ARC Journal of Pharmaceutical Sciences (AJPS)
Volume 4, Issue 2, 2018, PP 18-23
ISSN No.: 2455-1538
DOI: http://dx.doi.org/10.20431/2455-1538.0402003
www.arcjournals.org

Method Development and Validation of UV Spectro Photometric Method for Determination of Diazepam in its Pure and Pharmaceutical Dosage Form

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Abstract: *An UV spectrophotometric method for the quantitative determination of Diazepam in tablet was developed in present work. The Linearity, Intraday precision, Interday Precision, Ruggedness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. UV spectroscopic determination was carried out at an absorption maximum of 266.5 nm using methanol as solvent. The method was found to be linear and obeys beers law in the concentration range 2-20 µg/ml with a correlation coefficient 0.999. The developed method was validated as per ICH guidelines and was found to be accurate and precise. Thus the proposed method can be successfully applied for the estimation of Diazepam in pure and tablet dosage form.*

Keywords: *Diazepam, Linearity, Intraday precision, Interday Precision and Ruggedness*

1. INTRODUCTION

Diazepam (DZP), most commonly known by its trade name Valium, is a benzodiazepine and chemically it is 7-chloro-1, 3-dihydro-1-methyl-5- phenyl-1, 4- benzodiazepin-2-one. The structural formula of Diazepam with molecular formula C16H13ClN2O, is shown in Figure 1. It is commonly used to treat a wide range of conditions including anxiety, panic attacks, insomnia, seizures (including status epilepticus), muscle spasms (such as in tetanus cases), restless legs syndrome, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, opioid withdrawal syndrome, and Ménière's disease. However, it is a potent sedative- hypnotic, and is one of the most prescribed drugs in the world. It is also one of the top five most abused benzodiazepines, and misuse can lead to both psychological dependence and/or physical addiction1.

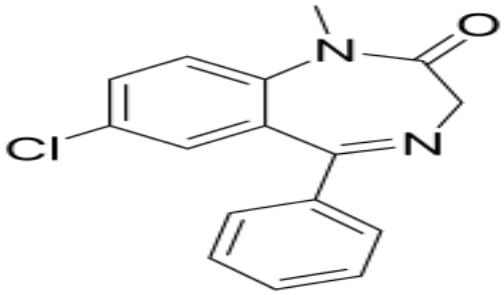

Fig1. *Structure of Diazepam*

Because of the therapeutic importance of DZP, many methods have been developed for its determination in pharmaceutical dosage forms and/or biological fluids. Literature survey reveals that various methods like Spectrophotometry2-4, gas liquid chromatography5, fluorimetry6, first derivative spectroscopy7, capillary electrophoresis8 and HPLC9-10 are reported for the estimation of Diazepam in single dosage form. However, most of these methods are tedious and involve expensive and sophisticated experimental set up which many ordinary quality control laboratories cannot afford. Spectrophotometry occurs in the forefront of the most sensitive and widely used analytical techniques.

In recent years, it has found wide applications for the determination of many important drugs11-14. Various spectrophotometric methods have been used for determination of DZP. The present investigation was carried out in the view of establishing a simple, rapid, accurate, economic, precise and robust UV method for estimation Diazepam in bulk and tablet dosage form using water as the solvent.

2. MATERIALS

2.1. Instrument

LABINDIA UV -3200 double beam uv-visible spectrophotometer with pair of 10 mm matched quartz cells.

2.2. Chemicals and Reagents

All the reagents and solvents were of analytical grade high purity deionized water. Loratidine was obtained as a gift from Spectrum Labs, Hyderabad. All other chemicals used were of analytical grade.

3. METHODS

3.1. Preparation of standard solution

3.1.1. Preparation of Standard Stock Solution

Standard stock solution of Diazepam drug was prepared by accurately weighing about 10mg of each drug in 10ml volumetric flask. The drugs were dissolved with 5ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent; results 1000µg/ml solution was obtained.

3.1.2. Preparation of Formulation Sample

Diazepam tablets (Placidox – 10mg) were purchased from local pharmacy. Ten tablets were weighed and average weighed was calculated. The tablet powder equivalent to 10mg of Diazepam was transferred in to a 10 ml. 3mL of methanol was added and sonicated for complete solubility. The volume was made up to the mark with methanol. From this sample stock solution of 1000µg/ml concentration final concentration 10µg/ml was prepared.

3.2. Measurement of Absorbance and Calibration Curve

The absorbance of the solutions containing DIAZEPAM at 100 µg/mL was determined in the UV range 200-400nm using an appropriate blank. The λ max was found to be 245.5nm. At this wavelength, calibration curve was drawn by plotting graph between absorbance and concentrations.

