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QUANTITATIVE DETERMINATION OF TARTARIC ACID IN TOLTERODINE TARTRATE BY ION CHROMATOGRAPHY USING CONDUCTIVITY DETECTION

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ABSTRACT

The present paper deals with the development and validation of analytical method based on a single column high performance Ion chromatography with cation suppressed conductivity detection, developed for quantitation of tartaric acid. The diluent used for the preparation of sample solution was water and injected into a standard chromatographic system connected with 250mm length, 4.0 mm ID and 5.0 µm particle size Metrosep A supp 5 Ion exchange column and suppressed conductivity detector. The developed analytical method was highly precise and accurate compared to titration methods and HPLC-UV detection methods to analyze the counter-ion tartaric acid in Tolterodine tartrate drug substance. Calibration curves were linear with correlation co-efficient of > 0.999 for tartaric acid. The % RSD of area response of tartaric acid in standard injections was below 1.0, which demonstrates the system precision. The % RSD of results in samples is below 1.0, which demonstrates the method precision of the test procedure. The accuracy of the method is also studied and observed between 99% and 101%. The developed method was validated according to ICH guidelines for the quantitative determination of tartaric acid in Tolterodine tartrate taken for the study.

Keywords: Tartaric acid, Tolterodine tartrate, Ion chromatography, Conductivity detector, Cation suppresser, Method validation

INTRODUCTION

Tolterodine is a cholinergic antagonist for the treatment of urinary incontinence. It is a follow up to terodiline, which was withdrawn due to side effects. Tolterodine has a safety profile in humans with no side effects. In the search for new drugs with improved side effect profile to treat an overactive bladder, a family of phenyl propyl amines as muscarinic receptor antagonists has been investigated[1]. A convenient synthesis for this drug was cited in literature[2] starting from 1-[2-(hydroxy-5-methyl) phenyl]-1-phenyl ethylene, accessible in high yield by alumina promoted *o*-alkenylation of *p*-cresol with phenyl acetylene. The quaternary ammonium compounds of such invention are preferably administered as salts with pharmaceutically acceptable acids like tartaric acid etc. The dosage of specific compound will very depending on its potency and the mode of administration[3].

There is variety of analytical methods for the determination of tartaric acid in different types of analytes like Fructus mume, orange juices, alcoholic beverages etc. The different reversed-phase high-performance liquid chromatography methods employ the detection techniques like UV, Chemiluminescence etc. Also there are various techniques like capillary electrophoresis, Attenuated total reflectance spectroscopy in addition to the classical titration techniques[4-10]. Tartaric acid has very less response at 210 nm wavelength in UV detection, whereas other techniques are not widely used in commercial laboratories. Classical

titration methods are not much accurate compared to instrumental techniques and are subjective for end point observations. So it has become necessary to quantify the counter ion tartaric acid with more accuracy and more precision than existing analytical methods. Use of Ion chromatography enables the quantification with more accuracy, precision and specific to counter ion analysis.

EXPERIMENTAL

Chemicals and Reagents:

Tolterodine tartrate is chemically described as (+)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl) -3-phenyl propylamine-L-hydrogen tartrate. Samples of this compound were received from manufacturing unit of bulk actives, CTO-6, Dr. Reddy's Laboratories Ltd. India. The chemical structure of the above mentioned drug substance is shown in Figure-1.

Analytical grade sodium carbonate and sodium hydrogen carbonate were purchased from s.d.fine chemicals, Mumbai, India. Analytical reagent grade sulfuric acid was purchased from Merck, Mumbai, India. Analytical grade tartaric acid and HPLC grade water used for the analysis were purchased from Qualigens Fine Chemicals, Mumbai, India.

Equipment:

The Ion Chromatography system purchased from Metrohm, Herisau, Switzerland used through out this study, which is equipped with 818 IC pump, 833 Liquid Handling unit, Sample injector with 20µL loop, 820 IC Separation center equipped with a Cation suppressor and Conductivity detector. Quantitation was performed from the output signal, monitored and processed using the IC Net 2.3 SR4 version software on Compaq computer (Digital Equipment Co). Dilutions were accomplished with Hamilton precision pipettes (Bonaduz, Switzerland).

