Development of Indirect Spctrophotometric Method for the Determination of Clonazepam in pharmaceutical preparation Using Resorcinol

تطوير طريقة طيفية غير مباشرة لتقدير الكلونازيبام في المستحضرات الصيدلانية باستخدام الريزورسينول

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Abstract :-

This paper describes the development of a spectrophotometric method for the determination of Clonrazepam after diazotization of its reduced product and coupling with Resorcinol in basic medium , to form an intense yellow – colored , water – soluble and stable azo-dye which shows a maximum absorption at 341 nm. Beer's law is obeyed over the concentration range 1.2 to 20 ppm with a molar absorptivity 4.5147×10^{4} $L.mol^{-1}.cm^{-1}$ and Sandell's sensitivity of $6.993\times10^{-2}\mu$ $g.cm^{-2}$. The relative error is ranged from -0.017 to 2.74 % and relative standard deviation from ±0.001 to ±0.2636 % , depending on the concentration level. The method has been applied successfully to the determination of Clonazepam in pharmaceutical preparation .

Key word: Clonazepam, Resorcinol, Coupling Reagent

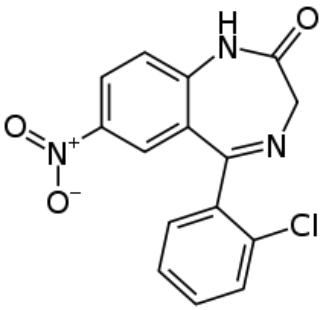
الخلاصة :-

تطوير طريقة طيفية لتقدير كميات متناهية في الصغر من الكلونازيبام بالاعتماد على ازوتة ناتج اختزال الكلونازيبام والاقتران مع الريزورسينول ككاشف اقتران في وسط قاعدي لتكوين صبغة أزوية ذات لون اصفر تعطي أعلى شدة امتصاص عند الطول الموجي341 نانوميتر .وكان حدود قانون بير من 1.2لى 20 جزء بالمليون وبلغت الامتصاصية المولارية 4.5147×10^{4} لتر. مول$^{-1}$.سم$^{-1}$ ودلالة ساندل 6.993×10^{-2} مايكروغرام . سم$^{-2}$والخطأ النسبي تراوح بين 0.017- و2.74 -% والانحراف القياسي النسبي بين 0.2636 و 0.001% اعتمادا على مستوى تركيز الدواء، تم تطبيق الطريقة بنجاح في تقديرالكلونازيبام بالأقراص الدوائية.

الكلمات المفتاحية: كلونازيبام، الريزورسينول ، اقتران أزوتي

INTRODUCTION

Clonazepam is a benzodiazepine drug having anxiolytic, anticonvulsant, muscle relaxant, sedative, and hypnotic properties.[1]Other names such as Ravotril, Rivatril, Rivotril, Clonex, Paxam, Petril or Kriadex are known throughout the rest of the world. Chemically, clonazepam is 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4benzodiazepin-2-one. It is a light yellow crystalline powder. It has a molecular weight of 315.72 and the following structural formula[2].



Clonazepam is an anticonvulsant agent widely used in the treatment of epilepsy in adults and children [3]. As a result of the therapeutic importance and widespread use of this compound,

the literature contain many reports dealing with its determination. For the biopharmacological, clinical and toxicological studies of these drugs, a rapid, sensitive and selective analytical method for its determination is essential. The British Pharmacopoeia[4] recommends non-aqueous titration with perchloric acid for the determination of clonazepam. For dosage forms, high-pressure liquid chromatography(HPLC) is recommended by US Pharmacopoeia [5]. Several methods for the assay of this compound have been reported by many authors, e.g., spectrometric methods [6-8], potentiometric methods [9,10], voltammetry [11], paleography [12], HPLC [12-14], gas chromatography coupled with mass spectrometry (GC-MS) [13,15].

Materials and methods

Instruments

All spectrophotometric measurements are performed on Shimadzu UV V-530, UV Visible recording spectrophotometer.

Reagents

All chemicals used are of analytical reagent grade.

Standard solution for clonazepam (10,000 µg/ml).

