Bulletin of Pharmaceutical Research 2013;3(1):29-33
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ROSUVASTATIN IN COMBINED BULK FORMS BY RP-HPLC USING ULTRAVIOLET DETECTION

Saurabh Kumar Banerjee* and Nitin M. Vasava

Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, SVKM's NMIMS, Shirpur Campus, Dhule-425 405, Maharashtra, India

**E-mail*: saurabhbanerjee15@gmail.com
Tel.: +91 9765362007.

Received: March 21, 2013 / Revised: April 01, 2013 / Accepted: April 02, 2013

The objective of the study was to develop simple RP-HPLC method for the simultaneous determination of amlodipine and rosuvastatin. In this method, kromasil C18 (100 mm, 4.6 mm, 5 μm) column was used. The mobile phase and flow rate used were {(acetonitrile 40, 55, 70, 40, 40): (phosphate buffer 60, 45, 30, 60, 60)}, (Time 0.5, 2.0, 3.0, 3.0, 2.0 min). UV detection was monitored at 239 nm. Calibration graphs were established for amlodipine and rosuvastatin. The average retention time for amlodipine and rosuvastatin was found to be 2.40±0.16 min and 4.28±0.04 min, respectively. The intraday and Interday precision expressed as percent relative standard deviation was below 2%. The validated HPLC method was found to be rapid, precise and accurate and can be readily utilized for analysis of amlodipine and rosuvastatin in bulk forms.

Key words: Amlodipine, Rosuvastatin, RP-HPLC, Method development, Validation.

INTRODUCTION

Amlodipine besylate, 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester (**Figure 1**), is a long-acting calcium channel blocker which is used as an anti-hypertensive and in the treatment of angina (EP, 2005; USP, 2007).

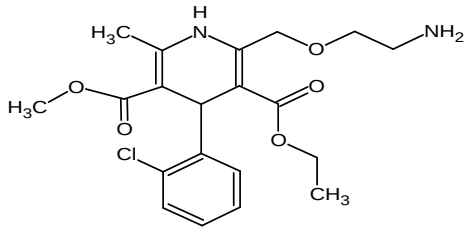

Fig. 1. Structure of amlodipine

Owing to widespread use of amlodipine in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of amlodipine individual and in combination are being investigated (Rahman and Azmi, 2001; Zarghi *et al* 2005; Dongre *et al* 2008). The most recent methods for the determination of amlodipine besylate include chromatographic, spectrophotometric and titrimetric techniques.

Rosuvastatin, (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid (**Figure 2**), is a member of the drug class of statins which is used to treat high cholesterol and related conditions, and to prevent cardiovascular disease (O'Neil, 2006; Srinivasa Rao *et al* 2011). In the literature, a capillary zone electrophoretic, UV spectrophotometric, LC/MS, and high performance liquid chromatography (HPLC) methods are reported for the analysis of rosuvastatin (Sane *et al* 2005; Gupta *et al* 2009; Kaila *et al* 2010). More accurate, simple and widely used HPLC method has been not reported for the simultaneous estimation of amlodipine and rosuvastatin in combination formulation.

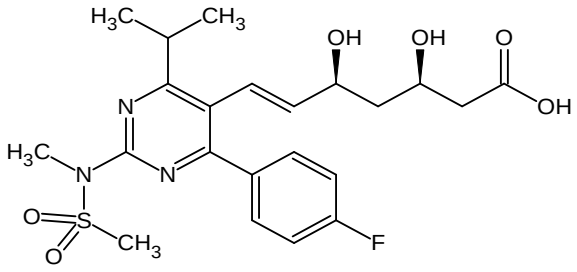

Fig. 2. Structure of rosuvastatin

So, in continuation of work done on method validation and simultaneous estimation of drugs by our research group (Shah *et al* 2011; Patil *et al* 2011; Chhabra *et al* 2012) and other scientists (Singh *et al* 2011; Basaveswara Rao *et al* 2012; Jain *et al* 2012), the present investigation was directed toward simultaneous estimation of amlodipine and rosuvastatin in combined bulk forms by RP-HPLC using ultraviolet detection.

