Original Article

DEVELOPMENT AND VALIDATION OF SIMPLE UV-SPECTROPHOTOMETRIC METHOD OF QUANTIZATION OF DIAZEPAM IN BULK DRUG AND SOLID DOSAGE FORMULATION USING MIXED SOLVENCY CONCEPT

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ABSTRACT

Objective: Commonly used organic solvents for spectrophotometric analysis of water-insoluble drugs are methanol, ethanol, chloroform, benzene, toluene etc. The main drawbacks of organic solvents include high cost, toxicity, and pollution. Organic solvents have numerous adverse effects caused by single exposure like dermatitis, headache, drowsiness, nausea, eye irritation and long-term exposure causes serious effects such as neurological disorder, chronic renal failure, and liver damage. They should be replaced by other eco-friendly alternative sources.

Methods: The present study is an attempt to show that solid can also be used to act as solvent precluding the use of organic solvents. A simple, safe and sensitive method of spectrophotometric determination of diazepam obeyed beers law in the concentration range of 5-25 mcg/ml at 306 nm.

Results: The results of analyses have been validated statistically for Linearity, accuracy, precision, LOD and LOQ. The results of validation parameters also indicated that proposed method was found to be accurate, precise, reproducible, sensitive, and suitable for routine quality control analysis for estimation of diazepam in bulk drug and solid dosage formulation.

Conclusion: A rapid, simple, and non-toxic UV spectrophotometric method has been developed for the determination and quantification of diazepam. The present method also validated as per ICH guidelines for linearity, precision, accuracy.

Keywords: Diazepam, UV-Spectrophotometry, Solid dosage formulation, Bulk drug, Mixed solvency concept

INTRODUCTION

Increasing the aqueous solubility of Insoluble and slightly soluble drugs has been done by various methods to avoid the usage of organic solvents. Because of toxicity, volatility, and also the high cost of organic solvents, an alternative method has been developed. Mixed solvency concept is one of the methods to enhance the aqueous solubility of less water-soluble drugs. Mixed solvency concept may be a proper choice to preclude the use of organic solvents. So there is a broad scope for mixed solvency concept in quantitative estimation of other less water-soluble drugs.

By application of this concept, the innumerable solvent system can be developed. Maheshwari [1-6] is one of the opinions that each substance possesses solubilizing power. He has given several eco-friendly methods in the area of drug estimations and formulations precluding the use of toxic organic solvents. The solubility of a large number of poorly soluble drugs has been enhanced by mixed solvency concept [1-21].

The present research work also provides an eco-friendly method to estimate spectrophotometrically, the diazepam drug in tablet formulations without the help of organic solvent.

Diazepam is chemically 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-1, 4-benzodiazepin-2-one. It is off-white to yellow, odorless, crystalline powder. It is freely soluble in chloroform, soluble in alcohol, practically insoluble in water. It is a benzodiazepine derivative used as an anxiolytic agent, hypnotic and muscle relaxant.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade diazepam was a gift from Ranbaxy Laboratories Ltd. and its dosage formulation VLB6002 and VLB6003 were purchased from local market, the expiry of which was not less than 1 y at the time of the study. All other chemicals were of analytical grade and obtained from BDH labs.

Instrumentation

Electrical Balance, UV Visible spectrophotometer (Model1800, Shimadzu, Japan) with 10–mm path length connected to a computer was used for spectrophotometric analysis.

Calibration curve

Standard stock solution of Diazepam was (500mcg/ml) prepared by weighing 50 mg of diazepam and transferred to a 100 ml volumetric flask and was dissolved in 20 ml blend of 25% phenol and 15% sodium benzoate then finally volume was made up to 100 ml of distilled water to get a concentration of 500 mcg/ml. Appropriate volumes of this solution were further diluted with distilled water to obtain final concentrations in the range of 5-25 mcg/ml. The absorptions of these standard solutions were noted at 306 nm against respective reagent blanks.

Preliminary solubility studies

To determine the solubility of the drug in distilled water and mixed solvent blend (containing 25% phenol and 15% sodium benzoate) at room temperature sufficient excess amount of the drug was added to a 25 ml capacity vial containing distilled water and the mixed solvent blend. After putting the vial cap and applying the aluminium seal, the vial was shaken mechanically for 12 h at room temperature (27 °C) in an orbital flask shaker. The solution was allowed to equilibrate for 24 h undisturbed and then filtration was done through Whatmann filter paper#41. The filtrate was appropriately diluted with distilled water to measure the absorbance at 306 nm against reagent blanks.