This solution is prepared by dissolving 0.5 g of clonazepam pure in ethanol volume is complete to 50 ml with ethanol in a volumetric flask

Reducing clonazepam (500 µg/ml).

Taking from standard clonazepam solution 5ml (10,000 µg/ml placed in a Baker then added 4 grams of zinc powder and 10 ml distilled water and 20 ml of 36% HCl Shake the mixture and cooled in a ice bath for 30 mints and the mixture is filtered using a Buechner funnel and the filtrate is placed in 100 ml volumetric flask then complete to the mark with distilled water and kept in a dark bottle.

Reducing clonazepam (100 µg/ml).

Taken 20 ml of a solution of 500 micrograms / ml of clonazepam reduced and add 7 solution ml 20% sodium carbonate white precipitate of zinc carbonate then solution is filtered in a volumetric flask 100 ml and complete the volume to the mark with distilled water and kept in a dark bottle [16] was prepared solution factor used in subsequent experiments concentration 50 micrograms / ml.

Resorcinol solution, 0.5%.

This solution is prepared by dissolving 0.5 g of resorcinol in 100 ml distilled water in a volumetric flask

Acetic acid solution, (1M)

Prepared by appropriate dilution of 99% concentrated acetic acid. solution to 100 ml with distilled water in a volumetric flask

Sodium nitrite solution, 1%.

Prepared by dissolving 1 g of sodium nitrite in 100 ml distilled water in a volumetric flask.

Sulphamic acid solution, 3%.

Prepared by dissolving 3 g of sulphamic acid in 100 ml distilled water in a volumetric flask.

Sodium hydroxide Solution 1M.

Prepared by dissolving 4 g of Sodium hydroxide in 100 ml distilled water in a volumetric flask. Form

Procedure and calibration graph

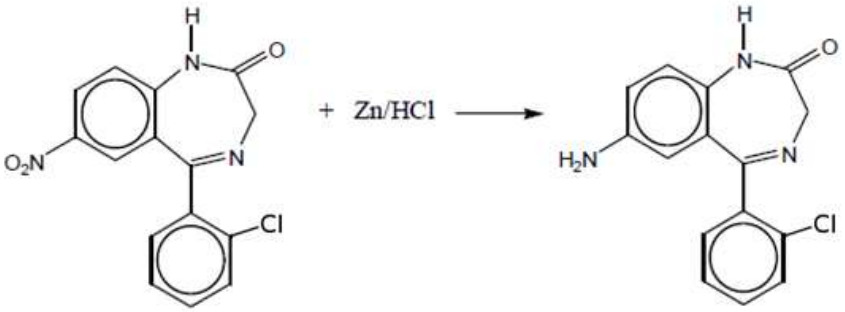
To a series of 25 ml volumetric flasks added, an increasing volume covering the concentration range (0.6-10) ml of reducing of clonazepam solution are transferred, followed by the addition of 1 ml of 1M of Acetic acid and 1 ml of 1% sodium nitrite solution and, shaking occasionally for 3 min, then a 0.5 ml of 3% sulphamic acid is added, with shaking and standing for 3 min. to remove the excess of nitrite ions, a 1 ml of 0.5% **,** Resorcinol reagent was added and a 1

ml of Sodium hydroxide Solution 1M was added, the flasks are diluted with distilled water to the mark. The absorbance was measured at 341 nm against the reagent blank. Beer's law is obeyed over the range of concentration 1.2 to 20 ppm clonazepam in 25 ml and a concentration above 20ppm in 25 ml gives a negative deviation (Fig.1).

