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A VALIDATED STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES IN QUETIAPINE FUMARATE

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ABSTRACT

A simple and accurate reverse phase liquid chromatographic method was developed for the determination of related substance and degradants of Quetiapine Fumarate bulk drug used as antipsychotic agent for the management of the manifestations of schizophrenia. Chromatographic separation between Quetiapine Fumarate its related substances and degradants was obtained from samples generated after stress degradation. The separation was achieved using a X-bridge C18, 150x4.6 mm, 3.5 µm column, mobile phase contains 5 mM ammonium Acetate as mobile phase A and Acetonitrile as Mobile phase B using a binary gradient mode with flow rate of the mobile phase kept at 1.0 ml/min. The sample concentration was 0.5 mg/ml. The column temperature was maintained at 40°C and the detection wavelength was 220 nm. The injection volume was 10 µL. The resolution between the critical pair of peaks (Impurity-B & analyte) was found to be greater than 4.5. The limit of detection (LOD) and limit of quantification (LOQ) of Impurity-A, Impurity-B and analyte were 27 ng mL^{-1} and 80 ng/ml, for Impurity-3 was 14 ng/ml and 40ng/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 and degradants during quality control of Quetiapine Fumarate active pharmaceutical ingredient.

Key words: *High performance liquid chromatography,Related substances and degradants ,Validation and quantification,Quetiapine Fumarate.*

INTRODUCTION

Quetiapine Fumarate is described chemically as 11-[4-[2-(2-Hydroxy ethoxy) ethyl]-1-piperazinyl] dibenzo (b, f) (1, 4) thiazepine hemifumarate (Fig.1). It is one of the recent "atypical" antipsychotic drug [1] used in treatment of patients with bipolar I disorders.

A liquid chromatography–atmospheric pressure chemical ionization mass spectrometry method for the quantitative determination of Quetiapine and its metabolites from human plasma samples was reported in the literature [2]. A liquid chromatographic method for the determination of Quetiapine and fluvoxamine from human plasma using UV detection has been developed and reported in the literature [3]. Two different methods capillary zone electrophoretic (CZE) method and spectrophotometric method for estimation of Quetiapine in commercial formulations reported [4]. An automated HPLC method with column switching was described for the determination of Quetiapine, Clozapine, Perazine, Olanzapine and metabolites in blood serum [5]. Two other liquid chromatographic methods were reported using UV and electrochemical

detection for estimation of Quetiapine in plasma samples. [6, 7] So far to our knowledge no stability indicating HPLC method for determination of related substances of Quetiapine Fumarate has been reported.

Attempts were made to develop a suitable stability indicating LC method that can be used to determine the related substances bulk samples of Quetiapine Fumarate. 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 which may be present in the bulk drug. The developed method was validated to ensure the compliance in accordance with ICH guidelines.

EXPERIMENTAL

Chemicals and Reagents

Samples of Quetiapine Fumarate and its related substances Impurity-A (Imp.A), Impurity-B (Imp.B) and Impurity-C (Imp.C) (Fig-2) were received from a business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India.

The HPLC grade acetonitrile was purchased from Rankem fine chemicals, India, Analytical Reagent grade ammonium Acetate, ammonium formate 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 (Digital Equipment Co).

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^{-1}.

RESULTS AND DISCUSSION

The objective of this work is to develop suitable stability indicating HPLC method for quantify related substances and degradation products that were present in Quetiapine Fumarate drug substance. The mixture of related compounds (Imp.A, Imp.B, Imp.C) and Quetiapine Fumarate was used in the method development. Different Reverse phase stationary phases were employed during method development namely Zorbax SB-C8, 250x4.6 mm, 5µm, (Agilent Technologies, USA), Luna C-18 150x4.6 mm, 3 µm (Phenomenex, USA) ; ACE C-18, 150x4.0 mm, 3 µm (Advanced chromatographic Technologies, Scotland), Zorbax SB-Phenyl, 150x4.6 mm, 5 µm (Agilent Technologies, USA), Zorbax SB-C18, 150x4.6 mm, 3.5µm, (Agilent Technologies), and X-bridge C18, 150x4.6 mm, 3.5 µm (Waters Corporation, Ireland). Different trails were made during the method development and the details were mentioned in the Table 1.

