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A VALIDATED STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES AND ASSAY OF TOLTERODINE TARTARATE

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ABSTRACT

A simple and accurate reverse phase liquid chromatographic method was developed for the determination of related substances, degradants and assay of Tolterodine Tartarate active pharmaceutical ingredient used in the treatment of urinary incontinence. A HPLC method was developed for the separation of related substances, degradants obtained from samples generated after stress degradation and also for the determination of assay of Tolterodine Tartarate. The separation was achieved using a Water X-terra MS C18, 150x4.6, 3.5 um column, mobile phase contains 0.05% TFA in water as mobile phase A and 0.05% TFA in Acetonitrile as Mobile phase B using a binary gradient mode with flow rate at 1.0 ml/min. The sample concentration was 0.5 mg/ml, detection wavelength was 220 nm. The injection volume was 10 µL. The resolution between the critical pair of peaks (Impurity-2 & Impurity-3) was found to be greater than 4.0. The limit of detection (LOD) and limit of quantification (LOQ) of Impurity-1, Impurity-2 and Impurity-3 were 66 ng/ml and 200 ng/ml respectively for 10 µl injection volume. The test solution and mobile phase was observed to be stable up to 24 h after the preparation. The validated method yielded good results of precision, linearity, accuracy, and robustness. The proposed method was found to be suitable and accurate for the quantitative determination related substances, degradants and assay of compound Tolterodine Tartarate active pharmaceutical ingredient.

Key words: High performance liquid chromatography, Related substances, degradants and assay ,Validation and quantification, Tolterodine Tartarate

INTRODUCTION

Tolterodine Tartarate is described chemically 2-(3-(diisopropylamino)-1- phenylpropyl)-4-methylphenol Tartarate (Fig.1). It is a new muscarinic receptor developed for the treatment of urinary urge incontinence and other symptoms of overactive bladder [1]. Tolterodine Tartarate is a potent muscarinic receptor antagonist that is equipotent to oxybutynin in the bladder, but less potent in salivary glands, with the aim of improving tolerability (less dry mouth) in patients with overactive bladder[2]

A liquid chromatography–tandem mass spectrometry for quanitation of Tolterodine and its 5-hydroxy methyl metabolite in plasma samples reported in the literatrue[3]. Determination of Assay of Tolterodine Tartarate in Plasma, serum and urine by Capillary Gas chromatography coupled with Mass spectrometer was also reported in the literature [4]. An enantio specific HPLC method for the determination of (S)-enantiomer impurities in (R)-tolterodine tartarate [6] and a validated chiral HPLC method for enantiomeric separation of above compound were reported [5]. A stability-indicating HPLC method for determination of Tolterodine Tartarate in dosage forms reported in literature[7] and analytical Method for the Determination of Related Components in Tolterodine Tartarate using LC[8]. So far to our knowledge the reported stability-indicating methods for related substance in active pharmaceutical ingredient are not suitable to

use with mass detection as they involve complex mobile phase mixtures. The proposed stability-indicating HPLC method for determination of related substances, degradants and assay of Tolterodine Tartrate API is simple, fast and compatible with LC-MS detector thus able to provide additional structural information.

Attempts were made to develop a suitable stability-indicating LC method can be used to determine the related substances assay of bulk samples of Tolterodine Tartarate. The present work describes a new, simple and accurate reverse phase liquid chromatographic method for the detection of the process-related impurities and degradation products generated from forced degradation studies that may be present in the bulk drug. The developed method was validated to ensure the compliance in accordance with ICH guidelines and also well suitable for studies on LC-MS.

EXPERIMENTAL

Chemicals and Reagents

Samples of Tolterodine Tartarate and its related substances Impurity-1 (Imp.1), Impurity-2 (Imp.2) and Impurity-3 (Imp.3) (Fig-2) were received from a business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India.

