



Selective and validated spectrophotometric methods for the determination of midazolam using *N*-Bromosuccinimide

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ABSTRACT

Simple and sensitive spectrophotometric methods for the determination of midazolam (MID) are proposed. The methods are based on the bromination of the drug using *N*-bromosuccinimide (NBS) and the excess oxidant is determined by either malachite green (MG) or safranin O (SO), the absorbances of which are measured at 617 or 522 nm for MID-MG and MID-SO, respectively. Under the optimized experimental conditions, Beer's law is obeyed in the concentration ranges of 15.00-30.00 and 10.00-50.00 $\mu\text{g mL}^{-1}$ with molar absorptivity of 8.88×10^3 and $1.18 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ for methods A and B, respectively. The limits of detection for methods A and B are 1.7368 and 0.5823 g mL^{-1} , respectively. No interference was observed from excipients commonly present in formulations. The proposed methods are applied successfully for the pharmaceutical formulations.

Key words: Spectrophotometry, Midazolam, *N*-Bromosuccinimide, Malachite green, Safranin O.

INTRODUCTION

Midazolam chemically, 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (Figure 1), belongs to a group of medicines known as benzodiazepines[1]. It is an injectable form of antidepressant which is used for sedation[2], medical procedures or dental procedures, anxiety relief and memory loss during surgeries[3]. The medication can relax the muscles and cause short time memory loss or sleepiness. Thus it is approved for use as an anesthesia before operation procedures[4]. Midazolam is superior to other benzodiazepines because of its rapid, nonpainful induction and lack of venous irritation. This medication is also approved for use in children[5].

A literature survey revealed that only few HPLC[6,7], RP-HPTLC[8], GC/MS[9], LC-MS[10,11] and spectrophotometric[12,13] methods are available for the determination of midazolam. An attempt has been made to develop rapid, sensitive and validated spectrophotometric methods for the determination of midazolam. The applicability of these methods also studied in their dosage forms.

EXPERIMENTAL SECTION

Apparatus

A SHIMADZU (Model No: UV 2550) UV-Visible spectrophotometer with 1 cm quartz cells was used for the absorbance measurements.

Reagents and solutions

All solutions were prepared with double distilled water. Chemicals used were of analytical reagent grade.

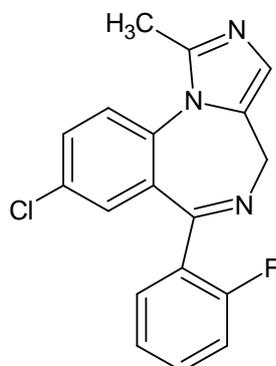


Figure 1

A solution of 0.5 % NBS was prepared by dissolving 0.5 g NBS in 100 mL distilled water. Solutions of MG (Merck India Ltd., Mumbai 0.25%), SO (sd fine chem. Ltd., India, 0.02%) and 2 M hydrochloric acid (Spectrochem Pvt. Ltd.) were prepared.

Standard drug solution

A 1000 $\mu\text{g mL}^{-1}$ standard drug solution was prepared by dissolving 0.1 g of MID in 100 mL ethanol [14]. The stock solution was diluted approximately to get working concentration.

*Procedure**Method A*

Different concentrations containing 5.00 - 30.00 $\mu\text{g mL}^{-1}$ of MID solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4 mL by adding adequate quantity of water. To each flask 1 mL of 2 M HCl and 1 mL of NBS solution (0.5 %) were added. The flasks were stoppered, content mixed and let stand for 15 min with occasional shaking. Finally, 1 mL of 0.25 % MG solution was added and the volume was diluted to 10 mL with water and mixed well. The absorbance of each solution was measured at 617 nm against a reagent blank.

