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Simultaneous estimation of atorvastatin calcium, glimepride and metformin hydrochloride in tablet formulation by RP-HPLC method

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Abstract

A rapid and sensitive high performance liquid chromatography method for determination of atorvastatin calcium, glimepride and metformin hydrochloride has been developed. The chromatography system used a reversed phase C18 column with UV detection at 248 nm. Mobile phase consisted of 45:35:20 (v/v) mixture of 0.01 M potassium di hydrogen phosphate (pH adjusted to 6.95 with orthophosphoric acid), acetonitrile and methanol at a flow rate of 1 ml/min. The calibration curve was linear in the concentration range of 4-22 µg/ml for atorvastatin calcium, 2.00-10.00 µg/ml for glimepride and 4-20 µg/ml for metformin hydrochloride. Percentage recoveries of three drugs were 101.21%, 102.21% and 100.41% for atorvastatin calcium, glimepride and metformin HCl respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of atorvastatin calcium, glimepride and metformin HCl in pharmaceutical dosage form. The method was validated with respect to linearity, precision and accuracy as per the International Conference on Harmonisation (ICH) guidelines.

Key words: Atorvastatin calcium, glimepride and metformin hydrochloride, HPLC analysis.

Introduction

Atorvastatin calcium chemically [R-(R,R*)]-2-(4-flurophenyl)-â,ä-dihydroxy-5(1-methylethyl)-3-phenyl-4-[phenylamino) carbonyl]-1H-pyrrole-1-heptanoicacid, calcium salt (2:1) trihydrate, is a synthetic HMG-CoA reductase inhibitor (Moffat et al. 2004). It has been demonstrated to be efficacious in reducing both cholesterol and triglycerides (Poswar et al. 1996) and (Page et al. 2002). Various analytical methods such as HPLC, GC-MS are reported for estimation of atorvastatin Calcium (Altuntas et al. 2004), (Gowri Sankar et al. 2005) and (McKenney et al. 1998).

Metformin

Metformin HCl is an oral biguanidine, which reduces the elevated blood glucose concentration in patients with diabetes but does not increase insulin secretion. It does not lower the blood glucose in nondiabetic subjects (Hermann et al. 1995). Augmentation of muscular glucose uptake and utilization, and reduction of increased hepatic glucose production through an antigluconergic action explain the blood glucose lowering effect (Hermann et al. 1979), (Denno et al. 1994). Metformin is safe and not teratogenic in many of the species studied (Tucker et al. 1981), (Pentikainen et al. 1979). Many HPLC methods for the analysis of metformin in plasma are reported. But most of the methods use ion pair reagent or cation exchange column (Zarghi et al. 2003), (Zhang et al. 2002), (Cheng et al. 2001) and (Bonfigli et al. 1999).

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Glimepride

Glimepride (GLM) is a sulfonylurea urea derivative chemically-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyyroline-1-oxamide) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea, widely used in patients with type 2 diabetes (non-insulin dependent diabetes mellitus) (Chakradhar et al. 2007). Method for estimation of glimepride in human plasma, various HPLC methods for the estimation of glimepride are reported (Salem et al. 2004), (Kovaríková et al. 2004) and (Khan et al. 2005).

At present no HPLC methods are reported for the simultaneous estimation of atorvastatin calcium, metformin HCl and glimepride in tablet formulation. Therefore, it was thought worthwhile to develop simple, precise, accurate reverse phase HPLC methods for simultaneous determination of atorvastatin calcium, metformin and glimepride in tablet formulation.

Materials and Methods

Drugs: Atorvastatin Calcium, Glimepride and Metformin Hydrochloride.

Chemicals and solvents: potassium di hydrogen phosphate, orthophosphoric acid was purchased from S.D. fine chemicals Ltd., India. Acetonitrile, methanol and water of HPLC grade were purchased from Qualigens Fine Chemicals, India. The gift samples of atorvastatin received from Intra-Lab India Pvt. Ltd, Bangalore, India. Glimepride and metformin HCl were the gift sample from Dr.Reddy's Laboratories Ltd, Hyderabad, India. The formulation (tablet) CD pro-2 was purchased from local market. Nylon syringe membrane filters (0.2 µm) were purchased from Sartoris, Germany.

HPLC system

The HPLC system consists of delivery pump (Shimadzu), reversed phase analytical column of Phenomenex Luna C18 (250 x 4.60 mm) internal diameter) 5 µm particle, Ryedone sample injector with a 20 µl loop volume and fixed wavelength detector (UV detector).

Chromatographic condition

The mobile phase consisted of 45:35:20 (v/v) mixture of 0.01 M potassium di hydrogen phosphate (pH adjusted to 6.95 with orthophosphoric acid), acetonitrile and methanol. The solution was filtered through a 0.2 µm membrane filters. The eluent was monitored with UV detector set at 248 nm with flow rate of 1 ml/min. Mobile phase was stirred on VCX 750 Microprocessor based Vibra cell Ultrasonic processor of 750 Watts and 20 KHz.

Standard solution and calibration curve

A standard stock solution of atorvastatin, metformin HCl and glimepride (10 mg each) were weighed accurately and separately transferred to 100 ml volumetric flasks. All three drugs were dissolved in 0.01 M potassium di hydrogen phosphate (pH adjusted to 6.95 with orthophosphoric acid), acetonitrile and methanol (45:35:20) (v/v) to prepare standard solutions containing 100 µg/ml. Subsequent dilutions are were made in mobile phase to give the concentrations 4, 8, 12, 18, 22 µg/ml for atorvastatin calcium, 2, 4, 6, 8 and 10 µg/ml for glimepride and 4, 8, 12, 16 and 20µg/ml for metformin HCl. The calibration curve was obtained by plotting the peak area of drug versus concentration.

