Supporting Information for op-00097q:

Stability-Indicating Specific and Accurate HPLC Method for Determination of Related Substances in Quetiapine Hemifumarate

A simple reversed-phase liquid chromatographic method was developed for the related substances determination in quetiapine hemifumarate, an antipsychotic drug. Forced degradation studies were performed on bulk sample of quetiapine hemifumarate using acid, base, oxidative hydrolysis, thermal stress and photolytic degradation. Considerable degradation of the drug substance was observed during oxidative and acid hydrolysis. The chromatographic method was fine tuned using the samples generated from forced degradation studies and eight process related impurities (Imp-1 to Imp-8) identified by process R&D team. Good resolution between the peaks corresponds to synthetic impurities and degradation products from the analyte were achieved on Zorbax eclipsed XDB C18 column. The stressed test solutions were assayed against the qualified working standard of quetiapine hemifumarate and the mass balance in each case was close to 99.9% indicating that the developed method was stability-indicating. Validation of the developed method was carried out as per ICH requirements.

Introduction

Quetiapine hemifumarate (1), a dibenzothiazepine derivative is described chemically as 2-[2-(4-dibenzo [b,f][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]-ethanol fumarate (2:1) has international approvals for the treatment of schizophrenia as well as for the treatment of acute manic episodes associated with bipolar I disorder as either monotherapy or adjunct therapy to lithium or divalproex [1-3]. An HPLC separation method for the determination of

impurities, a stability indicating reversed phase high-performance chromatographic (RP-HPLC) method for the determination of quetiapine, and few other methods for the quantitative determination of quetiapine in biological samples have been reported in the literature [4-9]. Reported methods were found to be limited as many of the impurities were not covered these methods. The analysis of crude quetiapine hemifumarate generated during the development has shown eight impurities (Imp-1 to Imp-8) whose area percentage ranged from 0.05 to 0.2% consistently. All these impurities are identified, synthesized and characterized with the help of LC-MS, IR and NMR. Isocratic reverse phase liquid chromatography method developed initially was not able to elute imp-8 due to its non-polar nature and to the best of our knowledge Imp-7 is not reported. In the present study, attempts were made to develop a more precise and accurate stability-indicating HPLC method, which can separate all the eight potential impurities (Imp-1 to Imp-8) generated during the synthesis and degradation impurities formed during the forced decomposition studies of quetiapine hemifumarate.

Experimental

Chemicals

Sample of Quetiapine hemifumarate and its eight potential process related impurities (Fig.1) were received from synthetic laboratory of Megafine Pharma (P) Ltd, Nashik, India. HPLC grade acetonitrile was purchased from Qualigen fine chemicals, Mumbai, India. Potassium dihydrogen orthophosphate was purchased from Merck, Mumbai, India. 1-Pentane sulphonic acid sodium salt was purchased from Merck, Mumbai, India. Potassium hydroxide was purchased from Merck, Mumbai, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Chemical name of the impurities

Imp-1. dibenzo[b,f][1,4]thiazepin-11(10H)-one,

Imp-2. 11-piperazin-1-yldibenzo[b,f][1,4]thiazepine ,

Imp-3. 2-(4-dibenzo[b,f][1,4]thiazepin-11-ylpiperazin-1-yl)ethanol

Imp-4. 11-(4-ethylpiperazin-1-yl)dibenzo[b,f][1,4] thiazepine

Imp-5.11-chlorodibenzo[b,f][1,4]thiazepine

Imp-6. phenyl[2-(phenylthio)phenyl] carbamate

Imp-7. N-methyl-N-phenyldibenzo[b,f] [1,4] thiazepin-11-amine

Imp-8. bis(dibenzo)piperazine

Equipment

The LC system used for method development, forced degradation studies and method validation was Agilent 1200 series (manufactured by Agilent technologies, 76337 Waldbronn Germany) with an Agilent photodiode array detector (PDA) and variable wavelength detector (VWD). The output signal was monitored and processed using Ezchrome Elite software version 3.2.1. The Zorbax Eclipse XDB C18 (250 mm length x 4.6 mm ID, 5µm particle size) column has been procured from Agilent technologies, made in USA and used for the method development, forced degradation studies and method validation.

