Stability-indicating HPLC determination of tolterodine tartrate in pharmaceutical dosage form

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A simple, selective, precise and stability-indicating high-performance liquid chromatographic (HPLC) method of analysis tolterodine tartrate in pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised a reversed-phase C18 column (250 × 4.6 mm), 5 µ with a mobile phase consisting of a mixture of buffer solution (2.88 g ammonium dihydrogen orthophosphate in 1 L of water) and methanol in the ratio of 40 : 60. Triethylamine (5 mL/L) was added to it and pH of mobile phase was adjusted to 7.0 ± 0.1 with orthophosphoric acid at a flow rate of 1.5 mL/min. Detection was carried out at 220 nm. The retention time of tolterodine was 6.49 min. The linear regression analysis data for the calibration plots showed good linear relationship with coefficient of regression value, \( r^2 = 0.99 \) in the concentration range 200.60-601.80 µg per mL. The value of correlation coefficient, slope and intercept were 1.0, 20.87 and –6.87, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. The samples degraded with hydrogen peroxide showed no additional peak. This indicates that the drug is susceptible to acid-base hydrolysis, photochemical and thermal degradation. Statistical analysis proves that the method is reproducible and selective for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Tolterodine tartrate, HPLC, Stability indicating, Degradation

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Tolterodine tartrate, chemically, \((R)-2-[3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol\) (Fig. 1) is used in treatment of urinary incontinence. The drug is listed in Merck index. Tolterodine tartrate blocks muscarinic receptors, which can be found on the muscle cell of the bladder wall. Stimulation of these receptors causes the bladder to contract and empty. When these receptors are blocked the muscle of the bladder wall contracts less. Literature survey reveals that a capillary solid phase extraction—tandem mass spectrometry, a capillary liquid chromatography—tandem mass spectrometry method and a gas chromatography-mass spectrometry method has been reported for the determination of tolterodine and its metabolites in plasma samples. But there is no stability indicating high-performance liquid chromatography (HPLC) method for the determination of tolterodine from its tablets or its pharmaceutical dosage form.

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. The hydrolytic and the photolytic stability tests are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of tolterodine tartrate is HPLC.

The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of tolterodine tartrate in the presence of its degradation products and related impurities as per ICH guideline.

Fig. 1—Chemical structure of tolterodine tartrate
Experimental Procedure

Reagents and Materials
Tolterodine tartrate was supplied by Sun Pharma India Ltd. and tablets (Label claim: 2 mg/tablet, product name: Roliten and manufacturer: Ranbaxy Laboratories Ltd.) were procured from the market. Methanol and water (HPLC grade), ammonium dihydrogen orthophosphate, triethylamine and orthophosphoric acid (all AR grade) were used.

Apparatus
The apparatus used was an Agilent 1100 series instrument equipped with an inbuilt solvent degasser, quaternary pump, photodiode array detector with variable injector and auto sampler. The column used was of stainless steel (250 × 4.6 mm) and packed with Inertsil ODS 3, (G.L.Sciences, Japan).

Method

Chromatographic conditions
Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of buffer solution (2.88 g ammonium dihydrogen orthophosphate in 1 L of water) and methanol in the ratio of 40 : 60. 5 mL of triethylamine per litre was added to it and pH of mobile phase was adjusted to 7.0 ± 0.1 with orthophosphoric acid at a flow rate of 1.5 mL/min. Detection was carried out at 220 nm. A 400 µg/mL of tolterodine tartrate of standard and sample preparation was injected. The injection volume was 20 µL for assay and degradation level.

Standard preparation
Working standard solution containing 400 µg/mL of tolterodine tartrate was prepared by weighing accurately 40 mg of tolterodine tartrate into a 100 mL volumetric flask. It was sonicated to dissolve in and diluted up to the mark with mobile phase.

Sample preparation
Twenty tablets containing tolterodine tatrarte were weighed and finely ground into a fine powder. A quantity of the powder equivalent to 20 mg of tolterodine tatrarte was weighed into a 50 mL volumetric flask. About 30 mL of mobile phase was added and sonicated for 20 min, and volume was made up with mobile phase. The solution was mixed well and centrifuged at 2500 rpm for 10 min. Clear supernatant solution was injected into the HPLC system.

