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ORIGINAL ARTICLE

Simultaneous determination of rosuvastatin and amlodipine in human plasma using tandem mass spectrometry: Application to disposition kinetics

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ABSTRACT

The liquid chromatography–tandem mass spectrometric assay method for the simultaneous determination of rosuvastatin and amlodipine in human plasma using deuterated analogs as internal standards has been developed and validated. The analytes were extracted from 100 μL aliquots of human plasma *via* liquid–liquid extraction using a mixture of ethyl acetate and *n*-hexane (80:20, v/v) as an extraction solvent. The optimized mobile phase was composed of 0.1% formic acid in 5 mM ammonium acetate, methanol, and acetonitrile (20:20:60, v/v/v) and delivered at a flow rate of 0.75 mL/min. The calibration curve obtained was linear ($R^2 \geqslant 0.999$) over the concentration range of 0.52–51.77 ng/mL for rosuvastatin and 0.10–10.07 ng/mL for amlodipine. A sample turnover rate of less than 2.5 min makes it an attractive procedure in high-throughput bioanalysis of rosuvastatin and amlodipine. The present method was found to be applicable to clinical studies and the results were authenticated by incurred sample reanalysis.

Introduction

Hypertension and hyperlipidaemia are major risk factors for the development of atherosclerosis and its associated conditions such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. Calcium antagonists have been used for decades as antihypertensive agents. On the other hand, 3-hydroxy-3-methylglutaryl coenzyme A

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(HMG-CoA) reductase inhibitors (statins) have been extensively used for the treatment of hyperlipidaemia because of their potent lipid lowering properties [1–3].

Rosuvastatin, a synthetic statin, was developed for the treatment of hyperlipidaemia [4,5]. The dose dependent peak plasma concentration (C_{max}) reached 3–5 h after oral administration of a 10- to 80-mg dose [6–8]. Amlodipine, a calcium antagonist, is prescribed for the treatment of hypertension and angina pectoris. It has a long elimination half-life and large volume of distribution. Low plasma concentrations (ng or pg) were reported after oral administration of amlodipine. The combination of rosuvastatin and amlodipine exerts more beneficial effects on cardiomyocyte hypertrophy and fibrosis [9,10]. Compared with the co-administration of each drug, the convenience of a fixed dose combination (FDC) tablet has the potential outcome to improve patient adherence and the management of cardiovascular risk, thereby improving clinical outcomes.

Many liquid chromatography–tandem mass spectrometric (LC/MS/MS) methods have been reported for the determination of rosuvastatin [11–18] individually or in combination with other drugs in biological samples. The major disadvantages of these methods include, less sensitivity [11], more sample volume (>0.25 mL) [11,13,14,19], longer chromatographic run time (>4 min) [11–18], complex with derivatization and expensive automated extraction procedure [13,18], and narrow linearity range not suitable for bioequivalence/pharmacokinetic application in humans at higher dose (0.1–30 ng/mL) [13,14]. Similarly, numerous LC/MS/MS methods are described in the literature to determine amlodipine in different biological fluids [19–31]. Among the applied methods, either the chromatographic run time was long (>4 min) [19,20,22,24,25,30,31], the plasma volume was high (>0.25 mL) [19,21–25,30] or the method was insensitive for bioequivalence/pharmacokinetic application [20,23,25,27,30,31].

Some methods [15,21,26–29] which can be applied for quantitation of one drug in biological fluids selectively and sensitively, cannot be applied satisfactorily for simultaneous determination of rosuvastatin and amlodipine. To investigate the safety and tolerability of rosuvastatin and amlodipine fixed dose combination (FDCs) and/or for comparative bioavailability and bioequivalence studies of rosuvastatin associated with amlodipine, it is necessary to perform the quantitation of rosuvastatin and amlodipine simultaneously. An effective bio-analytical method should gratify in terms of sensitivity, efficient extraction process, rapid chromatography and specific. To our knowledge, no LC/MS/MS method has been reported for the simultaneous determination of amlodipine and rosuvastatin in human plasma. The present work describes a simple, selective and sensitive method, which employs liquid–liquid extraction (LLE) technique for sample preparation and liquid chromatography with electrospray ionization–tandem mass spectrometry for simultaneous quantitation of rosuvastatin and amlodipine in human plasma. The method uses isotope labeled compound rosuvastatin d6 and amlodipine d4 maleate as internal standards (IS) for the quantitation of rosuvastatin and amlodipine, respectively to avoid the potential matrix effect related problems and variability in recovery between analyte and IS. The suggested assay was applied to a clinical study in humans following oral administration of rosuvastatin and amlodipine. Furthermore, assay reproducibility is demonstrated by conducting incurred sample reanalysis (ISR).

Experimental

Reagents and chemicals

Reference standards of amlodipine besylate (purity 99.95%), amlodipine d4 maleate (IS1; purity 99.35%) and rosuvastatin d6 sodium salt (IS2; purity 99.87%) were purchased from Vivan Life Sciences Ltd. (Mumbai, India), while rosuvastatin calcium (purity 95.40%) was from Hetero Drugs Ltd. (Hyderabad, India). Water used for the LC/MS/MS analysis was prepared from Milli Q water purification system procured from Millipore (Bangalore, India). HPLC grade acetonitrile and methanol were purchased from J.T Baker (Phillipsburg, USA); while ethyl acetate and *n*-hexane were from Merck Ltd. (Mumbai, India). Analytical grade formic acid and ammonium acetate were also purchased from Merck (Mumbai, India). The control human plasma sample was procured from Deccan's Pathological Labs (Hyderabad, India).

Preparation of stock and working solutions

Primary stock solutions (1 mg/mL) of rosuvastatin, amlodipine, IS1, and IS2 were prepared in methanol and these stocks were stored at 2–8 °C. Working solutions were prepared in a mixture of acetonitrile and water (50:50, v/v; diluent) for the purpose of plotting the calibration curve (CC) standards. Another set of working solutions were prepared in appropriate concentrations (using the same diluent) for quality control (QC) samples. A combined working solution for IS1 (500 ng/mL) and IS2 (50 ng/mL) was also prepared in diluent.

Preparation of calibration curve standards and quality control samples

Calibration samples were prepared by spiking 950 µL of control human plasma with the appropriate working standard solution of the each analyte (50 µL combined dilution of rosuvastatin and amlodipine). Calibration curve (CC) standards of analytes in blank plasma were prepared by spiking with an appropriate volume of the working solutions, giving final concentrations of 0.52, 1.04, 2.59, 5.19, 10.37, 20.75, 31.06, 41.41, and 51.77 ng/mL for rosuvastatin and 0.10, 0.20, 0.51, 1.01, 2.02, 4.04, 6.04, 8.06, and 10.07 ng/mL for amlodipine. The CC samples were analyzed along with the quality control (QC) samples for each batch of plasma samples. The QC samples were prepared at five different concentration levels of 0.52 (lower limit of quantification, LLOQ), 1.49 (low quality control, LQC), 6.19 (middle quality control, MQC-1), 25.78 (MQC-2) and 46.03 (high quality control, HQC) ng/mL for rosuvastatin and 0.10 (LLOQ), 0.29 (LQC), 1.20 (MQC-1), 5.02 (MQC-2) and 8.96 (HQC) ng/mL for amlodipine. All the prepared plasma samples were stored at −70 ± 10 °C.

Sample processing

All frozen subject samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature prior to analysis. The samples were vortexed for 10 s prior to spiking. A 100 µL aliquot of human plasma sample was mixed with 25 µL of the internal standard