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Development and Validation of High Performance Liquid Chromatography Method for the Determination of Diazepam in Pharmaceutical Formulation

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ABSTRACT:
A new, precise and accurate reversed - phase high performance liquid chromatography(R-HPLC) a method was developed for determination of diazepam. High performance liquid chromatography was carried out by isocratic technique on a reverse-phase using column C18 inertstil (150 *3.9 mm), 5µm column with a mobile phase consisting of phosphate buffer solution pH = 6 and methanol in (1:1) ratio the diluents 5cm^3 of sulfuric acid in 1000cm^3 of methanol. The flow rate was adjusted at 2cm^3/min, detection wavelength at 230nm, temperature at 40C^o and retention time was found to be 6.4 min. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of developed method according to ICH guidelines (ICH Q2 R1, 2005).The assay percentage of diazepam in tablet dosage form was found to be (98.61±0.89) %. The % of recovery was found to be (97.91-99.28) %. The limit of detection (LOD) and limit of quantization (LOQ) were found to be 0.000033mg/cm^3 and 0.000101mg/cm^3 respectively.

KEYWORDS: Isocratic, Reverse phase, Benzodiazepines, Anti-epileptic, Simulated analysis.

INTRODUCTION:

Diazepam (Figure 1) is chemically 7-chloro-1, 3- dihydro-1-methyl-5-phenyl-1, 4 benzodiazepin-2-one(USP, 2015). Off-white to yellow, crystalline powder, practical colorless. Freely soluble in chloroform, soluble in alcohol, practically insoluble in water(USP, 2015). The molecular formula is $C_{16}H_{13}ClN_2O$ and molecular weight is 284.74.

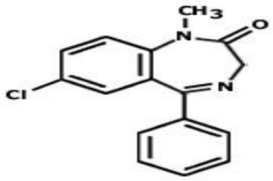

Fig (1): structure of diazepam.

Diazepam is a benzodiazepine antiepileptic drug. Like other drugs of this class, diazepam is the most widely- prescribed in the world today to treat anxiety disorders, alcohol with drawal symptoms or muscle spasms(Mattson HR et al.,1982). Diazepam is sometimes used with other medications to treat seizures.

Several techniques have been reported for the analysis of BZD, individually in pharmaceutical dosage forms potentiometric(Salem AA et al.,2003), polarography(Guadalupe GM et al.,1993), thin-layer chromatography (TLC) (Bakavoli M et al.,2003)and fluorimetry(Salem A et al.,2004).

Various Spectrophotometric methods have been reported for the determination of diazepam in active pharmaceutical ingredient dosage form and in biological fluids(Daharwal S J 2013) (Debabrata Ghosh et al.,2009). Various GC methods was also reported for determination of diazepam(Bruce A et al.,2009) (Nasiruddin et al.,2013). Various HPLC methods was also reported for determination of diazepam(Choudhury T et al.,2010) (Moghaddam KA et al.,2008) (Sruthi A et al.,2013) (Vishali P et al.,2011).

The objective of this research was to develop and validated an accurate, precise and selective reverse phase HPLC methods for the simulated analysis of Diazepam. Then validation of this method through elements such as accuracy, linearity, sensitivity, precision, limit of detection and limit of quantification.

MATERIALS and METHODS:

Material:

Chemicals and Reagent:

Methanol, HPLC grade from Duksan pure chemicals Korea, Sulfuric acid GR grade from Duksan pure chemicals, potassium dihydrogen phosphate, orthophosphoric acid, diazepam working standard (purity 99.6%) from Shanghai- Sudan Pharmaceutical CO-LTD, and diazepam tablet 5mg dosage from the local market.

Instrumental:

The instruments used for the study was. High-performance liquid chromatography (HPLC) SHIMADZU Japan, prominence–I LC-2030c.UV-VIS detector software LC solution.

Methods:

Diluents preparation

$5cm^3$ of sulfuric acid in $1000cm^3$ of methanol.

Preparation of buffer pH=6

6.8g of potassium dihydrogen phosphate and 1g sodium hydroxide were transferred to $1000cm^3$ volumetric flask and dissolved by purified water and completed to the mark by purified water. Adjusted to pH with orthophosphoric acid.