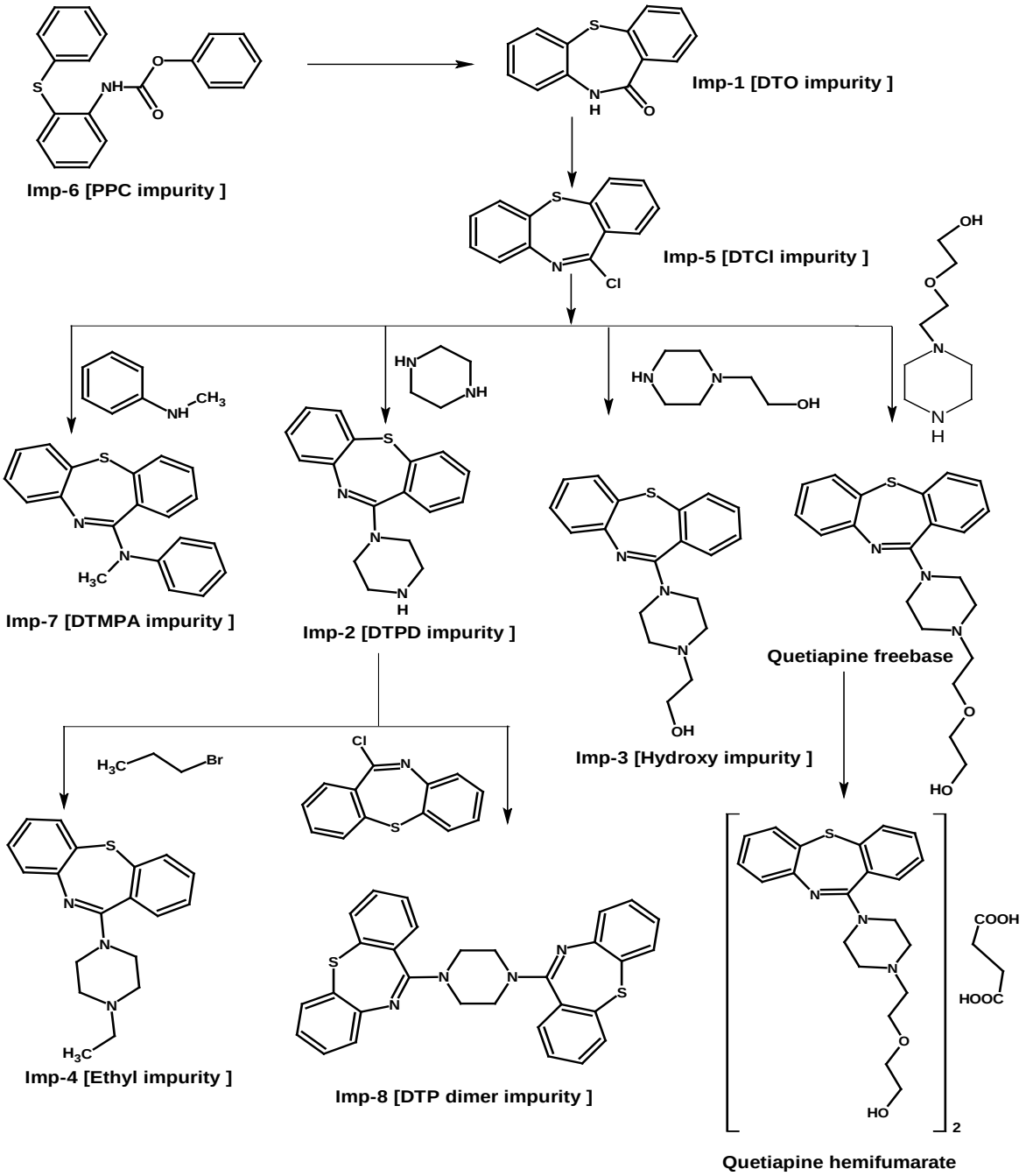


Fig.1. Synthetic scheme for quetiapine hemifumarate with formation of its process related impurities

Chromatographic conditions

The conditions established involve; the mobile phase solvent-A consists of a mixture of 0.025 M potassium dihydrogen orthophosphate and 0.01 M 1-pentane sulphonic acid sodium salt in water. The mobile phase solvent-B consists of a mixture of acetonitrile,

methanol and water in the ratio of 45:45:10 v/v. The flow rate of the mobile phase was kept at 1.0 mL min^{-1}. The HPLC gradient was set as: T/%B: 0/45, 5/45, 40/90, 55/90, 58/45 and 65/45. The column temperature was maintained at 40°C and the detection wavelength was 250 nm. The test concentration was about 1.0 mg mL^{-1} (i.e. 1000 µg mL^{-1}) and the injection volume was 20 µL for related substances determination. A degassed mixture of water and acetonitrile (20:80 v/v) was used as diluent during the standard and test sample preparation.