Chromatographic conditions:

The chromatographic column used was a Metrosep A Supp 5 column (250 x 4.0 mm, 5.0µm Particle size) having stationary phase of Polyvinyl alcohol with Quaternary Ammonium groups[11], that was safeguarded with Metrosep A Supp 4/5 guard column. The mobile phase used was a mixture of 1.0 mM sodium hydrogen carbonate, 6.0 mM sodium carbonate prepared in HPLC grade water, degassed and filtered. The flow rate of the mobile phase was set at 0.8 ml/min. The injection volume was 10 µL. Water was used as a diluent.

The Anion exchange chromatographic system is equipped by a cation exchange resin suppressor for chemical suppression. Chemical suppression reduces the background conductivity and replaces the counter ions in the sample i.e. all cation from the mobile phase are replaced by H^{+}. By this suppression reaction, an eluent with high conductivity is transferred to water and carbon di-oxide which is of low conductivity. Suppressor is regenerated after each run using a suppressor regenerator followed with suppressor rinsing with HPLC grade water. Suppressor regenerator used is 50 mM Sulphuric acid prepared in HPLC grade water. The detector interface was set with detector range 100µS/cm and detector full scale 20 µS/cm. The run time for each run is 20 min.

Preparation of solutions:

The test solutions are prepared by dissolving the test sample at concentration of 100 µg/ml. The standard solution of 31.5 µg/ml concentration of tartaric acid was prepared directly from 3150 µg/ml stock solution of tartaric acid. This solution correspond to the theoretical content of tartaric acid in Tolterodine tartrate i.e. 31.5% w/w.

Method development:

Various trials were performed for the method development of tartaric acid content in Tolterodine tartrate. The trials were done to separate the peak of interest from all other peaks from test solution. Also the objective of different trials was to retain the tartaric acid peak at considerable time in short run time. The details of trials and observations are captured in table-1. Finally, the conditions were achieved as per mentioned in section chromatographic conditions.

Method validation:

During method optimization, all chromatographic parameters were found to prove precision, accuracy, linearity, limit of detection and limit of quantitation of tartaric acid peaks, with appropriate analysis run

time. All calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas.

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[12,13]. To demonstrate the proposed Ion exchange chromatographic method as a stability indicating procedure for tartaric acid content, samples of Tolterodine tartrate were subjected to forced degradation in acid, alkaline, photolysis, thermal and oxidative conditions. In the optimized ion exchange chromatographic system, the sample solution was injected after subjecting to the stress degradation with strong acid (0.1M Hydrochloric acid), strong base (0.5 N sodium hydroxide), oxidative degradation with 3% hydrogen peroxide, thermal degradation at 105 °C h and photolytic degradation done by exposing the sample to the 1.2 million lux hours of UV light as per ICH guidelines[14].

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the homogeneous sample under prescribed conditions [12-13]. The system precision for tartaric acid was checked at standard concentration of 31.5 µg/ml (at specification level) and the percentage relative standard deviation of area was calculated. Method precision was checked for six different preparations of test samples. Each solution was prepared at the analyte concentration of 100 µg/ml. The percentage relative standard deviation of content of tartaric acid in six preparations was calculated. The intermediate precision of the method was also evaluated by different analyst and on different day.

The linearity of an analytical test procedure is its ability to obtain test results (within a given range), which is directly proportional to the concentration of the analyte in the sample[12,13]. The linearity of the method was checked at five different concentration levels from 10 µg/ml to 50 µg/ml of tartaric acid. Each linearity solution was injected in duplicate and the average area response was used for plotting calibration curve. The calibration curve was drawn by plotting the peak areas of tartaric acid against corresponding concentration. The correlation coefficient of the regression line of the calibration curve is also calculated.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the expected value found[12,13]. Standard addition and recovery experiments were conducted to determine accuracy of the quantitation of tartaric acid in bulk drug samples of Tolterodine tartrate. The study was carried out by addition of tartaric acid at 80%, 100% and 120% of specification level and injected to the chromatographic system in triplicate at each level. The % recoveries for tartaric acid were calculated from the slope and y-intercept of the calibration curve obtained.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [12-13]. The limit of detection for phosphate and phosphite was established by injecting a series of dilute solutions with known concentration and the signal-to-noise ratio for peak response of tartaric acid peak was calculated. The limit of detection was established at signal to noise ratio 3:1. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [12-13]. Limit of quantitation was estimated at signal to noise ratio 10:1.The solution stability was carried out by keeping both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 hours. The same sample solutions were analyzed at twelve hours interval up to the study period. Further, mobile phase stability was also carried out for two days by analyzing the freshly prepared sample solutions against freshly prepared reference standard solutions for six hours interval. Mobile phase prepared was kept constant during the study.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The flow rate of the mobile phase was 0.8 ml/ min in the method. To study the effect of flow rate on system precision, it was changed by 0.1 units to 0.7 ml/ min and 0.9 ml/min while the mobile phase components were held constant and the effect of flow rate was studied.

