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Short Communication

Stability Indicating Assay Method Development and Validation of Tolteridone Tartrate in Bulk Drug and Capsules

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Abstract

A stability indicating novel RP-HPLC method as developed for the quantification of Tolteridone tartrate in capsules. The separation was carried out by Athena C_{18} (250 mm × 4.6 mm) 5µ column using mobile phase phosphate buffer (pH 4.0), acetonitrile and methanol in the ratio of 40:20:40 v/v at a flow rate of 1 ml/min and detection at 240 nm. The retention time of Tolteridone tartrate was found to be 2.417 min and standard calibration plot was linear over a concentration range of 60-140 µg/ml with r^2 0.999. The method has been validated for accuracy, specificity, precision, robustness and ruggedness. Forced degradation studies were conducted according to ICH guidelines. The assay and recoveries of the drug was found to be 99.4% and 99.8 ± 0.2%.

Keywords: *Stability studies; Tolteridone tartrate (TOL); Capsules, RP-HPLC*

Tolteridone tartrate is a muscarinic receptor antagonist and it chemically described as (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine–L-hydrogen tartrate. It exists in two isomeric forms (R) and (S) [1]. It is used in the treatment of urinary urge incontinence and other symptoms of an overactive bladder [2]. Both urinary bladder contraction and salivation are mediated via cholinergic muscarinic receptors. After oral administration, Tolteridone is metabolized in the liver, resulting in the formation of the 5-hydroxy methyl derivative, a major pharmacologically active metabolite. The 5-hydroxy methyl metabolite, which exhibits an antimuscarinic activity similar to that of Tolteridone contributes significantly to the therapeutic effect. Both Tolteridone and 5-hydroxy methyl metabolite exhibit a high specificity for muscarinic receptors, either both show negligible activity or affinity for other neurotransmitter receptors and other potential cellular targets, such as calcium channels [3]. Literature studies indicated [4] a variety of analytical methods for analysis of drug in biological matrices, for example HP-electrospray ionization mass spectrometry (LC-ESI-MS), capillary solid phase extraction (SPE) coupled with electrospray tandem mass spectrometry and GC-MS. A variety of few methods have been reported for the determination of Tolteridone in formulations by UV-Spectrophotometry and HPLC [5]. But limited methods investigated for the determination of Tolteridone from its tablets or its pharmaceutical dosage form. As per ICH guidelines stability is a fundamental property of all products and the term "stability indicating assay" had been used describe a procedure which affords specific determination of a drug substance in the presence of its degradation products. The prime objective of studying the stability [6] of drug is to determine its shelf life. The various stress conditions specified for forced degradation studies include thermal, light, acidic, alkaline, hydrolysis and oxidative stress. Limited work was done on the stress studies of Tolteridone tartrate [7]. So an attempt was made to develop and validate a simple, precise, accurate and economical RP-HPLC method as per ICH guidelines.