Preparation of mobile phase

Mixture of $500cm^3$ buffer and $500cm^3$ methanol.

Preparation of standard stock solution

206.0mg diazepam standard (purity 99.6%) were weighed and transferred to $100cm^3$ volumetric flask by $5cm^3$ distilled water and $55cm^3$ of diluents and sonicated for one minute then cooled and completed to the mark by diluents.(Final concentration $2.06mg/cm^3$).

Preparation of placebo stock solution

63.31mg of diazepam placebo were weighed and transferred to $100cm^3$ volumetric flask by $5cm^3$ distilled water and $50cm^3$ of diluents and shacked for 30 minutes on mechanical shaker (RPM 270) and completed to the mark by diluents.

Average of tablet 68.31mg then the theoretical weight of placebo equivalent to 5mg of diazepam equal (68.31- 5) 63.31mg.

Specificity:

Diluents', mobile phase, placebo, standard solution (100%) and sample solution was injected on HPLC.

Linearity:

Aliquots 2.5, 5, 7.5, 10, 12.5 and $15cm^3$ of above solution ($2.06mg/cm^3$) to series $50cm^3$ volumetric flask and completed to the mark by diluents to obtain solutions had final concentrations 0.001023, 0.00206, 0.00304, 0.00412, 0.00515 and $0.00618mg/cm^3$ respectively (25% - 150%).

Accuracy:

The accuracy of the method was done by adding a known amount of standard solution to placebo across the range of linearity(50,100 and 150 %).

Precision:

The precision was done by preparation of a 100 % standard solution for both intermediate - precision and repeatability. Using different analysts each one prepared different standard solution.

Intermediate Precision: The intermediate precision was done by using 100% concentration solution

Repeatability: Six replicates in same concentration were analyzed in same day for repeatability

Limit of detection and the limit of quantification:

Detection limit, is the lowest concentration of analyzed sample that can be detected but not necessary quantized. It can be determined by preparing a solution that expected to produce a response that is approximately 3 to10 time's base line noise

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\ \sigma/S$$

σ = standard deviations of the response

S = mean of the slope of the calibration plot

Results and Discussion:

Specificity:

Showed no interference between the area under peak of diazepam and diluents, mobile phase, placebo or degradation product. The result showed in table(1) and **Figures No(2)(a, b, c, d, e)**.

