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Stress Degradation Behavior of Pregabalin, Identification of Degradation Impurities and Development of Stability Indicating UPLC Method

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***Abstract*— A novel, high-throughput, reverse phase-ultra performance liquid chromatographic (RP-UPLC) method has been developed for the quantification of Pregabalin and its related impurities in drug substance. The stability-indicating capability of the developed method is demonstrated using forced degradation samples from stress conditions such as hydrolysis, oxidation, thermal and photolytic degradation. The separation of known impurities and degradation impurities are accomplished using a phenyl-hexyl stationary phase with 100 mm length and 1.7 µm particle size in short run time (10 min). The developed method employs a linear gradient elution with phosphate buffer pH 6.2-acetonitrile as mobile phase, and is validated in accordance with International Conference on Harmonization requirements. During forced degradation it has been observed significant degradation of drug substance in base hydrolysis and oxidative stress conditions, and slight degradation in acid hydrolysis and thermal stress conditions. The ten major oxidative degradation impurities formed are identified by LC-MS, HR-MS and NMR techniques and the proposed structures are reported.**

***Index Terms*— Pregabalin, Ultra performance liquid chromatography (UPLC), Stability-indicating methods, Identification of forced degradation impurities by NMR & Mass, Validation.**

I. INTRODUCTION

Pregabalin, (S)-(+)-3-(aminomethyl)-5-methylhexanoic acid, is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults. Pregabalin binds to the α2δ (alpha2delta) subunit of the voltage-dependent calcium channel in the central nervous system and decreases the release of neurotransmitters including glutamate, norepinephrine, substance P and calcitonin gene related peptide, reducing the communication between nerves can contribute to the effect on pain and seizures.

Pregabalin, a gamma-aminobutyric acid analogue, is a prescription drug sold under the trade name Lyrica®. The maximum recommended dose is 100 mg three times a day (300 mg/day) in patients with creatinine clearance of at least 60 mL min-1. Begin dosing at 50 mg three times a day (150 mg/day). The dose may be increased to 300 mg/day within 1 week based on efficacy and tolerability. Pregabalin is eliminated primarily by renal excretion.

Few methods have been reported in literature for the determination of Pregabalin both in biological matrices and pharmaceuticals involving mass spectroscopy methods, pre-column derivatization methods, post-column derivatization methods, direct HPLC methods and spectrophotometric methods. To the best of our knowledge no method was reported for the determination of Pregabalin and its potential impurities in bulk drug using UPLC for regular analysis and stability studies.

Ultra performance liquid chromatography (UPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 µm particles for stationary phase. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. The objective of this research work was to develop a simple stability-indicating UPLC method for the related substances and assay determination of Pregabalin.

II. EXPERIMENTAL

A. Chemicals and Reagents

Pregabalin and its related impurities were synthesized and purified using column liquid chromatography by the process research department of Active Pharmaceutical Ingredients, IPDO, Dr. Reddy's Laboratories (Hyderabad, India). The UPLC-grade acetonitrile and AR-grade dipotassium hydrogen phosphate, monopotassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Rankem (Mumbai, India). Millipore purified water (Milli-Q Plus; Bangalore, India) was used to prepare the mobile phase and wash solvents.

B. Instrumentation

The method development attempts, forced degradation studies and subsequent validation of method were performed on Waters Acquity UPLC system with a diode array detector. The data were collected and processed using empower software. The photolytic degradation was carried out using Binder KBS240 photolytic chamber. The mass analysis of degradation products was performed on Agilent LCMS 6410 QqQ instrument and Waters Acquity HR-MS ultra performance liquid chromatography system coupled with a time of flight spectrometer. The NMR analysis was performed on Varian 400 MHz, oxford magnet, California, USA.

C. Chromatographic Conditions

The chromatographic separation was optimized on a Phenomenex kinetex phenyl-hexyl column with the dimensions of 100 mm x 2.1 mm and 1.7 µm particle size. The gradient LC method employs water:monopotassium dihydrogen phosphate:dipotassium hydrogen phosphate in the ratio of 100:0.136:0.05 (v/w/w) as mobile phase A and acetonitrile as mobile phase B. The UPLC gradient program was optimized as: time/% mobile phase B: 0/0, 7/50 and 10/0 with a post run time of 3 min. The flow rate was 0.30 mL min-1. The column temperature was maintained at 40°C and detection wavelength was set at 210 nm.

