Robust and Rugged Stability-Indicating HPLC Method for the Determination of PA-824 Assay and Its Related Impurities in Drug Substance and Drug Product

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Purpose
Determination of PA-824 Assay and its Related Impurities in Drug Substance and Drug Product

Methods
A reversed-phase high-performance liquid chromatographic method was developed and validated for the determination of assay and related substances in the drug substance and drug product. The method was developed using the Zorbax SB-CN 250 x 4.6 mm, 5µm using the gradient program with mobile phase A consisting of 0.05% of TFA in water and mobile phase B consisting of 0.05% TFA in Acetonitrile with flow rate of 1.0 mL/min. PA-824 and its impurities were monitored at 330 nm. Test samples of PA-824 were prepared with 0.16 mg/mL and 1.0 mg/mL concentrations for the estimation of assay and impurities respectively. Method was validated as per the ICH Q2(R1): Validation of Analytical Procedures.

Results
The process impurities and degradation impurities were well-resolved from the main peak and its impurities. Forced degradation studies show that PA-824 peak is pure and all degradation impurities generated were well separated from each other and from the PA-824 peak in all the stress conditions of oxidative, acid, base, hydrolytic, thermal, humidity, and photolytic degradations demonstrating the stability-indicating power of the method. The performance of the method was validated for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness, and robustness. Solution stability of standard and test samples of assay and impurities were established. Relative response factors, limit of quantification and limit of detection for all the six known impurities were established. The method is efficient for estimation of all degradation and process related impurities.

Conclusion
The analytical method developed for the estimation of Assay and Impurities of PA-824 conforms to all the requirements of ICH Q2(R1): Validation of Analytical Procedures.