RESULTS AND DISCUSSION

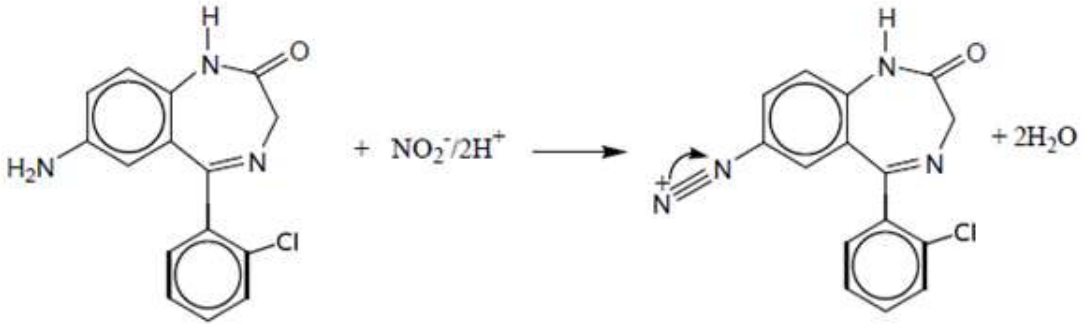
principle of method

Nitro group in clonazepam was reduced by zinc in acidic medium as in equation:



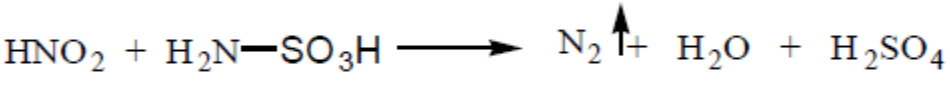
Clonazepam Reduced clonazepam

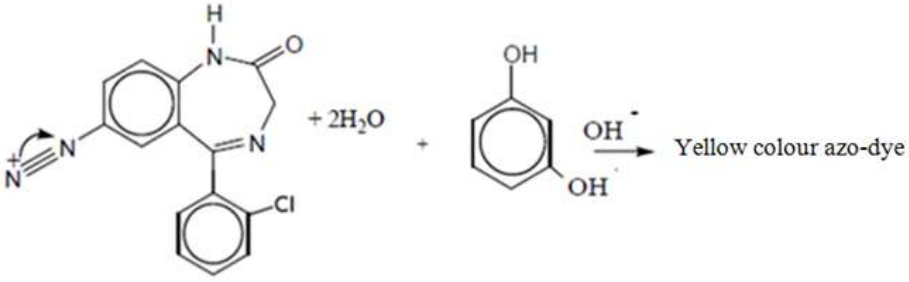
Then reduced clonazepam output with sodium nitrite in acidic medium



Reduced clonazepam Diazotized clonazepam

Remove excess of nitrite by sulphamic acid





Diazotized clonazepam resorcinol

Optimization of variables

During the investigation, 50 μg of reducing clonazepam is taken and the final volumes are brought to 100 ml with distilled water. The effect of various parameters on the absorption intensity of the colored complex is studied and the reaction conditions have been optimized.

Effect of quality and quantity of acid used for diazotization reaction

The effect of quality and quantity of acid on intensity of the coloured complex is examined. Different volumes (0.5-3.0) ml of 1M of different acid solutions are added to analiquot of solution containing 50 μg of reducing form of clonazepam. The intensities of absorption are read against the reagent blank. The results are shown in Table 1.

The results shown in Table 1 indicate that 1.0 ml of 1M **CH_3COOH** is considered as an optimum value therefore it is recommended for subsequent experiments.

Effect of sodium nitrite amount with the time

Different amounts of the 1% $NaNO_2$ solution are added and the time needed to complete the diazotization of reducing form of clonazepam is studied by standing of the solutions after adding sodium nitrite solution for different times, with occasional shaking ,then the other reagents are added and the absorbance is measured against the reagent blank. The results indicate that complete diazotization of clonazepam occurs after 3 min. when 1ml of 1% $NaNO_2$ solution is added because it gives the higher sensitivity, therefore it has been selected for subsequent experiments. The results are shown in Table 2.

Effect of sulphamic acid amount with the time

The effect of the amount of 3% sulphamic acid solution for removing the excess sodium nitrite with the standing time with occasional shaking are investigated. The results indicate that complete reaction of sulphamic acid with sodium nitrite occurs after 5 min. when 0.5 ml of 3% sulphamic acid solution. Therefore, the standing time 5 min. is recommended for the subsequent experiments. The results are shown in Table 3.

Effect of the Resorcinol reagent amount

The effect of the amount of 0.5% resorcinol reagent on a maximum formation of the colored complex is investigated. The results are shown in Table 4.

