AMERICAN JOURNAL OF PHARMTECH RESEARCH

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New Validated HPLC Method for the Estimation of Oxazepam In Pharmaceutical Formulation

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ABSTRACT

A simple, selective, linear, precise and accurate HPLC method was developed and validated for rapid assay of Oxazepam in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed. The chromatographic analysis was performed on a ODS 5 µm (4.6 mm x 15 cm) or equivalent column at ambient temperature. The mobile phase consisted of Methanol: Water in the ratio of 65:35v/v. The UV detection wavelength was 230nm and 100µl sample was injected. Flow rate was found to be 1.0. The retention time for Oxazepam was identified. The Average percentage recovery of the method was in the range of 0.5. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

Keywords: Oxazepam, HPLC, UV detection, Recovery, Precise.

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Received 19 January 2017, Accepted 02 February 2017

Please cite this article as: Sreeram *et al*., New Validated HPLC Method for the Estimation of Oxazepam In Pharmaceutical Formulation. American Journal of PharmTech Research 2017.

INTRODUCTION

Oxazepam[1] is a short-to-intermediate-acting 3-hydroxy benzodiazepine derivative.[23] Oxazepam is used extensively since the 1960s for the treatment of anxiety and insomnia and in the control of symptoms of alcohol withdrawal. It is a metabolite of diazepam, prazepam, and temazepam,[4] and has moderate amnesic, anxiolytic, anticonvulsant, hypnotic, sedative, and skeletal muscle relaxant properties compared to other benzodiazepines.[5] It is an intermediate-acting benzodiazepine with a slow onset of action,[6]. Physicians may use oxazepam outside its approved indications to treat social phobia, post-traumatic stress disorder, insomnia, premenstrual syndrome, and other conditions.[7] Oxazepam, along with diazepam, nitrazepam, and temazepam, were the four benzodiazepines listed on the pharmaceutical benefits scheme and represented 82% of the benzodiazepine prescriptions in Australia in 1990-1991.[8] Side effects due to rapid decrease in dose or abrupt withdrawal from oxazepam may include abdominal and muscle cramps, convulsions, depression, inability to fall asleep or stay asleep, sweating, tremors, or vomiting.[9] Benzodiazepines require special precautions if used in the elderly, during pregnancy, in children, alcohol- or drug-dependent individuals, and individuals with comorbid psychiatric disorders.[10] Benzodiazepines including oxazepam are lipophilic drugs and rapidly penetrate membranes, so rapidly cross over into the placenta with significant uptake of the drug. Use of benzodiazepines in late pregnancy, especially high doses, may result in floppy infant syndrome.[11]

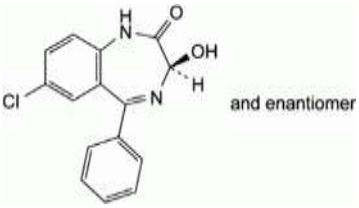

Figure : 1 Structure of Oxazepam

Ashish Ravindra Deshpande et.al.,[12] *described* HPLC method was validated which shows good separation for Oxazepam, its impurities and degradation products The drug substance, its impurities and degradation products were found well separated with gradient conditions having run time of 60 mins by using Zorbax Extended C-18 column from Agilent (250 x 4.6 mm, 5µ). The flow rate was kept 1.0 $mL.min^{-1}$. The gradient mobile phase consisted of A= 0.02M di-potassium hydrogen phosphate pH 10.5 and B= Acetonitrile (100%). Detection was performed at 235 nm using PDA detector. The method was validated for Specificity, LOD, LOQ, Linearity, Precision and Accuracy as per ICH guidelines [1,2] The stability indicating capability of the method was established by performing forced degradation study. The method was found to be reliable for its

intended purpose. V.D.N. kumar Abbaraju et.al.,[13] proposed a simple, selective, linear, precise and accurate HPLC method was developed and validated for rapid assay of Amoxycillin Trihydrate in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 0.3ml/min was employed. The chromatographic analysis was performed on a C18 5 µm (4.6 mm x 15 cm) or equivalent column at ambient temperature. The mobile phase consisted of Acetonitrile: phosphate buffer in the ratio of 5:95v/v. The UV detection wavelength was 230nm and 100µl sample was injected. Flow rate was found to be 1.0. The retention time for Amoxycillin Trihydrate was identified. The Average percentage recovery of the method was in the range of 0.5. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

MATERIALS AND 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:

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a ODS 5 µm (4.6 mm x 15 cm) or equivalent. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

Chemicals and Reagents

The reference sample of Oxazepam was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Methanol and water used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase

A mixture of Methanol and water buffer in the ratio of 65:35v/v was prepared and used as mobile phase.