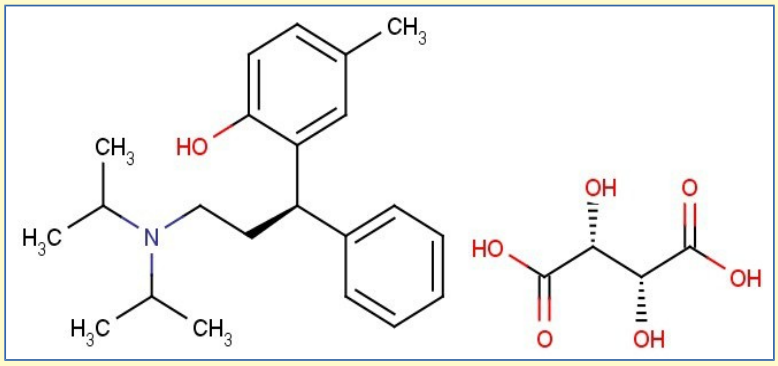

Figure 1: *Chemical structure of Tolteridone tartrate.*

Tolteridone tartrate of pharmaceutical grade was supplied as gift sample by Aurobindo Pharma, Hyderabad. TOL capsules were procured from local market. Acetonitrile, methanol and ammonium di hydrogen orthophosphate of HPLC grade were purchased from S.D fine chemicals Hyderabad. Liquid chromatography used was Schimadzu LC-2010 equipped with autosampler and photo diode array detector [8]. The chromatographic analysis was performed using LC solution software on Athena C 18 column (250 mm × 4.6 mm) 5µ column. In addition, digital micro balance electrolab and pH meter were used in this study. A mixture of phosphate buffer (pH 4.0), acetonirile and methanol in the ratio of 40:20:40 v/v was used as mobile phase and filtered through a 0.45 µm membrane filter and degassed. Flow rate was maintained at 1.0 ml/min with the detection at 240 nm.

Standard stock solution of TOL (100 µg/ml) was prepared by dissolving 100 mg of TOL with the mobile phase in 100 ml flask and further dilution was done by transferring 5 ml of the solution in to 50 ml flask and make up the volume with mobile phase. Twenty five capsules of Urotel-X each containing 4 mg of TOL were weighed, empty the capsules and finely powdered. A quantity of powder equivalent to 100 mg of TOL [9] was transferred to 100 ml flask and make up the volume with mobile phase, which contain concentration of 100 µg/ml. Both the standard and stock solutions were filtered through 0.45µ membrane filter. An aliquot of 20 µl of both standard and stock solutions were injected separately and chromatograms were recorded up to 10 min. Standard stock solutions in the concentration range of 60, 80, 100, 120 and 140 mcg were prepared. Five series of standard solutions containing 0.6 to 1.4 ml of standard stock solutions and make up to 10 ml with the mobile phase. The solutions were injected using a 20 µl and chromatograms were recorded. Calibration curves [10] were constructed by plotting peak area versus concentration and regression equations were computed for the drug.

The method of analysis was validated as per the guidelines of ICH and USP for the parameters like specificity, accuracy, linearity, precision, limit of detection, limit of quantitation, robustness and ruggedness. Forced degradation studies of the bulk drug carried out under conditions of acid, alkali, thermal, oxidation and UV. For heat and light study period was 10 days where as for acid, base and oxidative degradation it was about 48 hrs. There is no significant degradation of the sample was obtained in the acid, alkali, thermal, oxidation and UV [11]. In method precision, a homogenous sample of a single batch was analyzed six times and was checked whether the method gave consistent results for a single batch. The sample TOL was analyzed six times of the same batch as per analytical procedure. The % relative standard deviation was calculated. The % RSD of the TOL for six determinations was found to be 0.63% which is within the acceptance criteria limit and it is concluded that the method is precise. The system precision was carried out to ensure that the analytical system was working properly. Standard solution was injected six times in to the system and chromatogram was recorded. The % RSD [12] was found to be 0.29%.

