Journal of Nature, Life and Applied Sciences
Volume (6), Issue (2) : 30 Jun 2022
P: 120 - 130

AJSRP
ISSN: 2522-3356

مجلة العلوم الطبيعية والحياتية والتطبيقية
المجلد (6)، العدد (2) : 30 يونيو 2022م
ص: 120 - 130

Determination of Midazolam by analytical spectrophotometry

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Abstract: Rapid useful and easy spectrophotometric method for the quantitative analysis of (Midazolam) (MDZ) in raw material and (tablet) pharmaceutical formulation has described. This method is based on the formation of yellow (ion-pair complex) between (Midazolam) and (Bromocresol purple) in (Acetonitrile) (medium). Different parameters affecting the reaction such as: effect of solvents, the concentration of reagent, correlation ratio, time etc. were optimized. The formed complex was quantified spectrophotometrically at absorption maximum at 402 nm. The range of linearity was 3.26 – 26.06 µg/mL, regression analysis had a good correlation coefficient R^2 = 0.9998. The limit of detection (LOD) also limit of quantification (LOQ) were 0.89 µg/mL and 2.71 µg/mL respectively. The average percent (recovery) was (99.46 - 100.92)% for Midazolam.

This study was applied on Syrian pharmaceutical products: (DOMID-Oubari 7.5 mg). This method was successfully applied for (Midazolam) determination in tablets pharmaceutical formulation. The proposed method is direct, sensitive, simple, and doesn't require any (extraction) process. Thus, the method could be ready to apply in routine analysis and quality control.

Keywords: Midazolam (MDZ), Benzodiazepines (BDZ), Bromocresol purple.

تحديد الميدازولام بالطريقة الطيفية

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المستخلص: تم وصف طريقة طيفية بسيطة وسريعة للتحليل الكمي (للميدازولام) (MDZ) في المواد الخام والأشكال الصيدلانية للأقراص. تعتمد الطريقة على تكوين معقد زوج شاردي أصفر اللون بين (الميدازولام) و(البروموكريزول) الأرجواني في وسط من (الأسيتونيتريل). تم تحسين العوامل المختلفة التي تؤثر على التفاعل مثل: تأثير المذيبات، الوقت، تركيز الكاشف، نسبة الارتباط، إلخ. تم قياس كمية المعقد المتكون بالطيف المرئي عند طول موجة امتصاص أعظمي عند 402 نانومتر. كان المجال الخطي واقعاً بين 3.26 - 26.06 ميكروغرام/ مل، وأظهر تحليل الانحدار معامل ارتباط جيد R2 = 0.9998. أما حد الكشف (LOD) وحد القياس الكمي (LOQ) فكانا 0.89 ميكروغرام/ مل و2.71 ميكروغرام/ مل على التسلسل. كانت القيمة المتوسطة لنسبة الاسترداد (99.46 - 100.92)% للميدازولام.

تم تطبيق هذه الدراسة على العلامة التجارية الصيدلانية السورية: (DOMID-Oubari 7.5 mg). تم تطبيق الطريقة بنجاح لتحديد (الميدازولام) في المستحضر الصيدلاني للأقراص. الطريقة المدروسة سهلة وحساسة ومباشرة أي لا تتطلب استخلاص. وبالتالي، يمكن تطبيقها بسهولة لاختبارات مراقبة الجودة والتحليل الروتيني في معامل الأدوية.

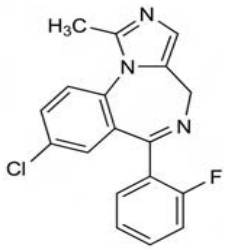
الكلمات المفتاحية: ميدازولام، بنزوديازبينات، بروموكريزول أرجواني.

INTRODUCTION.

Benzodiazepines (BDZ) are psychoactive therapeutic drugs with varying hypnotic, anxiolytic, sedative, muscle relaxant, anticonvulsant and amnesic properties, (BDZ) are brought on by slowing down the activity of the central nervous system. One representative benzodiazepine is midazolam (MDZ) [1]. Midazolam {8-chloro-6-(2-fluorophenyl)1- methyl-4H-imidazo[1, 5-a] [1, 4] benzodiazepine, it is a newer class of benzodiazepine derivatives which are characterized by an imidazole ring fused in the 1, 2-position to the diazepine ring [2, 3], as shown in Fig. (1, a). The pharmacological properties of midazolam are similar to classical benzodiazepines, but it differs from them in having a short duration action, due to rapid metabolic inactivation [3, 4, 5], also has short elimination half-life, used as induction or continuous agent for general anesthesia [6]. Midazolam is a premedicate, sedative and anesthetic induction Agent [7, 8]. It has about twice the sedative potency of diazepam. It also has anticonvulsant, muscle-relaxant properties, powerful anterograde amnesic action and sleep-inducing similar to that of diazepam [8, 9]. Several methods have been reported in the literature for the analysis of Midazolam such as High-performance liquid chromatographic (HPLC) [10, 11], Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) [12], Potentiometry [13]. Spectrometry method [14].