Optimized Chromatographic Conditions

Chromatographic separations were achieved only on X-bridge C18, 150x4.6 mm, 3.5 μm column using the mobile phase contains 5 mM ammonium acetate as mobile A and Acetonitrile as Mobile phase B in binary gradient using below conditions. Mobile phase composition maintained using Liner gradient consisting of %B/T: 0/35, 16/35, 25/90, 30/90 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). The column temperature was maintained at 40°C and the detection wavelength was 220 nm. The injection volume was 10 μL. The total analysis time for each run was 30 min. Good separations of all impurities and degradants within short run time were observed on X-bridge C18, 150X4.6 mm 3.5 μm column.

Typical retention times of Imp.A, Imp.B, and Imp.C is 5.5 min., 11.7 min. and 25.0 min. respectively. The system suitability [8] results were given in Table 2.

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 Quetiapine 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 0.5 N HCl), base hydrolysis (using 0.5 N NaOH), and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of the proposed method to separate Quetiapine from its degradation products. For heat and light studies, study period was 10 days where as for acid, base, and oxidative degradation it was 48 hours. To check and ensure the homogeneity of Quetiapine peak in the stressed sample solutions, diode array detector was employed.

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 system and method precision for 0.5 mg/ml Quetiapine Fumarate spiked with 0.10% of Imp.A, Imp.B and Imp.C with respect to analyte concentration the percentage relative standard deviation (%RSD) of method repeatability and system repeatability for impurities was confirms good precision of the method. Results were given in Table 3

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.A, Imp.B, Imp.C and Quetiapine Fumarate. The coefficient of regression of the calibration curve

was found to be greater than 0.99, thus confirming the excellent correlation existed between the peak area and concentration of the impurities

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.A, Imp.B and Imp.C were 27 ng/ml, 27 ng/ml and 14 ng/ml respectively 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.A, Imp.B and Imp.C were 80 ng/ml, 80 ng/ml and 40 ng/ml for 10 µl injection volume. The precision for Imp-A, Imp-B and Imp-C at LOQ level was good, the relative standard deviation was found to be below 3.5%.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification Imp.A, Imp.B and Imp.C. The study was carried out at LOQ, 0.1, 0.2 0.3, and 0.5 % of target analyte concentration (0.5 mg/ml) of Imp.A, Imp.B and Imp.C. The percentage recoveries of impurities were ranged from 90.7 to 103.9 in samples of Quetiapine Fumarate.

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, mobile phase ratio and column temperature, the resolution between Quetiapine, Imp.A, Imp.B and Imp.C 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 for 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 and degradation products in Quetiapine Fumarate was described. The behavior of Quetiapine Fumarate under various stress conditions were studied and presented. 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 : Results of different trails

Trial	HPLC conditions	Remarks	number
1	Column: Zorbax SB-C8, 250X4.6 mm, 5µ Mobile phase: 0.1% v/v TFA in water(MP-A) and 0.1% v/v TFA in Acetonitrile (MP-B) and Flow rate: 1.0 ml/min, Column temperature: 40 °C	Poor resolution between Impurities analyte peaks	
2	Column: Luna C-18 150X4.6 mm, 3 µm Mobile phase: 0.1% v/v TFA in water and 0.1% v/v TFA in Acetonitrile Flow rate: 1.0 ml/min, Column temperature: 40 °C	Poor resolution between Impurities and analyte peaks	
3	Column: Luna C-18 150X4.6mm, 3 µm Mobile phase: 10 mM Ammonium Acetate and (MP-A) and Acetonitrile MP-B) Flow rate: 1.0 ml/min Column temperature: 40 °C	Poor resolution between Imp.B and analyte Peak shape of Imp. A is broad	
4	Column: ACE C-18, 150X4.0 mm, 3 µm Mobile phase :10 mM Ammonium Acetate and and Acetonitrile MP-B) Flow rate: 1.0 ml/min Column temperature: 40 °C	Retention of Impurity-C is high, at high solvent concentration the resoluton of Imp.B and analyte is poor	(MP-A)
5	Column: Zorbax SB-Phenyl, 150x4.6 mm, 5 µm Mobile phase :10 mM Ammonium Acetate and (MP-A) and Acetonitrile MP-B) Flow rate: 1.0 ml/min, Column temperature: 40 °C	Peak tailing is more	
6	Column: Zorbax SB-C18, 150X4.6 mm, 3.5µm Mobile phase: 10 mM Ammonium Acetate and (MP-A) and Acetonitrile MP-B) Flow rate: 1.0 ml/min, Column temperature: 40 °C	Resolution between Imp.A and Imp.B is poor	
7	Column: X-bridge C18, 150X4.6 mm, 3.5 µm Mobile phase: 10 mM Ammonium formate (MP-A), Acetonitrile (MP-B) Flow rate: 1.0 ml/min, Column temperature: 40 °C	AnalytePeak shape is poor	
8	Column: X-bridge C18, 150X4.6 3.5 µm Mobile phase: 10 mM AmmoniumAcetate (MP-A), Acetonitrile (MP-B) Flow rate: 1.0 ml/min, Column temperature: 40 °C	Drifit in base line is high	