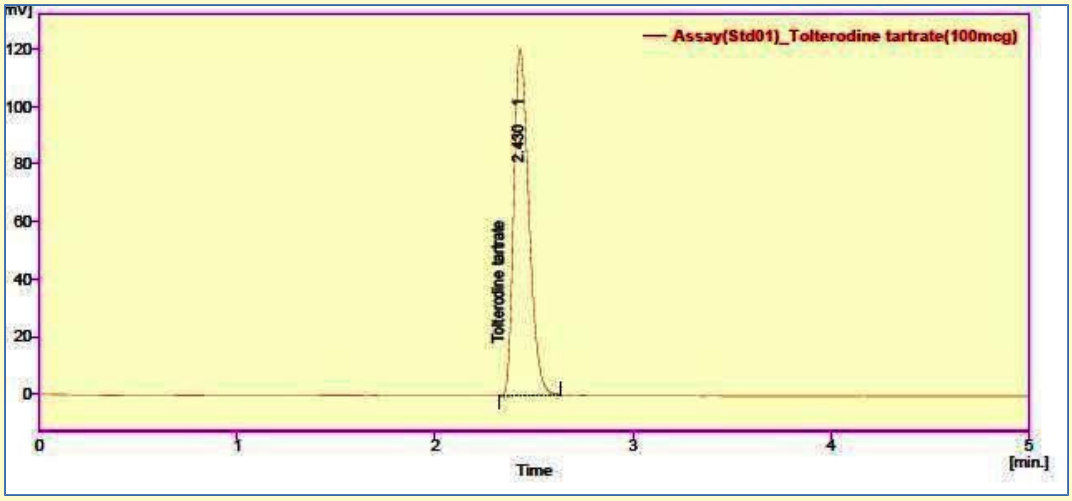

Figure 2: *A typical HPLC chromatogram of pure Tolteridone tartrate.*

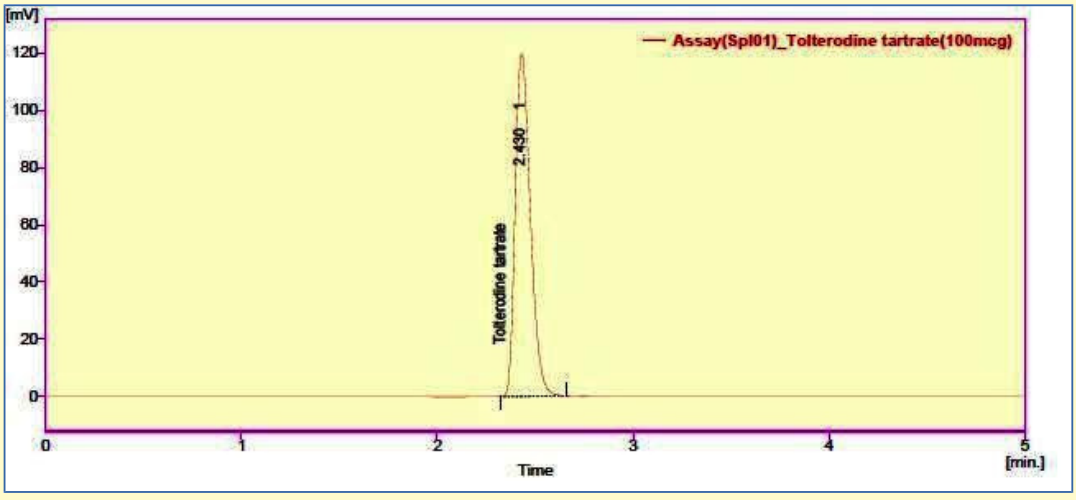

Figure 3: *A typical HPLC chromatogram of Tolteridone tartrate in sample formulation.*

Accuracy was investigated by spiking the drug with the sample at three different concentration levels i.e 80, 100 and 120%. The recovery obtained was in the range of 99.8 to 100.2% demonstrating that the method proposed is highly accurate. Percent recoveries for the marketed products were found to be within the acceptable limits. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The standard solutions were injected in to the chromatogram at slight changes of flow and wavelength. The effectiveness of the deliberated changes was observed on retention time of peak [13]. The statistical data gives no significant variations in the above parameters indicating the method is robust. Ruggedness was

performed by injecting same concentrations of standard and sample solutions, which were prepared and analyzed by different analyst. It is also expressed in terms of percentage recoveries of the drug. It was obtained as 98.5% and 99.2%, indicating that less variation of assay by two analysts. Range is the minimum and maximum concentration of sample at which the analytical procedure gives reproducible results. It can be determined by linearity, accuracy and precision studies [14]. The method was found acceptable across wide range of concentration 60-140 µg/ml. The limit of detection is the minimum concentration of the analyte that gives a measurable response; whereas limit of quantification is the minimum concentration that can be quantified accurately and precisely.