Robustness was not studied for the column temperature as the method employs the column equilibration at room temperature in analytical laboratory. The mobile phase used was highly basic due to presence of carbonates, so the difference of 0.2 pH unit was not considered appropriate to study the robustness.

RESULTS AND DISCUSSION

A simple, precise, linear and accurate analytical procedure was developed with ion exchange high performance liquid chromatography with conductivity detection which enables the determination and quantitation of tartaric acid in Tolterodine tartrate with simple standard and sample preparation at optimum costs.

Satisfactory peak shape and retention time was achieved with the mobile phase system. The typical chromatograms for standard solution and test solution are represented in figure-3 and figure-4 respectively.

During specificity study, no significant variations were observed to the quality control samples for tartaric acid content estimation. The system precision of the analytical method for tartaric acid was checked at the specification level (31.5 % with respect to analyte concentration). The % RSD of the results was found to be 0.49, which confirms good system precision. The method precision for the analytical procedure was checked with the six different test preparations of test sample and %RSD of the results was observed to be 0.25. The intermediate precision also performed by the different analysts on different days, which shows the % RSD less than 1.0.The limit of detection and limit of quantitation concentrations were found to be 100 ng/ml and 300 ng/ml respectively, where signal to noise ratio of 3:1 for LOD and 10:1 for LOQ were used as criteria, for 10 µL injection volume. Linearity graph was plotted over the range of concentration from 10 µg/ml to 50 µg /ml. Five different preparations of tartaric acid at 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml were injected. The calibration curve is plotted against concentration and area response of the peak. The correlation coefficient (r) for the tartaric acid peak was 0.9999 having regression equation y = 31.79x - 13.218. The results are mentioned in table-2 and the linearity graph is represented in figure-2. The proposed method was evaluated for recovery and estimated by the standard addition of tartaric acid standard to Tolterodine tartrate samples. The % recovery was calculated and observed to be between 98% and 102%.

No significant change was observed in sample analysis even after 48 h during the solution stability and mobile phase stability experiments. Hence, the method is proven to be stability indicating for the sample preparation and mobile phase system. The flow rate of the mobile phase was deliberately changed to 0.7 ml/min and 0.9 ml/min, while the mobile phase components were held constant and chromatograms were recorded for standard injection. The system precision was checked and observed to be below 1.0% RSD. The validation of analytical method also shows the satisfactory data for the tested parameters.

The developed method is stability-indicating and there was no interference of degradation impurities with tartaric acid peak. The newly developed method makes analysis procedure to be completed in shorter analysis time with good recovery, precision and sensitivity. Compared to other analytical methods, the results showed that the proposed method can be highly accurate, precise and robust. On the basis of these results, the method is concluded to be suitable for quality control of tartaric acid in Tolterodine tartrate drug substance.

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Table-1: Details of Method development trials with remarks

Trial No.	HPLC conditions	Remarks
1	Column: Metrosep Organic Acids, 100 mm Mobile phase: 0.2 mM HNO3 Flow rate: 0.7 ml/min	Early retention time and interference of blank peaks in tartaric acid peak
2	Column: Metrosep A Supp5, 250 mm Mobile phase: 3.2 mM Na_2CO_3+1.0 mM $NaHCO_3$ Flow rate: 0.7 ml/min	Peak eluted very late at ~24 min. It increases the run time for each run.
3	Column: Metrosep A Supp5, 250 mm Mobile phase: 6.0 mM Na_2CO_3+1.0 mM $NaHCO_3$ Flow rate: 0.8 ml/min	Optimum retention time and no interference of blank peaks in tartaric acid peak. Short run time for each run.