Preparation of solutions

Preparation of Standard solution:

Accurately weigh 20 mg of Oxazepam reference standard into a 100 ml volumetric flask.

Add 60 ml of solvent and ultrasonicate for 15 minutes. Cool and make up to volume with solvent. Dilute 10 ml of this solution to 50 ml with solvent. Further dilute 10 ml of this solution to 50 ml with solvent. Filter sample through a 0.45 µm filter.

Blank Preparation

Place unused swab in 10 ml of Solvent. Sonicate for 5 minutes. Squeeze, swab out well. Filter sample through a 0.45 µm filter.

Sample solution:

Place swab in 10 ml of Solvent (volume accurately determined) Sonicate for 5 minutes. Squeeze swab out well. Filter sample through a 0.45 µm filter. Inject the Blank, standard and sample preparation according to test the system suitability.

Detection of wavelength

The spectrum of 10 ppm solution of Oxazepam was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 230 nm was observed.

Choice of stationary phase and mobile phase

Finally the expected separation and peak shapes were obtained on ODS 5 µm (4.6 mm x 15 cm) or equivalent.

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 1.0 ml/min flow rate was ideal for elution of analyte.

Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte. in the presence of components that may be expected to be present. The results must show that the solvent solution (solution 1) and placebo solution (solution 2) must not contain any components which co-elute with the active compound peak (solution 3). Each of the degradation products and impurities must be well resolved from the active compound peak (at least baseline resolution > 1.5) and must elute within the specified assay run time. Determine the peak purity for each of the active peaks in at least the solutions 3 and 4 above. The purity angle must be **less than** the threshold angle. The solutions listed below were injected using the conditions specified in the method of analysis. No components are seen to co-elute with Oxazepam peak, and the peak Purity results indicate that Oxazepam peak can therefore be considered spectrally pure. The method employed is specific for the API Oxazepam in the product.