The HPLC grade acetonitrile was purchased from Qualigens fine chemicals, India, Trifluoro acetic acid (TFA) extra pure was purchased from Across organics, USA and HPLC grade water was produced internally by using Milli-Q, Millipore water purification system

Instrumentation

The LC system, used for method development and validation was from Agilent 1200 series (Agilent Technologies Inc., Palo Alto, CA, USA) consists of Quaternary gradient pump, auto sampler, Column oven and variable wavelength detector. The output signal was monitored and processed using chemstation software on Pentium computer (Hewlett Packard).

The LC system used in the degradation studies was an Agilent 1100 series (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with diode array detector. The output signal was monitored and processed using Chemstation software on Pentium computer.

Sample preparation

The stock solutions were prepared separately by dissolving the appropriate amounts of the related substances and compound in diluent, acetonitrile: water, 50:50 (v/v). The target analyte concentration was fixed as 0.5 mg /ml.

RESULTS AND DISCUSSION

Method development

The objective of this work is to develop simple and fast stability-indicating HPLC method for quantification of related substances and assay of Tolterodine Tartarate drug substance. The mixture of related compounds (Imp.1, Imp.2, Imp.3) and Tolterodine Tartarate was used in the method development. Different Reverse phase stationary phases were employed during method development namely Zorbax SB-C8, 150x4.6 mm, 5µm, (Agilent Technologies, USA), Zorbax Eclipse XDB C18, 250 X 4.6, 5µm(Agilent Technologies, USA), and Water X-terra MS C18, 150x 4.6, 3.5 um (Waters corporation, Ireland). Different kind mobile phases with pH ranges 5 to 3 were studied with combination of Acetonitrile. The resolution between Impurity-2 and Impurity-3 was critical also the tailing of peak was observed high.

Optimized Chromatographic Conditions

Chromatographic separations were achieved on Water X-terra MS C18, 150x 4.6, 3.5 um column using the mobile phase contains 0.05% TFA in water as mobile A and 0.05% TFA in Acetonitrile as Mobile phase B in binary gradient using below conditions. Mobile phase composition maintained using Liner gradient consisting of T/%B: 0/30, 15/80, 20/80 with flow rate of the mobile phase 1.0 ml/min. The test sample concentration was 0.5 mg/ml in diluent, Acetonitrile: water, 50:50 (v/v). Detection wavelength was 220 nm. The injection volume was 10 µL. The total analysis time for each run was 20 min. Good separations of all impurities and degradants within short run time were observed on Water X-terra MS C18, 3.5 µm column.

Typical retention times of Imp.1, Tolterodine, Imp.2, and Imp.3 is 4.9 min., 5.9 min.10.0 and 10.8 min. respectively. The system suitability[10] results were given in Table 1.

Method Validation

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.[9]. Specificity was tested by injecting the spiked sample of Tolterodine with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, identification of each impurity was confirmed with retention time as compared with those of pure standards.

Forced degradation studies were performed for bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of Photolytic degradation as per ICH Q1B, Thermal degradation (at 60° C), acid hydrolysis (using 1 N HCl), base hydrolysis (using 1 N NaOH), and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of the proposed method to separate Tolterodine from its degradation products. For heat and light studies, study period was 10 days where as for acid, base, and oxidative degradation it was about 48 hours. The samples degraded with light and heat showed no additional peaks. In the Acid and base significant degradation happened. To check and ensure the homogeneity of Toltorodine peak in the stressed sample solutions, diode array detector was employed. For content of product after degradation mass balance was considered, results found to be about 99.7%, which was calculated by adding together assay value and levels of degradation.