Bromocresol purple (BCP) is a Sulphonphthalein dye commonly used as indicator and spectrophotometric reagent.

Bromocresol purple (BCP) as shown in Fig. (1, b).



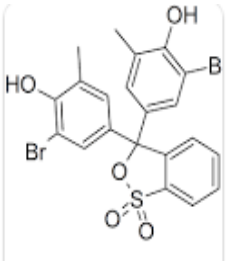


Fig. (1, a) **Fig. (1, b)**

Fig. (1, a): Structural formula of Midazolam

Fig. (1, b): Structural formula of Bromocresol purple

MATERIALS AND METHODS

Apparatus

All spectral measurements were carried out using a Spectro Scan 80 DV, UV/Vis spectrophotometer instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe).

Chemical regents:

Midazolam (MDZ) , Mw = 325.8 g/mol from (India), its purity 100.3%. Methanol and Bromocresol purple Mw = 540.22 g/mol from Merck (Germany). Acetonitrile from fine-chem limited (Mumbai).

Standard Preparation

Midazolam stock solution:

Stock solution 1×10^{-2} M of Midazolam (Mw = 325.8 g/mol) was prepared by dissolving 32.58 mg of raw material in volumetric flask 10 mL and complete the volume with Methanol, then 1 mL of the solution was taken to volumetric flask 10 mL and diluted with Acetonitrile to give concentration 1×10^{-3} M equivalent to 325.8 μg/mL. The working standard solutions of Midazolam were prepared by appropriate dilutions between (100 - 800) μL of 325.8 μg/mL solution in volumetric 10 mL flasks and added to each one of BCP 5×10^{-3} M equals to five times of Midazolam concentration then completed to volume with Acetonitrile to give concentrations between (3.26 –26.06) μg/mL of Midazolam.

Reagent stock solution:

Bromocresol purple 5×10^{-3} M was prepared by dissolving 135.055 mg of Bromocresol purple (Mw = 540.22 g/mol) in volumetric flask 50 mL and completing to volume with Acetonitrile.

Calibration Curve:

To construct the calibration curve, for each concentration five standard solutions were prepared and the absorbance was measured five times for each solution.

Sample preparation:

The same procedure is followed to prepare both DOMID-Oubari 20 and DORMITA-ELSAAD 10 (Syrian products). twenty tablets were weighed and finely powdered and an accurate weight equivalent to one tablet (Each tablet contained 7.5 mg Midazolam/tab.) for each product which was transferred to volumetric flask 10 ml, diluted to volume with Methanol, then 1 mL of the solution was taken to volumetric flask 10 mL and diluted to volume with Acetonitrile. 1 mL of the last solution was taken to volumetric flask 10 mL and added 0.8 mL of Bromocresol purple 5×10^{-3} M, then diluted to volume with Acetonitrile, to obtain equivalent theoretically to 7.5 μg/mL for (MDZ).

RESULTS.

Midazolam forms with Bromocresol purple at 25 ± 5 ºC yellow ion-pair complex. The stability of the complex was 2 hours. The result solution was studied in the range of wavelengths 300 - 550 nm

against a blank of BCP prepared in Acetonitrile, and then measured the absorbance at maximum wavelength 402 nm. We studied all the parameters of the colored result solutions to obtain the optimal conditions.

DISCUSSION.

This method depends on study a color ion-pair complex between the Midazolam and Bromocresol purple for the first time. It gives the lowest linearity range comparing with others spectrophotometric studies for color complexes, so we can consider it sensitive method. The results showed good results for percentage of recovery, accuracy and precision.

Stability of stock solution:

Time effect on standard stock solution of Midazolam in Acetonitrile was studied in three different concentrations 1×10^{-4}, 2×10^{-4} and 3×10^{-4} M. We did not notice any significant changes of the absorption during the measurement within 3 months.