MATERIALS AND METHODS

Chromatographic conditions

Analytical conditions were standardized through the LC system using kromasil C 18 column (100 mm, 4.6 mm, 5 μm). The mobile phase used was {(acetonitrile 40, 55, 70, 40, 40) **:** (phosphate buffer 60, 45, 30, 60, 60)}, (Time 0.5, 2.0, 3.0, 3.0, 2.0 min). UV detection was made at 239 nm. The volume of injection was fixed at 20 μl. All analyses were done at 30°C. The mobile phase was prepared fresh each day, vacuum-filtered through 0.50 μm millipore nylon filters.

Validation of the method

The developed method was validated as per ICH guidelines in terms of accuracy, specificity, linearity, precision (ICH, 1994). The precision (% relative standard deviation, %RSD) was expressed with respect to the intraday and interday variation in the expected drug concentration. After validation, the developed method was applied to pharmaceutical bulk forms containing amlodipine and rosuvastatin.

Linearity

Stock solution was prepared by dissolving 10 mg each of amlodipine and rosuvastatin in 10 ml volumetric flask with methanol. From the above stock solutions, dilutions were made to get the concentration in the range of 1-150 ppm of amlodipine and 0.5-100 ppm of rosuvastatin. A volume of 20 μl of each sample was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus corresponding drug concentration.

Precision

The precision of the proposed method was assessed as intermediate precision and repeatability by preparing three different sample solutions at low, medium and high concentrations, which were prepared freshly and daily analysed. These experiments were repeated over a 2-day period.

RESULTS AND DISCUSSION

HPLC method was found to be accurate, simple, economic and rapid for routine simultaneous estimation of amlodipine and rosuvastatin in bulk forms.

Optimization of the chromatographic conditions

Initially, the mobile phase used was acetonitrile:phosphate buffer (70:30%) then, ratio of the solvents were varied. At 70:30%, there was no good separation and at 50:50%, tailing of amlodipine was observed, at 30:70%, again there was no good separation. Gradient composition of mobile phase were tried in order to get better separation and good resolution. At time 0.5, 3.0, 3.0, 2.0, 7.0 min, acetonitrile (30, 40, 70, 30, 30) **:** (phosphate buffer 70, 60, 30, 70, 70)}, good separation of amlodipine and rosuvastatin was observed but the retention time was more. At time 0.5, 2.0, 3.0, 3.0, 2.0 min, acetonitrile (40, 55, 70, 40, 40) **:** (phosphate buffer 60, 45, 30, 60, 60)}, better resolution and less retention time was observed. Different values of pH of phosphate buffer were tried. Phosphate buffer with pH 3.0 has been selected for analysis. The isobestic wavelength 239 nm has been found to be optimum (**Figure 3**). The average retention time for amlodipine and rosuvastatin was found to be 2.40±0.16 min and 4.28±0.04 min, respectively (**Figure 4**).

Linearity

A linear calibration graph was obtained over six concentrations 10, 20, 30, 40, 50, 100 ppm (**Figure 5, 6**).

Precision

Intra-day precision of the method was determined by repeat analysis (three identical injections) at three concentration levels.

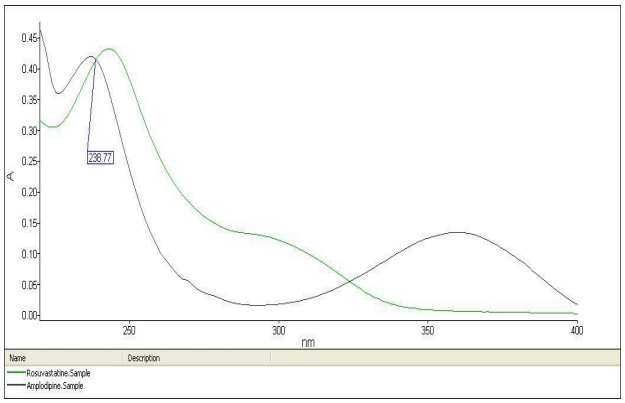

Fig. 3. λ_{max} of amlodipine and rosuvastatin (Isobestic point)

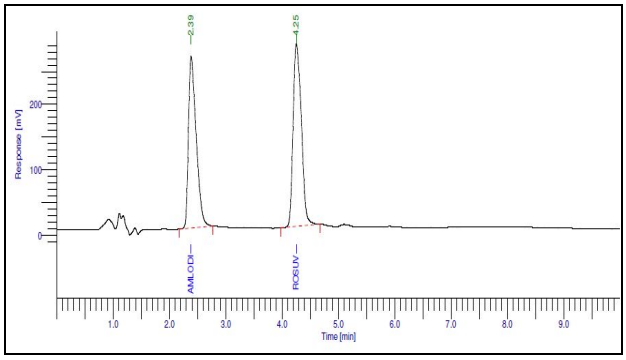

Fig. 4. Chromatogram of standard amlodipine and rosuvastatin (100 ppm each)

