Journal of Chromatographic Science 2012;50:368–372
doi:10.1093/chromsci/bms011

Article

A Stability-Indicating Ultra-Performance Liquid Chromatographic Method for Estimation of Related Substances and Degradants in Paliperidone Active Pharmaceutical Ingredient and its Pharmaceutical Dosage Forms

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Received 8 February 2011; revised 21 April 2011

A simple, linear gradient, rapid, precise and stability-indicating analytical method was developed for the estimation of related substances and degradants of paliperidone API and tablets. The chromatographic separations were achieved using an Acquity ultra-performance liquid chromatograph (BEH 100 mm, 2.1 mm, 1.7 μm C-18 column) employing 0.01 M potassium dihydrogen phosphate buffer (pH 2.0) as mobile phase A and acetonitrile–water (9:1) as mobile phase B. A linear gradient (mobile phase A, mobile phase B in the ratio of 84:16) with a 0.45 mL/min flow rate was chosen. All six impurities were eluted within five minutes of run time. The column temperature was maintained at 25°C and a detector wavelength of 238 nm was employed. Paliperidone was exposed to thermal, photolytic, hydrolytic and oxidative stress conditions. The stressed samples were analyzed by the proposed method. Considerable degradation of the analyte was observed when it was subjected to oxidative conditions and impurity F was found to be the major degradant. Peak homogeneity data of paliperidone obtained by photodiode array (PDA) detection demonstrated the specificity of the method in the presence of degradants. The method was validated with respect to linearity, precision, accuracy, ruggedness, robustness, limit of detection and limit of quantification.

Introduction

Paliperidone (9-hydroxy resperidone) is the major metabolite of risperidone (1, 2). It is a prolonged release oral, atypical antipsychotic licensed for the treatment of schizophrenia in adults (3). The recommended dose is 6 mg once daily, administered in the morning, although doses range from 3 to 12 mg once daily.

Although high-performance liquid chromatography (HPLC) is a well-established, reliable technique used to control the quality and consistency of active pharmaceutical ingredients (APIs) and dosage forms, it is often a slow technique because of the complexity of some of the samples, and it can still be improved.

Ultra-performance liquid chromatography (UPLC) is a new separation technique based upon the well-established principles of liquid chromatography, which utilizes sub-2 μm particles for the stationary phase. These particles operate at elevated mobile phase linear velocities to affect a dramatic increase in resolution, sensitivity and speed of analysis. Because of its speed and sensitivity, this technique has gained considerable attention in recent years for pharmaceutical and biomedical analysis (4).

The UPLC system will significantly decrease the time and cost per sample in the analytical process while improving the quality of the results. By outperforming traditional or optimized HPLC, the system allows chromatographers to work at higher efficiencies with a much wider range of linear velocities, flow rates and backpressures.

Liquid chromatographic methods using different detection techniques are described in the literature for the determination of risperidone and 9-hydroxy risperidone (paliperidone) in human serum and human plasma (5, 6, 7, 8, 9). Additionally, liquid chromatography–tandem mass spectrometry (LC–MS-MS) and LC with electrochemical detection methods are available for the determination of risperidone and 9-hydroxy risperidone enantiomers in human blood plasma and urine (10, 11). Few analytical methods using HPLC are described in patents for the estimation of impurities in paliperidone API (12, 13, 14). These methods require longer run times and do not represent all the impurities and degradants studied in this paper. To the best of our knowledge, no method available in the literature can separate Impurities, A, B, C, D, E and F using only a 5-minute run time. The method was validated as per ICH guidelines (15, 16).