The results shown in Table 4 indicated that 1 ml Resorcinol of reagent solution give the higher sensitivity value, therefore it has been selected for the subsequent experiments.

Effect of Base solution used (1M)

The results shown in Table 5 indicated that 1 ml Sodium hydroxide solution give the higher sensitivity value, therefore it has been selected for the subsequent experiments.

Effect of time

The effect of time on the development and stability of the colored complex for different amounts of reducing form of clonazepam is investigated under the optimum experimental conditions is established. Complete color formation occurs immediately after all reaction mixture are added and the absorbance of the complex remains constant for at least 90 minutes (Table 6).

Absorption spectra

Absorption spectra of the coloured complex formed from the reaction between diazotized reducing form of clonazepam and Resorcinol reagent in a base medium against its corresponding reagent blank shows maximum absorption at 341 nm in contrast to the Resorcinol reagent blank which have Non absorption at 341 nm (Fig.2).

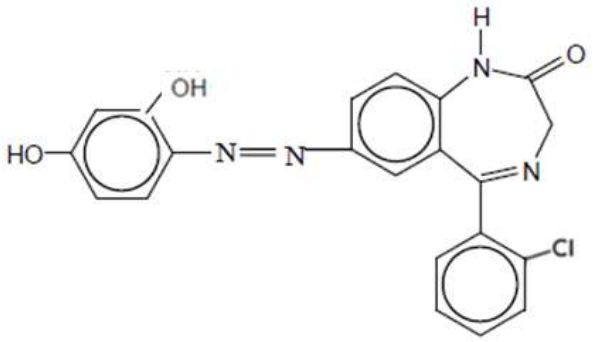
Accuracy and precision

To check the accuracy and precision of the calibration graph, clonazepam is determined at three different concentrations. The results shown in Table 7 indicated that the calibration graph is satisfactory.

Nature of clonazepam- Resorcinol reagent complex.

Job's method [17] and mole ratio method have been used in the determination of the reaction ratio of clonazepam with Resorcinol reagent. The obtained results (Fig.3 & Fig.4) showed that 1:1 clonazepam to Resorcinol reagent ratio is obtained.

From the above resulting data the probable azo dye formation is shown as follow:



Yellow azo-dye.

Application of the method

The proposed method was successfully applied to the determination of clonazepam in its pharmaceutical preparations and used tow methods to estimate of clonazepam direct and standard addition. The results which are shown in (Table 8,9& Fig.5)indicate that a good recovery was obtained.

Depending on the equation of a straight line when y=0 van x=2.003636, The size standard solution concentration 4ppm/100ml And using the relationship

C V=-Vs X

C= Concentration of the product solution Form (required)

V= Volume of the solution preparation clonazepam=2ml

X= Concentration of the standard solution of pure pharmaceutical substance added=4ppm

Vs= Standard solution size the drug

Comparison of the methods

Table 10 shows the comparison between some of analytical variables obtained from the present method with that of the recent spectrophotometric method.

REFERENCES

1. Cowen PJ; Green AR, Nutt DJ. *Nature* . (1981).290 (5801): 54–5.
2. Pinder, R. M., Brogden, R. N., Speight, T. M., and Avery, G. S. *Drugs*. (1976), 12,321.
3. Dahlin MG, Amark PE, Nergardh AR.,*Pediatr Neurol*; .(2003) 28(1): 48-52
4. The British Pharmacopoeia 98/34/EEC. London: The Stationery Offic\e, 2005
5. United States Pharmacopoeia,(2004) USP-27/NF-22. Rockville: Authority of the United States Pharmacopeia Convention,
6. El-Brashy A, Aly FA, Belal F. *Microchim Acta*,; (1993) 110(1-3): 55-60
7. Salem AA, Barsoum BN, Izake EL. *Anal Lett*; (2002). 35(10): 1631-1648