Preparation of Standard Solutions

A working standard solution of 1000 µg mL^{-1} and test sample solution of 1000 µg mL^{-1} was prepared for the determination of related substances analysis. A stock solution of impurity (mixture of Imp-1 to 8) at 150 µg mL^{-1} was also prepared in diluent.

Method Validation

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The specificity of the developed LC method for Quetiapine hemifumarate was carried out in the presence of its eight impurities viz., Imp-1 to Imp-8 as shown in Fig.1.

Stress degradation studies were performed on bulk drug substance to provide an indication of the stability-indicating property and specificity of the established method. Intentional stress conditions, such as photolytic degradation, thermal degradation (drug substance exposed in solid state at 105 °C), acid hydrolysis (using 5M hydrochloric acid, sample was refluxed in oil bath for 1 days), base hydrolysis (using 5M sodium hydroxide, sample was refluxed in oil bath for 1 days) and oxidative degradation (using 30%

hydrogen peroxide at RT) to evaluate the ability of the proposed method to separate quetiapine from its degradation products were applied as per ICH recommendations. For acid hydrolysis, base hydrolysis and oxidative degradation studies the period was 1 day, 1 day and 2 h respectively, where as for photolytic and thermal the period was 8 days. Photodiode array detector was employed to check and ensure the homogeneity and purity of quetiapine peak in all the stressed sample solutions.

Precision

The precision of the related substance method was checked by injecting six individual preparations of quetiapine hemifumarate spiked with 0.15% of all the eight impurities (Imp-1 to Imp-8) with respect to target analyte concentration (i.e. 1.0 mg mL^{-1}). The percentage of added impurities in six different spiked test preparations was calculated. The RSD was calculated for percentage of each impurity (Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8) in each spiked test preparation.

The intermediate precision of the method was also verified using different analyst, different day and different instrument number in the same laboratory. The results of method precision and intermediate precision were expressed in terms of percentage of impurities in six spiked test preparation. The percentage RSD of results of intermediate precision and results of method precision was calculated and compared with each other.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of all the eight impurities were determined using calibration curve method according to ICH Q2R1 recommendations by establishing the lowest concentration

that can be measured. Precision study was also carried out at the LOQ level by injecting six individual preparations of all the impurities and calculating RSD of the area.

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. Linearity test solutions for related substance method were prepared by diluting the impurity stock solution (as described above) to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 250% with respect to the impurities specification level of 0.15% (i.e. LOQ. 0.075, 0.150. 0.225, 0.300 and 0.375%). The calibration curve was drawn by plotting the peak areas of each impurity versus its respective concentration. The correlation coefficient, intercept and slope of the calibration curve were calculated & reported.

Accuracy

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 value found. The recovery experiments were conducted to determine accuracy of the related substance method for the quantification of all eight impurities in bulk drug samples. The study was carried out in triplicate at four concentration levels i.e. LOQ, 0.075, 0.15 and 0.225 % of the analyte concentration (1000 µg mL^{-1}). The percentage recoveries were calculated by using following formula:

$$\% \text{ Recovery} = \frac{\text{Amount recovered}}{\text{Amount added}} \times 100$$

Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between closely eluting impurities i.e. Imp-1 and Imp-2, Imp-4 and Imp-5, was evaluated. The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the resolution, the same was altered by 0.1 units i.e. from 0.9 to 1.1 mL min^{-1}. The effect of column temperature on resolution was studied at 37 and 43 °C instead of 40 °C, also the effect of buffer pH ± 0.1 (6.5 and 6.7 instead of 6.6) on resolution was studied. All the other mobile phase components were held constant as described above.