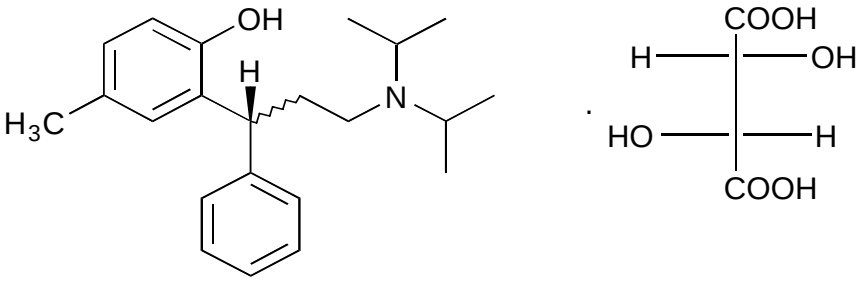

Fig.-1: Structure of Tolterodine tartrate

Table-2: Linearity results

Conc. in µg/ml	Area Response
10	309.815
20	616.332
30	942.388
40	1252.868
50	1581.067
Correlation coefficient (r)	**0.9999**

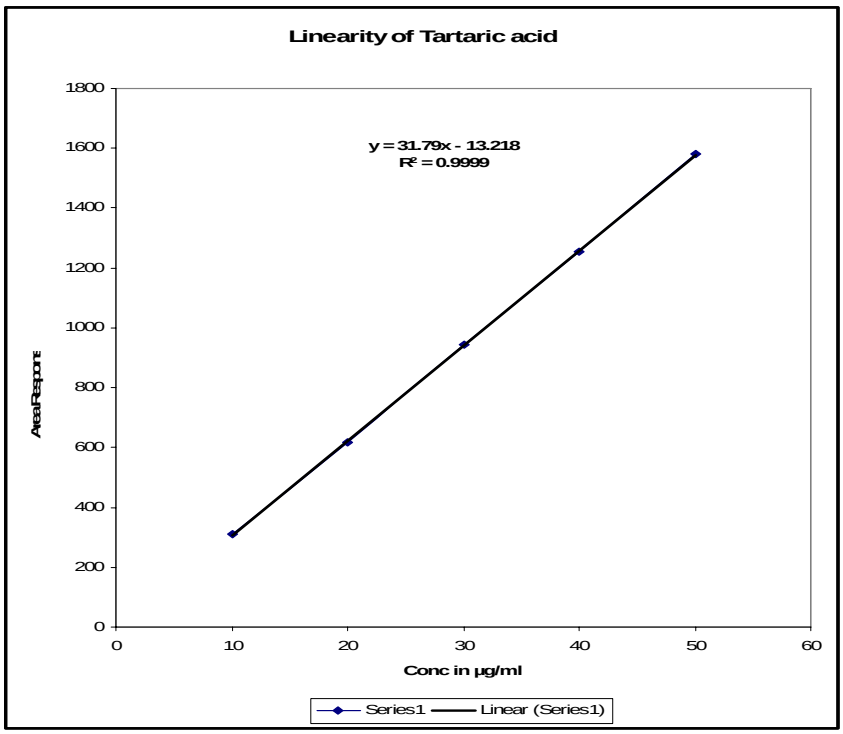

Fig.-2: Linearity graph

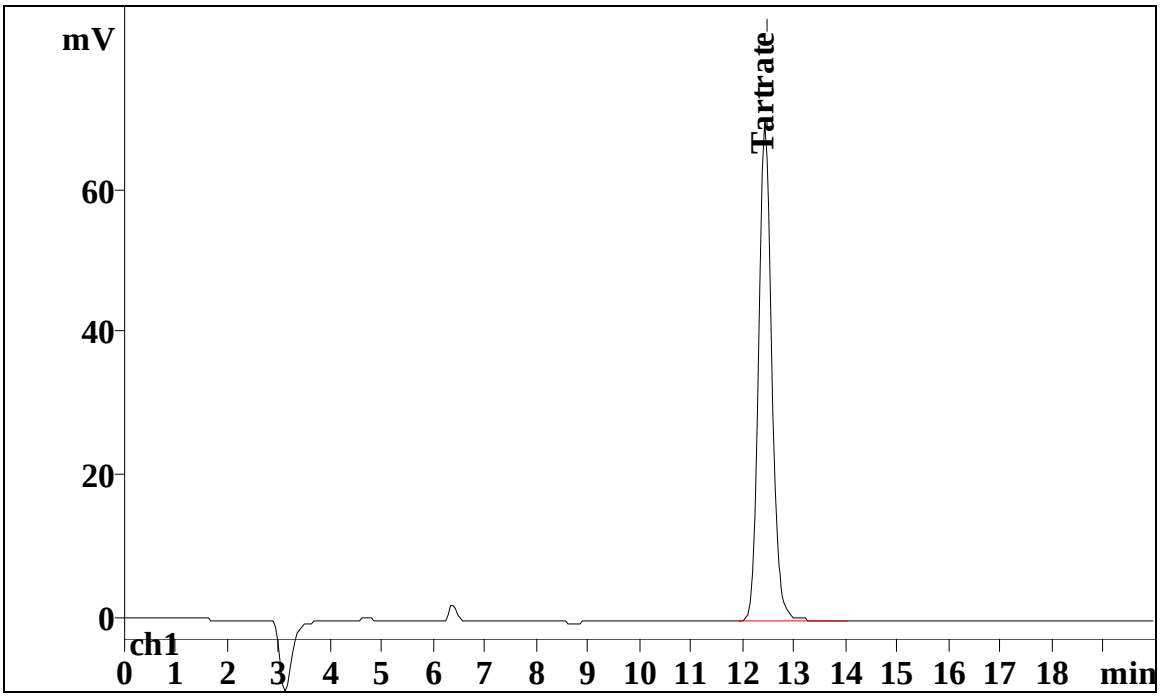

Fig.-3: Typical chromatogram for standard solution (conc. 31.5 µg/ml)