8. Salem AA, Barsoum BN, Izake EL. *Spectrochim Acta*, (2004) Part A; 60(4): 771-780
9. Nie L, Liu D, Yao S. *J. Pharm. Biomed. Anal.*; (1990). 8(4): 379-383
10. Salem AA, Barsoum BN, Izake EL. *Anal. Chim. Acta*.; (2003). 498(1-2): 79-91
11. Correia dos Santos MM, Famila V.. *Anal. Bioanal. Chem.*; (2002), 374(6): 1074-1081
12. Wilhelm M, Battista HJ, Obendorf D. *J. Chromatogr. A*; (2000). 897(1-2): 215-225
13. Cavedal LE, Mendes FD, Domingues CC, Patni AK, Monif T, Reyar S, Pereira AS, Mendes GD, De Nucci G., *J. Mass. Spectrom.*; (2007). 42(1): 81-88
14. Gandhi SV, Dhavale ND, Jadhav VZ, Sabnis SS. *J. AOAC Int.*; (2008)., 91(1): 33-38
15. Pujadas M, Pichini S, Civit E, Santamarina E, Perez K, De la Torre R. *J. Pharm. Biomed. Anal.*; (2007). 44(2): 594-601.
16. Al- Gharery , T. A. (2004)., M.SC., thesis, mosul university. (in Arabic).
17. Delvie, R. (1997). "Principles of Quantitative Chemical Analysis", International Edn., McGraw-Hill Inc., Singapore, 498 p.
18. Mitic S., Pavlovic A., Tošic S., Pecev E., Mitic M. and Stojkovic M. *Tropical Journal of Pharmaceutical Research;* (2012). 11 (1): 91-98.
19. Salem, A. A., Barsoum, B. N. and Izake, E. L.,*Analytica. Chemical. Acta.* (2003)., 498(1-2):79-91.
20. Patel V., Dave J., Patel F., Patel C. *IJPRBS*, (2012).1 (2): 62-70

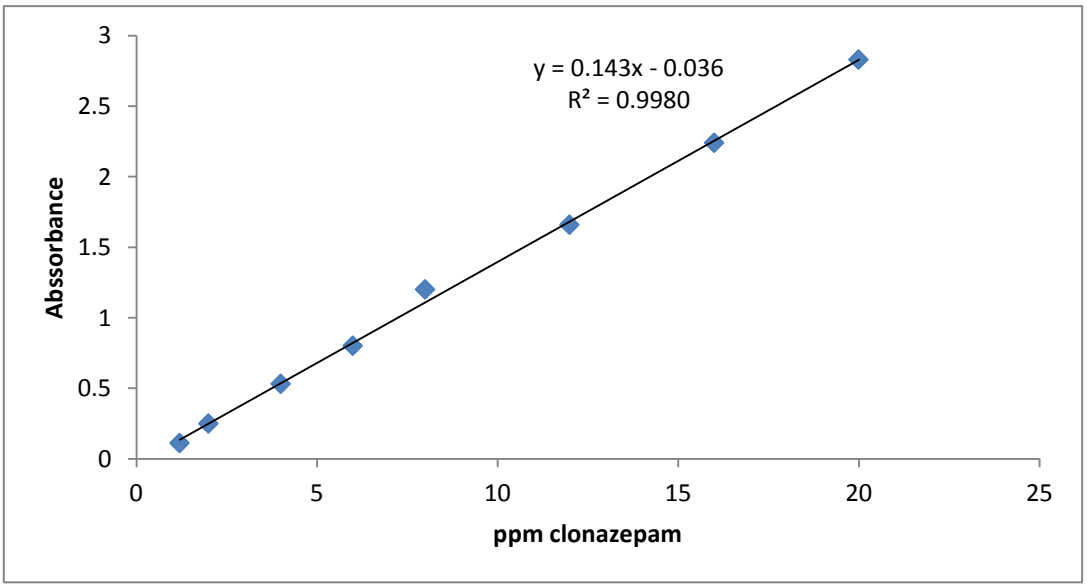


Fig. 1. Calibration graph for clonazepam determination

Table1:Effect of quality and quantity of acid on absorbance.

