Development and Validation of an Analytical Method for the Novel Long-Acting Inhaled β₂-agonist Bronchodilator; Indacaterol, in Biological Samples

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Introduction: Indacaterol is the first ultra-long-acting β₂-agonist that has been recently approved as a once-daily inhaled bronchodilator in chronic obstructive pulmonary disease (COPD) patients. Indacaterol has been formulated so that it is inhaled as a dry powder from a specific single-loaded inhaler device named brezhaler. For inhaled medicines, the use of pharmacokinetic methods to identify the relative lung and systemic bioavailability, as measures of efficacy and safety indices, respectively, requires a sensitive, reliable and robust analytical method for the drug determination in samples of various body fluids. Therefore, the objectives of the current work were to develop and validate two bio-analytical methods for indacaterol determination in human plasma and urine.

Methods: A simple, rapid liquid-liquid extraction method has been developed to extract indacaterol from 1 ml volume of either human plasma or urine. A 4 ml of ethyl acetate was used as an extraction solvent. The HPLC chromatographic conditions involved elution of indacaterol on a reversed phase C18 column with an isocratic mobile phase pumped at a flow rate of 1 ml/min. Only 5 μl injection volume was needed for the analysis after reconstituting the extracted indacaterol residue with a 200 μl of the mobile phase which was a mixture of water and methanol (50:70 v/v) acidified with 300 μl of formic acid. Formoterol was used as the internal standard (IS) in the analytical method. The detection system involved the tandem mass spectrometer which employed tandem mass spectrometry in the positive ion mode along with a multiple reactions monitoring (MRM). Indacaterol was detected at molecular ion m/z ratio of 393.3 and an MS/MS daughter at m/z ratio of 173.2. Whilst, the IS was monitored at molecular ion m/z ratio of 345.2 and an MS/MS daughter at m/z ratio of 149.1. The analytical run-times were 3 and 2.5 minutes in the plasma and urine methods, respectively. The international guidelines on the bio-analytical method validation were followed to validate the currently developed analytical methods in plasma and urine matrices.

Results: The results of the validation have shown that the lower limit of detection (LOD) for indacaterol was 0.050 ng/ml. The standard curves of the developed methods of indacaterol in plasma and urine were linear over a concentration range between 0.075 and 100 ng/ml with a lower limit of quantification (LLOQ) of 0.075 and a correlation coefficient ≥ 0.990. The specificity of the methods was established in 8 different batches of human plasma and 6 different batches of human urine. No biological matrices effect was detected. The intra-batch and inter-batch precision and accuracy were confirmed within ±20% (at LLOQ) and ±15% (at the Low, Mid and High quality control (QC) levels) after a 3-run validation process. The indacaterol samples’ short-term, long-term (freeze and thaw cycles) and auto-sampler stabilities were studied and reported.

Conclusion: Two specific, high performance liquid chromatographic methods coupled to tandem mass spectrometry detection (LC-MS/MS) have been developed and validated to provide a rapid determination of indacaterol in human plasma and urine samples. These methods are reproducible, accurate, precise and robust which will support future pharmacokinetic and clinical research studies.

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