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Original Article

Analytical method validation for the simultaneous determination of diazepam in bulk and tablet dosage form

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ABSTRACT

This paper describes an UV spectrophotometric validation method for the simultaneous determination of Diazepam. The study aimed to provide practical approaches for determining linearity, range, accuracy, precision, specificity, system suitability and limit of detection, lower limit of quantitation and robustness of this analytical method. All determinations were made in a wavelength of 231 nm in a solvent system of methanol: distilled water (1:1). The method was validated as per ICH guidelines and FDA guidance for industry. Here, obtained linear correlation coefficient was 0.999 and the mean recoveries were ≥98%. Hence, the method was found to be simple, rapid, economic, linear, accurate, precise, robust and reproducible and can be used for routine analysis of Diazepam in bulk and tablet dosage form.

Keywords: Accuracy, Anxiety, Assay, ICH guidelines, Linearity, UV Spectrophotometer.

INTRODUCTION

Diazepam ($C_{16}H_{13}ClN_2O$), is an anxiolytic and hypnotic drug of benzodiazepine-2-one group [1-3]. It is highly lipophilic in nature and is thus rapidly absorbed after oral administration [4]. Hence, Diazepam is freely soluble in chloroform and ethanol and slightly soluble in water [5]. It is effective in the symptomatic relief of tension and anxiety. Although Diazepam has an additional wide ranges of uses.

It acts like as an anticonvulsant, anesthesia. It is also used to treat patients having muscle spasms and pain, insomnia, epilepsy and seizure disorder, lessen the intensity of the withdrawal symptoms of alcohol or sedative–hypnotic drug [2-4], [6-8]. Diazepam potentiate GABAergic neurotransmission by modulating GABA type A ($GABA_A$) allosterically in essentially all areas of the central nervous system and regulates the entrance of chloride into the postsynaptic cells, which results in greater hyperpolarization of these cells and therefore leads to diminished synaptic transmission [2-3], [8].

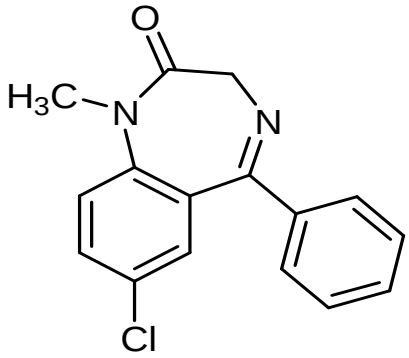

Figure 1: Diazepam [1]

Whenever the experimental conditions become change, an analytical method needs to be validated before their introduction into routine use. This work deals with development and validation of a new spectrophotometric method for the routine determination of Diazepam simultaneously in bulk and tablet dosage form to analysis the quality at laboratory. The work carries out to make the potency assay test affordable and economic. It has involved the sample preparation of Diazepam standard, placebo and marketed brands of Diazepam in biological matrix as well as provided

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practical approaches for determining linearity, range, accuracy, precision, specificity, system suitability and limit of detection, lower limit of quantitation and robustness of UV spectrophotometric method according to ICH guideline [9] and FDA guidance for industry [10].

MATERIALS AND METHOD

Apparatus and Instrument: A UV Visible Spectrophotometer (ANALYTIKJENE, Spectord 250 plus) was used. All weighing of ingredients were done on Electronic balance (COPLEY, England) and bath sonicator (COPLEY, England) was also used in study. Glass wares used in each procedure were washed properly with detergent and rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and materials: Pure drug samples of Diazepam standard was supplied as gift sample by Baximco Pharmaceutical Ltd (Bangladesh). All other chemicals and reagents used were of analytical grade.

Selection and standardization of the solvent: Several solvent systems were prepared for the selection of right solvent in which the absorbance of Diazepam was stable until 7 days. In methanol: distilled water (1:1), Diazepam absorbs bellow 240 nm and it has a very good absorptivity in entire UV range. Hence, methanol: Distilled water was selected as a possible solvent for analysis. The wavelength maximum of Diazepam was found to be 231 nm.

Preparation of standard stock solution: Standard stock solution of Diazepam was prepared by dissolving 13 mg of Diazepam in 250 ml of methanol+ Distilled water (1:1) solution and sonicated for 4 minutes in bath sonicator.

Linearity: Working sample solutions were prepared from standard stock solution to obtain 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 9, 11, 13 and 15µg/ml Diazepam concentration respectively. A calibration curve was plotted from linear response of these 3-15µg/ml concentrations range at 231nm wavelength in spectrophotometry system and linear correlation coefficient was determined.