Acid 1M	**Absorbance / ml of acid**					
	0.5	1.0	1.5	2.0	2.5	3.0
HCl	0.171	0.162	0.148	0.120	0.102	0.029
H_2SO_4	0.182	0.090	0.073	0.071	0.057	0.049
HNO_3	0.162	0.159	0.134	0.123	0.119	0.093
CH_3COOH	0.220	0.342	0.253	0.289	0.291	0.294
H_3PO_4	0.151	0.121	0.080	0.076	0.072	0.053

Table 2: Effect of the amount of sodium nitrite amount on absorbance.

ml of $NaNO_2$ solution(1%)	**Abs./min. standing time**					
	0	2.0	3.0	5.0	7.0	10
0.2	0.205	0.249	0.253	0.230	0.220	0.214
0.4	0.252	0.248	0.244	0.240	0.237	0.229
0.8	0.227	0.231	0.237	0.233	0.230	0.231
1.0	0.321	0.324	0.375	0.326	0.342	0.240
1.5	0.231	0.234	0.232	0.242	0.238	0.237

Table 3: Effect of the amount of sulphamic acid amount on absorbance.

ml of Salphamic Acid (3%)	**Abs./min. standing time**					
	0	2.0	3.0	5.0	7.0	10
0.5	0.380	0.391	0.399	0.416	0.223	0.112
0.7	0.315	0.332	0.340	0.347	0.291	0.220
1.0	0.337	0.349	0.355	0.351	0.301	0.229
1.5	0.310	0.319	0.340	0.350	0.195	0.013

Table 4: Effect of the amount of Resorcinol reagent amount on absorbance.

ml of 0.5% Resorcinol	**Abs.**
0.5	0.411
1.0	0.521
1.5	0.401
2.0	0.340
2.5	0.335
3.0	0.335

Table 3: Effect of the amount of Base on absorbance.

Basesolution used (1M)	Absorbance/ml of Base used					
	0.5	1.0	1.5	2.0	2.5	3.0
NaOH	0.390	0.530	0.450	0.410	0.401	0.260
Na2CO3	0.233	0.220	0.198	0.154	0.091	0.091
NH4OH	0.251	0.260	0.249	0.245	0.230	0.227
K2CO3	0.046	0.160	0.163	0.147	0.072	0.060

Table 6: Effect of time on the absorbance of complex

Time (min.)	Abs.
0	0.530
5	0.530
10	0.530
20	0.531
30	0.531
40	0.530
30	0.531
50	0.531
60	0.529
70	0.529
80	0.529
90	0.529

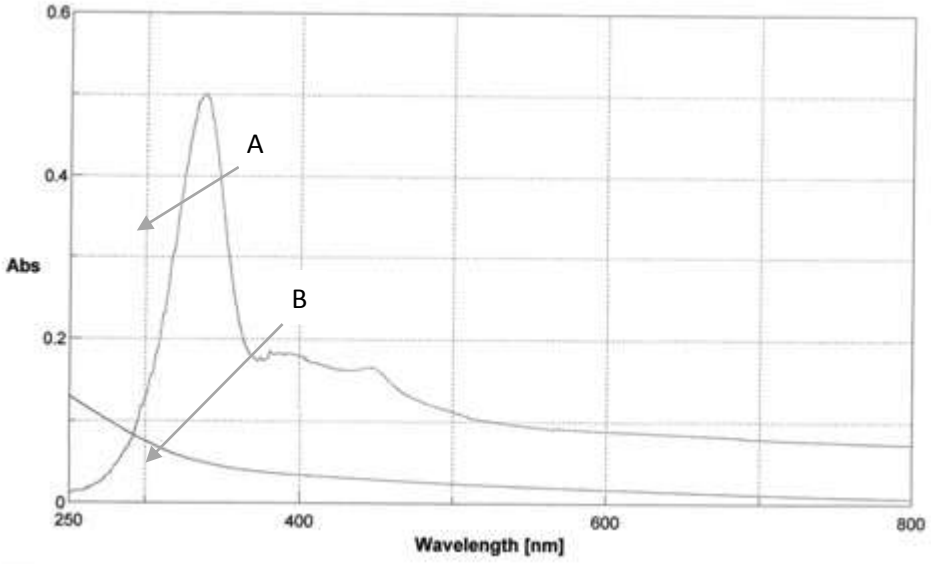


Fig. 2: (A) Absorption spectra of 4ppm of reducing form of clonazepam / 25 ml treated according to the optimum conditions and measured against, (B) blank

