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Research Article

VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF LORAZEPAM IN PHARMACEUTICAL FORMULATION

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ABSTRACT
A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Lorazepam in tablet dosage form. An Inertsil ODS Ultrasphere 5 µm column having 4.6 mm x 15cm internal diameter in isocratic mode with mobile phase containing Methanol : Water (65 : 35) was used. The flow rate was 1.0ml/min. and effluents were monitored at 230 nm. The retention time for Lorazepam was 6.8 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. The Mean percent recovery of Lorazepam from tablet formulation was found to be 102.00 %. This method gives linear response from 0.0007812 – 0.01 mg/swab therefore the method can detect the above concentration of API 0.0035 mg/ swab required by the method. The proposed method was successfully applied for the quantitative determination of Lorazepam in tablet formulation.
Key Words: Lorazepam, HPLC, Linearity, Validation,

INTRODUCTION
Lorazepam *(RS)*-9-chloro-6-(2-chlorophenyl)-4-hydroxy-2,5-diazabicyclo[5.4.0]undeca-5,8,10,12-tetraen-3-one is used for the short-term treatment of anxiety, insomnia, acute seizures including status epilepticus and sedation of hospitalized patients, as well as sedation of aggressive patients [1-4]
White or almost white, crystalline powder. Practically insoluble in water, sparingly soluble in ethanol (96 per cent), sparingly soluble or slightly soluble in methylene chloride. Lorazepam the molecular formula $C_{15}H_{10}Cl_2N_2O_2$ and Molar Mass was found that 321.2 g/mol.

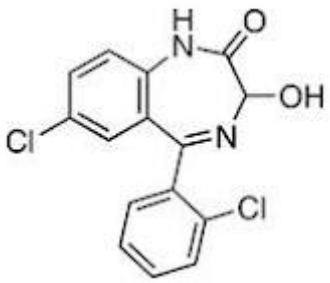

Figure :1 Lorazepam

Lorazepam is treatment of anxiety disorders, sedative, as hypnotics, onvulsant first line treatment for early stage of status epilepsy, pre-operation medication, tension, terminal agitation, alcohol withdrawal, serotonin syndrome and symptomatic treatment of nausea and vomiting associated with chemotherapy. Lorazepam has slightly metallic test, has half life of 7-16 hours and has poor water solubility. Lorazepam has Oral Bioavailability 85%, BCS Class-II, and its reaches within 2 hr after oral administration. The conditions mentioned above require immediate release of drug from the dosage form, which make lorazepam suitable candidate for fast dissolving tablets [5]

Several methods have been reported for the determination of benzodiazepines in various matrices using high-performance liquid chromatography (HPLC) [6,7], gas chromatography-mass spectrometry(GC-MS)[7,8], HPLC-MS[9], GC-tandem mass spectrometry[10], micellar electrokinetic capillary chromatography[11] and voltametry[12]. The fast dissolving tablet for lorazepam was made by direct compression technique, using synthetic superdisintegrants[13].

MATERIALS AND METHODS
Instrument:
The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS Ultrasphere 5 µm or equivalent ODS C-18 column with 4.6 mm x 15cm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.
Reagents and Materials:
Methanol of HPLC Grade was purchased from E-Merck, Mumbai, India. Lc grader water was obtained by double distillation and purification through Milli-Q water purification system.
The Mobile Phase:
A mixture of Methanol : Water in the ratio of 65 : 35 *v/v* was prepared and used as mobile phase.
Blank Preparation
Place unused swab in 10 ml of solvent. Sonicate for 5 minutes. Squeeze swab out well.
Filter through a 0.45 µm filter.
Preparation of Standard solution (Stock Solution)
A stock solution of lorazepam was prepared by Accurately weighing 50 mg of Lorazepam transferring to a 100 ml

volumetric flask, dissolving in 60 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. Appropriate aliquot of this solution was further diluted with solvent to 100 ml with solvent. Dilute 5 ml of this solution to 50 ml with solvent. Filter through a 0.45 µm filter. From (0.05 mg/ml) stock solution below, a series of standard solutions were prepared. Seven solutions containing 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.0015625 and 0.0007812 mg/swab of Lorazepam, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed,

Method development

For developing the method a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant.

Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose.

Detection of wavelength:

The spectrum of 10 ppm solution of lorazepam in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed.

Choice of stationary phase and mobile phase

Finally the expected separation and peak shapes were obtained on Chromosil C18 (250 mm ´ 4.6 mm, 5 μm) column.

Flow rate

Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 1.5 ml/min flow rate was ideal for elution of analyte.

Optimization of chromatographic conditions

Chromatographic conditions are required to be optimized. These optimized conditions were followed for the determination of Lorazepam in bulk samples and in its formulations.

Chromatographic conditions:

The mobile phase consisting of Methanol: water was filtered through 0.45 µm filter, degassed and were pumped from the solvent reservoir in the ratio of 65:35, V/V and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/minute and detection wave length was set at 230nm with a run time of 5.1min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Calibration Curve:

Appropriate aliquots of standard lorazepam stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.0015625 and 0.0007812 of lorazepam. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of lorazepam was constructed by plotting peak area ratio versus applied concentration of lorazepam and regression equation was computed. Results are shown in then **Figure: 2**. Similarly the sample solution was chromatographed and concentration of lorazepam in tablet sample was found out using regression equation. 50% MAC is equal to 0.0035 mg/swab and the method gives linear response from 0.0007812 – 0.01 mg/swab therefore the method can detect the above concentration of API 0.0035 mg/swab (50% MAC) required by the method. Results are tabulated in **Table: 1**

Validation of Proposed Method:

The proposed method was validated as per ICH guidelines (Hokanson, 1994). The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification and solution stability.

Specificity

The specificity of method was performed by comparing chromatograms of blank, standard and sample (prepared from formulation).

Linearity

Linearity was performed by preparing mixed standard solutions of lorazepam at different concentration levels including working concentration mentioned in experimental condition *i.e.* 12 ppm. The response was read and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.

Precision:

The precision will entail repeated testing of six samples prepared in the following manner. Six replicate injections of API MAC working standard solutions were injected according to the method of analysis. The percentages Recovery for the peak responses were determined. Accurately weigh 200 mg of Lorazepam reference standard into a 50 ml volumetric flask.

Add 20 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. (Solution 1 to be used for sample preparation). Dilute 10 µl to 10 ml with solvent. Filter through a 0.45 µm filter. Place 10 µl of solution 1 onto a specific surface area of stainless steel plate. Swab the surface area; take the swab stick and place into a 10 ml volumetric flask. Add 10ml of solvent and sonicate for 10 minutes. Filter through a 0.45 µm filter.. The % recovery should be greater than or equal to 65%. Results are shown in **Table:2**.

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 80%, 100% and 150%. The low values obtained for the SD (0.90) show the accuracy and reproducibility of the method. The high recoveries from solutions of lorazepam tablets (102.86-100.76) were indicative of the suitability of the method for the determination of lorazepam in tablets. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated. Results are shown in **Table:3**.

System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard

deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to Lorazepam for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability. Results are tabulated in **Table: 4**.

Limits of Detection and Limit of Quantification

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates that the analyte concentration is above or below a certain level. The Limits of Detection (LOD) and Quantification (LOQ) for lorazepam were determined experimentally by applying described method to dilute solutions. The results are shown in the **Table 5**.

Formulation

The proposed method was applied to the assay of commercial tablets containing Lorazepam. Sample was analyzed for five times after extracting the drug as mentioned in sample preparation for assay as described in the experimental section.