Table 1: Data of calibration curve

S. No.	Concentration (mcg/ml)	Stock solution in (ml)	Final volume with distilled water(ml)	Abs.(nm)	R^2
1	5	0.5	100	0.033	0.999
2	10	1.0	100	0.073	0.999
3	15	1.5	100	0.108	0.999
4	20	2.0	100	0.146	0.999
5	25	2.5	100	0.181	0.999

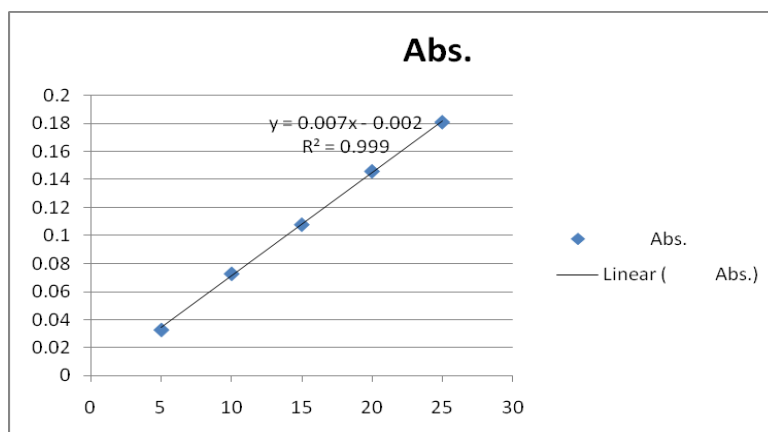

Fig. 1: Calibration curve of diazepam

Proposed method of analysis

20 tablets of Batch-I and Batch-II were accurately weighed and finely powdered. Amount of powder equivalent to 2.5 mg of bulk drug was transferred into 10 ml volumetric flask with 5 ml of the blend (25% phenol and 15% sod. benzoate) and the drug present in tablet powder was dissolved by sonication for 20 min. The flask was filled to the mark with distilled water and the resulting solution was filtered. One ml of the above filtrate was diluted to 10 ml to get 25 mcg/ml (expected). The method was followed as described under the analytical procedure and the absorbance was noted at 306 nm against the reagent blank. The drug content was calculated using the calibration curve. The Same procedure was repeated for the tablet formulation II. The results of the analysis are reported in table 2.

Table 2: Analysis data of diazepam tablet formulations with statistical evaluation (n=3)

Drug	Batch	Label claim mg/tab	% labelled claim[a] estimated	SD	%RSD	S. E.
Diazepam	I	5	101.89±0.39	0.380	0.372	0.219
Diazepam	II	5	101.49±0.21	0.201	0.198	0.116

Recovery studies

To perform the recovery studies standard diazepam drug was added (2.5 mg and 2 mg separately) to the pre-analyzed tablet powder equivalent to 2.5 mg of diazepam and the drug content was determined by the proposed method.

Results of the analysis were reported in table 3.

Table 3: Results of recovery studies with statistical evaluation n=3

Tablet formulation	Drug in pre-analyzed tablet powder (mg)	Amount of standard drug added(mg)	% recovery estimated (mean+-SD)	Percent coefficient of variation	Standard error
I	2.5	2.5	100.9±0.196	0.194	0.113
I	2.5	2	100.78±1.414	1.403	0.811
II	2.5	2.5	100.18±1.442	1.432	0.830
II	2.5	2	100.24±0.842	0.830	0.486

RESULTS AND DISCUSSION

The solubility of Diazepam in distilled water at room temperature was found to be 0.05 mg/ml. The solubility of Diazepam in the blend was more than 0.25 mg/ml.

It is evident from table 2 that the percent drug estimated in tablet formulation of Batch-I and of Batch-II were 101.89±0.39 and 101.49±0.21 respectively. The values are very close to100, indicating the accuracy of the proposed analytical method. Further table 3 shows that the range of percent recoveries varied from100.78±1.414 to100.24±0.842 which are again very close to 100, indicating the accuracy of the proposed method. The proposed analytical method is further supported by significantly small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error (table 3).

The limit of detection was found to be 0.2 mcg/ml and the limit of quantification was found to be 1mcg/ml. The criteria being the concentration should lay outside the range of o.2-1.0 for precise determination of diazepam.

CONCLUSION

A rapid, simple, and non-toxic UV spectrophotometric method has been developed for the determination and quantification of diazepam.

The present method also validated as per ICH guidelines for linearity, precision, accuracy. The results of all these parameters shows that the present UV spectrophotometric methods found to be precise, linear, rapid, and accurate and can be used for routine quality control analysis of diazepam in bulk drug and tablet dosage formulation in any laboratory. Phenol does not interfere above 300 nm.

CONFLICT OF INTERESTS

Declared none

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