Method B

Varying aliquots containing 10.00 - 50.00 $\mu\text{g mL}^{-1}$ of a standard MID solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. To each flask 1 mL of 2 M HCl and NBS solution (0.5 %) were added. The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Finally, 1 mL of 0.02 % SO solution was added to each flask then the volume was diluted to 10 mL with water, contents were mixed well and absorbance was measured against a reagent blank at 522 nm after 10 min. In either method, a standard graph was prepared by plotting the absorbance versus the concentration of MID.

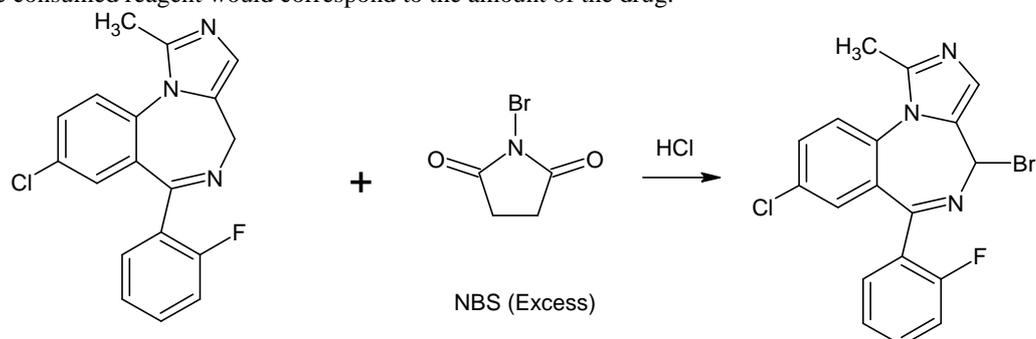
IV. Procedure for the assay of MID in pharmaceutical preparations

The commercial sample of injection containing the drug MID (Benzosed) was chosen for testing the suitability of the proposed methods. For this, MID injection of 5 mL containing 1 mg mL^{-1} MID was taken and the contents were transferred in to 100 mL volumetric flask. It was dissolved in ethanol and diluted to 100 mL with distilled water [15]. A convenient aliquot was then subjected to the analysis using the proposed methods.

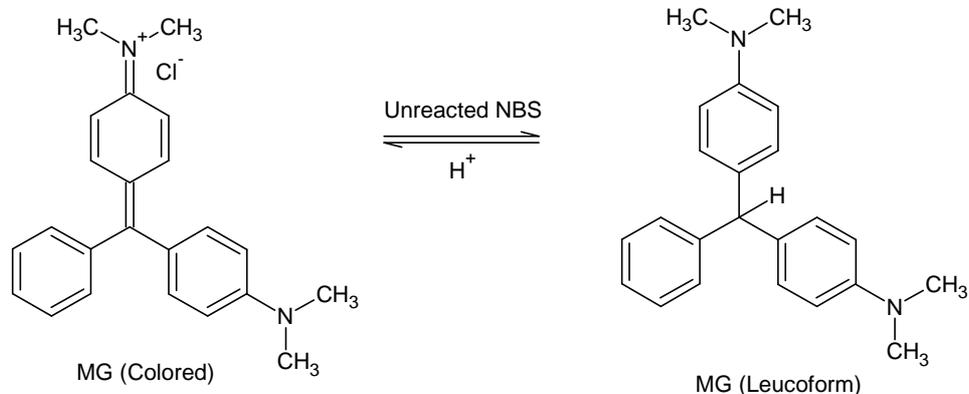
RESULTS AND DISCUSSION

The proposed methods mainly based on the bromination of MID by NBS followed by the bleaching action of NBS on the dyes. The drug undergoes bromination and the residual NBS is determined by discoloration being caused by the oxidative destruction of the dyes. In the present method, two dyes MG and SO have been used for the determination of MID (Scheme 1). The drug undergoes bromination and the residual NBS is determined by reacting