D. Method Development and Optimization

The core objective of the chromatographic method is to get a sharp peak shape for Pregabalin, and to separate all potential impurities and degradation products from the analyte in short run time, especially the critical pairs Pregabalin and Imp-B, and also Imp-D and Imp-E. Considering the pKa value of Pregabalin is 4.2 for the carboxylic group and 10.6 for the amine group, method development attempts were focused with phosphate buffer.

Satisfactory peak shape and resolution of closely eluting potential impurities were achieved on Phenomenex kinetex phenyl-hexyl column (100 mm x 2.1 mm, 1.7 µm), using phosphate buffer pH 6.2/acetonitrile as mobile phase. Kinetex Phenyl-Hexyl columns provide separations not achievable on C18 or C8 columns; such as increased retention for polar, aromatic compounds.

III. METHOD VALIDATION

A. Precision

Six individual measures of Pregabalin were performed with 0.15% w/w of each Imp-A, Imp-B, Imp-C, Imp-D and Imp-E to the reference of TAC. The percentage RSD of the content of impurities and the assay of Pregabalin in the precision study, including intermediate precision, were well within 3.9 and 0.41, respectively.

B. Limit of Detection and Limit of Quantification

The LOD and LOQ for each of the impurities were established by attaining signal-to-noise ratio of approximately 3:1 and 10:1 respectively. The limit of detection of Pregabalin and related impurities were less than or equal to 0.02% w/w (of TAC). The limit of quantification was less than or equal to 0.06% w/w (of TAC) for 1 µL injection volume.

C. Linearity

Excellent correlation was achieved for the regression line of Pregabalin and its related impurities at LOQ to 200% of the specification level. The correlation coefficient obtained for all the plots was greater than 0.999. The Y-intercept of each plot was below 2.3% of the response at 0.15% w/w level of the corresponding impurity.

D. Accuracy

The percentage recovery of each impurity falls in the range of 95.9 to 101.3%. The accuracy of the assay was evaluated in triplicate at three concentration levels, i.e. 8, 10 and 12 mg mL-1 of Pregabalin, corresponding to 80, 100 and 120% w/w of the TAC.

E. Robustness

In all deliberately varied chromatographic conditions — flow rate, wavelength, column temperature and mobile phase ratio by gradient change — the tailing factor of Pregabalin was less than 1.1 and the resolution for the critical pair Pregabalin and Imp-B was greater than 6.2, and for the critical pair Imp-D and Imp-E was greater than 4.5.

IV. RESULTS AND DISCUSSION

A. Forced Degradation Studies

The degradation of drug substance was not observed in water hydrolysis and photolytic stress conditions. Slight degradation was observed in acidic hydrolysis and thermal stress conditions, and significant degradation was observed in base hydrolysis and oxidative stress conditions. Pregabalin under acidic hydrolysis, base hydrolysis and thermal stress conditions led to the formation of known impurity-D. The drug substance Pregabalin under oxidative stress conditions led to the formation of ten unknown degradation impurities O1 to O10 along with known impurity-D.

B. Oxidative Degradation Pathways

The oxidative degradation pathways of Pregabalin were studied by subjecting the drug substance to oxidation with 10% hydrogen peroxide for 7 days. The oxidation of Pregabalin led to the continuous formation of mono hydroperoxide (191.1) as major degradant and lactam impurity (141.1) as minor degradant. The mono hydroperoxide was further converted to cyclized compound of mono hydroperoxide (173.1), dihydroperoxide (223.1) and mono hydroxy (175.1) compounds.

The mass balance of stressed samples was between 98.0 and 101.0% w/w when the RRF of the degradant was considered to be one, which confirms the specificity and stability indicating ability of the developed method.

V. CONCLUSION

The developed simple UPLC method for related substance and assay determination of Pregabalin is linear, precise, accurate and specific. The short run time of the developed method significantly saves lot of analysis time (~6 times faster) as well as the solvents cost (~3 times lesser). The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the Pregabalin drug substance during routine analysis and also for stability studies in view of its capability to separate degradation products.

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