Table 6: Data for the calibration graph for Clonazepam using the proposed method

Parameter	Value
Beer's law range (ppm)	1.2-20
r	0.999
r^2	0.998
ε, L mol-1 cm-1	4.5147×10^{4}
Sandell's, µg cm-2	6.993×10^{-2}
Intercept a	-0.036
the slope b, mL ppm	0.143
limit of detection LOD, ppm	3.3×10^{-5}
limit of quantitation LOQ, ppm	1.12×10^{-4}
Erel.%	-0.875**
RSD%	0.264**

For 4ppm of clonazepam
* Average of four determinations

Table 7: Accuracy and precision

Amount of clonazepam taken, ppm/25ml	Relative error*, %	Relative standard deviation*, %	Recovery(%)	Recovery(%)
2	+0.26	0.001	100.26	99.552
4	-0.875	0.264	99.125	
12	-0.729	0.017	99.271	

* Average of four\ determinations

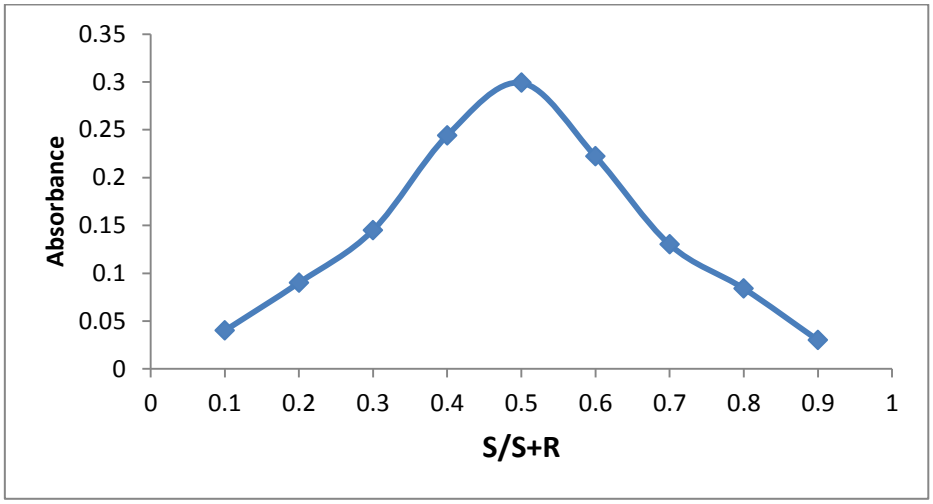


Fig. 3: Job's plot for clonazepam Resorcinol reagent.

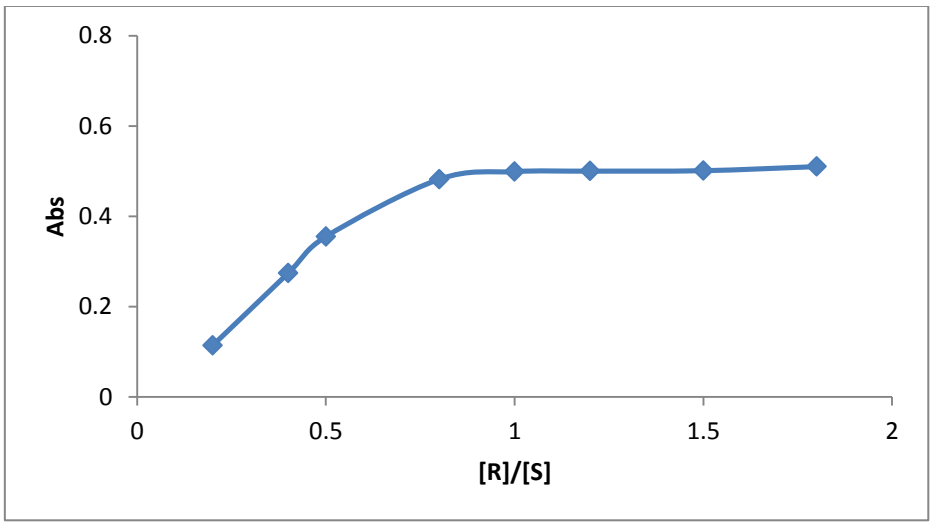


Fig. 4: Mole ratio's plot for clonazepam- Resorcinol reagent complex.