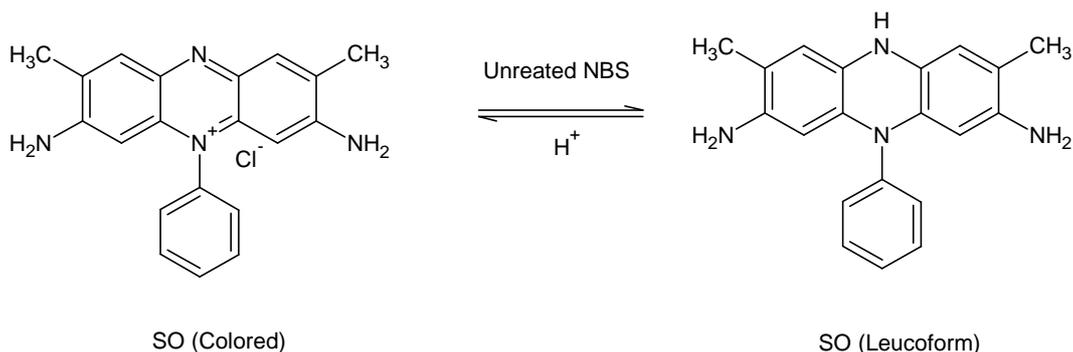
it with a fixed amount of either MG or SO and the reaction is found to be complete and quantitative in 15 min. According to the above mentioned reaction, NBS solution should be added in excess to react with the drug substance and the consumed reagent would correspond to the amount of the drug.



Method A



Method B



Scheme 1. Reaction of MID with NBS followed by MG and SO

Optimization of Reaction Conditions

Effect of NBS Concentration:

The effect of NBS concentration is studied by carrying out the reaction using 1 mL of different concentrations of NBS in the range of 0.25-1.5 % (w/v). It is observed that the maximum absorbance is obtained at a concentration of 0.5 % (w/v), and it is found that further increase in the concentration of the reagent does not have any effect on the reaction (Fig. 2).

Table 1 Spectral and Statistical data for the determination of Midazolam

Parameters	Method A	Method B
λ_{\max} nm	617	522
Beer's Law Limits ($\mu\text{g/ml}$)	15.00 – 30.00	10.00 -50.00
Molar Absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	8.88×10^3	1.18×10^4
Sandell's Sensitivity ($\mu\text{g cm}^{-2}$)	3.67×10^{-2}	2.75×10^{-2}
Limit of Detection* ($\mu\text{g mL}^{-1}$)	1.7368	0.5823
Limit of Quantification* ($\mu\text{g mL}^{-1}$)	5.2631	1.7647
Regression Equation**	$Y = a + b X$	$Y = a + b X$
Slope (b)	0.0095	0.0340
Intercept (a)	0.0757	0.0192
Correlation Coefficient (r)	0.9911	0.9953

* Limit of detection calculated according to ICH guidelines

** Y is the absorbance and X concentration in $\mu\text{g mL}^{-1}$ **Table 2 Evaluation of accuracy and precision****Method A**

Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
20.00	19.88	0.60	0.22	1.10
25.00	24.96	0.16	0.20	0.80
30.00	30.01	-0.03	0.09	0.29

Method B

Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
10.00	10.05	-0.50	0.11	1.09
20.00	19.93	0.35	0.06	0.33
30.00	29.86	0.46	0.08	0.29

* Mean value of five determinations

RE - Relative Error; SD - Standard Deviation; RSD - Relative Standard Deviation.

Table 3 Result of assay of formulation by the proposed method

Brand name	Labeled amount (mg)	Found* \pm SD using Method A	Found \pm SD using Method B
Benzosed	5	5.06 ± 0.23 $t = 0.68$	5.11 ± 0.28 $t = 0.38$

*Mean value of five determinations

Tabulated t value at 95% confidence level is 2.7

Method Validation**Quantification**

Regression analysis of the Beer's law data using the method of least squares is made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. A linear correlation is found in the graphs of absorbance versus concentration showed negligible intercept and is described by the equation: $Y = a + b X$ (where Y = absorbance; a = intercept; b = slope and X = concentration in $\mu\text{g mL}^{-1}$). The optical characteristics such as limits of detection (LOD) and quantification (LOQ) and sensitivity parameters such as molar absorptivity and Sandell's sensitivity are also given in Table 1.

