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Original Article

VALIDATED SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF MONTELUKAST SODIUM IN PURE AND DOSAGE FORMS USING N-BROMOSUCCINIMIDE AND DYES

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ABSTRACT

Objective: Simple, sensitive, precise, reproducible and validated spectrophotometric methods have been developed for the quantification of montelukast sodium as leukotriene receptor antagonist drug, in pure and dosage forms (tablets).

Methods: The methods use N-bromosuccinimide (NBS) as an oxidant and three dyes, amaranth, methylene blue, and indigo carmine, as auxiliary reagents. The three methods are based on oxidation reaction of montelukast sodium with a known excess of N-bromosuccinimide (NBS) in acid medium, followed by determination of unreacted NBS by the reaction with a fixed amount of dyes, amaranth, methylene blue, and indigo carmine followed by the measurement of the absorbance at 520, 664 and 610 nm, respectively.

Results: Under the optimum conditions, linear relationships with good correlation coefficients (0.9993-0.9996) were found over the concentration ranges of 0.5-10, 1.0-12 and 0.5-8.0 µg/ml with a limit of detection (LOD) of 0.15, 0.3 and 0.14 µg/ml using amaranth, methylene blue, and indigo carmine methods, respectively. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the common tablet excipients.

Conclusion: The proposed methods were validated in accordance with ICH guidelines and successfully applied to the analysis of montelukast sodium in dosage forms (tablets). The reliability of the methods was further ascertained by performing recovery studies using the standard addition method. Statistical comparison of the results obtained by applying the proposed methods with those of the reported method by applying student's t-test and F-test revealed good agreement.

Keywords: Montelukast sodium, N-bromosuccinimide, Spectrophotometry, Method validation, Dosage forms

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INTRODUCTION

Montelukast sodium is chemically designated as [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl]thio] methyl]cyclopropane acetic acid, sodium salt (MNT) (fig. 1) [1]. MNT is a leukotriene receptor antagonist used as an alternative to anti-inflammatory medications in the management and chronic treatment of asthma [2].

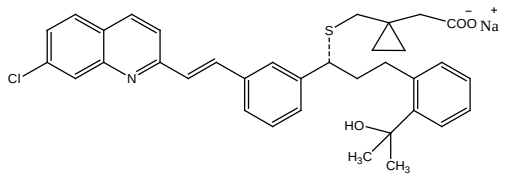


Fig. 1: The chemical structure of montelukast sodium (MNT)

The literature survey revealed that few methods were described for the determination of MNT in pure, tablet dosage forms and biological fluids such as spectrofluorimetry [2], electrochemical [3], electrophoresis [4, 5] and high-performance liquid chromatography [6-13]. Most of these reported methods are either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories.

In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, due to its simplicity, less expensive, less time consuming and reasonable sensitivity with significant economic advantages.

To the best of our knowledge, there are some methods have been reported for the quantification of MNT in commercial dosage forms using a spectrophotometric technique using bromothymol blue and bromocresol purple [14], $FeCl_3$ in HCl and orcinol in concentrated HCl [15], Wool fast blue [16], Fe^{3+}/1, 10-phenanthroline, MBTH and 2, 2'-bipyridyl [17], UV-spectrophotometry [18-21]. However, these previously reported methods suffer from one or more disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step; some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective and selective spectrophotometric method for the determination of MNT their pharmaceutical dosage forms.

From the foregoing paragraphs, N-bromosuccinimide (NBS) despite its strong oxidizing power, versatility, and high oxidation potential and stability in solution has not been applied for the assay of MNT in pure forms and tablets.