Table-2:System suitability report

Compound	USP Resolution (*Rs*)	USP tailing	No. of theoretical plates (*N*) USP Tangent method
Impurity-A	--	1.866	8142
Impurity-B	18.694	1.169	12275
Quetiapine	4.588	0.877	11249
Impurity-C	32.134	1.196	370785

Table-3 : Precision results

	IMP.A	IMP.B	IMP.C
Method precision (%RSD)	1.53	0.66	6.6
System precision (%RSD)	1.91	1.36	3.71

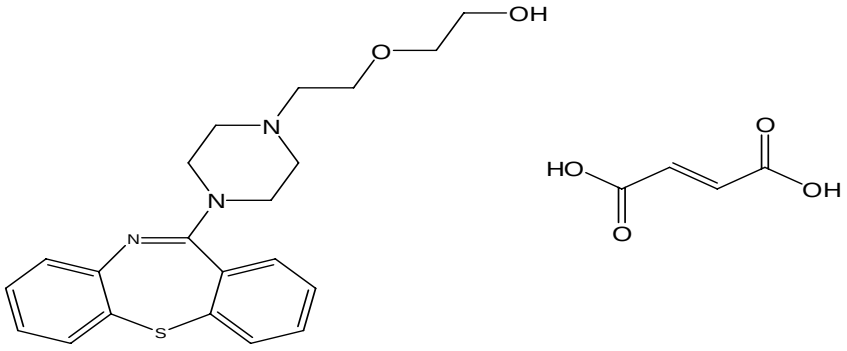
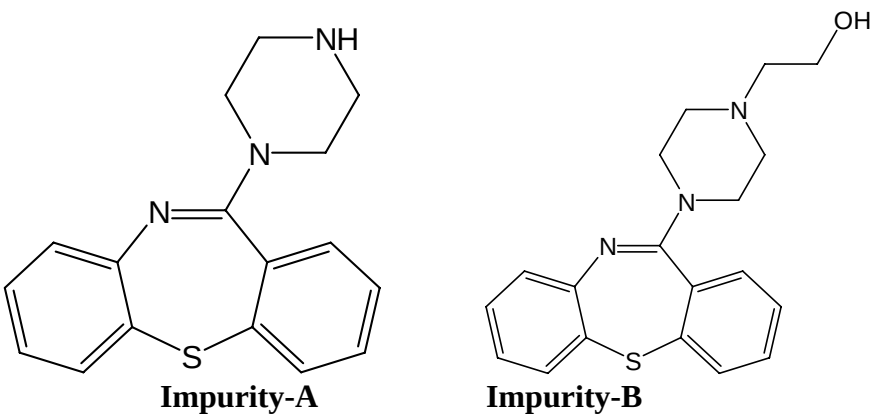


Fig-1 : Chemical structures of Quetiapine Fumarate: 11-[4-[2-(2-Hydroxy ethoxy) ethyl]-1-piperazinyl] dibenzo (b, f) (1, 4) thiazepine hemifumarate



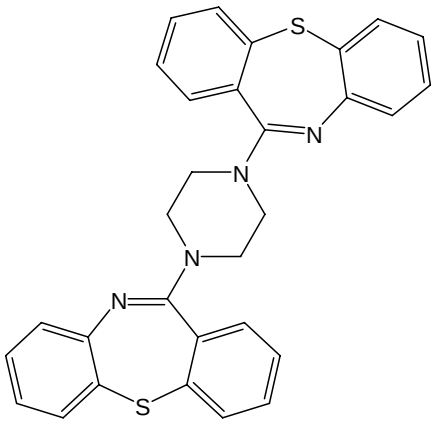


Fig-2 : Chemical structures of Impurity-A, Impurity -B and Impurity-C
Impurity-A : 11-Piperazinyl-dibenzo(b,f) (1,4)-thiazepine
Impurity-B : 11-[[4-(2-Hydroxyethyl)]-1-piperazinyl] dibenzo [b,f][1,4]thiazepine
Impurity-C : 11-[[4- dibenzo[b,f][1,4]thiazepine-11-yl]piperazinyl]dibenzo[b,f][1,4]thiazepine