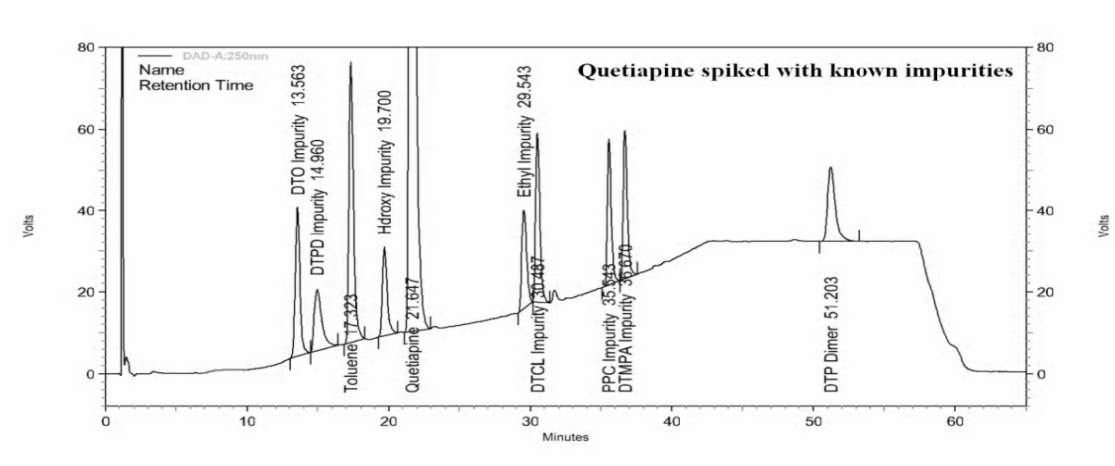
Results and Discussion

Optimization of Chromatographic Conditions

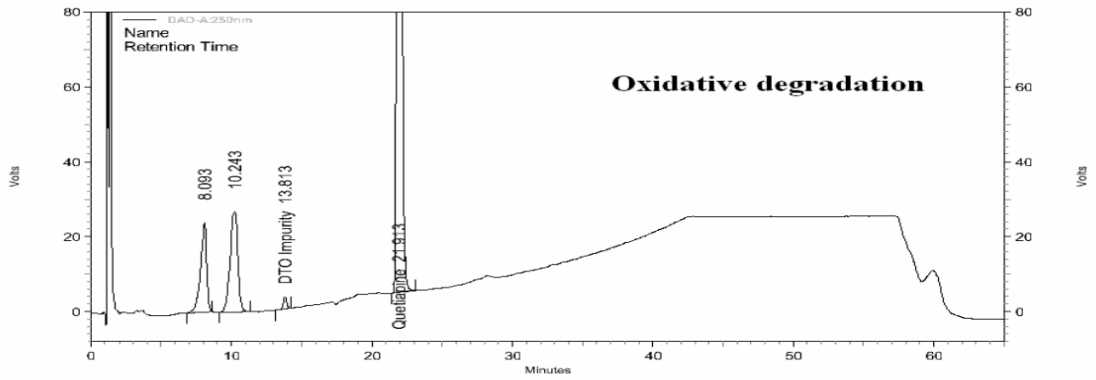
The main target of developing the chromatographic method is to detect and determine the potential impurity (Imp-8) present in bulk samples produced by Megafine Pharma (P) Ltd., and to achieve the separation of all eight impurities (Fig.1) along with the degradation products generated during stress studies from the analyte peak. Impurities were co-eluted by using different stationary phases like C8, Cyano and Phenyl and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (3.0-7.0) and using organic modifiers like acetonitrile and methanol in the mobile phase. Apart from the co-elution of impurities, poor peak shapes for some impurities and quetiapine were also noticed. Satisfactory chromatographic separation was achieved on Zorbax XDB C18 column using mobile phase consisting a mixture of 0.025M potassium dihydrogen orthophosphate and 0.01M 1-pentane sulphonic acid sodium salt dihydrate solution in water as solvent A and a mixture of acetonitrile, methanol and water in the ratio of 45:45:10 v/v as solvent B. The flow

rate of the mobile phase was kept at 1.0 mL min^{-1}. The HPLC gradient of mobile phase-B was kept as: T/%B: 0/45, 5/45, 40/90, 55/90, 58/45 and 65/45. In the optimized conditions the quetiapine, and all the eight impurities were well separated with a resolution greater than 1.5.