The present investigation aims to develop for the first time simple, sensitive, accurate, precise and cost-effective spectrophotometric methods for the determination of MNT in pure and dosage forms. The proposed methods employ N-bromosuccinimide which acts as brominating agent, and dyes; amaranth (AM), methylene blue (MB) and indigo carmine (IC), as auxiliary chromogenic reagents. No interference was observed in the assay of MNT from common excipients in levels found in dosage forms. These methods are validated by statistical data.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10

mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

Materials and reagents

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Reference standard of pure MNT and pharmaceutical formulations

Pure sample of MNT was kindly supplied by Delta Pharmaceutical Industries, Cairo, Egypt, with a purity of 99.70±0.52% by applying the reported method [14]. Montelair tablets, labeled to contain 5.0 mg MNT per tablet, product of Jedco Company, Cairo, Egypt. Delmonkast tablets, labeled to contain 10 mg MNT per tablet, product of Delta Pharmaceutical Industries, Cairo, Egypt were purchased from local pharmacies.

Preparation of stock standard solution

Stock standard solutions (100 µg/ml) and (1.0 × 10–3 mol /l) of MNT were prepared by dissolving 10 and 60.82 mg of pure MNT in bidistilled water and further diluted with bidistilled water to the mark in a 100 ml volumetric flask. The standard solutions were stable for at least 7.0 d when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range

Reagents

N-bromosuccinimide (NBS) (0.01 mol/l)

A stock solution of 0.02 mol/l NBS (Sigma-Aldrish) was freshly prepared by dissolving about 0.356 g of NBS in least amount of warm bidistilled water in a 100 ml measuring flask and then diluted to the mark with bidistilled water and standardized [22]. The solution was kept in an amber colored bottle and was diluted appropriately to get 200 µg/ml NBS for use in all methods. The NBS solution was stored in a refrigerator when not in use.

Potassium bromide (1.0% w/v)

A 1.0% w/v KBr solution was also prepared by dissolving 1.0 g of KBr in 100 ml water.

Hydrochloric acid (5.0 mol/l)

A 5.0 mol/l of HCl was prepared by diluting 43 ml of concentrated acid (Merck, Darmstadt, Germany, Sp. gr. 1.18, 37%) to 100 ml with bidistilled water and standardized as recommended previously [23] prior to use.

Dyes (1000 µg/ml)

Stock solutions (1000 µg/ml) amaranth (AM), methylene blue (MB) and indigocarmine (IC) were first prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100 ml calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of 200 µg/ml of dyes.

Recommended procedures

Different aliquots (0.05-1.0 ml), (0.1-1.2 ml), and (0.05-0.8 ml) of a standard 100 µg/ml MNT solution using AM, MB and IC methods, respectively, were transferred into a series of 10 ml calibrated flasks by means of a micro burette. To each flask 1.5 ml each of 5.0 mol/l HCl; 2.0 ml of NBS solution (200 µg/ml) and 1.0 ml of 1.0% (w/v) KBr were added successively. The flasks were stoppered, content mixed, and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.0 ml of (200 µg/ml) AM, MB and IC solution were added to each flask and mixed well, and then the volume was diluted to the mark with water. The absorbance of each solution was measured at 520, 664 and 610 nm for AM, MB and IC methods, respectively, after 3.0 min against a reagent blank. In all methods, a standard graph was prepared by plotting the absorbance versus the concentration of drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Procedure for tablets

The contents of twenty tablets of MNT were weighed accurately and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 20 mg MNT was dissolved in bidistilled water in a 100 ml volumetric flask with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with bidistilled water in a 100 ml measuring flask to give 200 µg/ml stock solution of VARD for analysis by spectrophotometric methods. A convenient aliquot was then subjected to analysis by the recommended procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

RESULTS AND DISCUSSION

Absorption spectra

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acid medium [24]. The proposed spectrophotometric methods are based on the reaction between MNT and measured excess of NBS and subsequent determination of the latter by reacting it with a fixed amount of AM, MB and IC dye, and measuring the absorbance at 520, 664 and 610 nm (fig. 2). These methods make use of the bleaching action of NBS on the dyes, the decolorization being caused by the oxidative destruction of the dyes. MNT when added in increasing concentrations to a fixed concentration of NBS consumes the latter and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentrations of MNT.