Impurities C [-(2-chloroethyl)-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido [1, 2-a] pyrimidin-4-one] and D [6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole] are key raw materials for the synthesis of paliperidone. Impurity E [3-(2-(4-(6-fluorobenzo [d]isoxazol-3-yl)piperidin-1-yl)ethyl)-2-methyl-7,8-dihydro-4H-pyrido[1, 2-a]pyrimidine-4,9(6H)-dione] is a process-related impurity. Impurities A [3-[2-[4-[(E)-(2,4-diflourophenyl) (hydroxy-imino)methyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-9-hydroxy-4H-pyrido[1, 2-a]pyrimidin-4-one] and B [3-[2-[4-[(Z)-(2,4-diflourophenyl)(hydroxy-imino)methyl] piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-9-hydroxy-4H-pyrido[1, 2-a]pyrimidin-4-one] are considered for development, because the corresponding impurities are listed in PharmEuropa for risperidone. Impurity F [4-(6-fluorobenzo[d] isoxazol-3-yl)-1-(2-(9-hydroxy-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1, 2-a]pyrimidin-3-yl)ethyl)piperidine-1-oxide] is a degradation product. The chemical structures and names of paliperidone and Impurities A, B, C, D, E and F are depicted in Figure 1. Although Impurities A, B and F are considered for method development and selectivity purposes, these impurities are not detected in the samples of paliperidone.

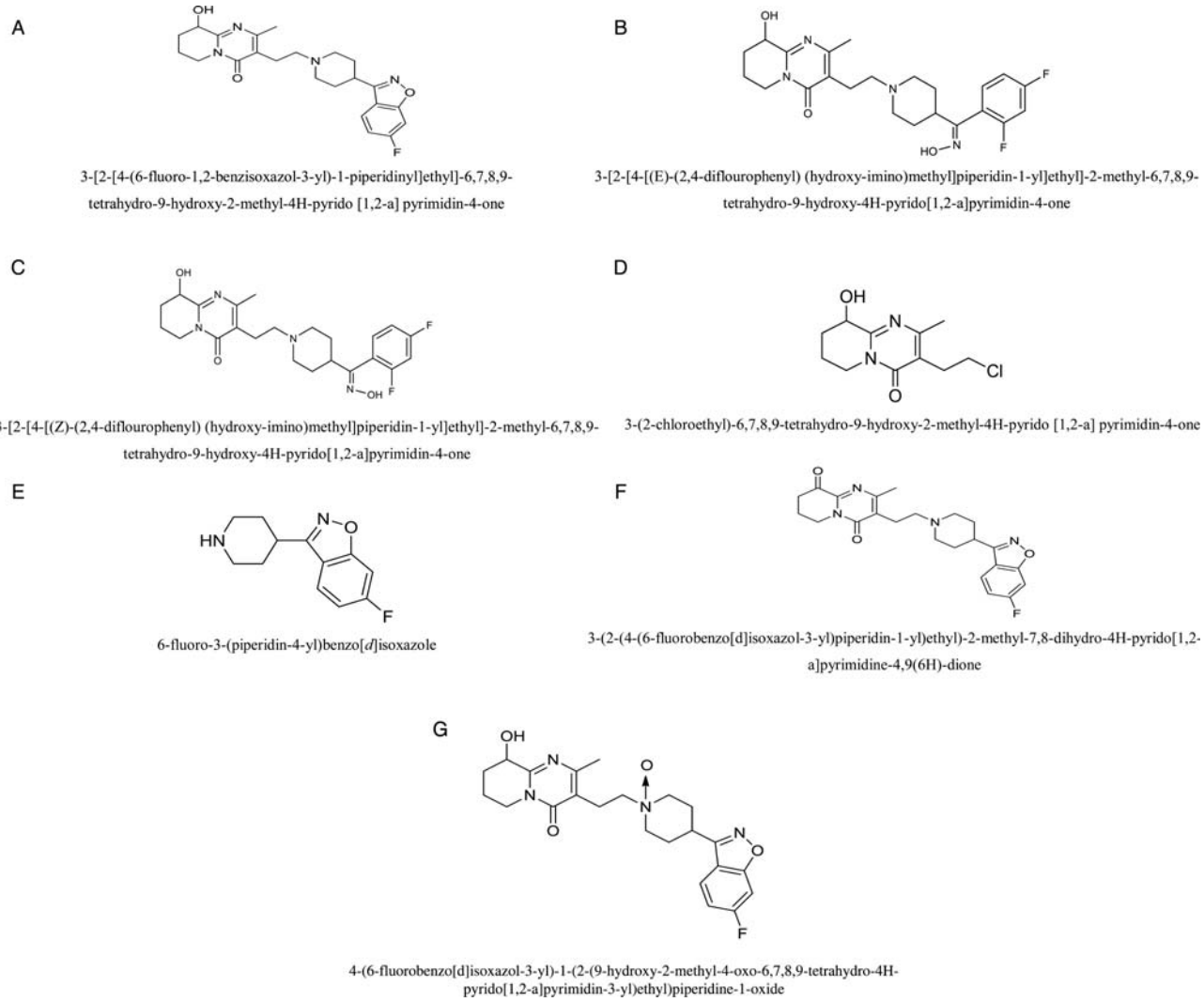

Figure 1. Chemical structure and name: (A) paliperidone; (B) Impurity A; (C) Impurity B; (D) Impurity C; (E) Impurity D; (F) Impurity E; (G) Impurity F.