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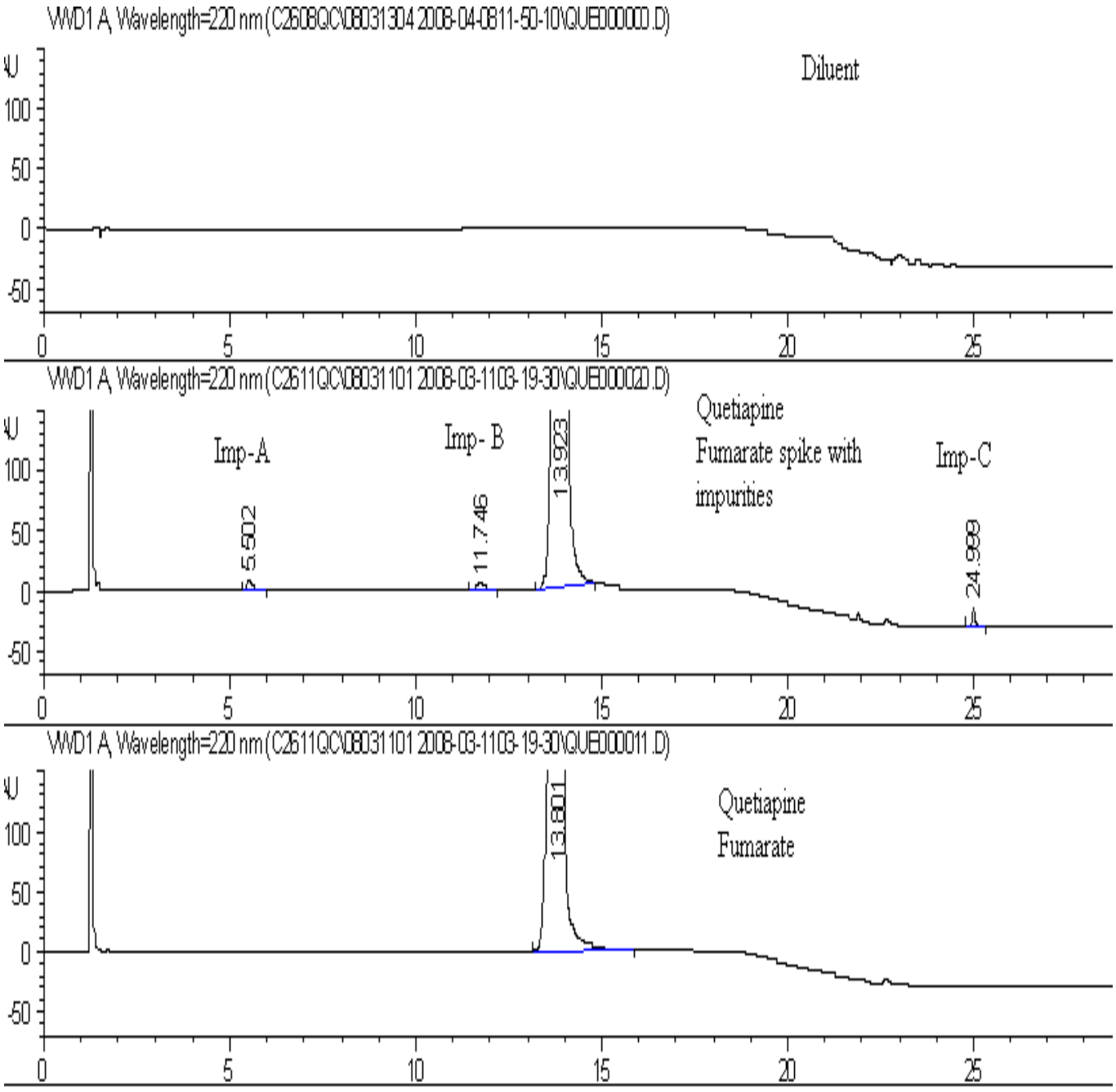

