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ORIGINAL ARTICLE

Simultaneous determination of atorvastatin, metformin and glimepiride in human plasma by LC–MS/MS and its application to a human pharmacokinetic study

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Abstract A simple, rapid and sensitive liquid chromatography-tandem mass spectrometric (LC–MS/MS) assay method has been developed and fully validated for the simultaneous quantification of atorvastatin, metformin and glimepiride in human plasma. Carbamazepine was used as internal standard (IS). The analytes were extracted from 200 μL aliquots of human plasma via protein precipitation using acetonitrile. The reconstituted samples were chromatographed on a Alltima HP C18 column by using a 60:40 (v/v) mixture of acetonitrile and 10 mM ammonium acetate (pH 3.0) as the mobile phase at a flow rate of 1.1 mL/min. The calibration curves obtained were linear ($r^2 \geq 0.99$) over the concentration range of 0.50–150.03 ng/mL for atorvastatin, 12.14–1207.50 ng/mL for metformin and 4.98–494.29 ng/mL for glimepiride. The API-4000 LC–MS/MS in multiple reaction monitoring (MRM) mode was used for detection. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. All the analytes were found to be stable in a battery of stability studies. The method is precise and sensitive enough for its intended purpose. A run time of 2.5 min for each sample made it possible to analyze more than 300 plasma samples per day. The developed assay method was successfully applied to a pharmacokinetic study in human male volunteers.

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1. Introduction

Type 2 diabetes is a complex metabolic disorder with two major biochemical defects, namely impaired insulin secretion and impaired insulin action at the periphery. Chronic hyperglycemia results from these defects. Current American

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Diabetes Association guidelines suggest that all adults with diabetes should be managed to achieve a low density lipoprotein (LDL) cholesterol less than 100 mg/dl employing statins as first-line therapy [1].

Atorvastatin is a lipid-lowering agent that specifically, competitively, and reversibly inhibits 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in the cholesterol biosynthesis [2,3]. US FDA (2006) has approved atorvastatin for use to reduce the risk of stroke and heart attack in people with type 2 diabetes without evidence of heart disease. Metformin is an orally administered biguanide that lowers glucose by reducing hepatic glucose production and gluconeogenesis and by enhancing peripheral insulin sensitivity [4–6]. Glimepiride is an oral sulfonylurea hypoglycemic agent indicated for the treatment of type 2 diabetes mellitus. The primary mechanism of action of glimepiride for lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic cells [7,8].

Hence, the combination of atorvastatin, metformin and glimepiride extends release complement each other and provides reduction in plasma cholesterol along with glycemic control, thereby providing a comprehensive control of diabetes and associated dyslipidemia. TRIPILL (Cipla Limited, Mumbai, India) is a fixed dose combination of metformin hydrochloride (500 mg), atorvastatin (10 mg) and glimepiride (2 mg). For many patients with type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target blood glucose levels and multiple drugs may be necessary to achieve adequate control [9,10]. In such cases metformin has been coadministered with glimepiride [4,9]. The combination of atorvastatin and metformin has greater benefit in improving liver injury in type 2 diabetes with hyperlipidemia [11].

As per the literature, several LC–MS/MS methods have been reported for the determination of atorvastatin [12–22], metformin [23–33] and glimepiride [34–38] individually or with some other drugs in biological samples. To date, no LC–MS/MS method has been reported for the simultaneous determination of atorvastatin, metformin and glimepiride in human plasma. Simultaneous determination of atorvastatin, metformin and glimepiride remains difficult using single mode of separation and extraction due to their different physicochemical properties and polarities. To address the pharmacokinetics of the new combined formulation, a sensitive and specific method that allows simultaneous measurement of atorvastatin, metformin and glimepiride in human plasma is needed. We felt that this simultaneous estimation method will help the researchers as the three drugs used in this method were available in market with fixed dose combination. The present work describes a simple, selective and sensitive method, which employs simple protein precipitation technique for sample preparation and liquid chromatography with electrospray ionization-tandem mass spectrometry for simultaneous quantitation of atorvastatin, metformin and glimepiride in human plasma. The application of this assay method to a clinical pharmacokinetic study in healthy male volunteers following oral administration of atorvastatin, metformin and glimepiride is described. The authenticity in the measurement of study data is demonstrated through incurred samples reanalysis.

2. Experimental

2.1. Chemicals and materials

The reference samples of atorvastatin calcium (97.90%), metformin hydrochloride (99.60%), glimepiride (99.40%) and IS (98.71%) were procured form Neucon Pharma Pvt. Ltd., (Goa, India). Chemical structures of these compounds are presented in Fig. 1. Water used for the LC–MS/MS analysis was prepared from Milli-Q water purification system procured from Millipore (Bangalore, India). Acetonitrile and methanol were of HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). Analytical grade ammonium acetate and acetic acid were purchased from Merck Ltd., (Mumbai, India). The control human plasma sample was procured from Deccan's Pathological Labs (Hyderabad, India).

2.2. Instrumentation and chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a Alltima HP C18 HL column (50 mm × 4.6 mm, 3 μm; Grace Davison, Deerfield, Ireland), a binary LC-20 AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20 A_3) were used for the study. Aliquots of the processed samples (25 μL) were injected into the column, which was kept at room temperature. The isocratic mobile phase, 60:40 (v/v) mixture of acetonitrile and 10 mM ammonium acetate (pH 3.00±0.05), was delivered at 1.1 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantitation was achieved with MS-MS detection in positive ion mode for all the analytes and the internal standard using an MDS Sciex API-4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ interface at 550 °C. The ion spray voltage was set at 4800 V. The source parameters viz. the nebulizer gas,

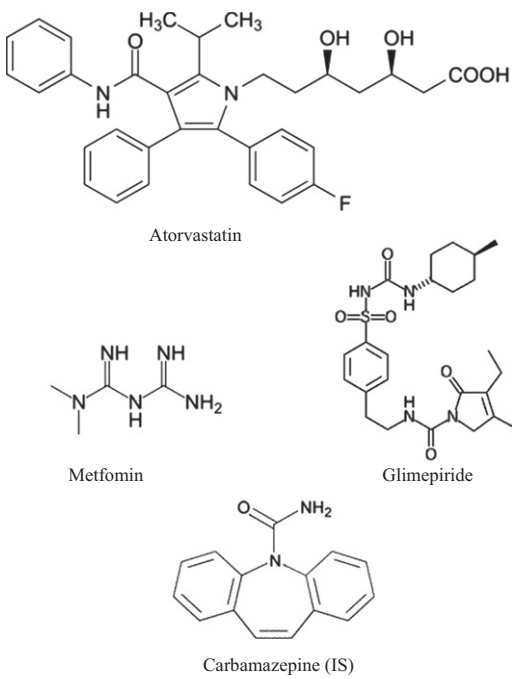

Fig. 1 Chemical structures of atorvastatin, metformin, glimepiride and carbamazepine (IS).