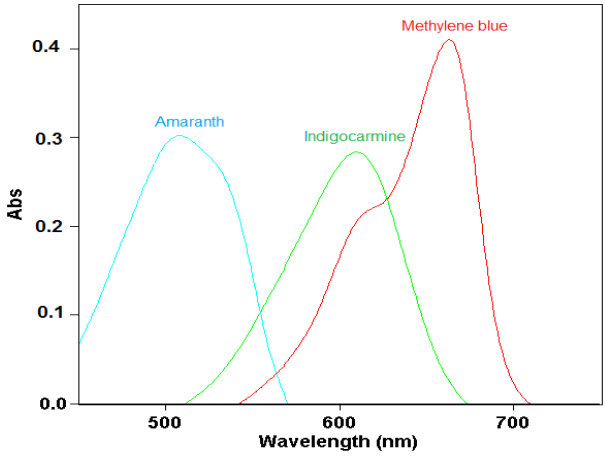
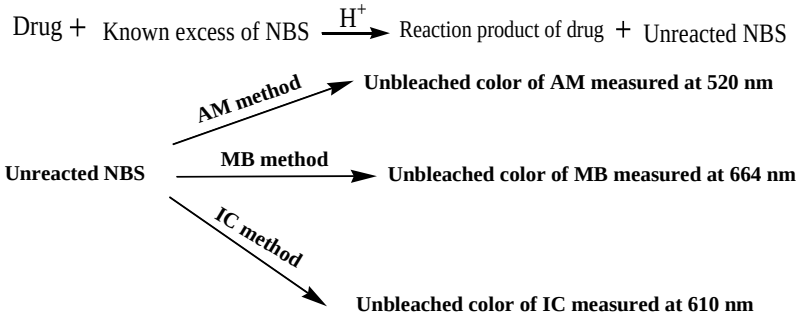


Fig. 2: Absorption spectra for the unreacted NBS that determined by reacting with a fixed amount of dyes and measuring the absorbance of unbleached dyes at 520, 664 and 610 nm for AM, MB and IC methods, respectively

Chemistry of the reactions

NBS is a strong oxidizing or brominating agent and perhaps the most important positive bromine containing organic compound used for the determination of many pharmaceutical compounds [25-29]. It is also used for the specific purpose of brominating alkenes at the allylic position [30]. The analytical reactions involved two steps; the first one was concerned with the bromination of MNT with a known excess amount of NBS in hydrochloric acid medium. The second step involved the determination of the excess residual NBS via its reaction with a fixed amount of AM, MB and IC dyes and measuring the absorbance at the respective λ_{max}. The tentative reaction scheme of spectrophotometric methods is shown in Scheme 1. In all methods, the absorbance increased linearly with increasing concentration of MNT. The latter methods make use of the bleaching action of NBS on dyes, the discoloration being caused by the oxidative destruction of the dye.



Scheme 1: Tentative reaction scheme for the proposed spectrophotometric methods

Selection of acid type and concentration

The reaction between MNT and NBS was performed in different acid media HCl, H_2SO_4, HNO_3, and CH_3-COOH solutions. Better results were suitable in hydrochloric acid medium. The effect of HCl concentration on the reaction between MNT and NBS was studied by varying the concentration of HCl keeping the concentrations of NBS and MNT fixed. The reaction was found to be rapid yielding a constant absorbance with maximum sensitivity and stability when the HCl concentration was 5.0 mol/l and maintained in the range of 0.25–3.0 ml of HCl (5.0 mol/l) in a total volume of 10 ml. The results indicated that, at 1.0-3.0 ml of HCl (5.0 mol/l), there were almost same absorbance values were obtained in the presence of MNT, the absorbance values obtained were constant and were almost the same as those of the reagent blank. At the acid volumes less than 1.0 ml, reaction led to go slower and incomplete. Therefore, 1.5 ml of HCl (5.0 mol/l) was used though out the study (fig. 3).