Fig-3 : HPLC chromatograms of blank run, Impurities spiked (Imp-A, Imp-B and Imp-C) in pure Quetiapine Fumarate and Quetiapine Fumarate samples

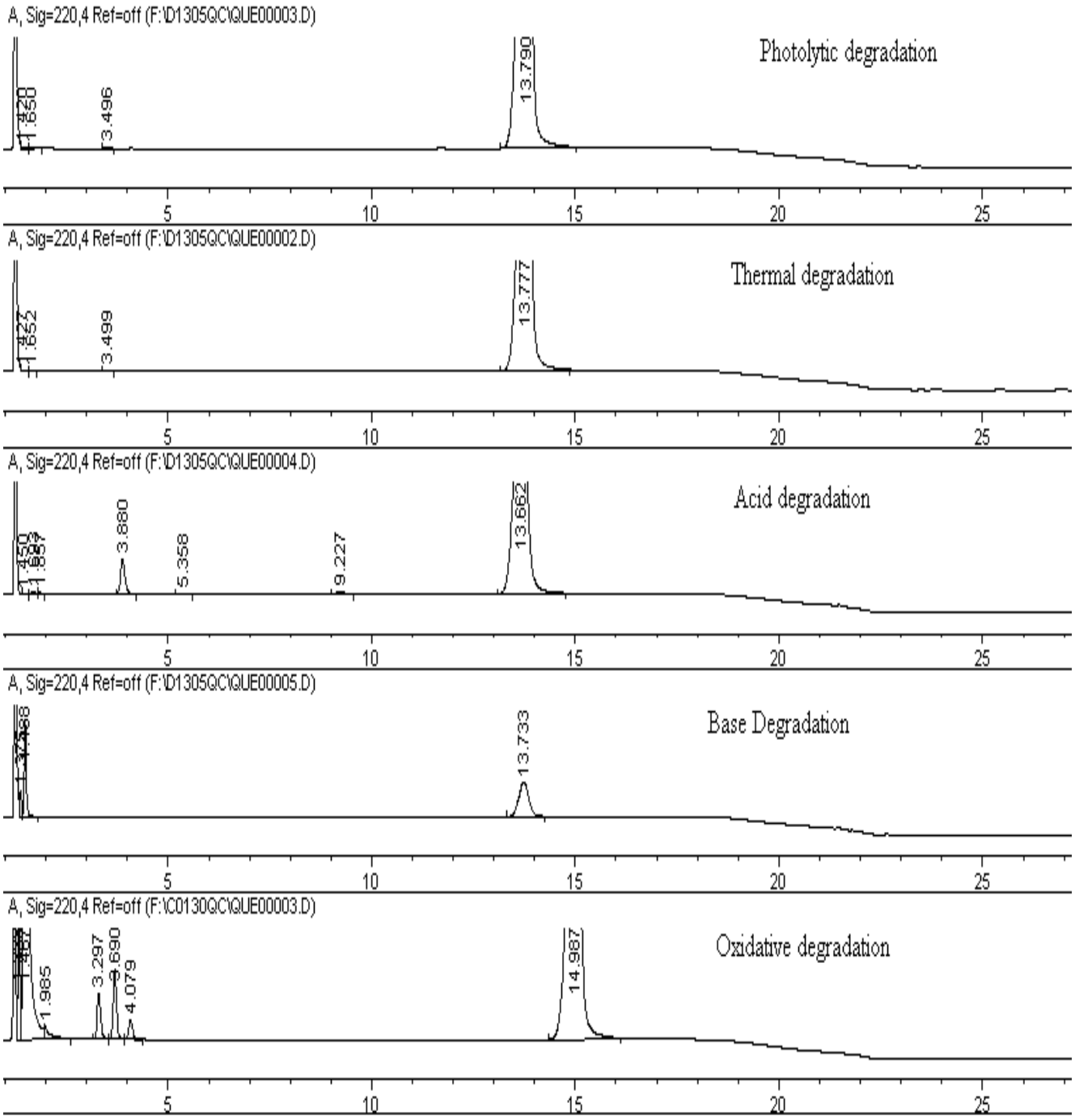

Fig-4: Typical HPLC chromatograms of stressed test samples of Quetiapine Fumarate

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