Effect of reagent concentration

To study the effect of reagent concentration on the colored complex solution, we made a series of 10 mL of separated volumetric flasks, by adding 1 mL of Midazolam 1×10^{-4} M equivalent to 10 µM and added between (0.025 – 1.2) mL of (BCP) 1×10^{-3} M, equivalent to (2.5 – 120) µM after completing the volume to 10 mL by Acetonitrile. The absorbance at 402 nm for every added (BCP) reagent was measured against the blank of Acetonitrile. It was found that the completed colored complex formation in the best condition was 200 µM of (BCP) equivalent to 0.2 mL of (BCP) which equal to five times of Midazolam concentration, as it is shown in Fig. 2.

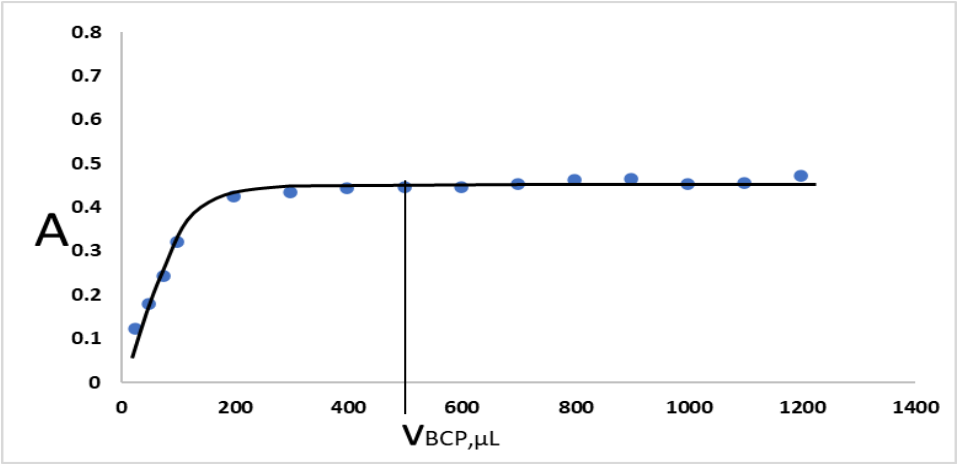


Fig. (2) Effect of reagent concentration.

Midazolam concentration 40 µM.

Correlation ratios by molecular ratio:

We have prepared a series of complex solutions MDZ-BCP in the medium of the Acetonitrile. The concentration of the reagent changes within the ratio (6.25 × 10-6 – 125 × 10-6) M while the concentration of Midazolam was constant in each solution and equal to 3 × 10-5 M. We measured the absorbance values of these solutions at the wavelength of the maximum absorbance 402 nm according to the used reagent percentage (using Acetonitrile as a blank). The absorption changes of the molecular ratio of the reagent to the Midazolam permitted us to measure correlation ratio, we obtained the curve A = f ([BCP]/[MDZ]) shown in Fig.3 where the correlation ratios are (1:1).

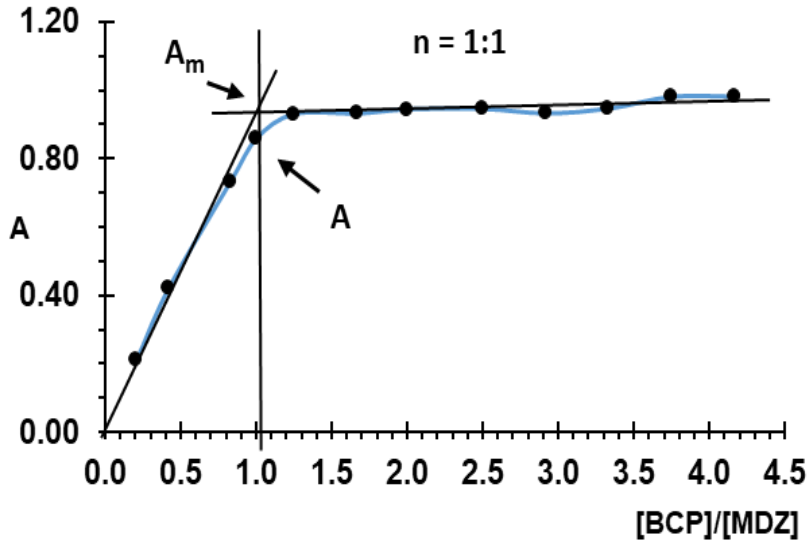


Fig. (3) Correlation molecular ratios (1:1).