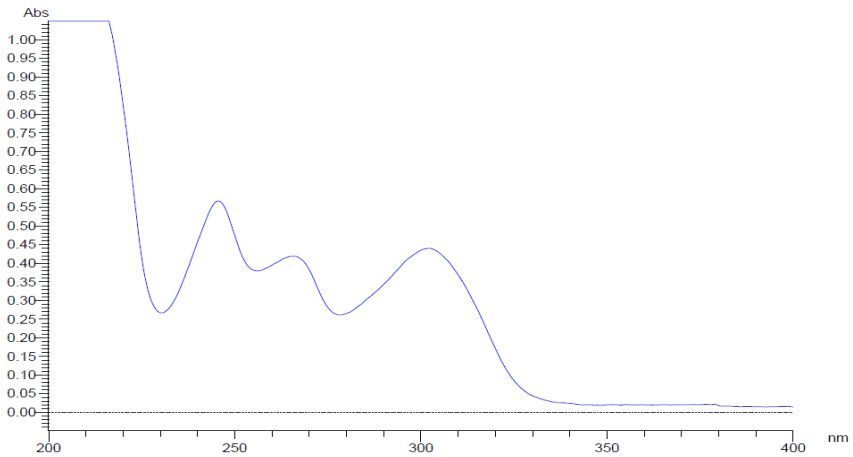

Fig2. *Determination of Wavelength of Loratadine*

4. METHOD VALIDATION

4.1. Linearity

The linearity of the method was demonstrated over the concentration range of 2-12 µg /mL. A Series of dilutions were made by using the stock solution. From the stock solution 0.2,0.4,0.6,0.8,1.0 and 1.2

mL were pipette out into a 10mL volumetric flasks and diluted with methanol and finally make up to the volume with methanol. The resulting solutions were labelled as 2, 4, 6,8,10 and 12 µg /mL. The linearity was calculated by the least square regression method

Table1. *Results of Linearity*

S. No	Concentration in µg/ml	Absorbance
1	2	0.234
2	4	0.381
3	6	0.536
4	8	0.694
5	10	0.835
6	12	0.986
	Slope:0.076 Intercept:0.0795 CorrelationCoefficient:0.9994	

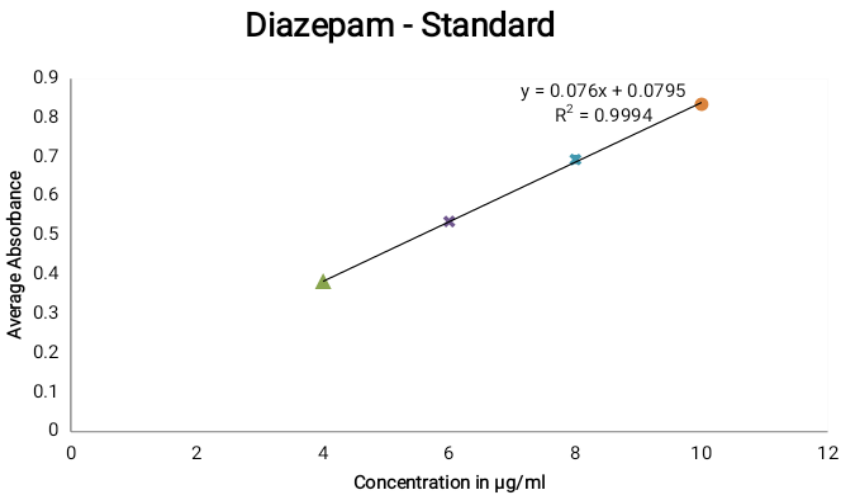

Fig3. *Calibration Curve of Diazepam*

4.2. Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as percent RSD. For this 6µg /mL concentration solution was measured six times in day and same was measured in next day also. The percent RSD was calculated. Table2. Results Precision

Table2. *Results of Intraday precision*

S.NO	Concentration in µg/ml	Absorbance
1	6	0.536
2	6	0.531
3	6	0.533
4	6	0.532
5	6	0.532
6	6	0.534
	RSD=0.336	

Table3. *Results of Interday Precision*

S. No	Concentration in µg/ml	Absorbance
1	6	0.538
2	6	0.539
3	6	0.537
4	6	0.538
5	6	0.535
6	6	0.539
	RSD:0.280	

4.3. Accuracy (Recovery)

The accuracy of the method was evaluated through standard addition method. In this, known amount of standard diazepam 4 µg/mL was added in pre-analyzed sample for 2, 4 and 6µg/mL in triplicate.

