Optimization, development and validation by UV spectrophotometric method for the determination of a solid semi-legal drug: Valium

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Abstract: *Valium is a tranquilizer of the benzodiazepine family; classified in the category of semi-legal drugs. The present work proposes a very simple, fast, efficient and reliable spectrophotometric method for the determining of this drug in Active Pharmaceutical Ingredient and tablet dosage formulation have been developed using absorbance ratio method and validated by using following experimental procedure for the validation of analysis method in chemistry guidelines. Parameters such as the analysis medium (proportion of solvents), the concentration of the molecule, the stability of time, the pH, and the temperature have been optimized. It shown from this optimization that 0.1 M hydrochloric acid at a concentration of 10 $\mu gmL-1$ allowed to have a maximum absorption at 242 nm with $T_{max} = 10$ min. The method is linear from 0 to 30 $\mu gmL-1$ with a correlation coefficient of 0.9999, a relatively low limit of detection (LOD = 0.101$\mu g/mL$) and limit of quantification (LOQ = 0.34 $\mu g/mL$). The % RSD was less than 2 % (0,05059 %) and the Ratio of similarity was found to be 9.89 It is reproducible in this range and can be used in case of possible seizures of Diazepam and routine quality control analysis in API and solid dosage formulation.*

Keywords: *Diazepam, UV-Spectrophotometric method, dosage formulation, Niger.*

I. Introduction

Valium is the second most consumed and sold drug in Niger **[1].** Valium is often called by its generic name, which is Diazepam, and is prescribed in cases of anxiety or in the treatment of manifestations related to alcohol withdrawal **[2].** It is a tranquilizer of the benzodiazepine family; classified in the category of semi-legal drugs witch is used sometimes with other medications to treat seizures. Its molecular weight is 284.8, chemical formula is$C_{16}H_{13}ClN_2O$. Its name according to IUPAC is 7-chloro-1-methyl-5-phenyl-3H-1, 4-benzodiazepine-2-one **[3].** The detection of this drug by the RDT (Rapid Diagnostic Test) **[4, 5, 6, 7, 8]** used at OCRTIS (Central Office for the Suppression of Illicit Traffic in Narcotics) is not often sufficiently reliable quantitatively.

Valium (Figure 1) used since the sixties (around 1963); it comes from all over the world in several forms (tablets, capsules and liquid) whose most common form is that of tablets. Commonly called D-5 or D-10 to specify its generic name that is Diazepam and its dosage (in mg) (**[9, 10, 11]**).

It is freely soluble in chloroform, soluble in ethanol, practically insoluble in water. Many methods for the evaluation of diazepam such as infrared spectrum, Mass spectrum, Thin layer chromatography, Gas chromatography, potentiometric, HPLC etc… In this study, the Spectrophotometric methods have been choose for the determination of diazepam in active pharmaceutical ingredient (API)(**[12,13,14]**)., dosage formand in seized materials.Some spectrophotometric UV methods were validated for determination of diazepam (**[15, 16,17, 18]**). A rapid, simple, sensitive and precise UV spectrophotometric method was developed for the quantitative assay of diazepam, which has been used in this study by usingProtocol for the validation of an analytical method in chemistry**[19]**. This method is rapid, simple, least cost and accurate as compared to other reported methods.
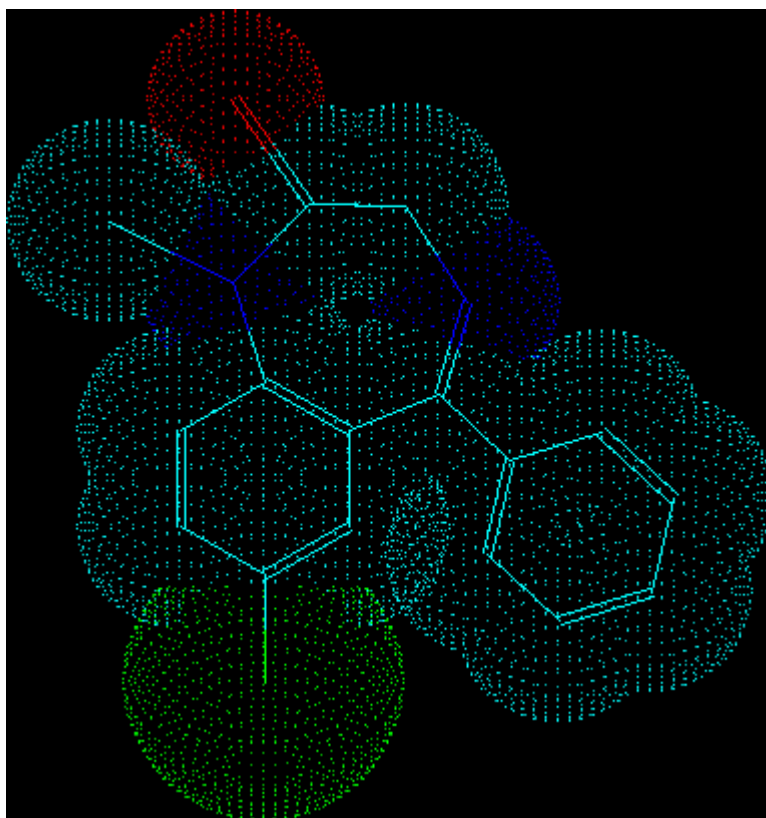

