A stability indicating RP-HPLC method for the determination of amitriptyline hydrochloride in pure and dosage forms


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ABSTRACT

A simple isocratic RP-HPLC method was developed for the determination of amitriptyline HCl in pure and dosage forms. A Waters HPLC equipped with Alliance 2695 separation module and 2487 dual wavelength UV-Visible detector was used in the present investigation. A mixture of potassium dihydrogen orthophosphate buffer of pH 3.0±0.05 and acetonitrile in the ratio 35:65 was used as mobile phase with a flow rate and injection volume were 0.8ml/min. and 20µl respectively. The data were acquired at 239nm. The proposed method was linear in the range of concentration 20-60µg/ml and the correlation coefficient is found to be 0.9990. The mean recovery of the substance was found to be 100.2%. The values of LOD and LOQ for amitriptyline HCl were found to be 0.015 and 0.052µg/ml respectively. The method has higher sensitivity towards the determination of the amitriptyline HCl. The developed method is found to be accurate and precise as indicated by recovery studies and % RSD not more than 2.0. The proposed method can be used as an alternative method for routine analysis in quality control.

Keywords: RP-HPLC, Amitriptyline HCl, Linearity, LOD, LOD, Correlation coefficient

INTRODUCTION

Amitriptyline HCl (ATL) is used to treat depression, mainly melancholic, endogenous, or when anxiety or insomnia coexists. It is the most widely used tricyclic antidepressant (TCA) and has at least equal efficacy against depression as the newer class of SSRIs according to a study from early 2001. Amitriptyline is used for a number of medical conditions including: depressive disorders, anxiety disorders, attention deficit hyperactivity disorder, migraine prophylaxis, eating disorders, bipolar disorder, post-herpetic neuralgia, and insomnia. It is also used as a preventive for patients with recurring biliary dyskinesia, and in the treatment of nocturnal enuresis in children. ATL may be prescribed for other conditions such as cyclic vomiting syndrome post-traumatic stress disorder (PTSD), chronic pain, tinnitus, chronic cough, carpal tunnel syndrome (CTS), fibromyalgia, vulvodynia, interstitial cystitis, male chronic pelvic pain syndrome, irritable bowel syndrome (IBS), diabetic peripheral neuropathy, neuropathic pain, laryngeal sensory neuropathy, chronic fatigue syndrome and painful paresthesias related to multiple sclerosis. Typically lower dosages are required for pain modification of 10 to 50 mg daily. A randomized controlled trial published in June 2005 found that amitriptyline was effective in functional dyspepsia that did not respond to a first-line treatment (famotidine or mosapride).

The chemical structure of the drug is 1-Propanamine,3-(10,11-dihydro-5,6-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-hydrochloride with molecular formula and molecular weight C20H21N.Cl and 313.86g/mole respectively. It is available in different brand names such as Amitrol, Elavil, Endep, Levate, Tryptizol, Vanatrip. Amitriptyline Hydrochloride contains not less than 99.0 percent and not more than 100.5 percent of C20H21N.HCl, calculated on the dried basis. The chemical structure of the drug is given in Fig.1. Several techniques have been adopted for the determination of amitriptyline, including spectrophotometry, high-performance liquid chromatography, gas chromatography, capillary electrophoresis and potentiometric method using ionselective electrodes fluorescence polarization immunoassay method, UV spectrophotometric method, combination with other drugs and UV method.

MATERIALS AND METHODS

Experimental

A Waters HPLC equipped with Alliance 2695 separation module and 2487 dual wavelength UV-Visible detector was used for the separation and quantification. An analytical column; Symmetry C18 (4.6 mm ID x 150mm, 3.5 µm, Make: XBridge) was used in the analysis. Chromatographic software Empower-2 was used for data collection and processing. Elico-SL159 model, 2nm high resolution, double beam, 1cm length quartz coated optics and wavelength range190-1100nm is used for measuring absorption spectrum.

Chemicals and Reagents

Amitriptyline and methanol of HPLC grade procured from Merck (India) were used. Potassium dihydrogen orthophosphate and orthophosphoric acid were all of AR grade (Merck, India) HPLC grade water obtained from Millipore system was used throughout the analysis. The investigated sample, amitriptyline HCl was obtained as a gift sample from Dr. Reddy’s Laboratory, Hyderabad, India.

About 7.0 grams of potassium dihydrogen phosphate is transferred into a beaker dissolved in 1000ml with HPLC water, sonicated, filtered through 0.45µm filter and the pH of the solution was adjusted to 3.0±0.05 by adding a few drops of ortho phosphoric acid. The mobile phase was prepared by mixing about 350ml buffer and 650 ml of HPLC grade acetonitrile. The mixer was degassed in ultrasonic water bath for 5 minutes, filtered through 0.45µm filter.

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Fig.1 The chemical structure of Amitriptyline HCl

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Preparation of standard and sample

About 10.0mg of 99.80 percent pure amitriptyline HCl was accurately weighed transferred into a 10ml volumetric flask, 7.0ml of diluent was added, sonicated to dissolve and filtered through 0.45µm filter. Further 0.4ml of the above solution was pipette out into 10ml volumetric flask and diluted to the mark with diluent, sonicated and filtered. The average weight of five amitriptyline HCl tablets was calculated, made them as homogeneous powder, an amount of the powder equivalent to 10 mg of amitriptyline HCl was weighed, transferred into a 10ml volumetric flask, dissolved in 7ml of diluent, sonicated to dissolve, made up to the mark, mixed well and filtered through 0.45µm filter. Further 0.4ml of the sample solution was pipette out into 10ml volumetric flask and diluted to the mark with diluent, sonicated and filtered.