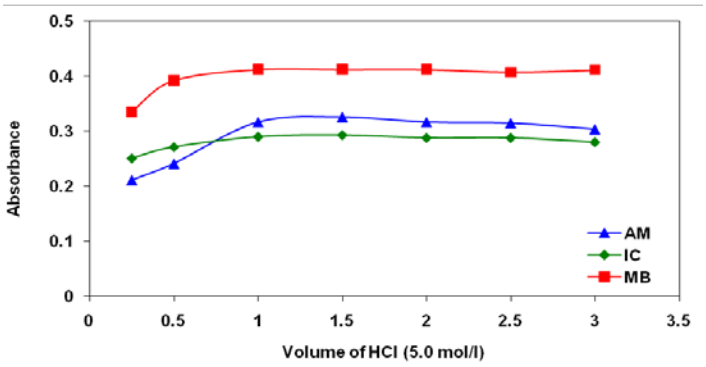


Fig. 3: Effect of volume of HCl (5.0 mol/l) of the oxidation product of MNT with NBS and dyes

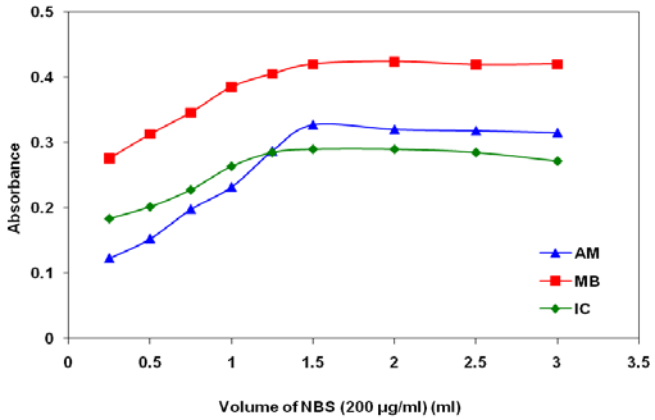


Fig. 4: Effect of volume of NBS (200 µg/ml) of the oxidation product of MNT with NBS and dyes in HCl medium

Effect of NBS concentration

To investigate the optimum concentration of NBS, different concentrations of NBS were treated in the range of 0.25–3.0 ml with a fixed concentration dyes in HCl medium and the absorbance was measured at optimum wavelength. It was found that maximum color intensity of the products was achieved with 2.0 ml of NBS (200 µg/ml) (fig. 4).

Effect of KBr concentration

The effect of KBr concentration was studied in the range of 0.5–2.5 ml. 1.0 ml of (1.0%, w/v) KBr was chosen as an optimum volume to accelerate the oxidation process.

Effect of dye concentration

The effect of AM, MB, and IC concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of NBS. The effect dye concentration was studied in the range of 0.25–3.0 ml of each dye (200 µg/ml). It was found that maximum color intensity of the oxidation products was achieved with 1.5 ml AM, MB and IC solution, respectively (fig. 5).

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 25 to 60 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at room temperature (25±2 °C). The effect of mixing time required completing oxidation of MNT and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min at room temperature (25±2 °C). The time required for complete oxidation of MNT is not critical and any delay up to 15 min in the determination of unreacted NBS had no effect on the absorbance. A 3.0 min standing time was found necessary for the complete bleaching of the dye color by the residual NBS was found necessary for complete reduction of residual NBS by all dyes and the absorbance of the unreacted dye was stable for at least 6.0 h, thereafter.

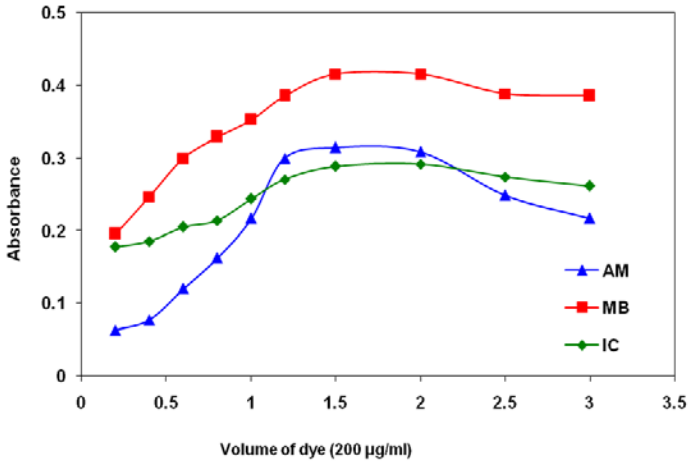


Fig. 5: Effect of volume of dyes (200 µg/ml) of the oxidation product of MNT with NBS and dyes in HCl medium