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions [9]. The method precision for 0.5 mg/ml Toltorodine Tartarate spiked with 0.15% of Imp.1, Imp.2 and Imp.3 with respect to analyte concentration the percentage relative standard deviation (%RSD) of method repeatability for impurities is found to be between 1.8 and 4.4%. For assay method precision was performed at 0.5mg/ml concentration, the relative standard deviation for Tolterodine peak is 0.15% confirm the good precision of the method for impurities and analyte.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample [9]. The linearity of the method was checked at six concentration levels i.e. from LOQ to 2500 ng/ml of Imp.1, Imp.2, Imp.2 and Toltorodine Tartarate. The coefficient of regression of the calibration curve was found to be greater than 0.99. Also linearity studies of Toltorodine Tartarate peaks was studied at 80%, 100% and 120% with respect to test sample concentration (0.5mg/ml), correlation coefficient was found to be 0.999 was thus confirming the excellent correlation existed between the peak area and concentration of the impurities and Toltorodine peak.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) represents the concentration of analyte that would yield a signal to noise ratio of 3 [9]. The limit of detection (LOD) of Imp.1, Imp.2 and Imp.3 were 66 ng/ml for 10 µl injection volume. The limit of quantification (LOQ) represents the concentration of analyte that would yield a signal to noise ratio of 10 [9]. The limit of quantification (LOQ) of Imp.1, Imp.2 and Imp.3 were 200 ng/ml for 10 µl injection volume. The precision for Imp-1, Imp-2 and Imp-3 at LOQ level was good, the relative standard deviation was found to be below 6.5%.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification Imp.1, Imp.2 and Imp.3. The study was carried out at LOQ, 0.08, 0.15, 0.3 and 0.5 % of target analyte concentration (0.5 mg/ml) of Imp.1, Imp.2 and Imp.3. The percentage

recoveries of impurities were ranged from 80% to 115% in samples of Toltorodine Tartarate. Recovery of Toltorodine Tartarate study carry out at 80% , 100% and 120% with respect to analyte concentration (0.5mg/ml) and the percent of recovery found to be between 99 and 100%.

Robustness

The robustness of an analytical procedure [9] is measure of its capability to remain unaffected by small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions viz. flow rate and mobile phase composition, the resolution between Toltorodine, Imp.1, Imp.2 and Imp.3 peaks was found to be > 4.0 illustrating the robustness of the method.

Solution stability and mobile phase stability

Solution stability was studied by keeping the test solution spiked with impurities in tightly capped volumetric flask at temperature $25^{o} \pm 2^{o}$ C on a laboratory bench for 24 h. Content of impurities was checked for every 6 h interval and compared with freshly prepared solution. No variation was observed in the content of impurities in sample solutions prepared in diluent were stable up to 24 h.

Mobile phase stability was carried out by evaluating the content of impurities in sample solution spiked with impurities, which were prepared freshly at every 6 h up to 24 h. The same mobile phase was used during the study period. No variation was observed in the content of impurities for the study period and it indicates prepared mobile phase was found to be stable up to 24 h.

CONCLUSION

In this study, the simple, accurate and well-defined stability indicating HPLC method for the determination of related substances, degradation products and assay of Toltorodine Tartarate was described. The behavior of Toltorodine Tartarate under various stress conditions were studied and presented. The proposed method is compatible with mass detector can be employed for studies on LC-MS. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies

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Table-1: System suitability report

Compound	USP Resolution (*Rs*)	USP tailing	No. of theoretical plates (*N*) USP Tangent method
Impurity 1	--	1.098	12605
Toltorodine	4.926	1.638	10059
Impurity 2	20.503	1.117	56324
Impurity 3	4.284	1.148	63176