a) **Chromatogram of quetiapine spiked with known impurities**



b) **Chromatogram of quetiapine test sample in oxidative degradation**



c) **Chromatogram of quetiapine test sample in acid degradation**

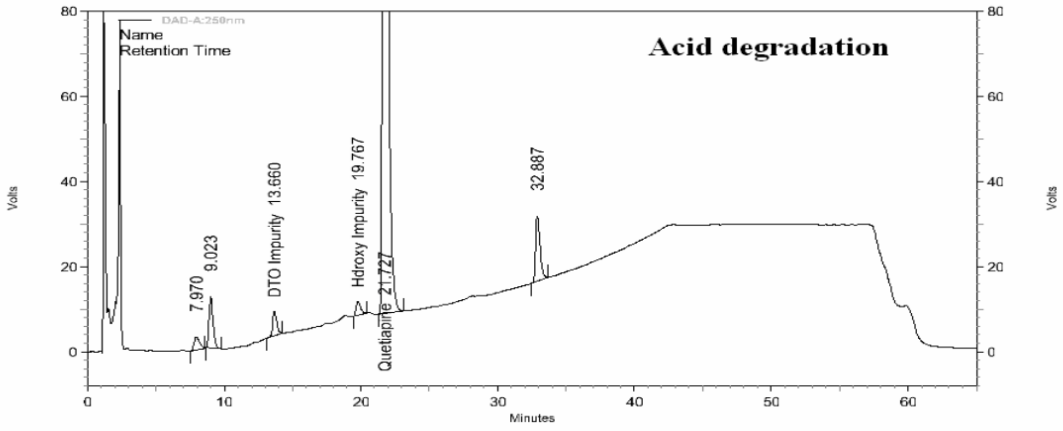


Fig.2. HPLC chromatograms of; a) quetiapine spiked with known impurities (Imp-1 to Imp-8), b) oxidative degradation, c) acid degradation.

Specificity

The typical retention times of Imp-1, Imp-2, Imp-3, quetiapine, Imp-4, Imp-5, Imp-6, Imp-7, and Imp-8 were about 13.6, 15.0, 19.7, 21.7, 29.5, 30.5, 35.5, 36.7and 51.2 min respectively (Fig 2a), and the developed LC method was found to be specific for quetiapine and its eight impurities, namely Imp-1, Imp-2, Imp-3, quetiapine, Imp-4, Imp-5, Imp-6, Imp-7, and Imp-8

Precision

The RSD percentage of content of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, and Imp-8 in method precision study was within 2.8% and in intermediate precision study was within 5.8%. The results of method precision and intermediate precision are compared with each other. The overall RSD (n=12) [15] for percentage of impurities (i.e. Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, and Imp-8) were found within the range of 3.16-5.92

LOD and LOQ

The LOD of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8 were 0.002, 0.003, 0.001, 0.001, 0.002, 0.003, 0.002 and 0.002% respectively (of analyte concentration i.e. 1000 µg mL^{-1}). The LOQ of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8 were 0.005, 0.010, 0.004, 0.004, 0.007, 0.008, 0.007 and 0.006% respectively (of analyte concentration i.e. 1000 µg mL^{-1}). The LOQ precision for all impurities at LOQ level was below 10 % RSD.

Linearity

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.375% for Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8. The correlation coefficient obtained was greater than 0.999.

Accuracy

The percentage recovery of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8 in bulk drugs samples ranged from 96.32 to 107.68%. HPLC chromatograms of blank, pure sample and all eight impurities spiked in quetiapine bulk drug sample were shown in Fig 2a.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and pH of buffer) the resolution between Imp-1 and Imp-2, Imp-4 and Imp-5 was greater than 1.5, while other impurities were greater than 2.0 illustrating the robustness of the method.

Conclusions

The method presented in this communication describes the development of specific and accurate HPLC method that separates eight potential impurities (Imp-1 to Imp-8) generated during the chemical synthesis and degradation impurities formed during the forced decomposition studies of quetiapine hemifumarate. The developed method was validated to ensure the compliance in accordance with ICH guideline. The behavior of quetiapine hemifumarate under various stress conditions was studied. The developed method could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies

References

1. Edward J W, Wilmington D (1989) US Pat No. 4879288
2. Campbell A, Yeghiayana S, Baladessarini J R, and Neumeyer LJ (1991) Psychopharmacology 103: 323-329
3. (a) Saller C F, Salama I A (1993) Psychopharmacology (1999) 112: 285-292 (b) Goldstein M J, Litwin C L, Sutton B E, Malick B J (1993) Psychopharmacology 112: 293-298 (c) Migler M B, Edward J W, Malick BJ (1993) Psychopharmacology 112: 299-307
4. Pullen R H, Palermo K M, Curtis M A (1992) J. Chromatogr 573: 49-57
5. Kansal V K, Lal K, Ahmed S, Leonov D (2006) WO Pat appl No. 113425
6. Fathalla B, Amina E, Manala E, Jenny J N (2008) J. Liq. Chromatogr. Related Technol. 31: 1283-1298
7. Julia S, Johannes K, Sebastian H, Christoph H (2006) J.Chromatogr. B 830: 342-348
8. Bharathi Ch, Prabahar K J, Prasad Ch, Srinivasa Rao M, Trinadhachary G N, Handa V K, Dandala R, Naidu A, (2008), Pharmazie, 63, 14-19.
9. Elzbieta U S, Lukasz K, Kamil E, Marek K, Magdalena G, Andrzej K, (2009), Pharm. Technol.,14, 27-37