Range: Determination of range was carried out by taking the 80%, 90%, 100%, 110% and 120% of the nominal concentration of the analyte in the sample to determine the linear correlation.

Precision

Repeatability: The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for Diazepam without changing the parameter of the proposed spectrophotometry method.

Intermediate precision: Intermediate precision of this analytical method was determined in terms of intra day and inter day variations (%RSD). Intraday precision (%RSD) was assessed by analyzing standard diazepam solutions within the calibration range, three times (1st, 3rd, and 8th hour) on the same day. Inter day precision (%RSD) was assessed by analyzing standard Diazepam solutions within the calibration range on four different days (1st, 3rd, 5th, and 7th day) within a week using the following formula.

$$\%RSD = \frac{\text{Standard deviation of Measurements}}{\text{mean value of measurement}} \times 100$$

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the following equations respectively.

$LOD = 3.3\delta/S$

$LOQ = 10\delta/S$

Where, δ: standard deviation of y-intercept and S: slope of calibration curve.

Accuracy : In order to check the accuracy of the proposed spectrophotometry method, recovery study was carried out by taking standard Diazepam mixture at 5 levels equivalent to 80%, 90%, 100%, 110%, 120% with the addition of a marketed Diazepam tablet and absorbances were determined at 231nm.

Specificity: Specificity study was carried out by spiking Diazepam standard with appropriate levels of placebo and two calibration curves were plotted from the linear response of different and fixed concentration range of Diazepam standard respectively with calculating linear correlation coefficient. A spectral scan of Diazepam standard, placebo, and marketed Diazepam tablet at 200-400nm range was carried out to check its resolution from nearest.

System Suitability: A system suitability test of the spectrophotometric system was performed with six replicate reading of standard preparation were taken and %RSD was calculated.

Robustness: Robustness experiment should expose the reliability of an analysis with respect to deliberate variations in method parameters. Here, robustness study was carried out in UV spectrophotometric system by taking absorbance of standard preperation for 6 times at 230, 231 and 232 nm wavelength.

Assay (Drug Content per Tablet): One weighted marketed tablet of Diazepam was taken into a 50ml volumetric flask and made up to mark with diluents after 2/3 drops of water addition. After 4 minutes sonication and filtration 1ml of this solution was withdrawn and made up to mark with diluents in a 10ml volumetric flask. After taking absorbance at 231 nm the amount of Diazepam in each brand was determined. This study was carried out for all 4 brands (D1, D2, D3, and D4) of diazepam and two samples for each brand using following equation.

Drug content (mg/tablet)=

$$\frac{sample\ absorbance}{standard\ absorbance} \times \frac{standard\ weight}{sample\ weight} \times avarage\ weight \times \frac{standard\ potency}{100}$$

RESULT

Table 1: Concentration VS Absorbance Table for Linearity Study

% of nominal concentration	Concentration (µg/ml)	Absorbance
0%	0	0
60%	3.096	0.3868
70%	3.612	0.4639
80%	4.128	0.5531
90%	4.644	0.6063
100%	5.16	0.6708
110%	5.676	0.7282
120%	6.192	0.8015
130%	6.708	0.8657
140%	7.224	0.9335
180%	9.288	1.1873
220%	11.352	1.448
260%	13.416	1.6999
300%	15.48	1.9854