Operating conditions

The analysis was carried out under the isocratic conditions. The data were acquired at 239nm for 50min. and processed by use of Empower software data handling system. A mixture of buffer and acetonitrile in the ratio 35:65 (v/v) was used as diluents in the preparation of analytical solutions. Amitriptyline HCl tablets was calculated, made them as homogeneous powder, and the powder equivalent to 10 mg of amitriptyline HCl was weighed, transferred into a 10ml volumetric flask, dissolved in 7ml of diluent, sonicated to dissolve, made up to the mark, mixed well and filtered through 0.45µm filter. Further 0.4ml of the sample solution was pipette out into 10ml volumetric flask and diluted to the mark with diluent, sonicated and filtered.

Linearity

The linearity plot of peak areas versus concentration was drawn for amitriptyline HCl and is presented in Fig.5. The linear regression data for the drug tested is given in Table 2. The data shown in Table 2 is confirmed that the detector response at 239 nm was linear over the range studied for the drug.

The limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined for amitriptyline HCl from the standard deviation of the peak area and slope of the linearity data. The values of LOD and LOQ for amitriptyline HCl were found to be 0.015 and 0.052µg/ml respectively. The results were depicted in Table 2.

Precision and Accuracy

System precision was verified using diluted solution standard solution, which was analysed for five times and R.S.D. of amitriptyline HCl peak areas was evaluated and found to be 0.404%. Precision of the method was studied for repeatability and intermediate precision. Repeatability was determined by analyzing five separate Amitriptyline HCl sample solutions the %R.S.D was found to be 0.404% and given in Table 2. The intermediate precision of the method was determined on five separate sample solutions prepared from same lot by spiking by different days. The %R.S.D was evaluated and found to be 0.410% which was within the acceptance criterion of NMT 10% R.S.D. The results are presented in Table-5. Accuracy of the method was
Table 5: Intermediate precision

<table>
<thead>
<tr>
<th>Injection</th>
<th>Concentration</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>40µg/ml</td>
<td>3002113</td>
</tr>
<tr>
<td>Injection-2</td>
<td>40µg/ml</td>
<td>2996759</td>
</tr>
<tr>
<td>Injection-3</td>
<td>40µg/ml</td>
<td>3003927</td>
</tr>
<tr>
<td>Injection-4</td>
<td>40µg/ml</td>
<td>302104</td>
</tr>
<tr>
<td>Injection-5</td>
<td>40µg/ml</td>
<td>3028438</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>3006668</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>12462.1</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.410</td>
</tr>
</tbody>
</table>

Table 6: Study of degradation of the drug in the presence of different degradation conditions

<table>
<thead>
<tr>
<th>Degradation Parameter</th>
<th>Peak Area of Standard</th>
<th>Peak Area Standard</th>
<th>% of Recovery</th>
<th>% of Drug Degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1M HCl</td>
<td>2780828</td>
<td>2990138</td>
<td>92.99</td>
<td>7.00</td>
</tr>
<tr>
<td>0.1 M NaOH</td>
<td>2721026</td>
<td>2990138</td>
<td>91.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Thermal</td>
<td>2601420</td>
<td>2990138</td>
<td>86.99</td>
<td>13.00</td>
</tr>
<tr>
<td>Peroxide</td>
<td>2541617</td>
<td>2990138</td>
<td>84.99</td>
<td>15.00</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The system suitable parameters such as tailing factor (1.3) and number of theoretical plates (2578) are found to be within the limits. A typical chromatogram for the standard and sample were presented in Fig.3 and Fig.4 respectively. The retention time of the component was found to be 2.256min. The intra-day precision or inter-day precision of a method was expressed in terms of statistical parameters such as standard deviation and %RSD. The %RSD was calculated for five replicate measurements and found to be less than 2.0. Inter-day precision of the method was determined by carrying out the experiment on different days using same instrument and same column under similar chromatographic conditions. The results are presented in Table-2. The proposed method was linear in the range of concentration 20-60µg/ml and the correlation coefficient is found to be 0.9990. A calibration curve was constructed by plotting concentration against peak area (Fig.5). The correlation coefficient, slope and intercept were presented in Table-3. The accuracy of the method was determined from recovery experiments. The recovery studies were carried out at three different concentration levels (50%, 100% and 150% of target concentration). The percentage recovery of the drug at three different concentration levels and the mean percent of recovery are presented in Table-4. Robustness of the proposed method is checked by making slight deliberate change in the flow rate and mobile phase composition is made to evaluate the impact on the method.

CONCLUSIONS

A simple isocratic RP-HPLC method was developed for the determination of Amitriptyline HCl in pharmaceutical formulations as per the ICH guidelines. The method has higher sensitivity towards the determination of the Amitriptyline HCl. The developed method is found to be accurate and precise as indicated by recovery studies and % RSD not more than 2.0. Recovery studies are performed at 50%, 100% and 150% concentration levels are found to be within the limits mentioned as per ICH Guidelines. The proposed method was found to be simple, precise, accurate and robust. Therefore the method can be used for routine analysis in quality control.

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REFERENCES


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