ABSTRACT
The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Amoxapine using mobile phase [mixture of Phosphate buffer pH-3.6 and Acetonitrile in the ratio of 35:65] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 298.0 nm was selected as the analytical wavelength. The retention time of Amoxapine was found to be 3.668. The linearity of the proposed method was investigated in the range of 20-100 µg/ml (r = 0.999). The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

KEYWORDS: HPLC, Amoxapine.

INTRODUCTION
Amoxapine[^1] is chemically 2-chloro-11-(piperazin-1-yl)dibenzo[b,f][1,4]oxazepine. It is freely soluble in ethanol. The molecular formula is C_{17}H_{16}ClN_{3}O and Molecular weight is 313.781 g/mol. Amoxapine is used in the treatment of major depressive disorder. Compared to other antidepressants it is believed to have a faster onset of action, with therapeutic effects seen within four to seven days.[^2]
The therapeutic importance of this compound justifies that, necessary to establish analytical methods for its determination in bulk and pharmaceutical formulation. Literature survey reveals that no analytical methods have been reported for the quantitative estimation of amoxapine. In the present work attempt has been made to develop a simple, accurate, sensitive, rapid and economic a RP-HPLC method for the quantitative estimation of amoxapine in bulk and pharmaceutical formulation.

As chromatographic methods of analysis is a pre-requisit for the marketing of most of formulations.

**Experimental**

**2.1 Chemicals and Reagents**

The working standard of Amoxapine was gifted from Watson Pharma Pvt Ltd (Goa). The tablet formulation of Amoxapine (Label claim: Amoxapine 50mg) was purchased from the local market. Distilled water was obtained from local market for analytical work and rinsing purpose.

**2.2 Instrument Used**

A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector; Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 μl injection of sample loop (manual). The output signal was monitored and integrated using software class LAB SOLUTIONS (Shimadzu).

**2.3 Preparation of Mobile Phase**

The HPLC grade Acetonitrile was filtered through 0.4 μm membrane filter paper. Buffer (0.585 gm. of anhydrous disodium hydrogen phosphate and 0.843gm. citric acid monohydrate in 650 ml distilled water) was filtered through 0.4 μm membrane filter paper. Mobile phase was prepared by mixing 350 ml of buffer with 650 ml of Acetonitrile and sonicated for 15 min.

**2.4 Preparation of Standard Stock Solution**

100 mg of standard Amoxapine was weighed accurately and transferred to 100 ml volumetric flask. Both the drugs were dissolved in 50 ml of mobile phase with sonication for 15 min and then volume was made up to the mark with mobile phase (solution–A). Further the stock
solutions were diluted to get 100 μg/ml final concentration of standard stock solution of drug (solution–B). This stock solution was filtered through 0.4 μ membrane filter paper.

2.5 Preparation of Calibration Curves
Appropriate dilutions were prepared separately and 20 μl of each was injected into the HPLC system and the chromatograms were recorded under the same chromatographic conditions as described below. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

2.6 Chromatographic Condition
The mobile phase containing both Buffer and aceto nitrile in the ratio of 35:65 was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1.0 ml/min and UV detection was carried out at 298.0 nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 μm membrane filter paper. All determinations were performed at constant column temperature (25°C).

2.7 Selection of Analytical Concentration Range
Appropriate aliquots were pipetted out from the standard stock solution (solution B- 100 μg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20-100 μg/ml of Amoxapine. Triplicate dilutions of each of the above mentioned concentrations was prepared separately and from these triplicate solutions, 20 μl of each concentration of the drug were injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

2.8 Analysis of Tablet Formulation
Twenty tablets each containing 50mg of Amoxapine weighed accurately and powdered. A quantity equivalent to 100 mg of Amoxapine was weighed accurately and transferred to 100 ml volumetric flask containing approximately 50 ml of mobile phase. The contents were sonicated for 15 min and volume was made up to the mark with the mobile phase. The resulting solution was filtered through a membrane filter. The solution obtained was then diluted with the mobile phase so as to obtain a concentration of 1000 μg/ml. Sample solution
was injected under the same chromatographic conditions and the chromatogram was recorded
in triplicate. The amount of Amoxapine present in tablet formulation was determined by
comparing the peak area from the standard. The results are furnished in Table 2.

2.9 Method Validation \[^{[3-5]}\]

The developed analytical method was subjected to validation with respect to various
parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD),
accuracy, precision, recovery studies and reproducibility as per the ICH guidelines.

**Linearity**

The standard curve was obtained in the concentration range of 20-100 μg/mL. The linearity
was evaluated by linear regression analysis using the least square method. It was found that
correlation coefficient and regression analysis are within the limits.