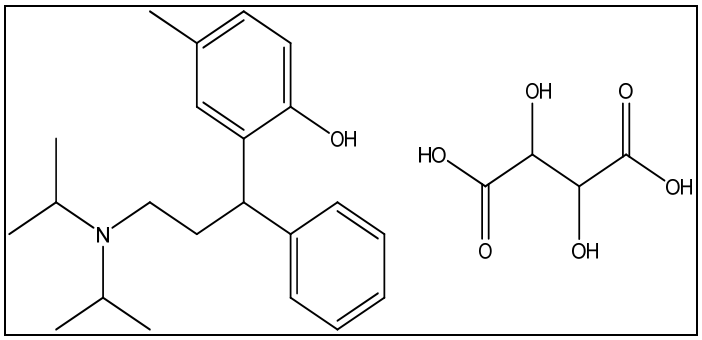
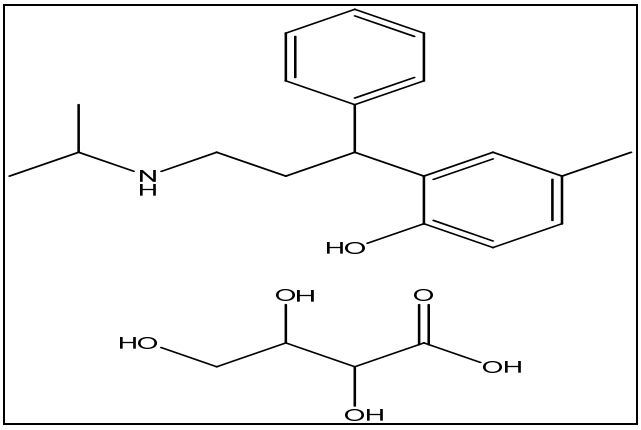
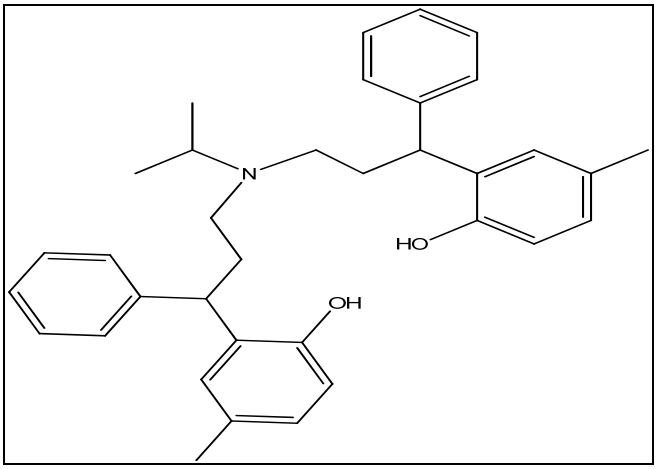


Fig-1 : Chemical structures of Tolterodine Tartarate

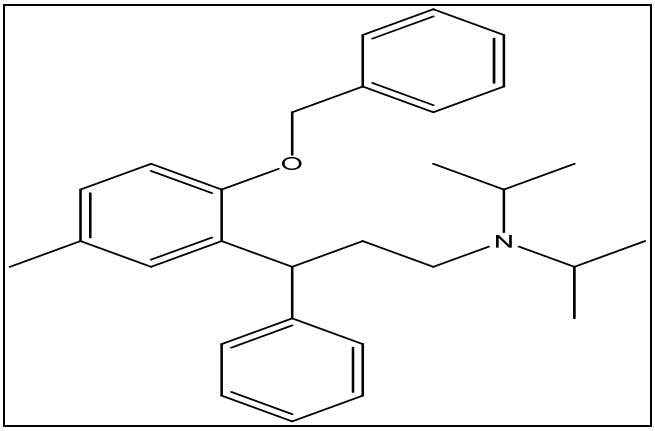
Tolterodine Tartarate**:** 2-(3-(diisopropylamino)-1- phenylpropyl)-4-methylphenol Tartarate



Impurity-1 : N-Isopropyl-3-(2-hydroxy-5-methyl phenyl)-3-phenyl propylamine-L-hydrogen tatrate



Impurity-2 : 2,2'-(3,3'-(isopropylazanediyl)bis(1-phenylpropane-3,1-diyl))bis(4-methylphenol)



Impurity-3: N,N-Diisopropyl-3-(2-benzyloxy-5-methyl phenyl)-3-phenyl propylamine
Fig-2 : Chemical structures of Impurity-1, Impurity -2 and Impurity-3