Figure.1: chemical structure of Diazepam (Red: O; Green: Cl; Blue: N; Cyan: C/-CH$_3$/-CH$_2$)

II. Material And Methods

A. EXPERIMENTALChemicalsandreagents

Pharmaceutical grade diazepam was a kind gift from Martin Dow Limited and it dosage formulation, Valium®10 mg Tablets were purchased from pharmacy, the expiry of which was not less than 3 year at the time of study. The hydrochloric Acid was purchased from VWR. hydrochloric Acid was be choosen in this study because most of seized materials containing diazepam are solid form so they would be ingested. This drug must be absorbed by stomach and this one contains and frees hydrochloric acid**.[20]**

B. Instrumentation

PH meter (BASIC 20 CRISON serial Pr: 646033) was used for measuring different concentration of HCl. Electrical analytical balance (Precisa 205 A Certified ISO 9001). UV visible spectrophotometer (Model EVOLUTION 300 UV-VIS) connected to HP computer (Compaq LE 2001 W home edition) loaded with its printer (HP laser jet P 1102) was used in these studies. A Distiller (wassermangelsicherung 2001/4), used for distillation of water. Used software package are:VISIONpro; Microcal Origin 6; Chem Sketch; CHNMR Viewer, 3D Viewer.

C. Optimization of some parameters

Some parameters have been optimized. Atfirst to search for the Location of Absorption maxima. Then prepare different concentrations of chosen acid to see what corresponding to the following analysis and its better time, which gives the best peak. Prepare all materials at least 24 hours before use.

D. Preparationofstandardstocksolution

Standard stock solution of Diazepam (1000 μg mL^{-1}) was prepared by weighing 100 mg of diazepam and transferred to a 100 mL volumetric flask and volume was made up to 100 mL with 0.1M Hydrochloric Acid to get a concentration of 1000 μg mL^{-1}, the prepared solution is wrapped up by aluminum paper to avoid swallowing the light. Appropriate volumes of this solution were further diluted to obtained final concentrations in range of 0 to 30 μg mL^{-1} (table 1). The spectrum of this solution was recorded using EVOLUTION 300 UV-VIS Spectrophotometer, in the range of 190-400 nm.

E. Pharmaceuticalformulation

Twenty tablets of Valium®10 mg were accurately weighed to have the average and finally fifteen of them have been powdered. A powder equivalent of 100 mg was transferred into a 100 mL volumetric flask wrapped up by aluminum paper to avoid swallowing the light with 0.1 M Hydrochloric acid and dissolved as describe under analytical procedure.

F. Validation

a. Linearity

The linearity of the analytical method is determined by taking concentration in between 0-30 μg mL^{-1}. From standard stock solution of diazepam (1000 μg mL^{-1}), pippete out aliquots of standard stock solution transferred to series of volumetric flasks and final volume made up to mark with 0.1 M hydrochloric acid as diluent to form solutions of 0 to 30 μg mL^{-1} of diazepam (table 1). These solutions were then taking absorbance at 242 nm against 0.1 M hydrochloric acid as blank and then calibration curve was plotted as absorbance via concentration to check the linear relationship between absorbance and concentration of diazepam. Acceptance Criteria: regression Coefficient should be greater than 0.995. [19]