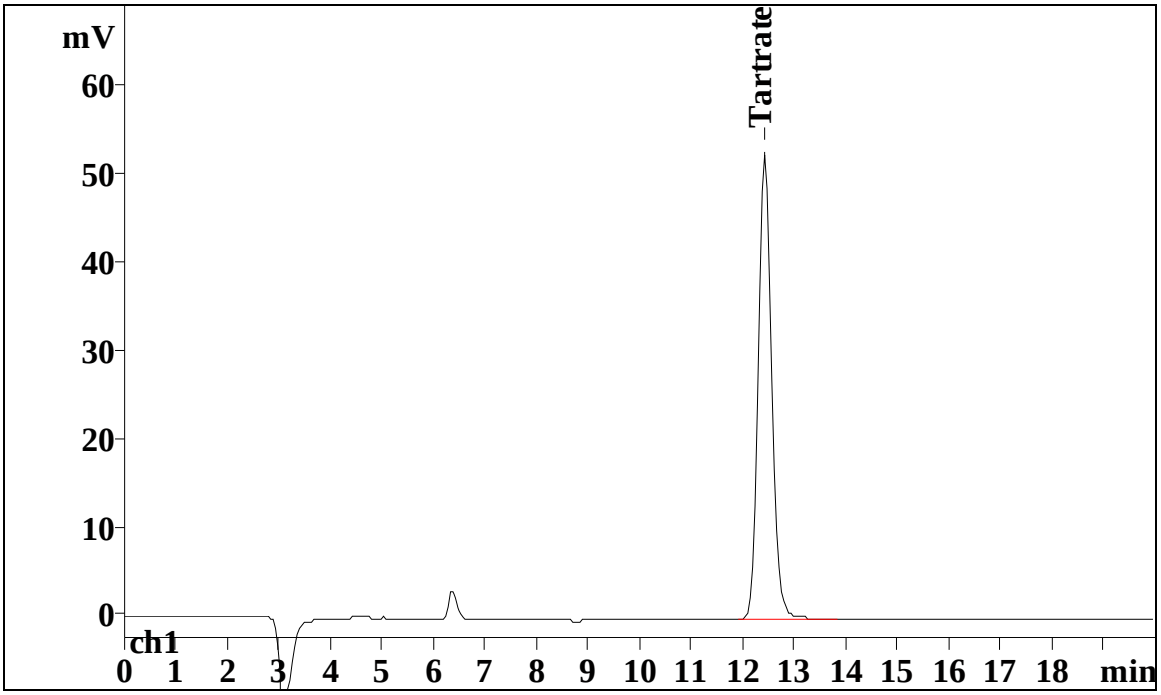

Fig.-4: Typical chromatogram for Tolterodine tartrate test solution (conc. 100 µg/ml)

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"Two percent of the people think; three percent of the people think they think; and ninety-five percent of the people would rather die than think."

- George Bernard Shaw