Table4. *Results of Accuracy*

S No	Spiked Level	Target (µg/ml)	Spiked (µg/ml)	Final (µg/ml)	Amount found (µg/ml)	% Recovered
1	50%	4	2	6	5.91	98.50
2		4	2	6	5.93	98.83
3		4	2	6	5.89	98.17
4	100%	4	4	8	7.98	99.75
5		4	4	8	7.91	98.875
6		4	4	8	7.86	98.25
7	150%	4	6	10	9.89	98.90
8		4	6	10	9.93	99.30
9		4	6	10	9.91	99.10

4.4. Limit of Detection (LOD)

The parameter LOD was determined on the basis of intercept and slope of the regression equation. The LOD for this method was found to be 0.07 µg/mL.

4.5. Limit of Quantification (LOQ)

The parameter LOQ was determined on the basis of intercept and slope of the regression equation. The LOQ for this method was found to be 0.25 µg/mL.

Table5. *LOD & LOQ*

LOD	0.07µg/ml
LOQ	0.25µg/ml

4.6. Ruggedness

Table6. *Results of Ruggedness*

S.NO	Concentration in µg/ml	Absorbance
1	6	0.539
2	6	0.542
3	6	0.542
4	6	0.541
5	6	0.546
6	6	0.549
	RSD:0.673	

5. Validation Parameters

Table7. *Validation Parameters*

Parameter	Result
Absorption Maxima(nm)	245.5nm
Linearity Range (µg/mL)	2-12 µg/Ml
Standard Regression Equation	y=0.076x+0.0795
Correlation Coefficient (r2)	0.9994
Accuracy(% Recovery ±Sd)	98.85±0.636
LOD (µg/mL)	0.07µg/Ml
LOQ (µg/mL)	0.25 µg/mL

6. Determination of Diazepam In Tablets

Diazepam tablets (valium10mg) were purchased from local pharmacy. Ten tablets were weighed and average weighed was calculated. The tablet powder equivalent to 10mg of Diazepam was transferred in to a 10ml. 3Ml of methanol was added and sonicated for complete solubility. The volume was made up to the mark with methanol. From this sample stock solution of 1000µg/ml concentration final concentration 10µg/ml was prepared.

6.1. Validation

Table8. *Assay of Diazepam in Tablets*

S. No	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1	Valium	Tablet	10mg	6µg/ml	5.92µg/ml	98.67

7. Discussion

For optimize the method by UV for diazepam, different solvents were tested such as Water and methanol. Due to greater solubility and reproducible readings of maximum absorbance, methanol was taken for further work. Standard diazepam (10mg) was accurately weighed and transferred to 10 mL volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain a concentration of 1000 µg/mL. By using this solution different concentrations of 2, 4,6,8,10,12 µg/mL were prepared and calibration curve was plotted by plotting graph between absorbance and concentration (Fig.2). The results of linearity are presented in table 1. The data was statistically validated by means of least square regression method. The detection and quantization limits as LOD (k=3.3) and LOQ (k=10) were calculated and these were found to be 0.1017 µg/mL and 0.3079 µg/mL respectively. The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicates the method was précised. The accuracy of the method was performed by standard addition method. The average recovery was found to be 98.5%, 98.95% and 99.1% respectively and the accuracy results showed good recovery with percent relative standard deviation (% RSD) is below 2.0. This indicates accuracy of proposed method was also applied for the assay of diazepam in tablets. The results as tabulated in Table 4. The results obtained were satisfactory and good agreement as per the ICH guidelines.

8. Conclusion

Although various methods have been developed for the estimation of Diazepam individually and in combinations with other drugs, no method has been published till now with a selected solvent. The present work done on this a simple, precise and accurate method by UV spectrophotometric method. The proposed methods can be successfully applied for diazepam assay in tablet dosage forms without any interference of excipients in quality control. Analysis of the tablets by this method were reproducible, reliable and in good agreement with ICH guideline The linearity and absorbance was determined, the concentration of Diazepam were then determined by comparing the absorbance sample with that of standard of Diazepam can be identified by their lambda maximum being 245.5 nm. The results obtained from UV method were reproducible and encouraging.

Acknowledgement

The authors are Thankful to Rev Dr. K. V. K. Rao and Dr. P. Venkateswara Rao, principal for providing the facilities to carry out this research work in St. Mary's Group of Institutions Guntur.

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Citation *A. Sireesha, et al., "Method Development and Validation of UV Spectro Photometric Method for Determination of Diazepam in its Pure and Pharmaceutical Dosage Form", ARC Journal of Pharmaceutical Sciences (AJPS) , vol. 4, no. 2, p. 18-23, 2018. http://dx.doi.org/10.20431/2455-1538.0402003*