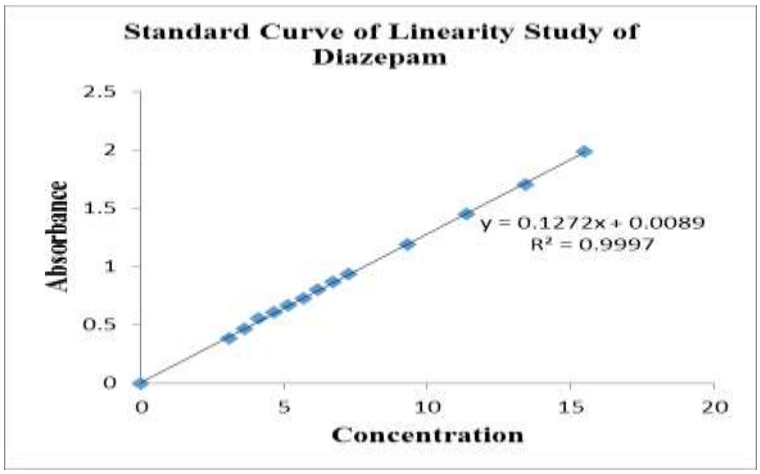

Figure 2: Linearity Study of Diazepam

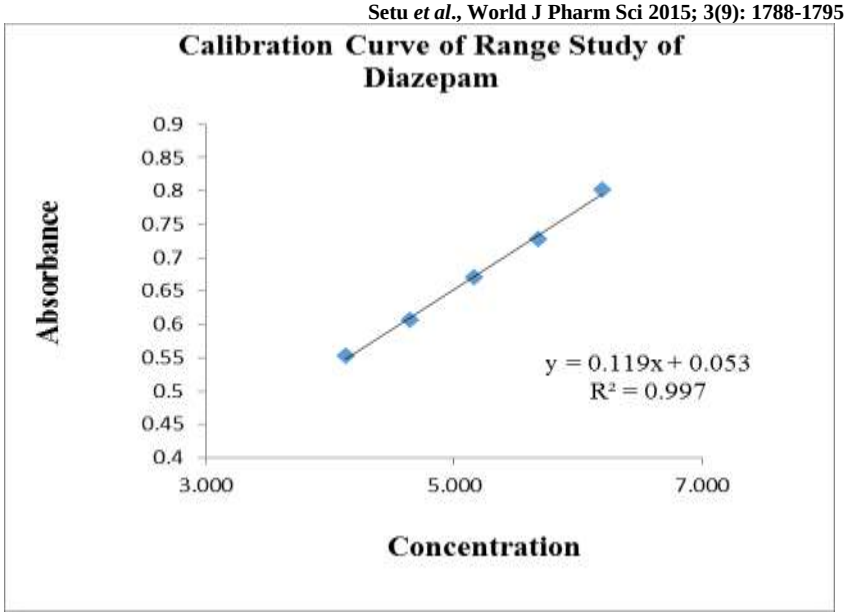

Figure 3: Range Study of Diazepam

Table 2: Evaluation of LOD and LOQ

Optical Characteristics	**Value**
$\lambda_{max.}$	231
Regression equation (Y= mx+C)	0.127x+0.008
Linearity range (µg/ml)	3-15
Slope	0.1268
Standard deviation of Y-intercept	0.006108
Correlation coefficient	0.999
LOD (µg/ml)	0.158953375
LOQ (µg/ml)	0.481676893

Table 3: Evaluation Data of Precision (Repeatability) Study

Absorbance No	**% Assay**
1	101.16
2	101.16
3	101.16
4	101.16
5	101.18
6	101.18
Mean	101.17
SD	0.0079
%RSD	0.0078

Table 4: Evaluation Data of Precision Study (Intra day + Inter day)

Sample No.	% Assay (Intra Day) 1st hour	% Assay (Intra Day) 3rd hour	% Assay (Intra Day) 8th hour	% Assay (Inter Day) 1st day	% Assay (Inter Day) 3rd day	% Assay (Inter Day) 5th day	% Assay (Inter Day) 7th day
1	98.47	100.78	92.92	100.50	101.87	99.34	101.59
2	98.30	100.72	92.90	97.00	100.96	100.71	101.02
3	98.24	100.79	92.71	96.88	97.10	99.96	100.68
4	98.15	100.51	92.58	98.94	101.15	101.09	99.02
5	98.15	100.55	92.60	100.29	100.24	99.20	102.16
6	98.11	100.55	92.70	100.22	100.50	100.24	99.67
Mean	98.23	100.65	92.74	98.97	100.30	100.09	100.69
SD	0.13	0.13	0.15	1.67	1.67	0.74	1.17
%RSD	0.14	0.13	0.16	1.68	1.66	0.74	1.17

Table 5: Evaluation Data of Accuracy Study

Sample no.	% Recovery Level	Absorbance	Concentration Given	Concentration Obtained	% Recovery
1	80%	1.1298	9.128	8.978	98.36
2	90%	1.2036	9.644	9.594	99.48
3	100%	1.2687	10.160	10.137	99.77
4	110%	1.3357	10.676	10.696	100.18
5	120%	1.4049	11.192	11.273	100.72

