *t* (2.57) and *F* (5.05) at confidence limit at 95% confidence level and five degrees of freedom (*p*=0.05)

CONCLUSION

This paper presents that RCL, BAC and HQ are as new diazo coupling reagent for the spectrophotometric determination of MDC. The proposed methods are selective as the free aromatic amine group preferentially interacts with nitrous acid to form the diazotized product which is later coupled with RCL, BAC or 8-HQ to form very stable dye stuff. The proposed method concerned with 8-HQ was superior as compared with that of the other coupling reagents and the performance order of the proposed methods is 8-HQ > BAC > RCL according to higher molar absorptivity and lower detection limits. The proposed methods provide adequate sensitivity, selectivity and free from interferences. Hence, the proposed methods are very suitable for routine analysis of MDC in quality control laboratories.

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