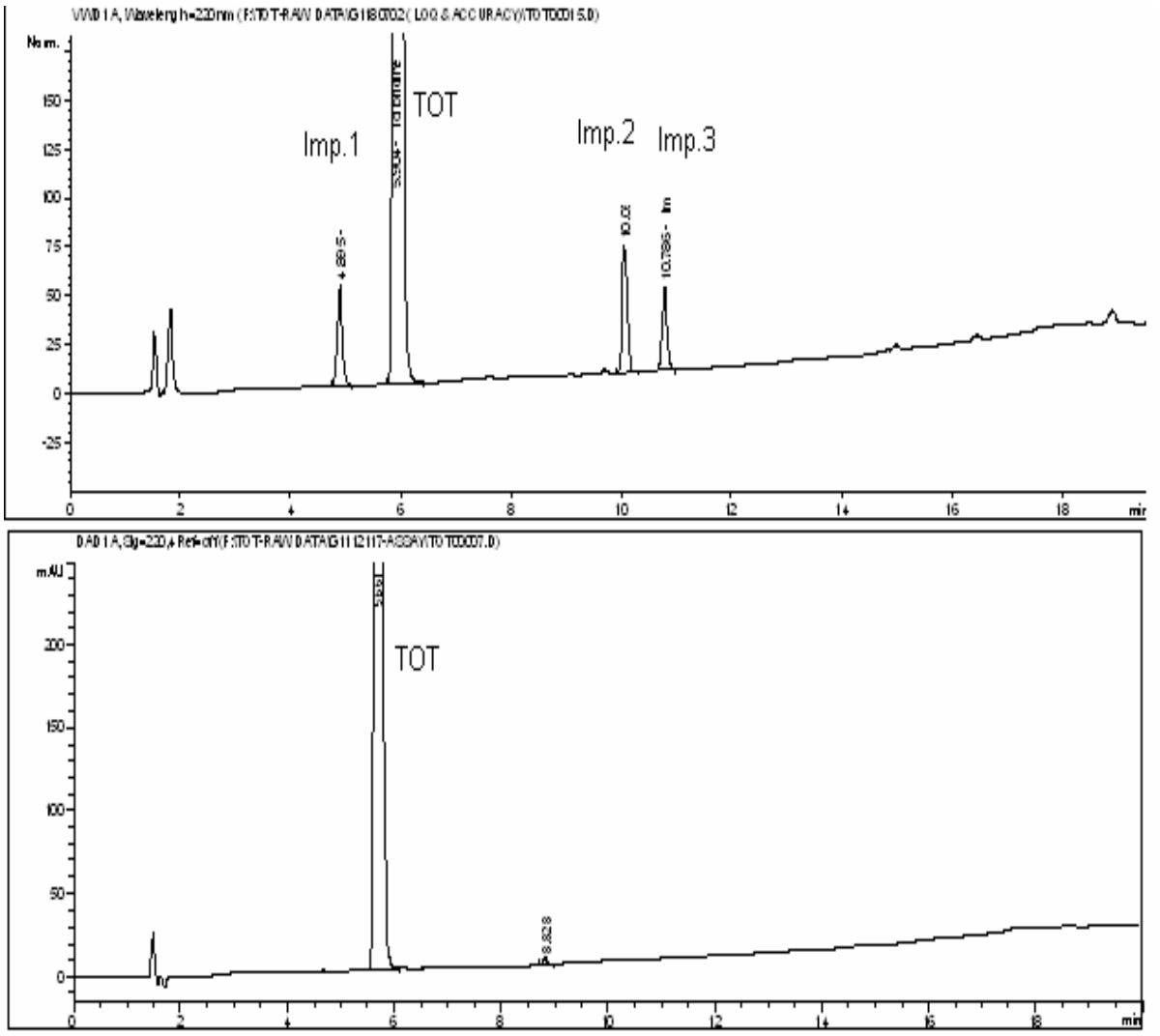


Fig-3: HPLC chromatograms of Impurities spiked (Imp-A, Imp-B and Imp-C) in pure Tolterodine Tartarate and Tolterodine Tartarate sample