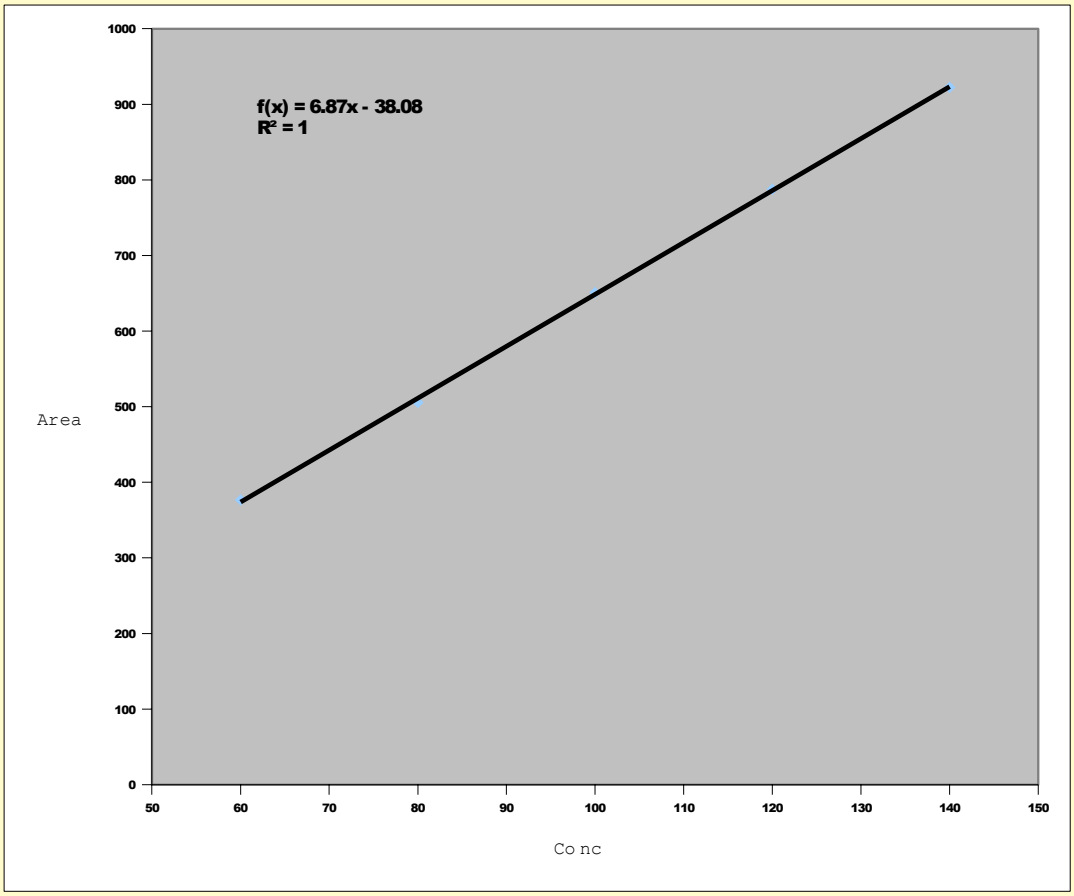

Figure 4: *Linearity of Tolteridone tartarate.*

Conclusion

In the proposed research study, stability of TOL was established based on tests utilizing ICH recommended stress conditions. Mild degradation was observed in acidic, alkali, thermal, light and oxidation conditions. Drug peak was well resolved from the peak of its degradation products as well as those of excipients in formulation. The validated method yielded good results of precision, linearity, accuracy and robustness. The proposed research study was found to be suitable and accurate for the method development and validation of TOL in pharmaceutical formulations. Statistical result and low % RSD values indicate that the method can be used for routine analysis of the drug.

Parameters	Tolteridone tartrate
Linearity range (mg/ml)	0.06 to 0.14
Correlation coefficient (r^2)	0.9999
Slope (m)	6.866
Intercept (c)	38.08
LOD	15.20 mcg/ml
LOQ	46.06 mcg/ml
Tailing factor (T)	1.611
Retention time (min)	2.417
Theoretical plates	4355
(%) RSD	0.63
(%) Accuracy	100.4
(%) Assay	99.4

Table 1: *Validation parameters of Tolteridone tartrate.*

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