Effect of sequence of addition

The optimum sequence of the addition was MNT–HCl–NBS–KBr and then dye. Other sequences gave lower absorbance values under the same experimental conditions.

Method validation

The proposed methods have been validated for linearity, sensitivity, accuracy, precision, robustness, ruggedness and recovery.

Linearity and sensitivity

Under the optimum conditions, a linear correlation was found between absorbance at λ_{max} and the concentration of MNT in the ranges of 0.5-10, 1.0-12 and 0.5-8.0 µg/ml using AM, MB and IC, respectively (fig. 6). The calibration graph is described by the Eqn. 1.

$$A = a+b\ C \quad \text{Eqn. 1}$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg/ml, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in table 1. For accurate determination, Ringbom concentration range [31] was calculated by plotting log concentration of drug in µg/ml against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of MNT and represented in table 1. Apparent molar absorptivity and Sandell's sensitivity values were calculated and illustrated in table 1 as per the current ICH guidelines [32]. The high molar absorptivity and lower Sandell sensitivity values reflect the good and high sensitivity of the proposed methods.

Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following Eqn. 2. and Eqn. 3. [31, 32]:

$$LOD = 3s/k \quad \text{Eqn. 2.}$$

$$LOQ = 10\ s/k \quad \text{Eqn. 3.}$$

Where s is the standard deviation of ten replicate determinations values of the reagent blank and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the limit of detection was found to be 0.15, 0.30, and 0.14 µg/ml for AM, MB, and IC methods, respectively. According to this equation, the limit of quantitation was found to be 0.5, 1.0, and 0.47 µg/ml for AM, MB, and IC methods, respectively.

The validity of the proposed methods was evaluated by statistical analysis [32] between the results achieved from the proposed methods and that of the reported method [14]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (table 1), there is no significant difference between the proposed and reported method [14] regarding accuracy and precision.

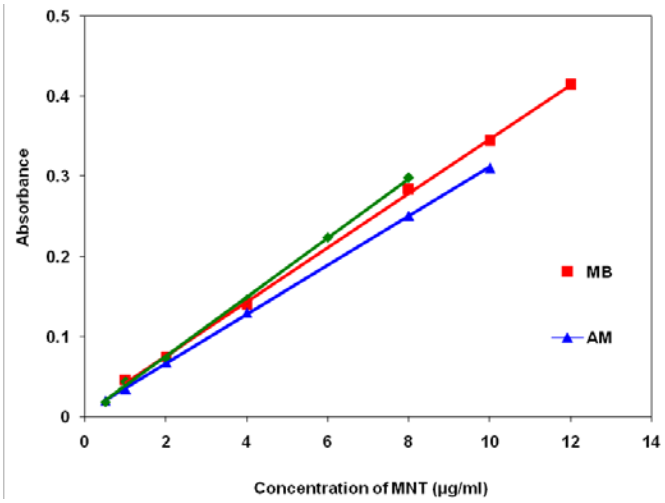


Fig. 6: Calibration curves for MNT using AM, MB and IC methods

Table 1: Analytical and regression parameters of the proposed spectrophotometric methods for determination of MNT