Assay

For analysis of the tablet dosage form, twenty CD pro2 tablets were weighed individually and their average weight was determined. Tablets were then crushed to a fine powder and powder equivalent to the weight of one tablet was transferred to 100 ml volumetric flask and dissolved in 0.01 M potassium di hydrogen phosphate (pH adjusted to 6.95 with orthophosphoric acid), acetonitrile and methanol (45:35:20) (v/v). The solution was vigorously stirred for 15 min and sonicated for 15 min, then filtered through 0.2µ membrane filter and washed with solvent. The solution was then diluted with the same solvent. Twenty µl of this solution was injected in triplicate under the specified conditions. The amounts

of atorvastatin, glimepride, and metformin HCl in tablet were calculated by extrapolating the peak area from the calibration curve.

The results are reported in Table 1.

Table 1. Results of HPLC assay.

Drug	Label claim (mg per tablet, n=6)	Amount found (mg)	Drug concentration (%)	S.D.	COV	S.E
ATR	10	09.98	99.85	0.54	0.523	0.256
GLM	2	1.99	99.98	0.16	0.131	0.092
MET	500	510.30	102.06	0.75	0.740	0.320

Validation of the assay

To study the accuracy, reproducibility and precision, recovery experiment was carried out. The recovery of the added standard was studied at three different levels. To an aliquot of the analyzed formulation a known concentration of standard solution was added. The content of atorvastatin calcium, glimepride, and metformin was determined (Table 2). The linearity of the standard curve was confirmed for atorvastatin calcium, Glimepride and metformin HCl by plotting peak area against concentration. From these calibration plots it was clear that response was a linear function of concentration over the ranges of 4-22 µg/ml for atorvastatin calcium, 2-10 µg/ml for glimepride and 4-20 µg/ml metformin HCl. The linear regression equations for

Atorvastatin calcium: $y = 3098 x + 96124$ ($n=6$, $r^2 = 0.998$)
Glimepride: $y = 59919 x + 272388$ ($n=6$, $r^2 = 0.999$)
Metformin HCl: $y = 3098 x + 96124$ ($n=6$, $r^2 = 0.999$)
where y is response (peak area) and x is the concentration in µg/ml.

Table 2. Results of recovery studies.

	Atorvastatin calcium			Glimepride			Metformin HCl		
Amount added (mg)	5	10	15	5	10	15	5	10	15
Amount found (mg)	15.10	20.23	25.31	7.09	12.52	17.18	505.10	510.84	515.37
Percentage Recovery	100.66	101.15	101.24	101.25	104.33	101.05	101.01	101.16	100.07
Mean	101.01			102.21			100.41		

Results and Discussion

Figure 1 shows typical chromatograms of three drugs. As per USP-XXIII, system suitability tests were carried out on freshly prepared standard stock solutions of drugs (Table 3). The calibration curve was linear in the range of 4-22 µg/ml for atorvastatin calcium, 2-10 µg/ml for glimepride and 4-20 µg/ml for metformin HCl. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.0100 µg, 1.05 µg for Atorvastatin calcium; 0.20 µg, 2.90 µg for glimepride and 0.100 µg, 2.15 µg for metformin HCl.

Table 3. System suitability parameters.

Drugs	Atorvastatin calcium	Glimepride	Metformin HCl
Concentration (µg/ml)	4-22	2-10	4-20
Theoretical plates	4593	11236	1090
Tailing factor	1.12	1.3	1.2
LOD (µg)	0.10	0.20	1.20
LOQ (µg)	1.05	2.90	2.15

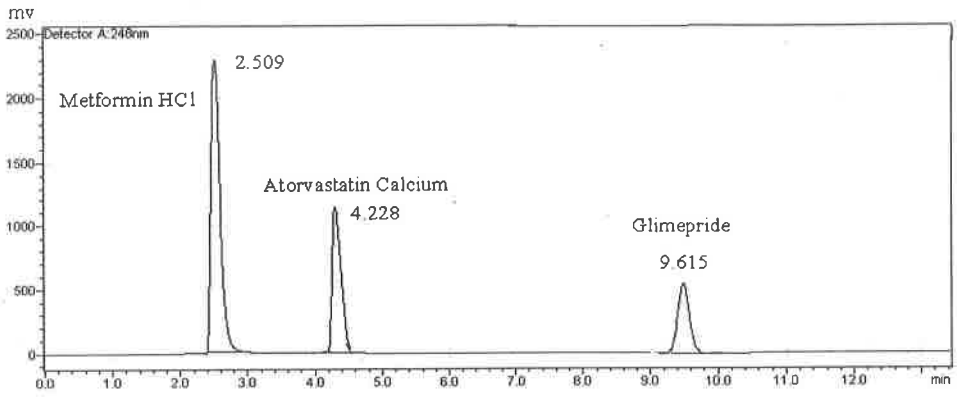

Figure 1. Chromatogram of Metformin HCl, Atorvastatin calcium and Glimepride.

Conclusion

The HPLC assay method described here is simple, precise and accurate for quantitation of in atorvastatin calcium, glimepride and metformin HCl in tablet dosage form. The sensitivity, simplicity and rapidity of the method were the main advantages of the method. The method can be conveniently used for the tablet dosage form. Hence, it can be conveniently adopted for routine quality control analysis in the combination formulation.

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