Table 6: Evaluation Data of Specificity Study

Sample No.	Concentration (Fixed Standard with Different Excipient Concentration-µg/ml)	Absorbance	Concentration (Different Standard with Fixed Excipient Concentration-µg/ml)	Absorbance
1	5.160	0.6709	4.128	0.5428
2	5.160	0.6799	4.644	0.6096
3	5.160	0.6779	5.160	0.677
4	5.160	0.6774	5.676	0.7401
5	5.160	0.6861	6.192	0.8012

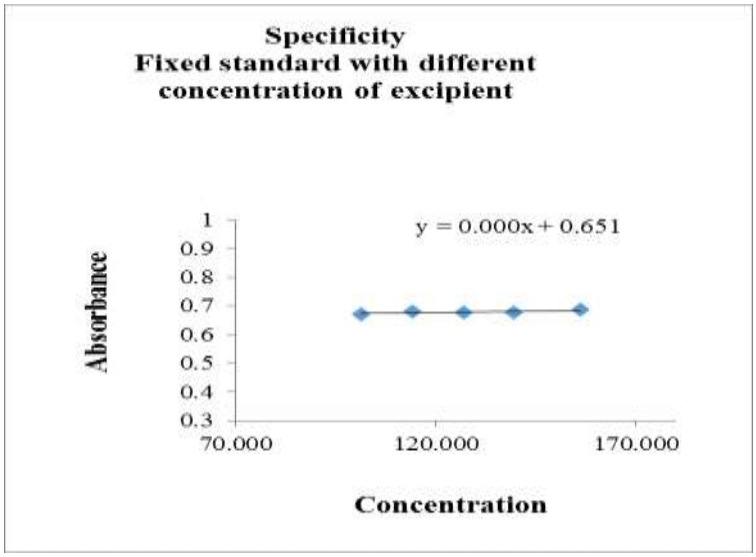

Figure 4: Specificity Study of Diazepam

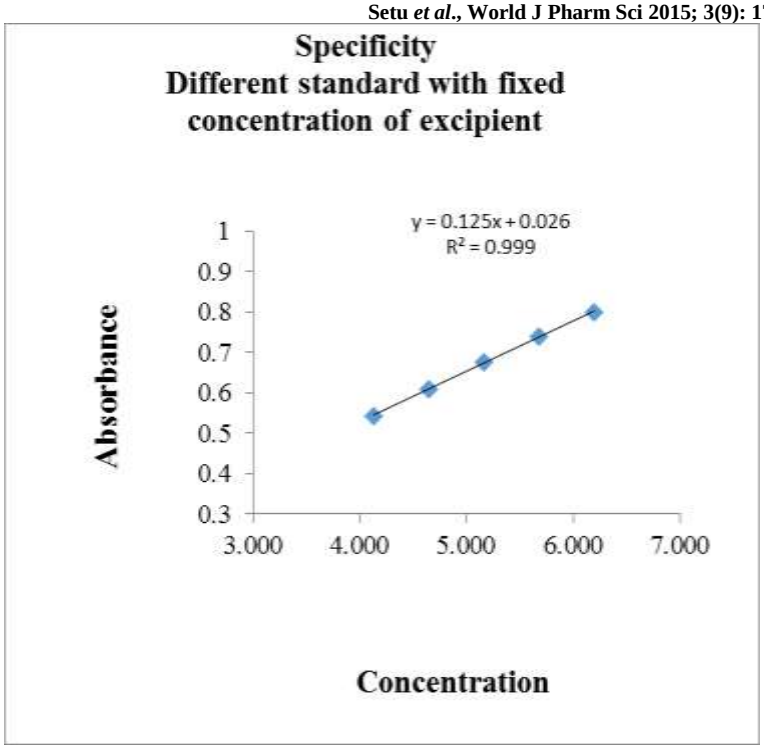

Figure 5: Specificity Study of Diazepam

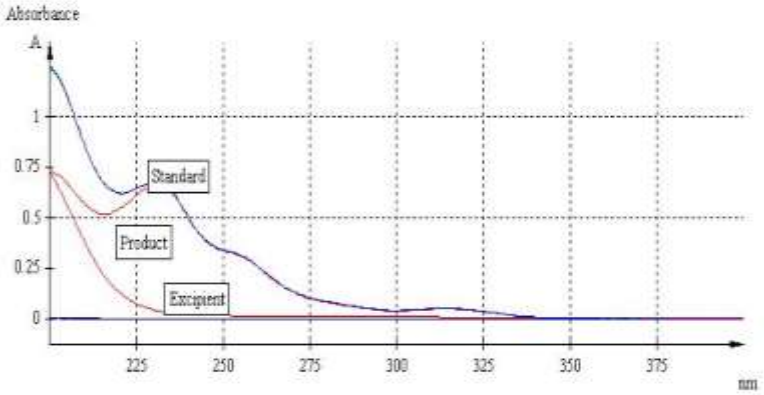

Figure 6: Overlay Figure of UV Spectrum of Diazepam Standard+ Placebo+ Marketed Product

