**INTRODUCTION**

Atropine is a tropane alkaloid extracted from deadly nightshade (*Atropa belladonna*), jimsonweed (*Datura stramonium*), mandrake (*Mandragora officinarum*) and other plants of the family Solanaceae. Atropine probably doesn’t pre-exist in the plant, but is formed from its isomer L-hyoscyamine by racemization during its extraction process. It is a secondary metabolite of these plants and serves as a drug with a wide variety of effects. It is a competitive antagonist for the muscarinic acetylcholine receptor. It is classified as an anticholinergic drug. Being potentially deadly, it derives its name from Atropos, one of the three Fates who, according to Greek mythology, choose how a person was to die. Atropine is a core medicine in the World Health Organization’s “Essential Drugs List”, which is list of minimum medical needs for a basic health care system.

Atropine is a racemic mixture of D-hyoscyamine and L-hyoscyamine, with most of its physiological effects due to L-hyoscyamine. Its pharmacological effects are due to binding to muscarinic acetylcholine receptors. It is an antimuscarinic agent.

**ABSTRACT**

An isocratic RP-HPLC method was developed for the estimation of atropine sulphate in bulk. The separation was achieved with a C18 column using mobile phase comprising of methanol:5 mmol potassium dihydrogen phosphate buffer (50:50, v/v), the pH of which was kept unadjusted. The flow rate was kept at 1 mL min⁻¹ and analysts was screened with UV detector at 264 nm. The retention time for atropine sulphate was found to be 4.797 minutes. The LOD and LOQ for atropine sulphate were found to be 23.07 and 69.91 ng mL⁻¹, respectively. The described method was precise and sensitive.

**Key words:** Atropine Sulphate, Reverse Phase HPLC.

**Fig. 1: Structure of Atropine sulphate**

The most common atropine compound used in medicine is atropine sulfate (C₁₇H₂₃NO₃)₂⁻\(\text{H}_₂\text{SO}_₄\cdot\text{H}_₂\text{O}\), the full chemical name is 1αH, 5αH-Tropan-3-α 01 (±)-tropate(ester), sulfate monohydrate. The structure of atropine sulphate is shown in Fig. 1.

There are various methods reported for estimation of atropine sulphate by LC-MS, enantio-separation by capillary electrophoresis, chiral separation (12), with fluorescence detection, with conductometric detection, by cation exchange, using ion-pair chromatography. However, there is single method reported for the of atropine sulphate in a pharmaceutical dosage form. Thus, an improved LC-UV method was developed and validated.
EXPERIMENTAL

Instrumentation and Chromatographic Conditions

The LC system (Analytical, India) consisted of a solvent delivery module (ALC), analytical manual injector 2010 fitted with a 20 μL injection loop and a UV detector (ASPD). The column used was Grace Smart C18 5 micron {250 mm x 4.6 mm i.d.}. The mobile phase was prepared by mixing methanol: 5mmol potassium dihydrogen phosphate buffer in the ratio 50:50, v/v; and the pH was unadjusted. The flow rate was kept at 1 mL min⁻¹. The detection wavelength was set to 264 nm. Operation, data acquisition and analysis were performed using Analchrom software.

These chromatographic conditions were developed by following a series of experiments in an effort to elute atropine sulphate at a retention time that is suitable for analysis with better peak shape 918).

Reagents

A reference standard of atropine sulphate was procured from Intas Pharmaceuticals (Ahmedabad, Gujarat, India). Methanol was of LC grade (Rankem, Delhi), ertho-phosphoric acid (Rankem, Delhi) and potassium dihydrogen phosphate buffer (S. D. fine Chem., Mumbai) were analytical reagent grade. Deionized water was prepared using a triple stage water purifier system; that was further filtered through 0.2 micron filter.

Preparation of Calibration Standards and Quality Control Samples

Standard stock solution (500 µg mL⁻¹) of atropine was prepared in mobile phase. The solution was kept at 4°C. Further dilutions were prepared in mobile phase to obtain working standards in a concentration range of 0.5 – 16.00 µg mL⁻¹.

The quality control samples were prepared in mobile phase to obtain concentration range of 3-12 µg mL⁻¹ for atropine sulphate.