Figure:2 Chromatogram of Standard Drug

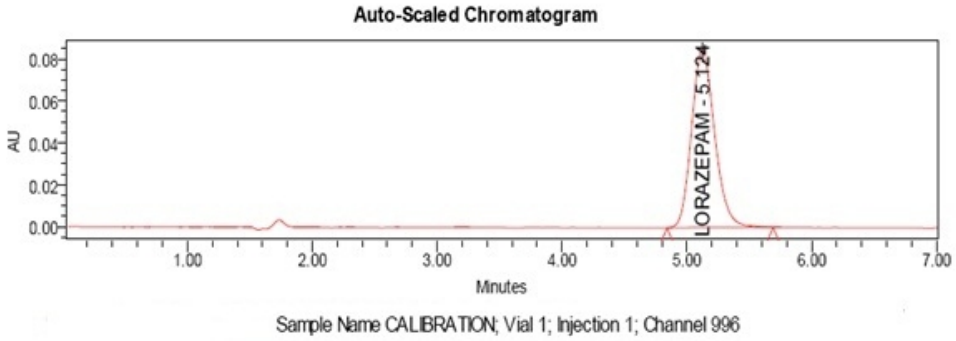

Figure:3 Linearity Studies

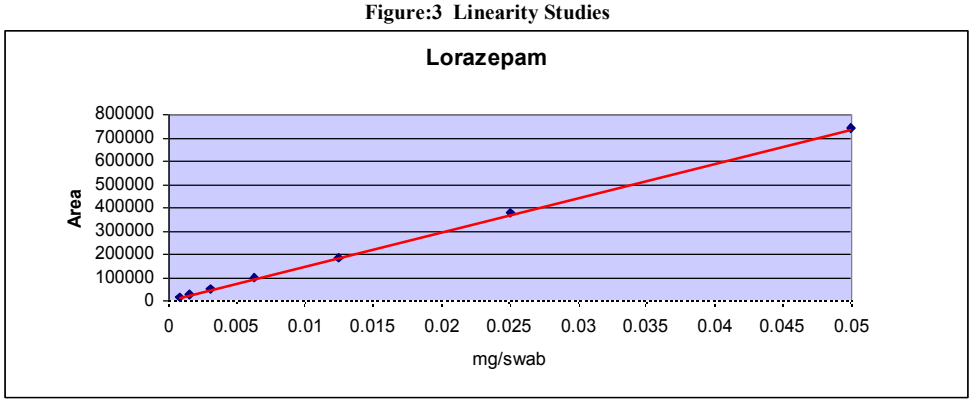

Table:1 Results of Linear Response

Conc (mg/swab)	Area 1	Area 2	Average
0.05	739551	737007	738279
0.025	373766	375607	374687
0.0125	189365	178148	183757
0.00625	95758	95453	95606
0.003125	46969	46814	46892
0.0015625	24829	24150	24490
0.0007812	10817	10654	10736

Table:2 Precision Studies

Sample	% Recovery
1	93
2	97
3	84
4	91
5	92
6	87
Mean	91

Table: 3.Percent Recovery

Level	% Recovery
80%	102.86
	102.76
	103.51
100%	102.79
	101.85
	100.80
150%	100.76
	101.16
	101.58
Mean	102.00

Table: 4 System Suitability results

Sample	Lorazepam Area
1	757397
2	748222
3	746061
4	750917
5	749914
6	744731
Mean	749540
% RSD	0.6

Table: 5 LOD and LOQ

Compound	LOD (µg/ml-1)	LOQ (µg/ml-1)
Lorazepam	35	55
Lorazepam – B	0.032	0.048

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Methanol : Water in the ratio of 65:35 v/v and 1.0 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 230nm at which much better detector responses for drug was obtained. As it was shown in **Fig. 2**. The retention times were 5.1 min for Lorazepam. The number of theoretical plates was found to be 6528.10, which indicates efficient performance of the column. A system suitability test was applied to representative chromatograms for various parameters. Thus, the system meets suitable criteria. The calibration curve was obtained and it was found to be linear. Seven point's graph was constructed**.** The standard deviation of the slope and intercept were low. Calibration curve **(Fig.3)** found to be linear with 0.0007812 - 0.1mg/swab. The results obtained were within acceptable limits where capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Low values of standard deviation denoted very good repeatability of the measurement. The Mean % recovery was found to be 91%. This indicates good method precision. Standard addition method at 80%, 100% and 150% to the proposed HPLC method is carried out to find the Accuracy of the Lorazepam. The results showed good recoveries ranging from 102.86-100.76%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an R.S.D < 2.0%, which satisfied the acceptance criteria set for the study.

CONCLUSION

The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Lorazepam in tablet formulation. All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Lorazepam in bulk and in pharmaceutical formulations without interference and with good sensitivity

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