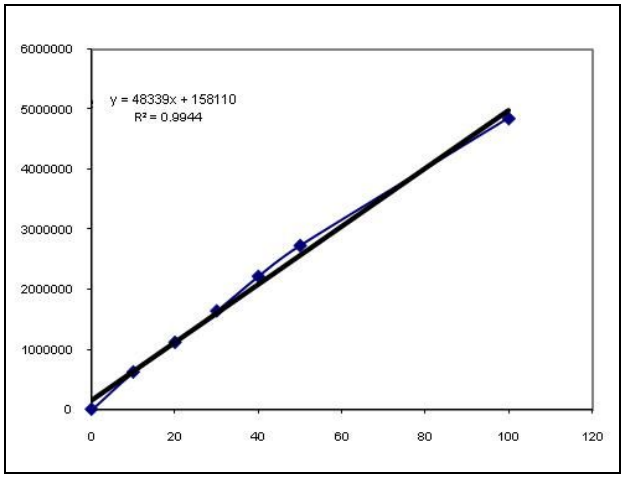

Fig. 5. Linearity curve of amlodipine

Inter-day precision was established by performing the analysis next day on a freshly prepared solution. The low RSD values of table indicated the ruggedness of the method (**Table 1, 2**).

Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amounts of amlodipine and rosuvastatin were added to a pre quantified sample solution of its dosage form and the amounts were estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range. Accuracy was evaluated at three different concentrations equivalent to 80, 100 and 120% of the active ingredient by calculating the recovery with %RSD (**Table 3**).

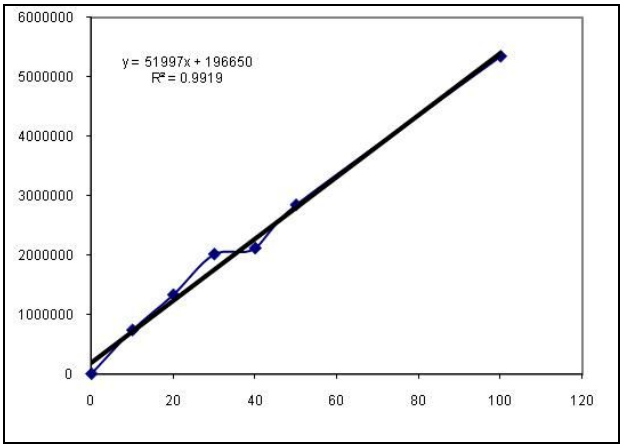

Fig. 6. Linearity curve of rosuvastatin

Repeatability

The peak area of 40 ppm drug solution was analyzed six times on the same day. The % RSD was calculated for the resultant peak area (**Table 4**).

Robustness

The HPLC method was found to be robust as the results were not significantly affected by slight variation in the extraction time, composition of mobile phase, flow rate and wavelength.

CONCLUSION

The proposed method is simple, accurate, rapid, economical and selective for the simultaneous estimation of Amlodipine and Rosuvastatin in bulk form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. By HPLC method, analysis can be done within 10 min with the use of simple solvents.

Hence, developed HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulations.

Table 1. Precision studies of amlodipine

Interday						
Conc. (ppm)	**I**	**II**	**III**	**Mean**	**±SD**	**%RSD**
10	297573.69	296663.83	292229.82	295489.11	2859.06	0.97
30	1183686.78	1193521.24	1173709.80	1188604.01	6954.01	0.59
50	2518405.27	2504416.23	2530877.87	2517899.79	13238.06	0.53
Intraday						
Conc. (ppm)	**I**	**II**	**III**	**Mean**	**±SD**	**%RSD**
10	313786.59	307883.21	312242.86	311304.22	3061.58	0.98
30	1226229.06	1223540.03	1243170.93	1230980.01	10642.92	0.86
50	2736451.63	2758890.17	2749090.18	2748143.99	11249.15	0.41