Correlation ratios by continuous variation:

We have prepared a series of complex solutions MDZ-BCP in the medium of the Acetonitrile. The concentration of the reagent and the concentration of Midazolam changes in solutions between (0 - 1) × 10-4 M where the sum of both concentrations remains constant and equal to 1 × 10-4 M.

We measured the absorbance values of these solutions at the wavelength of the maximum absorbance 402 nm according to the used reagent percentage of the formed complex in terms of molecular fraction of Midazolam. We obtained the curve A = f ([BCP]/{[BCP] + [MDZ]}) shown in Fig. 4: where the correlation ratios are (1:1).

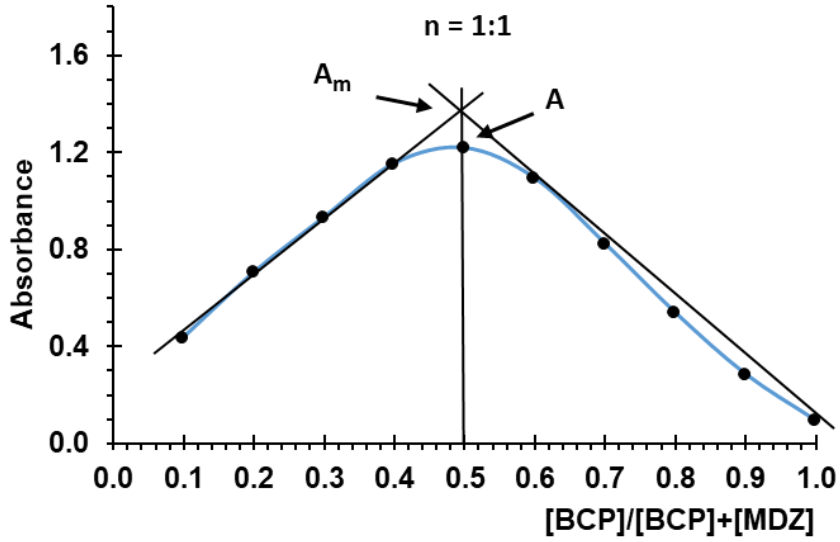


Fig. (4) Correlation ratio by continuous variation (1:1).

Calculation of formation constant for the (MDZ: BCP) complex

The conditional stability constant (Kf) of the ion-pair complexes were calculated from molecular ratio and the continuous variation curves. Data using the following equation:

$$K_f = \frac{\mathrm{A}/\mathrm{A_m}}{\left[1 - \frac{A}{A_m}\right]^{n+2} C_M(n)^n}$$

Where Am and A are the observed maximum absorbance and the absorbance value when all the Midazolam is completely associated with Bromocresol purple, respectively. CM is the mole concentration of Midazolam at the maximum absorbance and n is the stoichiometry which dye ion associates with Midazolam. The log Kf

values for MDZ-BCP ion-pair association at correlation ratio (1:1) by molecular ratio and continuous variation were 7.32 and 7.11 respectively, where the average logKf is 7.21.

Method's validation:

The validity and suitability of the proposed method was assessed by linearity (evaluated by regression equation), limit of detection (LOD), limit of quantification (LOQ), accuracy (reported as percent%), precision (reported as RSD%), robustness, and Sandell′s sensitivity.

Linearity:

We studied the linearity of Midazolam concentrations at the optimal conditions we made a series of 10 mL of separated volumetric flasks, each one contains 0.8 mL of BCP 5 × 10-3 M, and variable concentrations of MDZ stock solution 1 × 10-3 M, and completed to 10 mL with Acetonitrile, finally we

measured the absorbance at 402 nm for each concentration against the blank of BCP in Acetonitrile. Fig. 5 presents the MDZ spectra. The range of linearity was obeyed to Beer's law in concentration (3.25 - 26.06) µg/mL the linearity curve is presented in Fig. 6.

Limit of detection (LOD) and limit of quantification (LOQ):

In spite of the measurement LOD and LOQ, five concentrations were analyzed in five replicates. LOD and LOQ for Midazolam were calculated by using the following equations:

$$LOD = \frac{3.3 \times SD}{m} ; LOQ = \frac{10 \times SD}{m}$$

Where SD, is the standard deviation of y intercepts of regression lines and m is the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ)

were to be 0.894 and 2.710 μg/mL respectively.

Table (1) Precision and accuracy for determination of Midazolam.

