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METHOD DEVELOPMENT AND VALIDATION OF DIAZEPAM IN TABLET DOSAGE FORM BY HPLC

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ABSTRACT

To establish A RP-HPLC method for determination of diazepam and his decomposition products present in pharmaceutical dosage form. Various parameters that can potentially affect the analytical process were investigated and optimised. LC operating conditions were also optimised, and the chromatographic separation was performed on a Lichrospher 100 RP column at 25 degrees C using methanol, acetonitrile and (KH_2PO_4) (pH 3.0; 0.05M). Diazepam showed good linear at the range of 42–78 µg/ml (0.9995) with short run time. The recovery was found to be in the range of 98.1–100.8%. The LOD and LOQ for estimation of DZP were found to be 1.49µg/ml and 4.80µg/ml, respectively. The intra- and inter-day coefficients of variation were less than 1.4% (R.S.D.). The proposed method is simple, economic and accurate. It can provide reliable evidence for development and quality control.

KEY WORDS

HPLC, Diazepam, Pharmaceutical dosage form and Validation.

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INTRODUCTION

Diazepam (Figure No.1) is a benzodiazepine (BZD) generally used for a number of medical reasons. Chemically it is 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-1, 4-benzodiazepine-2-one.The molecular formula is $C_{16}H_{13}ClN_2O$. The main reason for a prescription of Diazepam is for the treatment of anxiety and panic disorders. Diazepam (DZP) is also routinely prescribed as the standard first-line treatment for acute convulsions and prolonged status epilepticus[1,2]. Diazepam has been determined in combinaison with other drugs using UV

Spectroscopy[3] , gas chromatography[4], gas chromatography–mass spectrometry (GC–MS),[5] and high-performance liquid chromatographic–mass spectrometry (LC–MS)[6,7] in pharmaceutical preparations.

However, all of these methods have limitations such as long run times and difficult detection methods such as mass detectors[8, 9]. In this study, efforts have focused on developing a HPLC simple and easy method with short run time and a small amount in the organic phase as mobile phase with UV detection at 230 nm. Process has been optimized and validated according to the guidelines of the International Conference on Harmonization (ICH)[10].

MATERIALS AND METHOD

Chemicals and reagents

An analytically pure sample of diazepam was procured as gift sample from Roche pharmaceutical (Morocco). Acetonitrile and methanol (HPLC grade) were procured from Merck Specialist. Ultra pure water (HPLC-grade) was obtained from Merck. Potassium dihydrogen phosphate (AR grade, purity 99.6%) was procured from Merck. Tablet formulations Valium (Roche in Morocco.) was procured from a local pharmacy with labeled amount 2 mg per tablet. Other chemicals used were of analytical grade.

Instrumentation and optimisation of chromatographic conditions

The HPLC system used for quantification of Diazeam consisted of a LaChrom L-7100 Merck Hitachi Pump, LaChrom L-7200 Merck Hitachi Autosampler and LaChrom L-7400 Merck Hitachi UV Detector. The chromatogram peaks were quantified by means of PC Multi- System Manager Software (Merck-Hitachi Model D-7000). LC parameters are optimized by investigating the influence of the mobile phase, column temperature and detection wavelength. The initial separation is carried out on Lichrospher 100 RP reserved-phase column with mixtures of methanol, acetonitril and water while the mobile phase with an isocratic elution method. Because the peak shapes are unsatisfactory, a small amount of phosphate buffer is added to the mobile phase in order to suppress the ionization of these compounds, sharpen peak shapes and improve analytical sensitivity and resolution. The optimum mobile phase was composed of methanol, Acetonitrile, KH_2PO_4 (pH 3.0; 0.05M) in ratio of 40:20:40 (v/v/v) that was set at a flow rate of 1.5 ml/min. The mobile phase was degassed in an ultrasonic bath prior to use and filtered through 0.45 µm membrane filter before pumping into HPLC system. The injection volume was 20 µl, and a chromatographic peak was detected at 230 nm. Chromatography separation for analyte was achieved on Lichrospher 100 RP analytical column with 125 × 4.0 mm i.d. and 5 µm particle size maintained at ambient temperature.

Preparation of mobile phase

Mobile phase was a mixture of 400 ml of methanol and 200ml of acetonitrile and 400ml Potassium dihydrogen phosphate 0.05M adjusted to pH 3.0 with ortho phosphoric acid.

Mobile phase was filtered through a 0.45 µm nylon filter and degassed for 5 min using an ultrasonicator.

Preparation of standard solution

Accurately weighed about 120 mg of Diazepam standard was taken in a 200 ml volumetric flask and was dissolved in 20ml with distilled water and it was diluted up to mark with mobile phase. 10 ml of this solution was further diluted to 100 ml with mobile phase.