Table 2. Precision studies of rosuvastatin

Interday						
Conc. (ppm)	**I**	**II**	**III**	**Mean**	**±SD**	**%RSD**
10	501886.53	508858.15	505273.96	505339.55	3486.27	0.69
30	1562916.94	1545859.52	1557682.51	1554388.23	12061.42	0.78
50	2812006.79	2834272.39	2838099.44	2828126.21	14090.36	0.50
Intraday						
Conc. (ppm)	**I**	**II**	**III**	**Mean**	**±SD**	**%RSD**
10	534151.65	537484.46	539466.22	537034.11	2685.75	0.50
30	1615581.5	1620816.27	1648000.36	1628132.71	17403.83	1.07
50	3130738.37	3110299.25	3114658.12	3118565.25	10765.16	0.35

Table 3. Results of recovery studies

Level of recovery	**% Recovery found (Amlodipine)**	**% Recovery found (Rosuvastatin)**	**% RSD (Amlodipine)**	**% RSD (Rosuvastatin)**
80%	99.77	99.87	0.18	0.21
100%	99.52	99.34	0.41	0.25
120%	99.40	100.04	0.49	0.33

Table 4. Results of repeatability studies

Drug	**Conc. (ppm)**	**% RDS**
Amlodipine	40	0.92
Rosuvastatin	40	1.39

REFERENCES

Basaveswara Rao MV, Prasanthi V, Sivanadh M, Venkata Rao G. Newer RP-HPLC method for the determination of doxazosin in human plasma and formulation. *Bull. Pharm. Res.* 2012;2(1):1-4.

Chhabra GS, Bhalodiya HK, Banerjee SK. LC-MS-MS method validation of pregabalin in human plasma. *Bull. Pharm. Res.* 2012;2(2):66-9.

Dongre VG, Shah SB, Karmuse PP, Phadke M, Jadhav VK. Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC. *J. Pharm. Biomed. Anal.* 2008;46(3):583-6. [DOI: 10.1016/j.jpba.2007.11.006]

European Pharmacopoeia 5.0, Council of Europe: Strasbourg, France, 2005; 2369-70.

Gupta A, Mishra P, Shah K. Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. *E-J. Chem.* 2009;6(1);89-92. [DOI: 10.1155/2009/956712]

ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1),

Current *Step 4* version. Parent Guideline dated 27 October,1994.

Jain N, Jain R, Jain DK, Jain SK. RP-HPLC method for simultaneous estimation of esomeprazole magnesium and domperidone in a tablet dosage form. *Bull. Pharm. Res.* 2012;2(3):134-9.

Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT, Shah AK. A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. *Indian J. Pharm. Sci.* 2010;72(5):592-8. [DOI: 10.4103/0250-474X.78526]

O'Neil MJ. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th edition, Merck & Co., Inc.: NJ, USA, 2006.

Patil MG, Banerjee SK, Bonde CG, Chhabra GS. UV spectrophotometric method development for the determination of desvenlafaxine succinate in tablet formulation. *Bull. Pharm. Res.* 2011;1(1):40-3.

Rahman N, Azmi SNH. Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. *Farmaco* 2001; 56(10):731-5 [DOI: 10.1016/S0014-827X(01)01093-X]

Sane RT, Kamat SS, Menon SN, Inamdar SR, Mote MR. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography. *J. Planar Chromatogr. - Modern TLC* 2005;18(103):194-8. [DOI: 10.1556/JPC.18.20 05.3.4]

Shah J, Banerjee SK, Chhabra GS. UV spectrophotometric method development and validation for entacapone in bulk and formulation. *Bull. Pharm. Res.* 2011;1(2):7-9.

Singh S, Yadav AK, Gautam H. Simultaneous estimation of valsartan and hydrochlorothiazide in solid dosage form using UV spectroscopy. *Bull. Pharm. Res.* 2011;1(3):10-12.

Srinivasa Rao K, Prasad T, Mohanta GP, Manna PK. An overview of statins as hypolipidemic drugs. *Int. J. Pharm. Sci. Drug Res.* 2011;3(3):178-83.

The United States Pharmacopoeia 30, National Formulary 25, The United States Pharmacopoeial Convention: Rockville, USA, 2007; 3126.

Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies. *Farmaco* 2005;60(9):789-92. [DOI: 10.1016/j.farmac.2005.06.012]

*****