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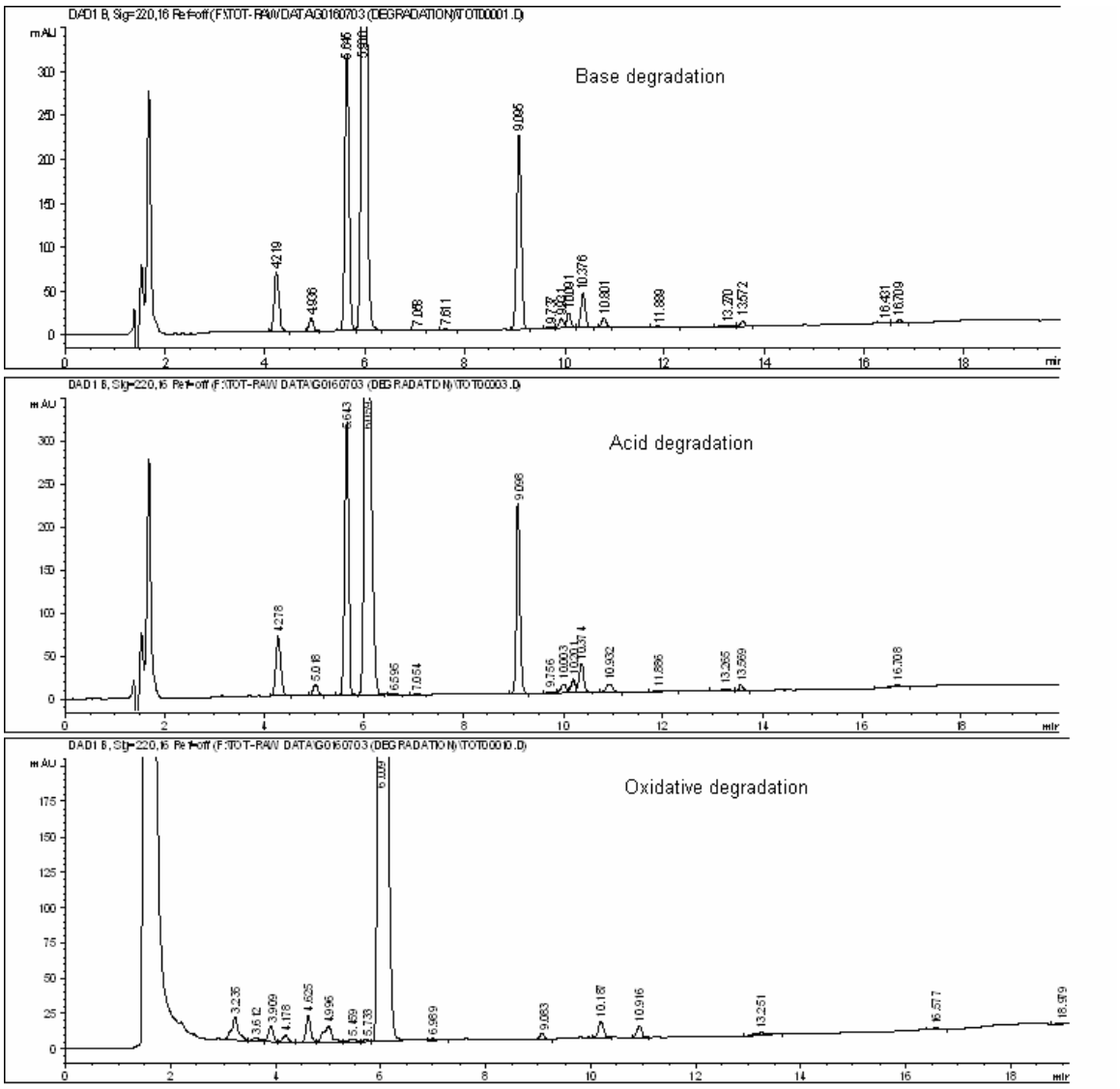

Fig-4: Typical HPLC chromatograms of stressed test samples of Tolterodine Tartarate

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"Progress is impossible without change, and those who cannot change their minds cannot change anything."

- George Bernard Shaw