Preparation of sample solution

Twenty tablets of diazepam hydrochloride were weighed and ground into a fine powder. A quantity of powder equivalent to 2500 mg of valium 2 mg was weighed and transferred into a 100 ml volumetric flask and was dissolved in 10ml with distilled water. 10 ml of this solution was further diluted to 50 ml with mobile phase. Filtered solution through 0.45 µm Teflon Syringe Filter.

Method validation

Specificity

Specificity of proposed method was determined by checking blank and placebo interference at the retention time of Diazepam peak. Identification of Diazepam peak in sample solution was confirmed by comparing retention time of Diazepam peak with retention time of solution standard of Diazepam.

Linearity

Linearity of the method was evaluated by using 5 linearity solutions of different concentrations. Accurately measured aliquots of solution standard were taken in five different 200 mL volumetric flask and diluted up to the mark with the mobile phase such that the final concentrations of Diazepam were 42 μg ml^{-1}, 51 μg ml^{-1}, 60 μg ml^{-1}, 69 μg ml^{-1} and 78 μg ml^{-1}. A 20 μl aliquot of each linearity solution was injected in Triplicate[10].

Accuracy

The accuracy of the method was determined by calculating recoveries of Diazepam by the standard addition method. Known amount of standard of Diazepam was spiked to placebo in three different levels (70%, 100% and 130% of sample concentration) and prepared three spiked samples of each level (Total 9 determinations as per ICH guideline.) These spiked samples were analyzed against solution standard and the amount of Diazepam recovered in three different levels was calculated.

Instrumental precision

The instrumental precision was checked by injecting six replicates of solution standard containing Diazepam (60.0 μg ml^{-1}) and calculated the percentage RSD of retention time and area responses of Diazepam.

Method precision (repeatability)

The method precision of the proposed method was determined by preparing six different sample solutions of same batch and analyzed against standard solutions. Assay values of these all six samples were calculated.

Intermediate precision (reproducibility)

The intermediate precision of the proposed method was evaluated by preparing six different sample solutions of same concentrations as prepared in method precision and analyzed against standard solutions on different days. Assay values of all the six samples were calculated.

Robustness

Robustness of method is its ability to remain unaffected by small changes in method parameters. Robustness of proposed method was demonstrated by making slight changes in method parameters like flow rate (±5%), column temperature (±2 °C), detection wavelength (±5 nm), and mobile phase composition (±5% organic phase).

Filter compatibility

To check the compatibility of filter paper used to filter sample solution, the sample solution was divided into two parts. One part of solution was centrifuged and other part of solution was filtered through different types of filter papers such as 0.45 μm PTFE syringe filter, 0.45 μm PVDF filter and 0.45 μm Teflon syringe filter. Results of centrifuged sample and filtered samples were compared.

Solution stability

The solution stability of sample solution and standard solution were evaluated by comparison of assay value of freshly prepared samples and stored samples (at room temperature for 72 h). Standard solution and sample solution were prepared as mentioned in chromatographic conditions. Sample solution was analyzed and assay value was calculated against standard solution. Both the solutions (standard and sample solution) were kept at room temperature for 24 h. After 24 h these stored samples were reanalyzed against freshly prepared standard solution and the assay values were compared.

RESULTS AND DISCUSSION

In this method to optimize chromatographic parameters several mobile phase compositions were tried. A satisfactory separation, good peak symmetry and to achieve good retention time was obtained with mobile phase consisting a mixture of methanol, acetonitrile, Potassium dihydrogen phosphate buffer (KH_2PO_4) (pH 3.0; 0.05M) in ratio of 40:20:40 (v/v/v) that was set at a flow rate of 1.5 ml/min was found to be optimum and further optimized by adjusting pH 3.0 by adding orthophosphoric acid. The suitability of the mobile phase decided on the basis of the sensitivity of the assay, time required for the analysis, ease of preparation, and use of readily available cost effective solvents. The composition of methanol, acetonitrile, Potassium dihydrogen phosphate buffer (pH 3.0; 0.05M) in ratio of 40:20:40

(v/v/v) gave the best results. The proposed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision, robustness, solution stability and filter paper compatibility. All results of validation parameters meet the limits of ICH guidelines. Chromatograms of blank, placebo of diazepam tablet and diazepam are shown in Figure No.2 respectively.

Specificity

It was observed that there was no interference from blank and placebo at the retention time of Diazepam peak. Retention time of Diazepam peak in sample solution matches the retention time of Diazepam peak in standard solution. These results indicate that proposed method gives uniform and pure peak of Diazepam.

Linearity

A calibration curve was obtained by plotting area response versus concentration. Correlation coefficient obtained from graph was 0.9995. Linearity curve of Diazepam is shown in Figure No.3.