Raw material	Theoretical concentration (µg/mL)	*Observed concentration (µg/mL)	SD µg/mL	*Precision (RSD %)	Accuracy (%)
Midazolam	6.52	6.58	0.08	1.22	100.92
	13.03	12.96	0.56	4.32	99.46
	22.81	22.98	0.28	1.22	100.75

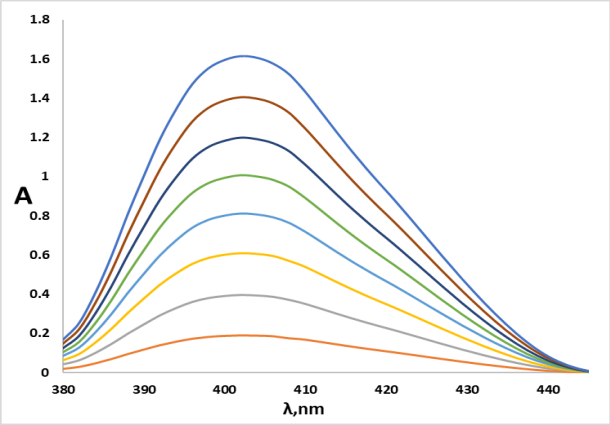


Fig. (5) spectra of (MDZ-BCP):

C_1: 3.26 μg/mL, C_2: 6.52 μg/mL,

C_3: 9.77 μg/mL, C_4: 13.03μg/mL,

C_5: 16.29 μg/mL, C_6: 19.55 μg/mL,

C_7: 22.81 μg/mL, C_8: 26.06 μg/mL.

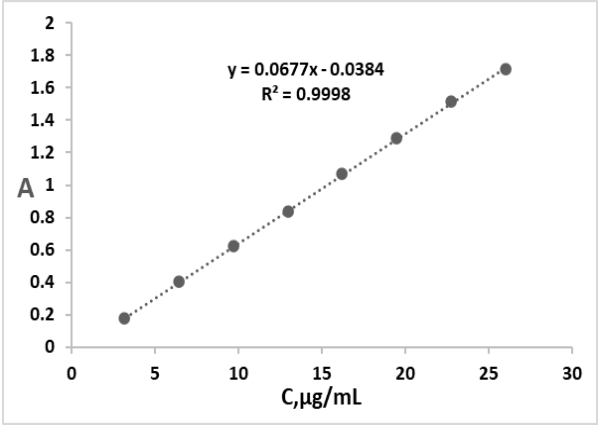


Fig. (6) Calibration curve for (MDZ-BCP):

C_1: 3.26 μg/mL, C_2: 6.52 μg/mL,

C_3: 9.77 μg/mL, C_4: 13.03 μg/mL,

C_5: 16.29 μg/mL, C_6: 19.55 μg/mL, C_7: 22.81 μg/mL, C_8: 26.06 μg/mL.

n = 5 repetition for each concentration.

* $\bar{x}$: mean of five replicated determinations, Accuracy (%) = (practical concentration/theoretical concentration) × 100,

*Precision (RSD%) = (standard deviation/average concentration) ×100.

Accuracy:

To determine the accuracy and precision of the proposed method, five replicate determinations were carried out on three different concentrations of standards (MDZ). The validation results are presented in table 1.

Precision:

In order to demonstrate the precision of the proposed method, intra-day and inter-day variability studies were performed at three different concentrations (3.26, 6.52, and 16.29) μg/mL for Midazolam at the same day and also at three days. Method efficiency was tested in terms of RSD% for both intra-day and inter-day precisions.

The precision was ascertained by carrying out five replicates of standard Midazolam under study and the mean was calculated. The results are showed in Table 2. The RSD% results were not more than 4.95during the determination in one day or three days, where the method is considered precise.

Table (2) Intra-day and inter-day precision for determination of Midazolam.

Intra-day							
Sample	Concentration μg/mL	Found concentration µg/mL					
		* Time I	Precision RSD%	* Time II	Precision RSD%	* Time III	Precision RSD%
Midazolam	3.26	3.16	1.97	3.22	0.41	3.20	1.44
	6.52	6.44	1.81	6.45	0.77	6.75	0.82
	16.29	16.39	3.74	16.77	0.85	16.62	1.09

Inter-day							
Sample	Concentration μg/mL	Found concentration µg/mL					
		* Time I	Precision RSD%	* Time II	Precision RSD%	* Time III	Precision RSD%
Midazolam	3.26	3.18	0.74	3.22	0.58	3.32	0.31
	6.52	6.45	0.24	6.52	2.81	6.50	0.53
	16.29	16.34	1.44	16.37	4.95	16.40	2.72

*n = 5.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to maintain unaffected results by a very small variation of some parameters and provides an indication of its reliability during normal usage. The studied variables parameters were slit scan speed and the wavelength which performed at concentration (3.26, 6.52, 16.29 μg/mL) for Midazolam Table 3.

