LC–MS–MS Simultaneous Determination of Atorvastatin and Ezetimibe in Human Plasma

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Abstract

Atorvastatin and ezetimibe are lipid-lowering drugs prescribed for the treatment of hypercholesterolemia. An LC–MS–MS method has been developed and validated for the simultaneous estimation of atorvastatin and ezetimibe in human plasma using pitavastatin as an internal standard. Liquid–liquid extraction was used for the purification and preconcentration of analytes from human plasma matrix. The chromatographic separation was achieved within 3.0 min by an isocratic mobile phase consisting of 0.2% formic acid in water–acetonitrile (30:70, v/v), flowing through Agilent Eclipse-plus C18, 100 3 4.6 mm, 3.5 mm analytical column, at a flow rate of 0.6 mL min⁻¹. Multiple reaction monitoring transitions were measured in the positive ion mode for atorvastatin and internal standard, while ezetimibe was measured in negative ion mode. A detailed validation of the method was performed as per US-FDA guidelines and the standard curves were found to be linear in the range of 0.2–30.0 ng mL⁻¹ with a mean correlation coefficient >0.999 for both drugs. In human plasma, atorvastatin and ezetimibe were stable for at least 36 days at −70±58°C and 6 h at ambient temperature. After extraction from plasma, the reconstituted samples of atorvastatin and ezetimibe were stable in an autosampler at ambient temperature for 6 h. Also, the cited drugs were stable in plasma samples upon subjecting to three freeze thaw cycles. The method is simple, specific, sensitive, precise, accurate and suitable for bioequivalence and pharmacokinetic studies of this combination.

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