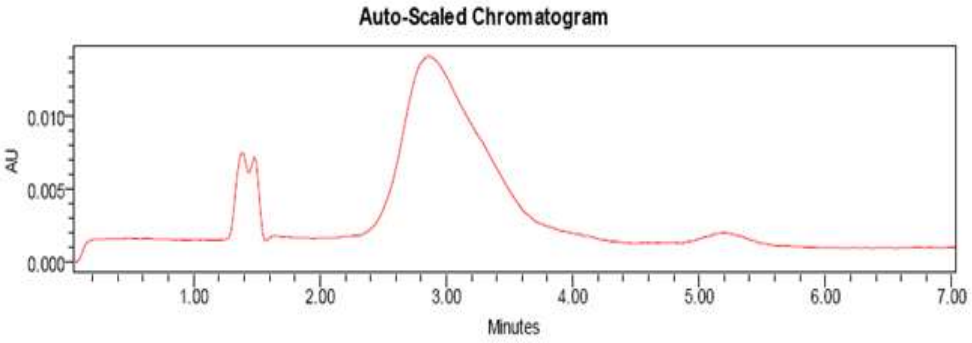

Figure: 2 Chromatogram 1: Solvent – no significant peaks detected

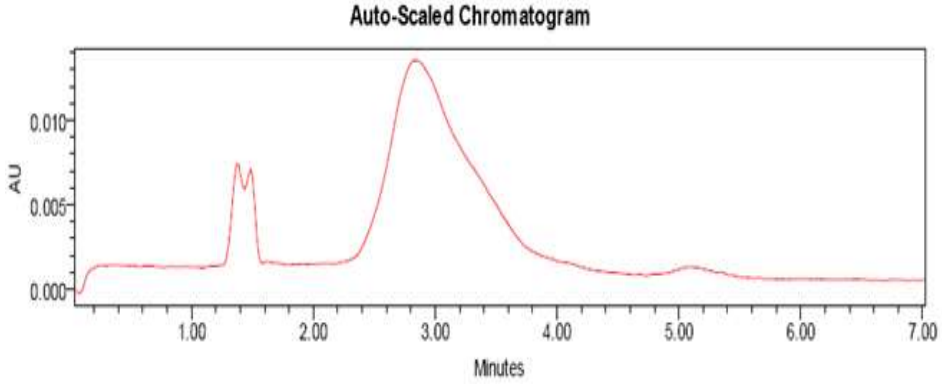

Figure: 3 Chromatogram 2: Placebo – no significant peaks detected

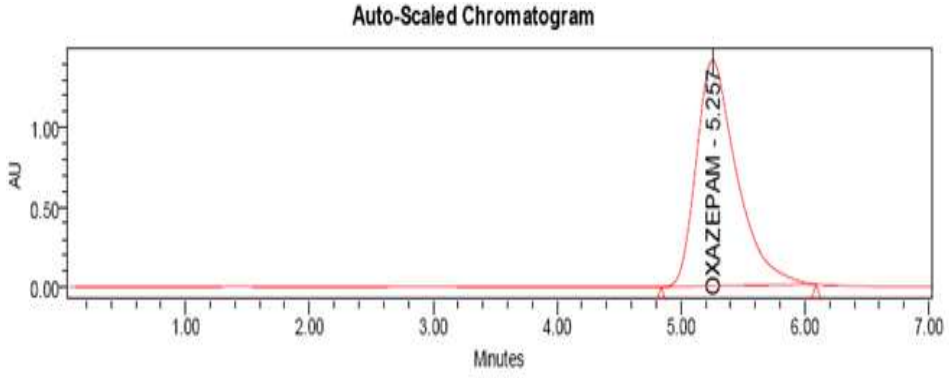

Figure: 4 Chromatogram 3: API – peak due to Oxazepam eluted at about 5 mins

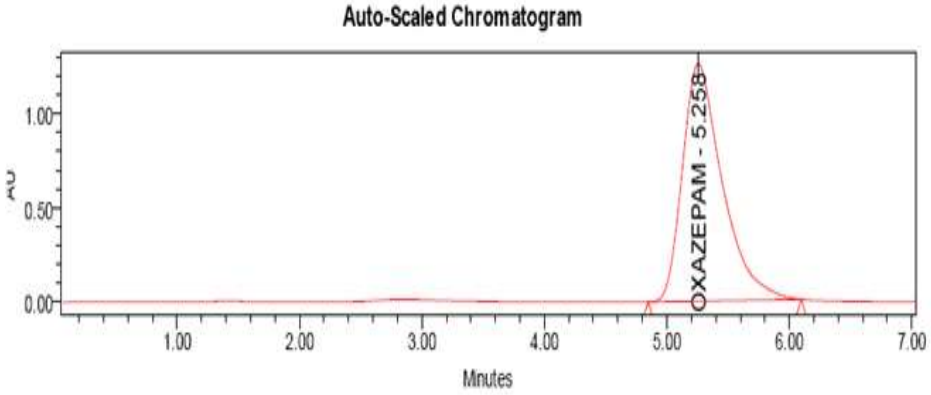

Figure: 5 Chromatogram 4: Product - peak due to Oxazepam eluted at about 5 min

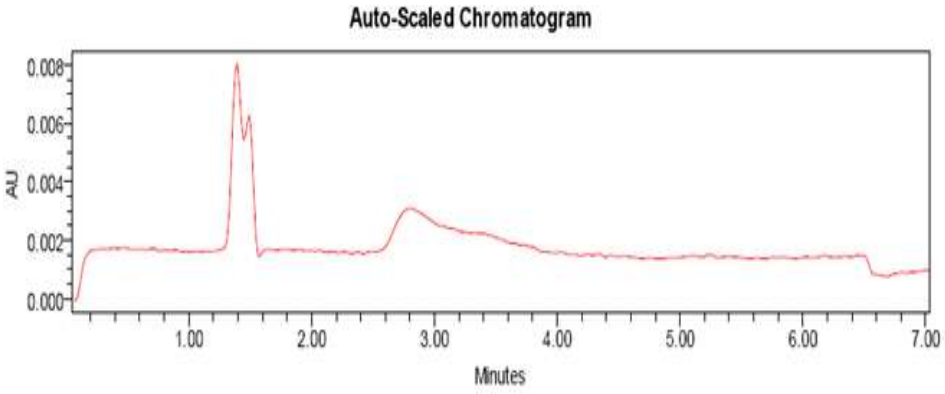

Figure: 6 Chromatogram 5: Detergent - no significant peaks detected

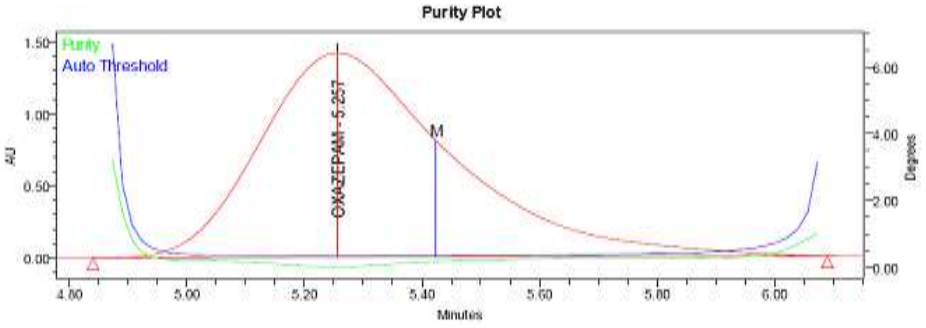

Figure: 7 Peak Purity 1: Purity Angle <Threshold: 0.114 < 0.353

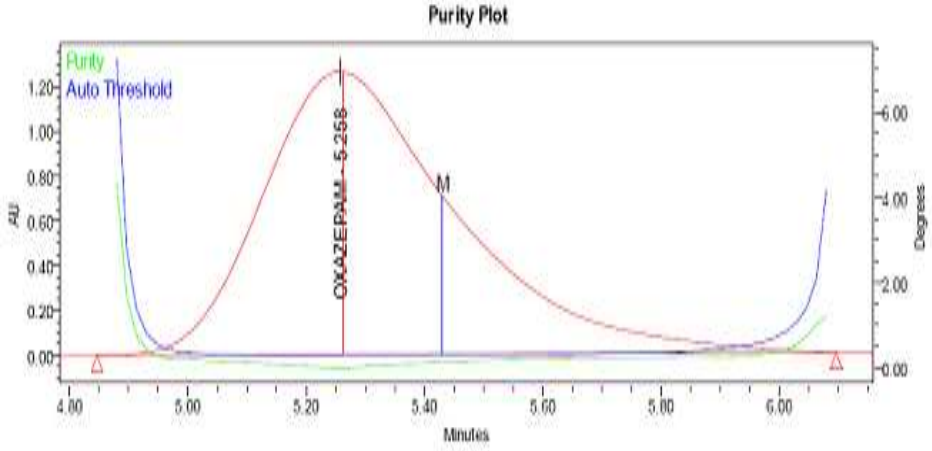

Figure : 8 Peak Purity 2: Purity Angle <Threshold: 0.094 < 0.305

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 Oxazepam for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability.

Table 1: Results for System Suitability

Sample	Oxazepam Area
1	30441241
2	30642188
3	30681207
4	30803539
5	30905469
6	30758664
Mean	30705385
% RSD	0.5

Detection Limit

The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected. The maximum allowance carryover of Oxazepam is 0.082 mg as determined in the Cleaning Validation Matrix. The range of standard solutions above was also injected twice and the average result was used in treatment of results. Ten solutions containing 0.2, 0.1, 0.02, 0.01, 0.002, 0.001, 0.0002, 0.0001, 0.00002, and 0.00001 mg/swab of Oxazepam, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, The detection limit must be capable of detecting the API at 50% MAC. 50% MAC is equal to 0.04 mg/swab and the method gives linear response from 0.00001 – 0.2 mg/swab therefore the method can detect the above concentration of API 0.04 mg/swab (50% MAC) required by the method.

Preparation of Standard solution (Working Standard)

Accurately weigh 20 mg of Oxazepam reference standard and quantitatively transfer to a 100 ml volumetric flask (0.2 mg/ml). Add 60 ml of solvent and sonicate for 15 minutes. Cool and make up to volume with solvent. Filter through a 0.45 µm filter before use, discarding the first few mls of filtrate. From (0.2 mg/ml) stock solution below, a series of standard solutions were prepared as follows 0.1, 0.02, 0.01, 0.002, 0.001, 0.0002, 0.0001, 0.00002, 0.00001 mg/ swab respectively.