Parameters	AM	MB	IC
Beer's law limits, µg/ml	0.5-10	1.0-12	0.5-8.0
Ringboom limits, µg/ml	1.0-8.0	2.0-10	1.0-7.0
Molar absorptivity, x 10^4 L/mol. cm	2.058	2.258	2.31
Sandell sensitivity, ng/cm^2	29.55	26.93	26.32
Regression equation[a]			
Intercept (a)	0.0039	0.0059	0.0011
Standard deviation of intercept (S_a)	0.008	0.009	0.006
Slope (b)	0.0308	0.0341	0.037
Standard deviation of slope (S_b)	0.01	0.014	0.009
Correlation coefficient, (r)	0.9996	0.9993	0.9994
mean±SD	99.89±1.50	99.62±1.60	100.63±1.04
RSD%	1.50	1.61	1.03
RE%	1.58	1.69	1.08
Limit of detection, µg/ml	0.15	0.30	0.14
Limit of quantification, µg/ml	0.50	1.0	0.47
Calculated *t*-value[b]	0.24	0.52	0.71
Calculated *F*-value [b]	1.33	1.51	1.56

[a]$A = a+bC$, where C is the concentration in µg ml^{-1}, A is the absorbance units, a is the intercept, b is the slope, [b]The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Table 2: Results of intra-day and inter-day accuracy and precision study for MNT obtained by the proposed methods

Method	Added (µg/ml)	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
AM	3.0	99.00	0.50	-1.0	2.97±0.016	100.50	0.60	0.50	3.015±0.019
	6.0	99.50	0.82	-0.50	5.97±0.051	99.20	0.96	-0.80	5.952±0.06
	9.0	99.80	1.30	-0.20	8.982±0.123	99.70	1.15	-0.30	8.973±0.108
MB	4.0	99.30	0.60	-0.70	3.972±0.025	99.00	0.38	-1.0	3.96±0.016
	8.0	99.10	0.90	-0.90	7.928±0.075	99.70	0.75	-0.30	7.976±0.063
	12	100.50	1.50	0.50	12.06±0.19	98.80	1.10	-1.20	11.856±0.137
IC	2.0	99.40	0.45	-0.60	1.988±0.009	99.20	0.55	-0.80	1.984±0.011
	4.0	100.70	0.83	0.70	4.028±0.035	99.30	0.80	-0.70	3.972±0.033
	6.0	98.70	1.80	-1.30	5.922±0.112	99.40	1.60	-0.60	5.964±0.1

[a]RSD%, percentage relative standard deviation; RE%, percentage relative error, [b]mean±standard error, confidence limit at 95% and five degrees of freedom (t = 2.571).

Accuracy and precision

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of MNT were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in table 2. Lower values of the relative standard deviation (RSD%) and percentage relative error (RE%) indicate the precision and accuracy of the proposed methods. The percentage relative error was calculated using the following equation (Eqn. 4.):

$$\mathbf{RE\%} = \frac{\mathbf{(Found - Added)}}{\mathbf{Added}} \times \mathbf{100} \quad \text{Eqn. 4}$$

The assay procedure was repeated six times, and percentage relative standard deviation (RSD%) values were obtained within the same day to evaluate the repeatability (intra-day precision) and over five

different days to evaluate intermediate precision (inter-day precision). For the same concentrations of MNT inter-and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of MNT were evaluated to ascertain the accuracy of the methods. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Robustness and ruggedness

For the evaluation of method robustness, the volume of NBS was slightly altered (2.0±0.5 ml) and the reaction time (after adding NBS, time varied was 5.0±2.0 min) were slightly varied deliberately in the three methods. The analysis was performed with altered conditions by taking three different concentrations of MNT and the methods were found to remain unaffected as shown by the RSD values in the ranges of 0.78-2.50%. Methods ruggedness was expressed as the RSD of the same procedure applied by three different analysts as well as using three different instruments (spectrophotometers). The inter-analysts RSD were in the ranges 0.80-2.50%, whereas the inter-instruments RSD ranged from 0.70-2.30%, suggesting that the developed methods were rugged. The results are shown in table 3.