b. Precision

The precision of the system is determined by assay of ten determinations at 1 μg mL^{-1} test concentration and relative standard deviation (%RSD) is calculated. The results of precision study were reported in terms of percentage relative standard deviation. Acceptance Criteria: The Relative Standard Deviation should not be more than 2%. [19]

c. Accuracy

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. The accuracy of developed method was carried out by calculating the percentage recovery of diazepam by standard addition method at three different levels i.e. 80 %, 100 % and 120 %.[11]

d. ProcedureforpreparationofsampleSolution

It was carried out at three levels 80 %, 100 % and 120 % of the nominal concentration. 80% Accuracy solution (8 μg mL^{-1}): It was prepared by diluting 0.4 ml of the stock solution up to 50 ml with 0.1 N hydrochloric acid. 100% Accuracy solution (10 μg mL^{-1}): It was prepared by diluting 0.5 ml of the stock solution up to 50 ml with 0.1 M hydrochloric acid. 120% Accuracy solution (12 μg mL^{-1}): It was prepared by diluting 0.6 ml of the stock solution up to 50 ml with 0.1 M hydrochloric acid. The sample was prepared in triplicate and analyzed by using UV spectrophotometer at wavelength 242 nm. Acceptance Criteria: Mean recovery should be in the range of 99-101 %.[12, 13].

e. Limitofdetection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following experimental procedure for the validation of analysis method in chemistry guidelines. LOD = 3 × σ Where, σ = Standard deviation of the response. [21].

f. Limitofquantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following experimental procedure for the validation of analysis method in chemistry guidelines. LOQ = 10 × σ Where σ = Standard deviation of the response. [21]

g. Ratio of similarity

The Ratio of similarity allowed indicating the validity of introducing the limit of detection. Ratio of similarity can be calculated using following experimental procedure for the validation of analysis method in chemistry guidelines. : $R' = \frac{\bar{x}}{LOD}$ Where $\bar{x}$ = average of ten determination and LOD is the limit of detection. Acceptance Criteria: The Ratio of Similarity R should be between 4 and 10.[19]

III. Resultand Discussion method development and optimization

a. Location ofAbsorptionmaxima

For the selection of analytical wavelength, 10 µg mL^{-1} solution of diazepam obtained by adding 0,25 mg of powder of Diazepam in a flask of 25 mL of HCl 0,1 M. Then it was scanned in the spectrum mode from 190 nm to 400 nm. HCl 0,1 M was been selected because this one is in human stomach. From the spectra of drug, we found three (3) peaks with their corresponding wavelengths (242, 284 and 366 nm) (figure 2). The best λmax was found to be 242 nm (molar extinction coefficient A1% =1020). So it was selected for the rest of analysis. **[12]**
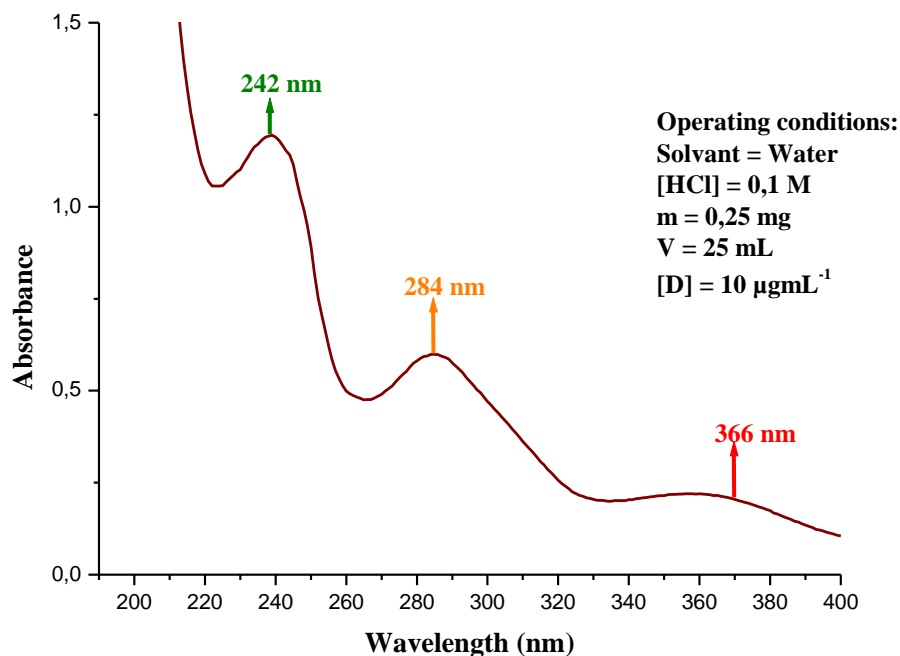