Table 7: Evaluation Data of System Suitability Study

Absorbance No	**231nm**
1	0.674
2	0.6744
3	0.6741
4	0.6733
5	0.6742
6	0.6708
Mean	0.673467
SD	0.00136
%RSD	0.202

Table 8: Evaluation Data of Robustness Study

Sample No	230nm	231nm	232nm
S1	0.6625	0.6665	0.6671
S2	0.6625	0.6665	0.6668
S3	0.6620	0.6660	0.6667
S4	0.6624	0.6665	0.6670
S5	0.6620	0.6660	0.6668
S6	0.6622	0.6664	0.6670
Mean	0.6623	0.6663	0.6669
SD	0.0002	0.00025	0.00015
%RSD	0.04	0.04	0.02

Table 9: Drug Content of Diazepam (Validated Method)

Sample No	mg/tablet	Percentage
D1 sample 1	4.74	94.8%
D1 sample 2	4.91	98.2%
D2 sample 1	4.98	99.6%
D2 sample 2	4.87	97.4%
D3 sample 1	4.95	99%
D3 sample 2	5.01	100.2%
D4 sample 1	4.87	97.4%
D4 sample 2	4.85	97%

DISCUSSION

The results of UV analysis have been shown in tables 1-9. A linear correlation coefficient value (0.999) obtained between absorbance and concentration and the linear regression equation was "y= 0.1272x + 0.0089" (table 1 & figure 2) of Diazepam indicated that the validated analytical method was linear. The proposed analytical method was found precise within its range of 80 to 120 % of the nominal concentration, with having a linear regression equation of, y = 0.119 x + 0.05 and the regression coefficient was 0.997 (figure 3). The minimum concentration levels at which Diazepam can be reliably detected (LOD) and quantified (LOQ) were found to be 0.159 µg/ml and 0.482 µg/ml respectively determined by the standard deviation of the response and the slope (table 2). The % RSD values of repeatability study, intra day and inter day % assay were ≤ 2% revealed that the proposed method was precise and repeatable (table 3 & 4). The recovery experiment was performed by marketed product and standard Diazepam addition. The mean recoveries were 98.36%, 99.48%, 99.77%, 100.18%, and 100.72%, which (≥90%) proved that the method was accurate (table 5). Two experiments were performed for the determination of specificity of proposed method: One was done by using several samples consisting of fixed standard Diazepam and different excipient concentration (table 6 & figure 4). Here different excipient concentration did not affect the absorbance of standard Diazepam and another was accomplished by using several samples consisting of fixed excipient concentration and different standard Diazepam concentration (table 6 & Figure 5). Here the obtained linear correlation value (0.999) with the linear regression equation of, y= 0.125x + 0.026 indicated the method was specific. A spectral scan within 200-400 nm range of standard Diazepam, placebo and marketed Diazepam tablet showed that excipient effect was indifferent on Diazepam (figure 6). % RSD of system suitability study was as low as 0.202 (table 7). This satisfactory value made the method acceptable and established that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system during the operation. In case of robustness experiment all variance conditions were applied resulted in unaffected values with low % RSD (≤1%) concluded the analytical method as robust (table 8).

A potency assay tests of all four brands of Diazepam were performed in validated analytical method by using UV spectrophotometer. According to USP-29, Diazepam tablets must contain not lower than 95.0% and not more than 105.0% of the leveled amount of drug. All the brand products met the criteria with the new analytical method (table 9).

CONCLUSION

The proposed analytical method was validated successfully, as accordance to ICH Q2B guideline and FDA guideline for industry, which concluded that the method was linear, specific, precise, accurate, robust and having stability indicating characteristics. The developed UV spectrophotometry method can be used in laboratories with very high accuracy and a wide linear range as well as with its time saving and cost effective properties. Hence, the developed method can be used for routine quantitative simultaneous estimation of Diazepam in bulk drug and in pharmaceutical dosage forms without interference.

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