Table 3: Results of method robustness and ruggedness (all values in RSD%) studies for MNT

Methods	Nominal amount concentration (µg/ml)	RSD%[a]			
		Robustness		Ruggedness	
		Variable alerted[b]			
		NBS volume	Reaction time	Different analysts	Different instruments
AM	3.0	1.05	0.78	0.80	0.70
	6.0	1.70	1.15	1.30	1.30
	9.0	2.30	1.70	1.90	2.10
MB	4.0	0.85	0.90	1.20	0.80
	8.0	1.40	1.80	1.94	1.50
	12	2.10	2.40	2.50	2.0
IC	2.0	1.10	1.0	0.90	0.80
	4.0	1.70	1.90	1.70	1.40
	6.0	2.50	2.20	2.25	2.30

[a]Mean of three determinations, [b]Volume of NBS is (2.0±0.5 ml) and reaction time is (5.0±2.0 min) were used

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, a recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of MNT (50, 100 and 150% of the level present in the tablet) to a fixed amount of MNT in tablet powder (pre-analyzed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from Eqn. 5.

$$\% \text{ Recovery} = \frac{[C_F - C_T]}{C_p} \times 100 \quad \text{.... Eqn. 5}$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in table 4 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets, which did not interfere in the assay.

Table 4: Application of the standard addition technique for the determination of MNT in pharmaceutical formulations using the proposed methods

Sample	Taken (µg/ml)	AM		MB		IC		Reported method [2]
		Added (µg/ml)	Recovery[a] (%)	Added(µg/ml)	Recovery[a](%)	Added(µg/ml)	Recovery[a](%)	
MontelairTablets	2.0	-	99.10	-	99.30	-	99.50	
		2.0	99.40	2.0	99.50	3.0	98.90	
		4.0	100.70	4.0	99.00	6.0	100.80	
		6.0	98.80	6.0	100.50	9.0	99.20	
mean±SD			99.50±0.84		99.00±0.65		99.53±0.93	99.63±0.59
RSD%			0.84		0.64		0.93	0.59
V			0.70		0.42		0.86	0.35
S. E			0.42		0.33		0.46	0.26
t-value[b]			0.28		1.60		0.20	
F-value[b]			2.03		1.21		2.48	
Delmonkast Tablets	2.0	-	100.30	-	99.40	-	98.60	
		2.0	99.30	2.0	99.20	3.0	100.90	
		4.0	98.70	4.0	100.50	6.0	99.00	
		6.0	100.70	6.0	98.50	9.0	99.70	
mean±SD			99.75±0.91		99.40±0.83		99.55±1.01	99.50±0.68
R. S. D%			0.91		0.83		1.01	0.68
V			0.84		0.69		1.02	0.46
S. E			0.46		0.41		0.50	0.30
t-value[b]			0.49		0.21		0.09	
F-value[b]			1.79		1.49		2.21	

[a]Average of six determinations, [b]Theoretical values of t and F are 2.571 and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Application of pharmaceutical formulations (tablets)

The proposed methods were applied to the determination of MNT in tablets dosage forms. The results in table 4 showed that the methods are successful for the determination of MNT and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of MNT by the proposed methods and the reported method [14] for the same batch of material is presented in table 4. The results agree well with the label claim and also were in agreement with the results obtained by the reported method [14]. When the results were statistically compared with those of the reported method by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [33]. Hence, no significant difference between the proposed methods and the reported method at the 95 % confidence level with respect to accuracy and precision.

CONCLUSION

Three new, useful simple, rapid, and cost-effective spectrophotometric methods have been developed for the determination of MNT in pure and tablets dosage forms using NBS as a brominating agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for MNT. The assay methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of MNT in pure and dosage forms.

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AUTHOR'S CONTRIBUTIONS

Prof. Dr. Ragaa El Sheikh has generated the research idea and interpreted the data and helped to draft the manuscript. Prof. Dr. Wafaa El Sayed Hassan has suggested the research idea and participated in the design of the study. Miss. Rowaida A. Fahmy was prepared the solutions, carried out the experiments, interpreted the data and helped to draft the manuscript. Prof. Dr. Ayman A. Gouda helped in check spelling, reducing the plagiarism, interpreting the data, reviewed the manuscript and submit the manuscript for publication.

CONFLICTS OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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