Figure 2:Determination of the Absorption maxima ofDiazepam by UV spectrum

b. Preparation of different molar concentration of Hydrochloric Acid and optimization of better peak vs times corresponding

Different concentrations between 0.01 to 2 M of Hydrochloric acid were carefully prepared from 37 % of Hydrochloric acid in bottle of 500 mL with distilled water and. This was done to see the optimal results for the seized substances containing Diazepam. A powder equivalent of 0.25 mg was transferred into a 25 mL volumetric flask to have 10 µg$mL^{-1}$with each concentration of Hydrochloric acid and then wrapped up by aluminum paper to avoid swallowing the light**[12, 13, 14]**. They will be dissolved as describe under analytical procedure. (Table 1).

Table 1: 0ptimisation of the molar concentration of HCl

SR.N°	[D] in µgmL^{-1}	[HCl] in M	pH [HCl]	Abs.	T°
1	10	0,01	1,86	1,097	
2	10	0,02	1,62	1,260	
3	10	0,05	1,20	1,114	
4	10	0,1	1,03	1,379	
5	10	0,15	0,89	1,209	
6	10	0,20	0,78	1,119	25,4
7	10	0,30	0,68	1,091	
8	10	0,50	0,54	1,364	
9	10	0,60	0,48	1,313	
10	10	1	0,33	1,290	
11	10	2	0,12	1,065	

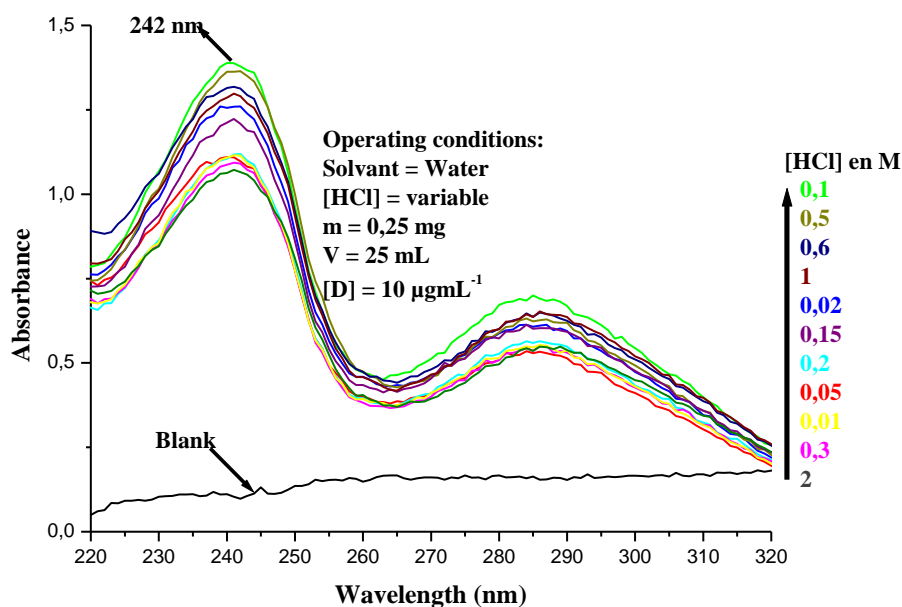**Figure 3: Absorption Spectrum ofDiazepam 10 µgmL^{-1} in different concentrations of HCl**