Table (1) Result of specificity for diazepam

Diluents	No interference
Mobile phase	No interference
Placebo	No interference

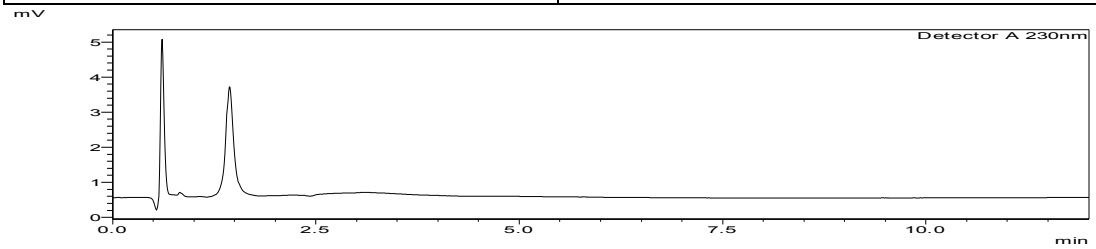

Fig (2-a) Chromatogram of M-ph

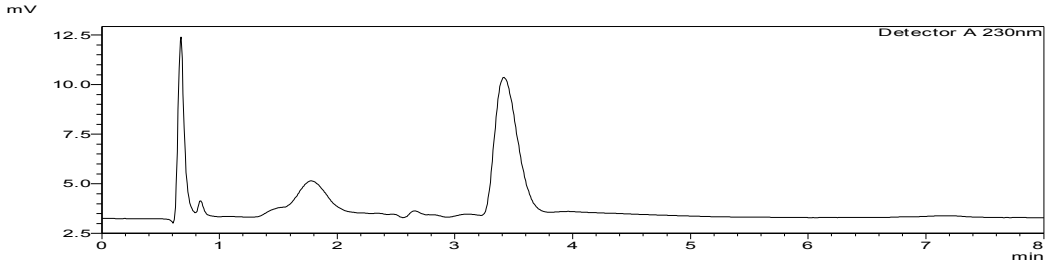

Fig (2-b) Chromatogram of diluents

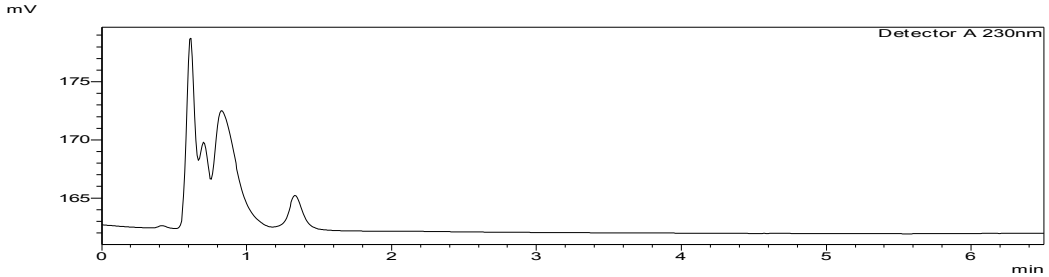

Fig (2-c) Chromatogram of placebo solution for diazepam.

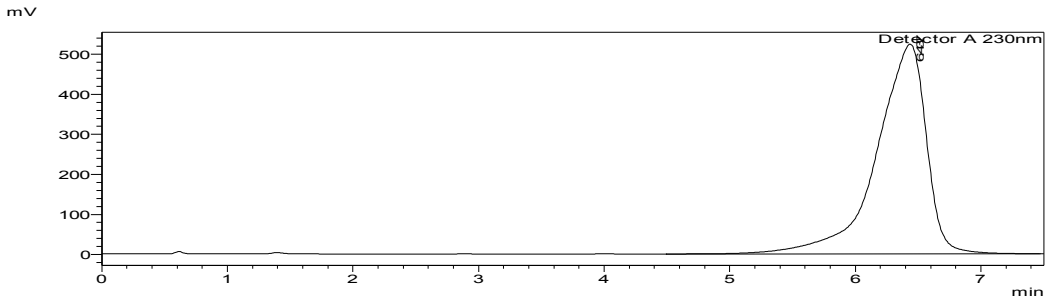

Fig (2-d) Chromatogram of standard.

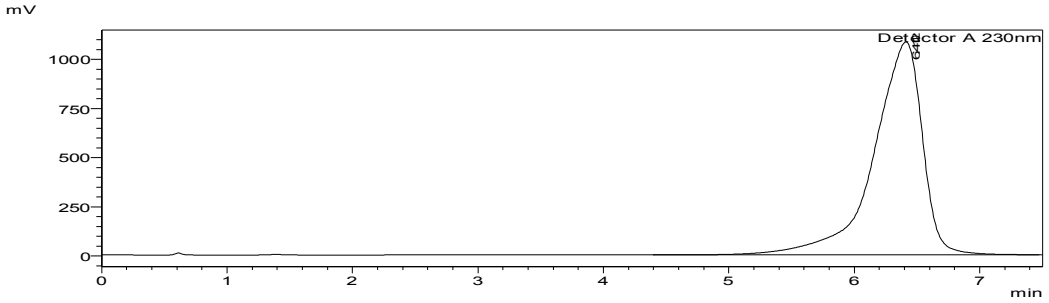

Fig (2-e) Chromatogram of sample.

Linearity:

The linearity was determined by terms of correlation coefficient and results are given in **Figure No 3 and Table No 2**. The correlation coefficient ($R^2 \geq 0.99$) demonstrates linearity. In addition, the y-intercept must not be significantly different from zero according to ICH guide lines.

The method has linear responding in the range of concentration (25% to 150%).