Induced degradation of tolterodine tartrate

(i) Acid and base-induced degradation
Tablet powder equivalent to 20 mg of tolterodine tartrate was transferred to 50 mL volumetric flask. To it, 25 mL of mobile phase was added and sonicated for 20 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase and mixed well. This solution was centrifuged at 2500 rpm for 10 min. This supernatant solution was injected into HPLC system. This acidic and basic forced degradation was performed in the dark in order to exclude the possible degradative effect of light.

(ii) Hydrogen peroxide-induced degradation
The method described above (i) was followed except that 5 mL of 3% H2O2 was added in place of HCl.

(iii) Photochemical degradation
The method described above (i) was followed without the addition of H2O2 or HCl/NaOH. The tablet powder used was previously kept in UV light for 24 h.

(iv) Thermal degradation
The method described above (i) was followed without the addition of H2O2 or HCl/NaOH.

Detection of impurities
The method described under sample preparation was followed.

Results and Discussion

Method development
The chromatographic conditions were optimized with a view to develop a stability-indicating assay method. Three different columns were tried as under chromatographic conditions namely, Hypersil CPS (Cyanopropyl silane); 250 × 4.6 mm; 5 µ Thermoquest (U.K.), Hypersil APS (Amino propyl silane); 250 × 4.6 mm; 5 µ Thermoquest (U.K.) and Inertsil ODS 3; 250 × 4.6 mm; 5 µ (G.L.Sciences, Japan). The columns Hypersil CPS and Hypersil APS gave good peak shape but a lower retention with low peak purity. Inertsil ODS 3 column gave a good peak shape with response at affordable retention time with peak purity of tolterodine on higher side.

Also, two different pH were tried on Inertsil ODS 3 column, these were pH 6.0 and 7.0. These pH had
minimal effect on tolterodine retention time, tailing factor, peak purity and theoretical plates. An additional mobile-phase in the ratio of 0.05 M KH2PO4 buffer : acetonitrile (50:50), pH of the mobile-phase adjusted to 5.0 with orthophosphoric acid at the flow rate of 1.5 mL/min using Inertsil ODS 3; 250 × 4.6 mm; 5 µ (G.L.Sciences, Japan) at 220 nm was attempted. The retention time of tolterodine was on lower side with theoretical plates and peak purity but a good peak symmetry or tailing factor. Hence, the final chromatographic conditions comprised a reversed-phase C18 column (250 × 4.6 mm), 5 µ with a mobile phase consisting of a mixture of buffer solution (2.88 g ammonium dihydrogen orthophosphate in 1 L of water) and methanol in the ratio of 40 : 60. Triethylamine (5 mL/L) was added to it and pH of mobile phase was adjusted to 7.0 ± 0.1 with orthophosphoric acid at a flow rate of 1.5 mL/min. Detection was carried out at 220 nm. It was also identified that the peak at retention time of 1.63 min was of tartaric acid under these conditions.

Calibration curves

The linearity of response for tolterodine tartrate assay method was determined by preparing and injecting solutions with concentrations of about 200.60 to 601.80 µg/mL of toltetodine tartrate using tolterodine tartrate working standard. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied for tolterodine tartrate with coefficient of regression, \( r^2 \) value as 0.99. The value of correlation coefficient, slope and intercept were 0.9999, 20.87 and –6.87, respectively.

Validation of the method

**Precision**

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same injection (400 µg/mL for standard preparation).

Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (400 µg/mL for sample preparation). It showed very low% relative standard deviation (% RSD) of peak area of tolterodine.

The %RSD for repeatability of standard preparation is 0.06%, whereas the %RSD for repeatability of sample preparation is 0.35%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ±2.0% and mean recovery between 98.0 and 102.0%. Table 1 represents the precision data obtained for the method.

**Ruggedness and robustness of the method**

Ruggedness and robustness of the method were determined by analysing same sample blend at normal operating conditions and also by changing some operating analytical conditions such as HPLC column make, mobile phase composition, flow rate, detector and analyst.