Experimental

Chemicals

Active pharmaceutical ingredient standards and samples were supplied by Dr. Reddy's Laboratories Limited, IPDO (Hyderabad, India). Invega, the extended release tablets of paliperidone, were purchased from Janssen Pharmaceuticals. The HPLC grade acetonitrile and analytical grade ortho-phosphoric acid were purchased from Merck (Darmstadt, Germany). Water was prepared by using a Millipore Milli-Q Plus water purification system.

Chromatographic conditions and equipment

LC was carried out on a Waters Aquity UPLC with a photodiode array detector. The output signal was monitored and processed using Empower Software. The chromatographic column was an Acquity UPLC BEH C-18 column (100 mm, 2.1 mm, and 1.7 μm particle size). The separation was achieved using a linear gradient method. Mobile phase A was 0.01M Potassium dihydrogen phosphate buffer and mobile phase B contained a mixture of water and acetonitrile in the ratio of 10: 90 (*v*/*v*).

The flow rate of mobile phase was 0.45 mL/min. The UPLC linear gradient program was set as: time (min) / % solution B: 0.01/16, 6/16. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 238 nm. The injection volume was 5.0 μL. The diluent was a solution of acetonitrile and methanol (1:1).

Preparation of solutions

Sample preparation

Ten milligrams of the test sample were placed in a 10-mL volumetric flask, dissolved and diluted to the mark with diluent.

An adequate number of paliperidone tablets were chosen, the two upper layers were carefully removed, and the tablets were ground to a fine powder using a mortar and pestle. The resulting powder was transferred into a sufficient quantity of diluent needed to obtain a 1.0 mg/mL concentration of paliperidone and sonicated for 15–20 minutes. The resulting solution was filtered and the filtrate was used for analysis.

Standard preparation

Ten milligrams of the paliperidone standard was transferred to a 10-mL volumetric flask, dissolved and diluted up to the mark with diluent.

Specificity–Forced degradation studies

Forced degradation studies were performed on paliperidone to prove the stability-indicating property of the method. The stress conditions employed for the degradation study of paliperidone included light exposure (carried out as per ICH Q1B), heat (70°C), acid hydrolysis (1 N HCl, kept at constant stirring under reflux conditions at 60°C for 24 hours), base hydrolysis (0.5 N NaOH, kept at constant stirring under reflux conditions at room temperature for 3 hours), water hydrolysis (kept at constant stirring under reflux conditions at room temperature for 24 hours) and oxidation (3% H2O2, kept at constant stirring at room temperature for 2 hours). For heat and light studies, the monitoring period was 10 days. Peak purity of the principal peak in the chromatogram of the diluted stressed samples of paliperidone was assessed using a photo diode array detector.

Method Validation

Linearity of response

Linearity of the response for all impurities was carried out at concentration levels from limit of quantification (LOQ) to 150% of the specification limit (0.15%), each with respect to the concentration of paliperidone.

Precision

The repeatability of the related-substance method was checked by a six-fold analysis of 1.0 mg/mL paliperidone spiked with 0.15% of each of the six impurities. The relative standard deviation (RSD) (%) of the peak area was calculated for each impurity.

Inter- and intra-day variation and analyst variation were studied to determine the intermediate precision of the proposed method. Intra-day precision was determined by a six-fold analysis of 1.0 mg/mL paliperidone spiked with 0.15% of each of the six impurities. Different analysts prepared different solutions on different days. The RSD (%) of peak area was calculated for each impurity.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of impurities in the paliperidone sample. The study was carried out in triplicate at 0.075%, 0.15% and 0.1875% of the analyte concentration (1.0 mg/mL). The percent recovery for Impurities A, B, C, D, E and F were calculated.