According to Figure 2, there was excellent peak of each concentration of HCl (Figure 3). The best peak was found with [HCl] 0, 1 M. So, carefully dilute 8.35 mL of 37 % of Hydrochloric acid to 1000 mL with distilled water for the following analysis. After this; the stability time has been optimized; this was illustrated by figure 3.
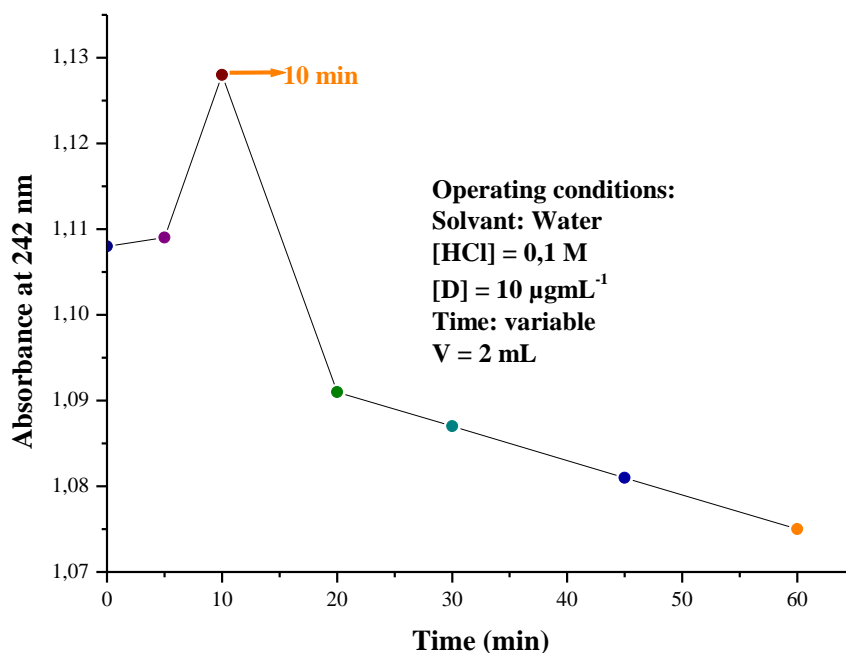

Figure 4: Absorption time of Diazepam 10 µgmL^{-1} in HCl 0,1 M

According to above Figure, the best peak was found at 10 min.
The present paper describes the application of absorption ratio method to estimation of diazepam in API and solid dosage form. The method was validated for the linearity, accuracy, precision, LOD and LOQ.

c. Validation of the method

1.   Linearity

The calibration curve was taken in the range of 0-30 µg mL^{-1} for diazepam at λmax 242nm. The diazepam was found to be linear within concentration range of 0–30 µg mL^{-1} with regression coefficient of 0.9999 by absorbance ratio method (Table 2).]The result is higher than Ali Abdul Mutur one which has found in 2016[16] but it is approximately equalwith Chakraborty's one[18]. There was an excellent correlation between absorbance and concentration (Figure 5). [19]

Table 2: Data curve calibration

Sr. N°	Concentration en µgmL^{-1}	Wavelength	Absorbance	R^2
1	0		0,131	
2	1		0,21	
3	5		0,573	
4	8		0,84	
5	10	242 nm	1,02	0,9999
6	12		1,203	
7	16		1,528	
8	20		1,903	
9	24		2,238	
10	30		2,761	

Operating conditions:
Solvant = Water
[HCl] = 0,1 M
[D] = variable
t = 10 min

$A = 8{,}78 \times 10^{-2}\,[D] + 13{,}4 \times 10^{-2}$
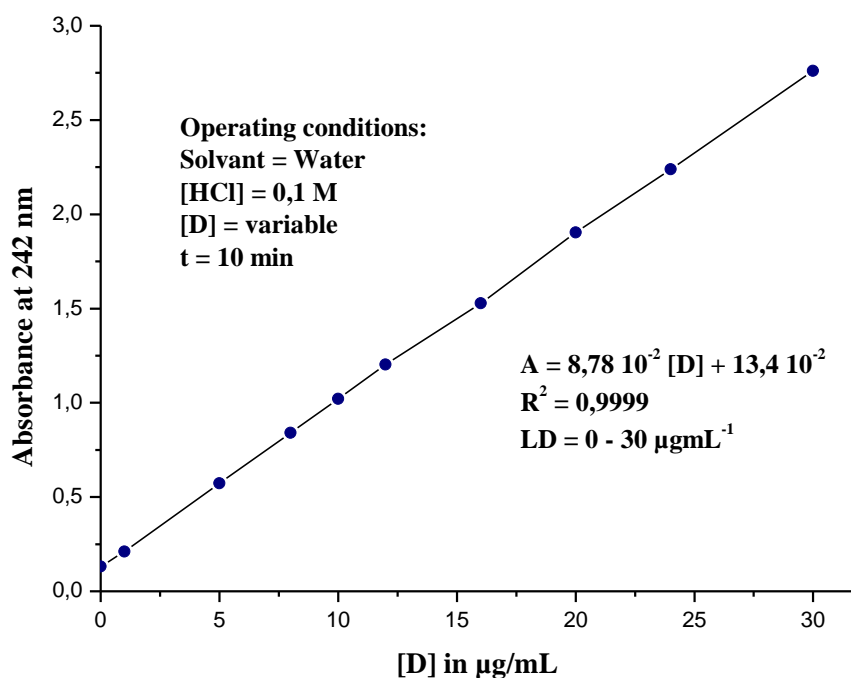$R^2 = 0{,}9999$
$LD = 0 - 30\ \mu gmL^{-1}$