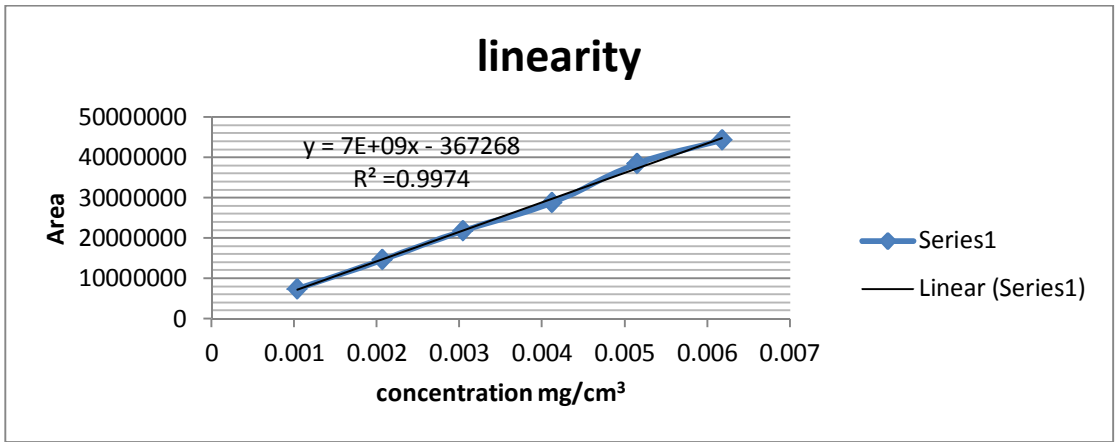

Fig (3) calibration carve of diazepam

R^2 = 0.9974 and intercept =367268

(Acceptance criteria for Linearity ≥ 0.99)

Table (2): Calibration curve of diazepam.

%sample concentration	Concentration	Area	SD of area under peak	RSD
25%	0.00103	7346443		
		7357302		
		7375565		
Average		7359770	14717.03058	0.1999659
50%	0.00206	14601239		
		14610328		
		14693989		
Average		14635185.33	51127.8387	0.3493488
75%	0.00304	21822159		
		21854549		
		21888025		
Average		21854911	32934.49213	0.1506961
100%	0.00412	28790453		
		28934159		
		28882182		
Average		28868931.33	72763.58	0.2520481
125%	0.00515	38491365		
		38465630		
		38720046		
Average		38559013.67	140050.4618	0.3632107
150%	0.00618	44568798		
		44367004		
		44374379		
Average		44436727	114436.268	0.2575263

Accuracy:

Accuracy is closeness of test results with the true value which is express as % of recovery. Results are given in Table.3

Table (3): Accuracy result of diazepam.

%	Actual Conc. mg/cm^3	Area std with placebo	Estimated conc. mg/cm^3	Area of std only	Recovery %
50	0.00206	14086629	0.0020133	14413577	97.73
		14081717	0.0020112	14423726	97.63
		14090460	0.0020081	14454429	97.48
Average	0.00206	14086269	0.002011	14430577.3	97.61
100	0.00412	28533952	0.00418572	28774953	99.16
		28571737	0.00419036	28780269	99.28
		28631236	0.00419823	28814122	99.35
Average	0.00412	28578975	0.0041914	28789781	99.27
150	0.00618	43241862	0.0061211	43657785	99.05

		43213658	0.0061152	43671812	98.95
		43217602	0.0061114	43702599	98.89
Average	0.00618	43224374.0	0.006116	43677398.7	98.96
Mean					98.61
SD					0.88
RSD %					0.89

Precision:

Intermediate Precision:

Result of intermediate precession of two analysts were found to be in the range (28869992-28789781) %. Results are shown in Table (5).

Table (5): Intermediate precision of diazepam.

Run	First analyst	Second analyst
1	28887016	28774953
2	28855897	28780269
3	28867064	28814122
Mean	28869992	28789781
Mean	28829887	
SD	56717.74	
RSD%	0.196732	

Repeatability:

Results were found to be within acceptable limits (RSD <2) as shown in table 4**.**

Table (4): Repeatability result of the system of diazepam.

NO	Area	Tailing factor	NO. Theoretical plate(USP)	Separation factor	USP Width	Resolution (USP)
1	28887016	0.703	10456	7.415	0.645	11.968
2	28855897	0.702	10414	7.424	0.648	11.952
3	28867064	0.702	10437	7.421	0.647	11.739
4	28811603	0.703	10489	7.418	0.645	11.676
5	28924492	0.702	10401	7.421	0.648	11.721
6	28882859	0.702	10375	7.414	0.648	11.935
Mean	28871489	0.702333	10428.7	7.418833	0.6468	11.8318

Limit of detection and the limit of quantification:

The limit of detection (LOD) may be expressed as:

σ = 71005

S = 7E+09

LOD = 3.3* 71005/ (7E+09) = 0.000033 mg/cm^3

Limit of Quantization can be determined in the same manner but using the formula

LOQ = 10 *71005/ (7E+09) = 0.000101 mg/cm^3.