Limit of detection and limit of quantification

The limit of detection (LOD) and LOQ for Impurities A, B, C, D, E and F were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. The LOQ values were confirmed by performing precision and accuracy verification.

Robustness

To determine the robustness of the method, experimental conditions were purposely altered and the resolution between the impurities was evaluated. The flow rate of the mobile phase was 0.45 mL/min. To study the effect of flow rate on the resolution parameter, it was changed by 0.05 units from 0.40 to 0.50 mL/min. The effect of column temperature on resolution parameter was studied at 20°C and 30°C instead of 25°C. In all the previously varied conditions, the components of the mobile phase were held constant.

Results and Discussion

Method development and optimization

The primary criteria for the development of a successful UPLC method for the determination of related substances and degradants in paliperidone was that the method should be able to determine related substances and degradants within five minutes of runtime and should be accurate, reproducible, robust, indicative of stability, free of interference from degradation products andimpurities and straightforward enough for routine use in a quality control laboratory. One of the patents for the synthesis of paliperidone states a reversed-phase HPLC method for the estimation of Impurities C and D. Impurities A, B, E and F were not considered for analysis. The total run time for this method was approximately 60 minutes and further analysis using this methodology showed that Impurities E and F were co-eluting, which clearly indicates that the method is not indicative of stability, because Impurity F is the major degradant of oxidation. As a result, further trials were completed using UPLC by using an Acquity UPLC BEH C-18 column (100 mm, 2.1 mm, 1.7 μm.) and 0.01M potassium dihydrogen phosphate buffer (pH 2.0) as the buffer. The pH of the buffer was found to be critical, because a pH of more than 2.0 (pH > 2.2) led to the close elution of Impurities A, B, C and D. Initial efforts were made to reduce the tailing factor of paliperidone, which was more than 2.0, by adding triethylamine and diethylamine to the buffer. However, this led to the co-elution of Impurities E and F. As a result, the addition of triethylamine or diethylamine was avoided. Optimum separation with minimum run time was obtained by employing a linear gradient of mobile phase A and mobile phase B in the ratio of 84:16, where mobile phase A was 0.01M potassium dihydrogen phosphate buffer (pH 2.0) and mobile phase B was acetonitrile–water (9:1). The flow rate was set at 0.45 mL/min. All impurities and paliperidone showed sufficient response at 238 nm and the analytical column was maintained at 25°C during the analysis. System suitability parameters were evaluated for paliperidone and its six impurities. The tailing factor for all six impurities was found to be less than 2.0. The resolution of paliperidone and the six potential impurities was greater than 1.3 for all pairs of compounds. The chromatogram representing paliperidone spiked with Impurities A, B, C, D, E and F at 0.15% is shown in Figure 2.

Specificity–Forced degradation studies

Paliperidone was not degraded under heat, light or water hydrolysis conditions. The sample was degraded under basic and oxidative conditions. The major degradation product that formed under oxidative conditions was Impurity F, whereas the major degradation product formed during basic hydrolysis was Impurity E.

The peak purity test results derived from the PDA detector confirmed that the paliperidone peak was pure and homogeneous in all the analyzed stress samples. This indicates that the method is specific and stability-indicating.

Linearity of response

A linear calibration plot of the method was obtained over the tested calibration ranges, i.e., LOQ to 200% for Impurities A, B, C, D, E and F. Correlation coefficient (r), slope and y-intercept for the impurities are presented in Table I. The correlation coefficient obtained was greater than 0.999, indicating a linear response of the impurities.

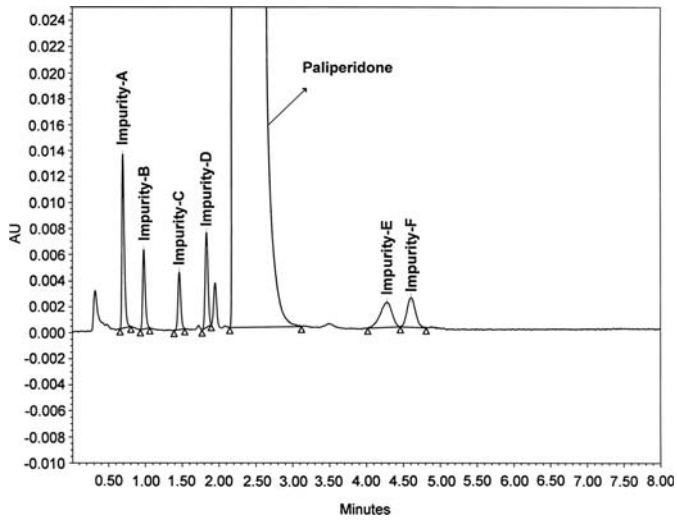

Figure 2. Chromatogram representing paliperidone spiked with Impurities A, B, C, D, E and F.