Figure 5: **CalibrationCurveofDiazepam**

2.   Precision

The precision of an analytical method is determined by assaying ten determinations at test concentration (10 µg mL^{-1}). % Relative Standard Deviation (% RSD) calculates statistically (Table 3). It was found to be less than 2% (0,05059747 %) indicate the high precision of the propose method. The precision of an analytical method is determined by assaying ten determinations at test concentration (10 µg mL^{-1}). [19]

Table 3: Precision Data of the proposed method

Sr. N°	Concentration en µgmL-1	Absorbance	Absorbance average	SD	% RSD
1	10	1,021			
2	10	1,02			
3	10	1,021			
4	10	1,02			
5	10	1,021	1,0206	0,0005164	0,05059747
6	10	1,021			
7	10	1,02			
8	10	1,021			
9	10	1,02			
10	10	1,021			

3. Accuracy

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 8, 10 and 12 µg mL^{-1} (that is to say 80%, 100% and 120% of the nominal concentration) of diazepam. The average percentage recoveries for three different concentrations were found to be 100.057 % as shown in Table 4.[11, 17]

Table 4: Method Accuracy from Recovery Assay

Sr. N°	Sampleidentity	[ ] en µgmL^{-1}	Absorbance	Absorbance average	Amount Recovered in µgmL^{-1}	% Recovered	% Recovered
1	Accuracy 80 %	8	0,839				
		8	0,842	0,8406	8,00634921	100,08	
		8	0,841				
2	Accuracy 100 %	10	1,021				
		10	1,020	1,0206	10,0065359	100,065	100,057
		10	1,021				
3	Accuracy 120 %	12	1,204				
		12	1,203	1,2033	12,003325	100,027	
		12	1,203				

4. LODandLOQ

The limit of detection was found to be 0.101 µg mL^{-1} and the limit of quantification was found to be 0.34 µg mL^{-1}. The criteria being the concentration should lie outside the range 0.101– 0.34 for precise determination of diazepam.[16, 17]

5. Ratio of similarity

The Ratio of similarity was found to be 9.89. This value means the concentration used is appropriate for the determination of results and analytical methodology. [19]

Table 5: Precision Data of the proposed method for the least concentration

Sr. N°	C$_E$ (µgmL^{-1})	C$_R$ (µgmL^{-1})	C$_R$ average	Abs.	Abs. Average	SD (µgmL^{-1})	Ratio	LOD (µgmL^{-1})	LOQ (µgmL^{-1})
1	1	0,9962		0,21					
2	1	1,0057		0,212					
3	1	1,0057		0,212					
4	1	1,0057	1	0,212	0,2108	0,03369	9,89	0,101	0,3369
5	1	0,9677		0,204					
6	1	0,9915		0,209					
7	1	0,9345		0,197					
8	1	1,0484		0,221					
9	1	1,0484		0,221					
10	1	0,9962		0,21					

IV. Conclusion

The use of UV spectrophotometric allowed us to develop a rapid, simple, least economic, fast and non-toxic methods for the determination and quantification of diazepam. The present methods also validated as per experimental procedure for the validation of analysis method in chemistry guidelines for linearity, precision, accuracy, LOD and LOQ. The results of all these parameters were shows that the present UV-spectrophotometric methods found to be precise, linear, rapid, less reagent, time consuming and accurate and can be used for routine quality control analysis of diazepam and seized substances witch containing diazepam in dosage formulation in any laboratory.

Acknowledgements

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