Table (3) Robustness test.

Initial conditions	Measured deviation	Found Concentration	$\bar{x}$ μg/mL	SD µg/mL	%RSD	Percent (%)
Step size 0.5 nm	2 nm	3.26	3.25	0.04	1.23	99.69
		6.52	6.54	0.09	1.38	100.31
		16.29	16.53	0.38	2.30	101.47
	1 nm	3.26	3.26	0.05	1.53	100.00
		6.52	6.49	0.08	1.23	99.54
		16.29	16.43	0.80	4.87	100.86
Scan speed medium	Slow	3.26	3.29	0.04	1.22	100.92
		6.52	6.48	0.08	1.23	99.39
		16.29	16.35	0.48	2.94	100.37
	Fast	3.26	3.25	0.04	1.23	99.69
		6.52	6.49	0.09	1.39	99.54
		16.29	16.53	0.38	2.30	101.47
Wavelength 402 nm	+2 nm	3.26	3.31	0.07	2.11	101.53
		6.52	6.46	0.13	2.01	99.08
		16.29	16.12	0.69	4.28	98.96
	-2 nm	3.26	3.26	0.07	2.15	100.00
		6.52	6.52	0.17	2.61	100.00
		16.29	15.91	0.65	4.09	97.67

*n = 5.

Sensitivity Sandell's and molar absorptivity ε:

Sensitivity of the proposed method for Midazolam was determined by calculating Sandell's sensitivity (SS), it was to be SS = 0.0313 µg/cm^2.The mean molar absorptivity ε was found equal to 20767 L/mol.cm.

RECOVERY:

The recovery was studied by three addition standards of Midazolam for DOMID-Oubari 20 product. Table 4 presents the recoveries results.

Table (4) Recoveries of Midazoalm in (Domid-Oubari 20) Syrian product

Product	Pharmaceutical dosage	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery%	SD µg/mL	RSD%	Recovery Average%
DOMID-Oubari 20	Midazolam 7.5 mg/tab.	7.5	6.00	13.57	100.52	0.21	1.55	100.37
			7.50	15.16	101.07	0.31	2.04	
			8.96	16.38	99.51	0.68	4.15	

$\bar{x}$ Mean for five separate determinations were performed and calculated the mean.

APPLICATIONS:

The method was applied for identification and quantitative determination of Midazolam in (DOMID - Oubari 20), (DORMITA - ELSAAD 10) Syrian pharmaceutical products. The samples were prepared as mention before in the section of samples preparation and analyzed. Quantitative analysis was done by using calibration curve.

The relative standard deviations RSD% of the quantitative results were in the range of 1.70 - 2.53% for (DOMID- Oubari 20) and 1.48 - 2.59% for (DORMITA-ELSAAD 10).

Table 5 presents the determination results of Midazolam in (DOMID-Oubari 20), (DORMITA-ELSAAD 10) products for two different batches.

Table (5) Results of Midazolam in (DOMID-OUBARI, 20) and (DORMITA-ELSAAD 10) tablets.

No. of batche	DOMID-Oubari 7.5 mg/tab				DORMITA-ELSaad 7.5 mg/tab			
	Concentration *$\bar{x}$mg/tab	SD mg/tab	RSD %	Per%	Concentration $\bar{x}$mg/tab	SD mg/tab	RSD %	Per %
1	7.63	0.13	1.70	101.7	7.44	0.11	1.48	99.2
2	7.51	0.19	2.53	100.1	7.33	0.19	2.59	97.7
Range of concentration	7.51 - 7.63							
Range RSD%	1.70 - 2.53				1.48 - 2.59			
Range Per%	101.13 - 101.73				97.73 - 99.20			

*$\bar{x}$ Mean for five replicates.

CONCLUSION.

We developed a new spectrophotometric method which is suitable for the quantification and identification of Midazolam in raw material and tablets formulation. This method can be simply and

successfully used in routine analyses. The proposed method is sensitive, simple, specific, rapid, a few costs and could be applied easily for quality control tests of Midazolam.

ACKNOWLEDGEMENT:

The Ministry of High Education in Syria financially and technically supported this work through department of Chemistry, Faculty of Science, University of Aleppo, Syria.

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