Table 8: Analytical applications.

clonazepam amount, ppm	Absorbance of standard solution	Domina (mg/ tablet) -syria	
		Absorbance	Recovery(%)
4	0.532	**0.525**	**98.68**
6	0.801	0.803	100.25
12	1.660	1.671	100.66

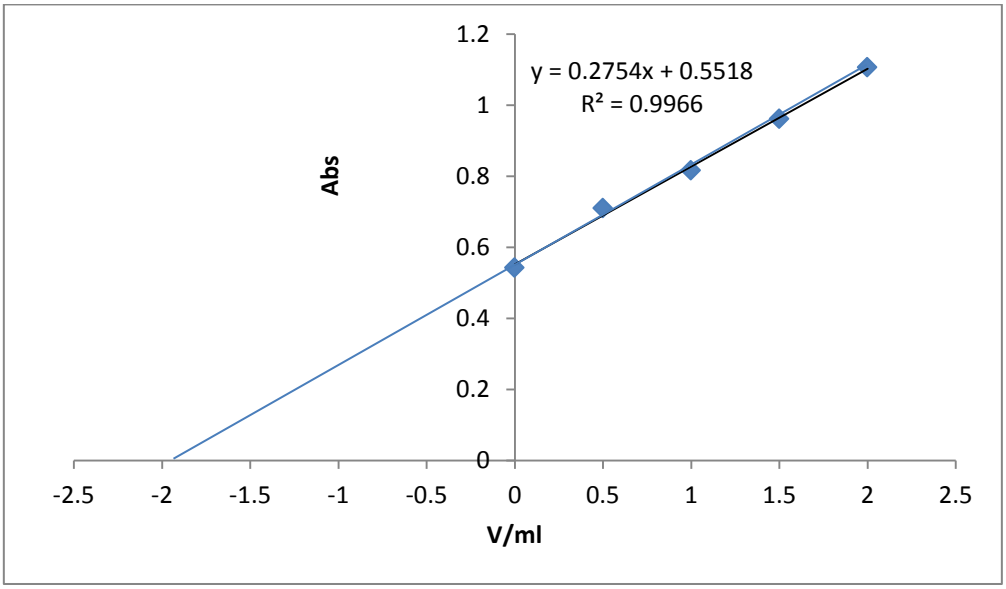


Fig.5.Standard addition method for determination of clonazepam tablets.

Table 10: Analytical applications(Standard addition method).

Sample preparation	Taken ppm	Found ppm	Relative error*, %	Recovery(%)
Tablets	4ppm	3.9927 ppm	-0.1825	99.817

Table 11: Comparison of methods.

Analytical parameters	Present method	Literature method	Literature method	Literature method
Type of method	Azo coupling	Ligand-Exchange [18]	Ion-selective electrodes[19]	UV Spectrophotometric [20]
Reagent	Diazotized Resorcinol	Ethanolic sodium	----------	HCL-NaOH
λmax (nm)	341	425	----------	309
Colour of the dye	**Yellow**	----------	----------	----------
Beer's law range (ppm)	1.2-20	0.32-4.10	0.32-31.57	4-28
Molar Absorptivity ($l.mol^{-1}.cm^{-1}$)	4.5147×10^{4}	----------	----------	----------
LOD (ppm)	3.3×10^{-5}	0.24	0.79	0.22
LOQ, ppm	1.12×10^{-4}	--------	---------	0.66
Recovery(%)	99.55	97.32	98.7	98.81-101.03%
Application of method	Determination of clonazepam in tablets.	Human serum	Determination of clonazepam in tablets .	Determination of clonazepam in tablets