Precision

The % RSD of percent area of Impurities A, B, C, D, E and F was found to be less than 5%, confirming the good precision of the method. The results are tabulated in Table I.

Accuracy

The percentage recovery of Impurities A, B, C, D, E and F ranged from 90 to 103. The percentage recovery of the impurities is listed in Table I. All the impurities are within the acceptance limit.

LOD and LOQ

The LOD of Impurities A, B, C, D, E and F were 0.00001, 0.00003, 0.00005, 0.00003, 0.00015 and 0.00008 mg/mL, respectively, with respect to paliperidone concentration, for a 5 µL injection volume.

The LOQ of Impurities A, B, C, D, E and F were 0.00005, 0.0001, 0.0002, 0.0001, 0.0005 and 0.0003 mg/mL, respectively, with respect to paliperidone concentration, for a 5 µL injection volume.

Robustness

In all of the deliberately varied chromatographic conditions (flow rate and column temperature), all analytes were adequately resolved and elution orders remained unchanged. Resolution between Impurity E and Impurity F was greater than 1.1 and resolution between all other components was

Table I
Validation Data

Parameter	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F
Linearity						
r	0.9996	0.9997	0.99998	0.9995	0.9992	0.9992
Slope	277.1	139.1	110.0	186.2	101.0	149.7
Y-Intercept	398.9	49.6	168.6	-125.6	335.9	1131.5
Accuracy (%Recovery)						
LOQ (n = 3)	91.6	93.3	95.6	90.5	98.3	92.6
50% (n = 3)	94.5	94.7	100.7	89.9	102.6	102.1
100% (n = 3)	102.0	98.5	98.3	97.6	101.5	99.9
150% (n = 3)	99.9	95.6	97.6	95.5	100.0	93.8
Precision (%RSD)						
LOQ (n = 6)	2.5	3.9	4.6	3.5	5.0	3.7
100% (n = 6)	1.9	2.2	2.2	1.3	2.9	1.0
150% (n = 6)	0.7	1.3	2.0	0.4	1.2	1.1
Rugged ness: Different day and analyst (%RSD)						
100% (n = 6)	2.0	1.4	3.8	2.6	2.6	2.8
Robustness (Resolution)						
Actual flow 0.45mL/min	4.5	7.1	5.2	1.5	5.8	1.3
Different flow 0.40mL/min	4.3	7.0	5.6	1.5	5.7	1.2
Different flow 0.50mL/min	4.3	6.7	4.9	1.6	5.7	1.1
Column temperature 22°C	4.3	6.8	5.8	1.5	5.8	1.1
Column temperature 30°C	3.9	6.4	4.8	1.6	5.7	1.3
Limit of Detection	0.00001 mg/mL	0.00003 mg/mL	0.00005 mg/mL	0.00003 mg/mL	0.00015 mg/mL	0.00008 mg/mL
Limit of Quantification	0.00005 mg/mL	0.0001 mg/mL	0.0002 mg/mL	0.0001 mg/mL	0.0005 mg/mL	0.0003 mg/mL

greater than 1.5 for all flow rates (0.40, 0.50 mL/min) and column temperatures (20°C, 30°C). The resolutions between the impurities under various conditions are listed in Table I.

Conclusion

A simple, specific liquid chromatographic method using UPLC was developed for the quantification of related substances and degradants of paliperidone API and its pharmaceutical forms. This method was validated and found to be specific, precise, accurate, linear and rugged for the detection and quantification of related substances and degradants of paliperidone.

Acknowledgment

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd. for supporting this work. Cooperation from colleagues of Research & Development and Analytical Research & Development of Dr. Reddy's Laboratories Ltd. is appreciated.

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