Accuracy and Precision

The validity of the proposed methods is studied by performing recovery studies. Precisions and accuracy are examined by analysis of MID with the concentrations of 20.00, 25.00 and 30.00 $\mu\text{g mL}^{-1}$ for method A and 10.00, 20.00 and 30.00 $\mu\text{g mL}^{-1}$ for method B. Five replicate measurements are recorded at each concentration level. Mean recovery values \pm SD, RSD % and E_r % are found for both the methods and are found to be satisfactory. The obtained results are summarized in Table 2.

Interference study

In the pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Under the optimum reaction conditions, to a known amount of the drug, excipients

such as starch, glucose, lactose and stearic acid are added in different concentrations and analyzed. The results in Table 3 revealed that no interference is encountered from any of these excipients.

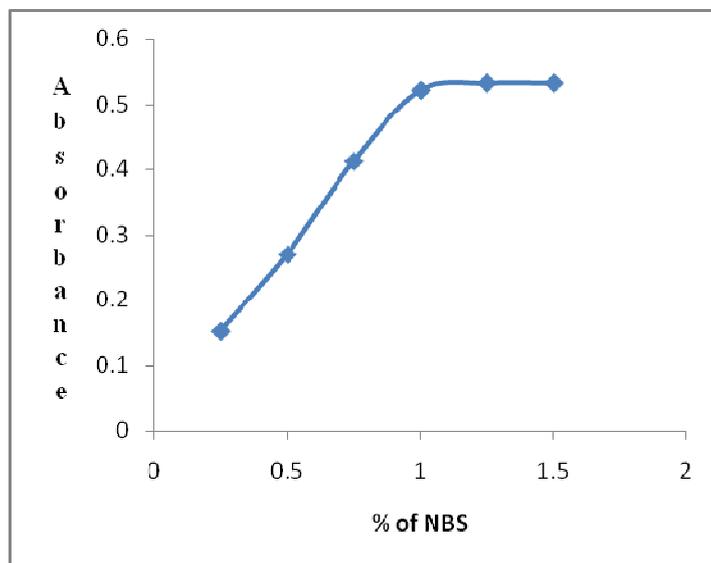


Figure 2. Effect of NBS concentration

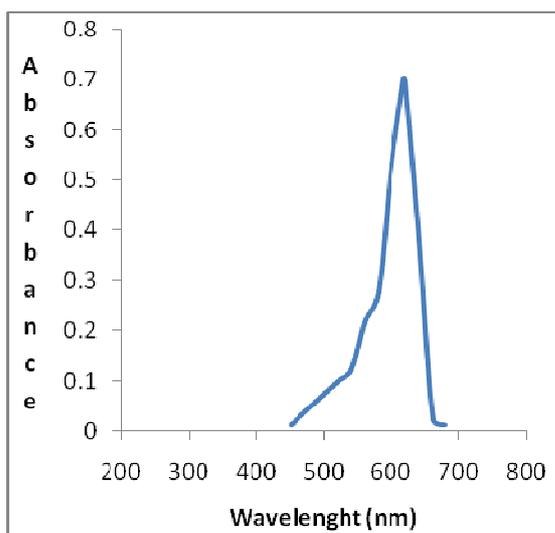


Figure 3. Absorption spectrum for Method A

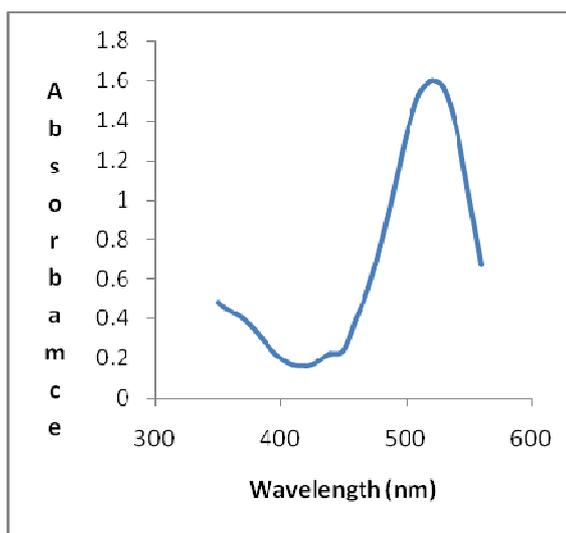


Figure 4. Absorption spectrum for Method B

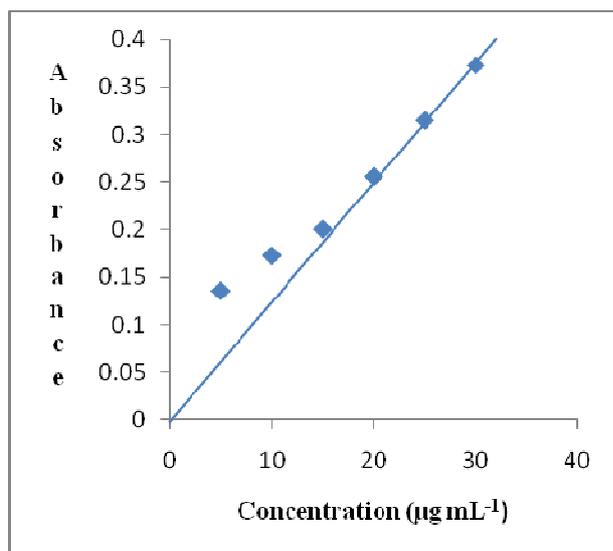


Figure 5. Calibration curve for Method A

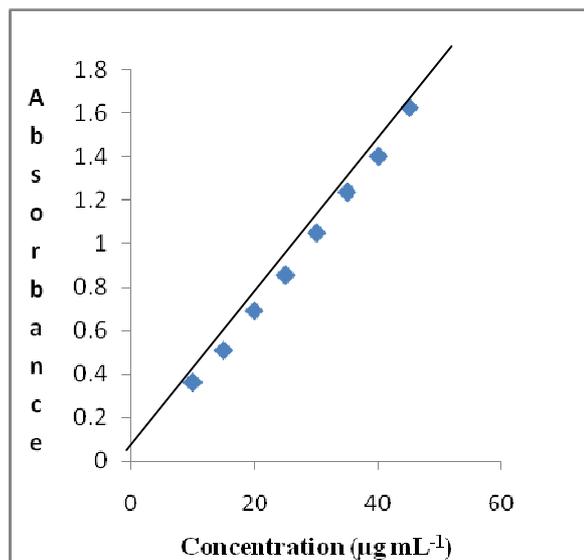


Figure 6. Calibration curve for Method B

Applications

Recovery studies are performed with the sample containing various amounts of MID. The results of recovery studies (Table 2) revealed that, other constituents present in the formulation do not interfere in the method. The proposed methods are applied successfully to determine MID in dosage form. Table 3 gives the result of the determination, from which it is clear that there is a close agreement between the results obtained by the proposed methods and label claim. In student's t-test no significant difference is found between the calculated and tabulated values in respect to accuracy and precision.

CONCLUSION

A simple and accurate method for the determination of MID has been developed and validated as per the ICH guidelines [16]. Methods are easy to perform and do not contain any stringent experimental variables which effect the reliability of the results. The methods are quite selective as the drug contains. The commonly used excipients and additives in the preparation of tablets are found not to interfere in the analysis. The proposed methods thus can be used as effective tool to analyze MID in pure and dosage forms.

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