Accuracy

The percentage recoveries of Diazepam from tablet samples were calculated. Recovery ranged between 98.1% and 100.8%, which shows that there is no interference with excipients. Percentage recovery values were calculated and the results were shown in Table No.1.

Instrumental precision

The percent relative standard deviations (RSD) for six replicate of standard solution were calculated for diazepam Figure No.4 and presented in the Table No.2. The Instrumental precision was found to be 0.25% and 0.33% for retention time and area response respectively.

Method precision

Percent relative standard deviation of Assay values for six samples were found to be 1.07%. The low RSD values indicate that the proposed method is precise or repeatable. Results of Method precision are shown in Table No.3.

Intermediate precision or reproducibility

% RSD of assay values of 12 samples (method and intermediate precision sample) were found to be 1.40%. The closeness of assay results and percent RSD values indicate that the proposed method is reproducible.

LOD and LOQ

LOD and LOQ for Diazepam were estimated by injecting a series of dilute solutions with known concentration. The parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation. The LOD and LOQ of the drug were found to be 1.49µg/ml and 4.80µg/ml respectively.

Robustness

It was observed in Table No.4 that by making changes in chromatographic parameters, absolute difference between percent assay under altered condition and mean percent assay obtained during repeatability was not more than 2.0%. % RSD of area response and retention time were below 2%.

Filter compatibility

The percent assay values were calculated for centrifuged and filtered samples. The results obtained using filter papers were compared with results obtained with centrifuged sample. Absolute difference between results for filtered solutions and centrifuged solutions was not more than 2.0%. It was observed that filter paper does not adsorb drug substance during filtration of sample solutions.

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 72 h at an interval of 24 h at room temperature. The results show that for solutions, the retention time and peak area of diazepam hydrochloride remained unchanged and no significant degradation within the indicated period, this indicates that both solutions were stable for 48h.

Table No.1: Recovery results of Diazepam

S.No	Accuracy level	Sample preparations	Added amount of diazepam (mg ml^{-1})	Recovered amount of diazepam (mg ml^{-1})	% Recovery	Mean % recovery	% RSD
1	Accuracy (70%)	Preparation-1	0.0426	0.0418	98.12	98.28	0.14
		Preparation-2	0.0429	0.0422	98.37		
		Preparation-3	0.0421	0.0414	98.34		
2	Accuracy (100%)	Preparation-1	0.0601	0.0597	99.33	99.06	0.62
		Preparation-2	0.0605	0.0595	98.35		
		Preparation-3	0.0586	0.0583	99.49		
3	Accuracy (130%)	Preparation-1	0.0763	0.0769	100.79	100.10	0.88
		Preparation-2	0.0788	0.0781	99.11		
		Preparation-3	0.0772	0.0775	100.39		

Table No.2: Instrumental precision results of Diazepam

S.No	Concentration (µg/ml)	Retention time (min)	Area response
1	60.232	4.25	4778205
		4.24	4800958
2	60.232	4.23	4817212
3	60.232		
4	60.232	4.23	4778064
		4.22	4800986
5	60.232	4.22	4782924
6	60.232		
Mean	---	4.231	4793058
% RSD		0.25	0.33

Table No.3: Method Precision results of Diazepam

S.No	Concentration (µg/ml)	Area response
1	57.32	4421367
2	57.47	4429898
3	57.80	4451613
4	56.53	4351785
5	56.43	4345797
6	57.75	4447416
Mean		4407979
% RSD		1.07

Table No.4: Results of robustness study

S.No	Method parameter	Altered condition	% Assay	% RSD
1	Flow rate	1.425 ml min^{-1}	99.11	1.15
		1.50 ml min^{-1}	99.71	1.06
		1.575 ml min^{-1}	101.69	0.85
2	Temperature	23 °C	98.56	1.17
		25 °C	99.71	1.06
		27 °C	99.28	0.74
3	Wavelength (nm)	225 nm	98.17	0.95
		230 nm	99.71	1.06
		235 nm	99.82	1.11
4	Mobile phase composition (Methanol: Acetonitrile: KH_2PO_4 (pH 3.0; 0.05M) (v/v/v)	41:19:40	98.34	1.22
		40:20:40	99.71	1.06
		40:21:40	99.13	0.79