RESULTS AND DISCUSSION

Method Validation

The proposed method was validated with respect to stability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to the ICH Guidelines19.

Calibration and linearity for Validation

A standard curve of six points was plotted. Standards were prepared in the concentration range of 0.5-16 µg mL⁻¹ for atropine sulphate. Quality control samples MQC (medium quality control) and HQC (high quality control) fro atropine sulphate.

A calibration curve of the drug was constructed and linearity was assessed by least square regression analysis. The correlation coefficient (r²) was determined which was found to be 0.999 or better (20). The acceptance criteria of standard concentration were ± 15% deviation from the nominal value except LLOQ, which was set as ± 20%.

<table>
<thead>
<tr>
<th>Spiked Conc. (µg mL⁻¹)</th>
<th>Found2 (µg mL⁻¹)</th>
<th>Precision % CV</th>
<th>Accuracya %Bias</th>
<th>Intra-day</th>
<th></th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.976 ± 0.006</td>
<td>0.594</td>
<td>-2.4</td>
<td>0.978 ± 0.023</td>
<td>2.347</td>
<td>-2.2</td>
</tr>
<tr>
<td>4</td>
<td>3.999 ± 0.023</td>
<td>0.578</td>
<td>-0.025</td>
<td>4.026 ± 0.024</td>
<td>0.605</td>
<td>+0.65</td>
</tr>
<tr>
<td>16</td>
<td>15.94 ± 0.079</td>
<td>0.495</td>
<td>-0.376</td>
<td>15.93 ± 0.01</td>
<td>0.07</td>
<td>-0.438</td>
</tr>
</tbody>
</table>

a – Mean ± standard deviation
b – Bias % = [(found-spiked)/spiked] x 100
Precision and Accuracy

Precision and accuracy determined by the analysis of three concentrations chosen from the high, medium and low range of the standard curves for all the three drugs (1, 4, and 16 µg mL⁻¹). Triplicates of each samples (n=9) were analyzed on day 1 to determine intra-day precision and accuracy. Inter-day precision and accuracy were determined by triplicate samples of these concentrations on day 1 to 8. Mean, standard deviation and relative standard deviation were calculated from these concentration values and used in the estimation of intra- and inter-day precision. Accuracy (Bias) is expressed as the percent difference between calculated mean concentrations relative to the nominal concentration. A precision (%CV) ≤5% and an accuracy (%bias) ≤ ± 15% are acceptable. The RSD values of intra- and inter-day studies varied from 0.9884 to 4.56% which showed that the precision of method was satisfactory (Table 1).

Method precision was determined from the results from seven independent determinations at 100% of the sample concentrations of atropine sulphate.

Stability Studies

Stability of the standard solutions of atropine sulphate was evaluated under different storage conditions. The long-term stability was assessed after storage of stock solutions at 4°C for 8 days. The stock solutions of individual drugs as well as mixed standard solutions were analysed on 3rd and 8th day and the response were compared with the response obtained from the analysis of stock solutions on 1st day. The results showed that the retention time and peak area were almost unchanged (RSD % < 5) and that no significant degradation was observed within the given period, indicating the solutions are stable for at least 8 days.

Specificity

Specificity, described as the ability of a method of discriminate the analytes from all potential interfering substances was evaluated by preparing the analytical placebo as well as blank mobile phase, and it was confirmed that there were no peaks obtained at the same retention time of the drugs. The representative chromatograms show no other peaks at the retention time of analytes, which confirms the specificity of the method.

Linearity

The linearity was determined by plotting the graph between peak area and concentration of the drug. The peak area responses for six concentrations were determined. The concentrations used for analysis were in the range of 0.5 - 16 µg mL⁻¹. The linearity curve was defined by the following equations: \( y = 19.7595 + 0.9325 \times \) and \( r^2 = 0.9999 \) (21). The y is the peak area and x is the concentration expressed in µ g mL⁻¹.

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantification (LOQ) the method based on the residual standard deviation of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curves was studied using samples containing the analytes in the range of 0.5-16 µg mL⁻¹. The LOD and LOQ for atropine sulphate were found to be 23.07 and 69.91 ng mL⁻¹, respectively.

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REFERENCES