**Precision**

The precision was assessed in terms of intra-day and inter-day variation. The intra-day and
inter-day variation in the peak area of drug solution was calculated in terms of coefficient of
variation (C.V.). The results are furnished in Table 4.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ for Amoxapine were predicted basing on the parameters of standard error
of estimate and slope, calculated from linearity of the response data of Amoxapine.

**Robustness**

The robustness was checked by changing the flow rate to 0.9 and 1.1 ml/min.

**Accuracy**

The accuracy of the HPLC method was assessed by adding known amount of standard drug
solution to a pre-analyzed tablet formulation. The recovery studies were carried out in
triplicate. The accuracy was expressed in terms of recovery at three levels 80%, 100% and
120%. The results are furnished in Table 5.

**RESULTS AND DISCUSSION**

Optimization of the chromatographic conditions were carried out with various combinations
of buffer and acetonitrile and by observing the peak parameters, the run time of the method
was set at 10 min, Amoxapine appeared on the typical chromatogram at 3.668 min, which
indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 20-100 µg/ml. The regression equation of Amoxapine concentration over its peak area ratio was found to be \( Y = 51496x - 4761 \) (\( r=0.999 \)) where \( Y \) is the peak area ratio and \( x \) is the concentration of Amoxapine (Fig. 2). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 2%. The tailing factor was found to be 1.495, which indicates good shape of peak. The number of theoretical plates was found to be 7905, which indicates efficient performance of the column.

The limit of detection and limit of quantitation was found to be 5.2323 µg/ml and 1.5855µg/ml which indicates the sensitivity of the method. The use of buffer and acetonitrille in the ratio of 35:65 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Amoxapine ranging from 99.96-99.98 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

Table 1. Calibration data of the method.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>1021353</td>
</tr>
<tr>
<td>40</td>
<td>2042706</td>
</tr>
<tr>
<td>60</td>
<td>3064059</td>
</tr>
<tr>
<td>80</td>
<td>4185410</td>
</tr>
<tr>
<td>100</td>
<td>5106765</td>
</tr>
</tbody>
</table>

Table 2. Assay of Amoxapine.

<table>
<thead>
<tr>
<th>Components</th>
<th>Mean*</th>
<th>Standard deviation*</th>
<th>Co-efficient of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMOXAPINE</td>
<td>99.96</td>
<td>0.017885</td>
<td>0.017887</td>
<td>0.0073</td>
</tr>
</tbody>
</table>

*\( n = 6 \)
Table 3. System suitability parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>20-100 (µg/ml)</td>
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<tr>
<td>Slope</td>
<td>51496</td>
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<tr>
<td>Intercept</td>
<td>4761</td>
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<tr>
<td>Regression coefficient ($r^2$)</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>5.2323</td>
</tr>
<tr>
<td>Limit of Quantification(µg/ml)</td>
<td>1.5855</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>3.668</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.495</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>7905</td>
</tr>
</tbody>
</table>

Table 4. Precision of the proposed HPLC method.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Mean Intra day</th>
<th>Mean Inter day</th>
<th>Standard deviation Intra day</th>
<th>Standard deviation Inter day</th>
<th>Co-efficient of variation Intra day</th>
<th>Co-efficient of variation Inter day</th>
<th>Standard error Intra day</th>
<th>Standard error Inter day</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>99.93</td>
<td>99.94</td>
<td>0.0352</td>
<td>0.03376</td>
<td>0.0043</td>
<td>0.01378</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>99.95</td>
<td>99.97</td>
<td>0.0173</td>
<td>0.02049</td>
<td>0.0070</td>
<td>0.00836</td>
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</tr>
<tr>
<td>80</td>
<td>99.95</td>
<td>99.96</td>
<td>0.0194</td>
<td>0.02236</td>
<td>0.0079</td>
<td>0.00913</td>
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</tr>
</tbody>
</table>

*n = 6

Fig. 1 Chromatogram showing retention times of Amoxapine respectively.

Table 5. Recovery studies of the proposed HPLC method.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Level of % Recovery</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Co-efficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>99.96</td>
<td>0.0173</td>
<td>0.0173</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>99.98</td>
<td>0.01</td>
<td>0.0100</td>
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<tr>
<td>3</td>
<td>120</td>
<td>99.96</td>
<td>0.01</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

*n = 3
Fig. 2. Calibration curve of Amoxapine at 298 nm in Acetonitrile and Buffer by RP-HPLC Method.

CONCLUSION

The proposed HPLC method was found to be simple, sensitive, accurate and precise for determination of Amoxapine. The method utilizes easily available and cheap solvent for analysis of Amoxapine hence the method was also economic for estimation of Amoxapine.

ACKNOWLEDGEMENT

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REFERENCES