Table 2: Results Average Area

Conc. (mg/swab)	Area I	Area II	Average Area
0.2	30780141	30925809	30852975
0.1	15251586	15329360	15290473
0.02	3172295	3143466	3157881
0.01	1601050	1605440	1603245
0.002	321404	321453	321429
0.001	164499	165371	164935
0.0002	35565	34655	35110
0.0001	20816	20086	20451

0.00002	5760	6390	6075
0.00001	2922	4007	3465

Treatment of results

The graph below is plotted from the Result above:

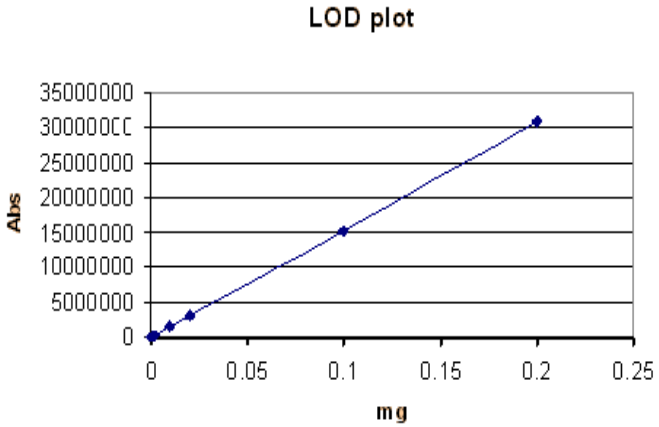

Figure: 9 Plot area

Method Precision

The percentage of a test procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision % recovery of a known amount of API in the sample after swabbing. An amount of material (predetermined limit) is placed on a specific surface area (stainless steel) and swabbed as outlined in SOP Cleaning Validation Cleaning using the specified solvent and specified material. The precision of the analytical method is determined by assaying the swabs and calculating the % Recovery of the API results. The precision will entail repeated testing of six samples prepared in the following manner. Six replicate injections of API MAC 10 % working standard solution were injected according to the method of analysis. The percentages Recovery for the peak responses were determined.

Standard solution

Accurately weigh 200 mg of Oxazepam reference standard into a 50 ml volumetric flask. Add 30 ml of solvent and sonicate for 15 minutes. Cool and make up to volume with solvent. (Solution 1 to be use for sample preparation) Dilute 10 µl to 5 ml with solvent. Filter sample through a 0.45 µm filter.

Sample Preparation

Place 10 µl of solution 1 on to specific surface area of stainless steel plate Swab the surface area; take the swab stick and place into 10 ml volumetric flask, Add 10ml of Solvent and Sonicate for 10

minutes. Filter sample through a 0.45 µm filter. The precision will entail repeated testing of six samples prepared in the following manner: The %recovery should be greater than or equal to 65%. The analytical system complies with the requirements specified by the method precision

Table: 3 Method precision results

Sample	% Recovery
1	67
2	80
3	68
4	76
5	66
6	73
Mean	72

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 65:35v/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 230 nm at which much better detector responses for drug was obtained. The regressions of the plots were computed by least square regression method. Linearity results are presented in Figure 9. The calibration curve was obtained for a series of concentrations and it was found to be linear. The results obtained were within acceptable limits where capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. The retention time 2.7 min for Oxazepam is identified. The number of theoretical plates was found to be 6324.16. It was found that there was no interference due to excipients in the tablet formulation and also showed good correlation between the retention times of standard and sample. The maximum allowance carryover of Oxazepam is 0.082 mg as determined in the Cleaning Validation Matrix The R.S.D. for intraday precision studies and that of interday precision were well within the acceptable criteria of not more than 2.0. Standard addition method at 50%, 100% and 150% to the proposed HPLC method was carried. 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:

A RP-HPLC method has been developed and validated for the determination of Oxazepam in tablet dosage form. This method is simple, rapid, accurate, precise, and specific. Its

chromatographic runtime of 10 mins allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Oxazepam in pharmaceutical dosage forms.

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