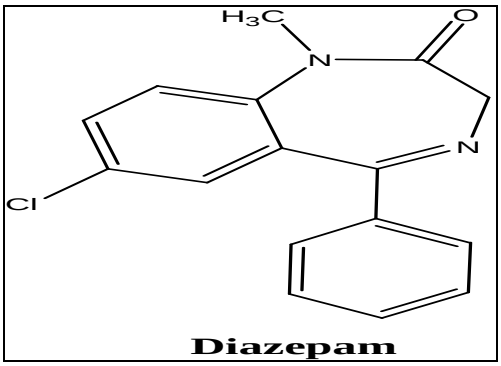
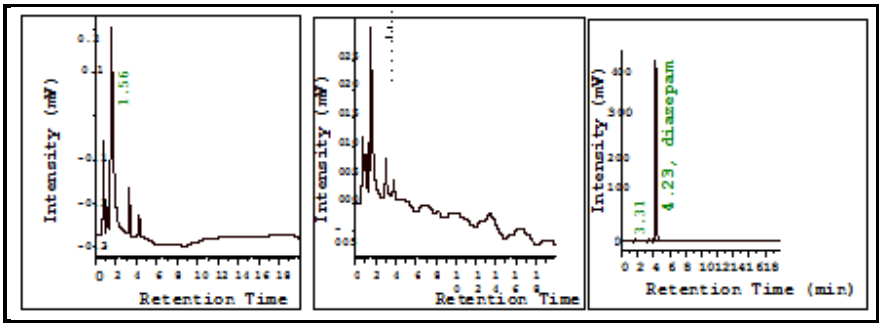


Figure No.1: Structure of diazepam



(2a) (2b) (2c)

Figure No.2: Chromatograms of blank (2a), Placebo of diazepam tablet (2b) and Diazepam (2c)

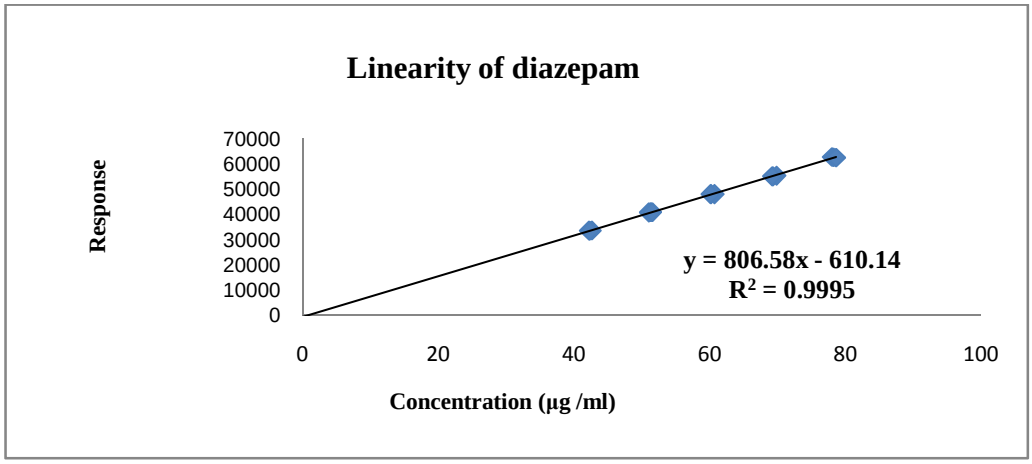


Figure No.3: Linearity curve of Diazepam

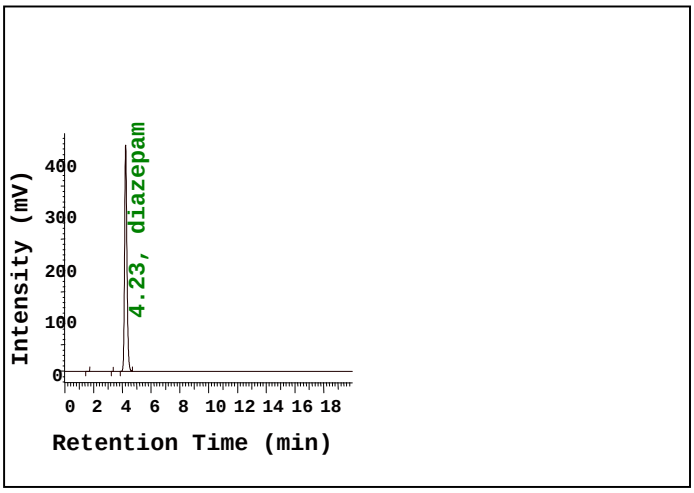


Figure No.4: Chromatogram of standard solution of Diazepam

CONCLUSION

Our objective was to develop and validate an analytical method for the quantitation of diazepam in bulk and tablet dosage form. We accomplished this using reversed phase chromatography. The assay provides a linear response across a wide range of concentrations and it utilizes a mobile phase which can be easily prepared and diluent is economic, readily available. The proposed method can be used for the quality control of diazepam hydrochloride in bulk preparations of the drug and, in pharmaceutical dosage forms without interference of excipients.

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