Results showed that detection limit and quantization limit for diazepam by using this method is 0.000033 mg/cm^3 to 0.000101 mg/cm^3 respectively. These data show that the proposed method is sensitive for the determination of diazepam.

Conclusion:

In the present work, a simple and sensitive HPLC method has been developed for the determination of diazepam. The method was completely validated by using sensitivity, stability, specificity, linearity, accuracy and precision parameters for determination of Diazepam in the pharmaceutical tablet formulations. This method can easily, conveniently and accurately adopted for routine quality control analysis of diazepam in pharmaceutical dosage forms

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REFERENCES:

ICHQ2 (R1) Harmonized triptite guideline. (2005) Validation of analytical procedure: text and methodology International Conference on Harmonization ICH. Genava.

Bakavoli M & Kaykhaii M. (2003) Quantitative determination of diazepam, nitrazepam and flunitrazepam in tablets using thin-laye chromatography-densitometry technique. J Pharm Biomed Anal; 31(6): 1185-1189.

Bruce A. Goldberger, Chris W. Chronister & Michele L.(2009) Merves. Quantitation of Benzodiazepines in Blood and Urine Using Gas Chromatography-Mass Spectrometry (GC-MS), *Springer Protocols,* 10, 1007.

Choudhury T, Ghosh J & Bagchi D. (2010) Reverse phase high performance liquid chromatographic method and method validation of imipramine by using single mobile phase. Int J Pharma Sci Tech; 4(1): 54-62.

Daharwal S J. (2013) Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Diazepam and Propranolol in Bulk Drug and Its Formulations. *Asian J. Pharm*, 3, 01-03.

Debabrata Ghosh, Dastidart & Biswanath SA, (2009) comparative study of UV – spectrophotometric and first order UV – spectrophotometry methods for the estimation of diazepam in the presence of tween 20 and propylene glycols. AAPS Pharmscitech 10(4), 1396 – 1400.

Guadalupe GM, Garcia A & Gonzalez I. (1993) Extraction and electrochemical quantification of the active ingredients (diazepam) in pharmaceutical products. Talanta,. 40: 1775.

Mattson HR, Woodbury DM, Penry JK & Schmidt RP, .(1982) Antiepileptic Drug, Raven Press, New York. 497.

Moghaddam KA, SolataniUddin MNF, Samanidou VF & Papadoyannis IN.(2008) Development and validation of an HPLC method for the determination of benzodiazepines and tricyclic antidepressants in biological fluids after sequential SPE. J Sep Sci, 31(13): 2358-70.

Nasiruddin, Mohammad, Victoria F Samanidou and Ioannis N Papadoyannis.(2013) Bio-Sample Preparation and Gas Chromatographic Determination of Benzodiazepines, *J Chromatogr Sci,* 10, 1093.

Salem A, Barsoum B & Izake E. (2004) Spectrophotometric and fluorimetric determination of diazepam, bromazepam and clonazepam in pharmaceutical and urine simples. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy; 60(4):771-780.

Salem AA, Barsoum BN & Izake EL.(2003) Potentiometric determination of diazepam, bromazepam and clonazepam using solid contact ion-selective electrodes. Analytica Chimica Acta, 498(1-2), 79 91.

Sruthi A, Tejaswi P, Thanuja N, Sudheer Kumar D & Vivek Sagar P.(2013) Simple RP- HPLC method for estimation of diazepam in tablet dosage form, *Journal of Pharmacy Research,* 6(1), 140- 144.

United states pharmacopeia. (2015) USP 38, The national formulary NF 33, United states pharmacopeia convention ,pp 3073.

Vishali P, Michale , Avinash T Gatade, Dr & Ramesh T Sane.(2011) Validated HPTLC content uniformity test for the determination of diazepam in tablet dosage forms. International Journal of Pharmaceutical Research and Development. 3(6), 95 – 103.