The parameters and results of normal operating condition (original) against changed conditions are included in Table 2. The low value of % RSD obtained after introducing the deliberate changes in parameters alters the results of tolterodine tartrate to 0.51% of method precision study, which is not a significant change. The ruggedness and robustness of the method is established, as the % deviation from mean assay value obtained from precision study is less than ±2.0%.

**Recovery studies**

Recovery study was performed by spiking 30, 50 and 70% of tolterodine tartrate working standard to a pre-analysed sample. The pre-analysed sample was

<table>
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<th>Sample preparation</th>
<th>% Assay</th>
<th>% Deviation from mean assay value</th>
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<tr>
<td>1</td>
<td>97.53</td>
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<tr>
<td>2</td>
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<td>0.16</td>
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<td>3</td>
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<tr>
<td>4</td>
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<td>6</td>
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<tr>
<td>Mean</td>
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<td></td>
</tr>
<tr>
<td>± SD</td>
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<td>% RSD</td>
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<table>
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<th>Parameter</th>
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<th>Changed conditions</th>
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<td>Hypersil BDS C18;</td>
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<td>4.6 × 250 mm, 5µ</td>
</tr>
<tr>
<td>Flow rate</td>
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<td>1.1 m/L / min</td>
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<tr>
<td>Mobile-phase composition</td>
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<tr>
<td></td>
<td>methanol</td>
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<td></td>
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<td>425 : 575</td>
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<td>Analyst II</td>
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<td>% assay, tolterodine tartrate</td>
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<td>98.38%</td>
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</tbody>
</table>

% RSD from mean assay value obtained in method precision studies is 0.51% of tolterodine tartrate.
weighed in such a way that final concentration was half or 50% of the sample preparation before spiking. The percentage sum level of pre-analysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosage nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range.

\[
\text{% Recovery} = \frac{\text{% Amount recovered}}{\text{% Sum level}} \times 100
\]

The results of recovery experiments are given in Table 3. The results indicate that the individual recovery of tolterodine tartrate ranges from 100.54 to 101.87% with mean recovery of 101.17% and % relative standard deviation of 0.47%. The recovery of tolterodine tartrate by proposed method is satisfactory as % relative standard deviation is not more than ±2.0% and mean recovery lies between 98.0 and 102.0%.

**Analysis of the marketed formulation**

The drug content was found to be 97.88% with a %RSD of 0.35%. It is noted that degradation of tolterodine tartrate had occurred in the marketed formulation that were analysed by this method with single maximum degradation as 0.24% and total degradation as 0.24%. The low RSD value indicated the suitability of this method for routine analysis of tolterodine tartrate in pharmaceutical dosage form.

**Stability-indicating property**

The values of % assay and % degradation with stress conditions are given in Table 4 with respective figures shown in Fig. 2. The no treatment sample (as control) has been evaluated relative to the standard concentration, whereas rest of the stressed condition samples (Sr. No. 2 to 6) are evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method. The chromatogram of no treatment sample (as control) showed additional peak at retention time (RT) of 4.36 min. The chromatogram of acid degraded sample showed additional peak at RT of 4.22 min. The chromatogram of alkali degraded sample showed additional peak at RT of 4.31 and 4.83 min. The chromatogram of thermal degraded sample showed additional peak at RT of 4.30 min. The chromatogram of photochemical degraded sample showed additional peak at RT of 4.29 min. The sample degraded with hydrogen peroxide showed no additional peak. Rest of the peaks, if any, was from its blank or placebo in each of these specified conditions. In each induced degradation sample where additional peaks were observed, the response of the drug was different from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis, thermal and photochemical degradation. The lower RT of the degraded components indicated that they were more polar than the analyte itself. In each of the stress condition, the peak purity of tolterodine tartrate as determined by diode array detector was greater than 980.

**Detection of impurities**

The sample solution showed additional peak other than principal peak at RT of 4.36 min with % single
maximum degradation as 0.24% and total degradation as 0.24%. Hence, related impurity is present in the market sample.

**Conclusion**

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of tolterodine tartrate in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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**References**