RP-HPLC Estimation of Trospium Chloride in Tablet Dosage Forms

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Abstract: A rapid, sensitive and precise HPLC method was developed for the estimation of trospium chloride in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase Azilent C₁₈ column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 60:40v/v. The flow rate was 1 ml/min and the detection wave length 215 nm. The linearity was found in the range of 10-150 μg/ml with a correlation coefficient of 1.0000. The proposed method was validated for its sensitivity, linearity, accuracy and precision. This method was employed for routine quality control analysis of trospium chloride in tablet dosage forms.

Keywords: Trospium chloride, Estimation, Tablets, RP-HPLC.

Introduction
Trospium chloride, (1,3,5)-3-[Hydroxy diphenyl acetyl]oxy] spiro [8 azoniabicyclo [3.2.1.] octane-8,1-pyrolidinium] chloride¹ (Figure 1), is a quaternary ammonium antimuscaranic agent with actions similar to atropine. It antagonizes the effect of acetylcholine on muscarinic receptors in cholinergically innervated organs including the bladder. Its parasympatholytic action reduces the tonus of smooth muscle in the bladder. As a result, it is used for management of urinary frequency, urgency and incontinence in detrusor instability and the elimination half-life²,³ has been reported to be between 10 and 20 hrs. A few UV spectrophotometry⁴, fluorimetric⁵, HPLC⁶, LC-MS⁷,⁸ methods were reported earlier for the determination of trospium chloride in biological samples. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of trospium chloride in bulk samples and in tablet dosage forms.

Experimental
The reference sample of trospium chloride was obtained from Divya Labs, Hyderabad. HPLC grade water and acetonitrile were purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.
Chromatographic conditions
The analysis of the drug was carried out on a waters HPLC system equipped with a reverse phase C\textsubscript{18} column (150×4.6mm; 5μm), a 2695 binary pump, an automatic injector with 20 μL injection loop and a 2487 dual absorbance detector and running on Waters empower software.

![Chemical structure of trospium chloride](image)

Figure 1: Chemical structure of trospium chloride.

A mixture of phosphate buffer and acetonitrile in the ratio of 60:40 v/v was found to be the most suitable mobile phase for ideal separation of trospium chloride. The solvent mixture was filtered through a 0.45 μ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 1500 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 215 nm. The run time was set at 4 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 2.48 min. A typical chromatogram showing the separation of the drug is given in Figure 2.

![Typical chromatogram showing separation of trospium chloride](image)

Figure 2: Typical chromatogram showing separation of trospium chloride.
**Calibration plot**

About 25 mg of trospium chloride was weighed accurately, transferred into a 100 ml volumetric flask and dissolved 25 ml of a 60:40 v/v mixture of phosphate buffer and acetonitrile (Diluent) was added to the flask. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 250 µg/ml solution. From this, a working standard solution of the drug (100 µg/ml) was prepared by diluting 4 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10 to 150 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 1ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-150 µg/ml of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of trospium chloride in tablet dosage forms.

**Validation of the proposed method**

The specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of trospium chloride. Solutions containing 50, 100 and 150 µg/ml of trospium chloride was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 2. The system suitability parameters are given in Table 3.

| Table 1: Calibration data of the method. |
|-----------------------------------------|
| Concentration (µg/ml) | Mean peak area (n=6) |
|------------------------|----------------------|
| 10                     | 202424               |
| 25                     | 506052               |
| 50                     | 1012226              |
| 75                     | 1518384              |
| 100                    | 2024248              |
| 125                    | 2530300              |
| 150                    | 3036493              |

| Table 2: Precision of the proposed HPLC method. |
|------------------------------------------------|
| Concentration of trospium chloride (µg/ml) | Measured concentration of trospium chloride (µg/ml) |
| Intra-day | Inter-day |
| Mean (n=3) | % C.V. | Mean (n=3) | % C.V. |
|-----------|--------|-----------|--------|
| 50        | 50.27  | 0.45      | 49.97  | 1.21   |
| 100       | 99.5   | 0.69      | 99.65  | 1.81   |
| 150       | 149.94 | 0.03      | 149.06 | 0.57   |
**Table 3: System suitability parameters.**

| Parameter            | Result |
|----------------------|--------|
| Theoretical plates (N) | 2150   |
| Tailing Factor       | 1.10   |
| Peak asymmetry       | 1.2    |
| LOD (µg/ml)          | 0.07   |
| LOQ (µg/ml)          | 0.21   |

*Estimation of trospium chloride in tablet dosage forms*

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate trospium chloride in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of trospium chloride was transferred to a 100 ml volumetric flask and extracted with small amount of diluent. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. From the filtrate, 4 ml of aliquot was taken in a separate 10 ml volumetric flask, the contents made up to the volume. The solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 4.

**Table 4: Recovery from dosage forms.**

| Formulation | Label claim (mg) | Amount found (mg) (n=6) | % Amount found |
|-------------|------------------|-------------------------|----------------|
| Brand-1     | 20               | 19.80                   | 99.00          |
| Brand-2     | 60               | 59.86                   | 99.77          |

**Results and Discussion**

In the proposed method, the retention time of trospium chloride was found to be 2.48 min. Quantification was linear in the concentration range of 10-150 µg/ml. The regression equation of the linearity plot of concentration of trospium chloride over its peak area was found to be $Y=20243X+43.07 \ (r=1.0000)$, where $X$ is the concentration of trospium chloride (µg/ml) and $Y$ is the corresponding peak area. The number of theoretical plates calculated was 2150, which indicates efficient performance of the column. The limit of detection and limit of quantitation were found to be 0.07 µg/ml and 0.21 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 60:40 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.
Conclusion
The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of trospium chloride and can be reliably adopted for routine quality control analysis of trospium chloride in its tablet dosage forms.

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