Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

AN ISOCRATIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ASPIRIN, RAMIPRIL AND SIMVASTATIN BY RP-HPLC

A.K.M. PAWAR*, K. SREEKANTH, A.B.N. NAGESWARA RAO, D. GOWRI SANKAR

University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530003. Email: akmpawarau@rediffmail.com

Received: 20 Oct 2011, Revised and Accepted: 5 Dec 2011

ABSTRACT

A simple isocratic Liquid Chromatographic method was developed and validated for the simultaneous quantitative estimation of Aspirin (ASP), Ramipril (RAM) and Simvastatin (SIM). Is a Spanish polypill under phase II clinical trials in its bulk and simulated formulation. Chromatography was carried on Lichrosphere 100 RP-18 (5 µm, 250 mm x 4.60 mm I.D.) column with mobile phase comprising of acetonitrile and pH-2.5 buffer (0.1% orthophosphoric acid adjusted with TEA) in the ratio 70:30 v/v. The flow rate was 1.0 ml/min with UV detection at 225 nm. The retention times of ASP, RAM and SIM were found to be 4.075 min, 6.850 min, and 17.400 min respectively. The different validation parameters such as linearity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) guidelines. In the linearity study, the regression equation and coefficient of correlation for ASP, RAM and SIM were found to be y=13548x+5412(R^2=0.999), y=933x-180(R^2=0.999) and y=14880x+1505.3 (R^2=0.999). The proposed method is highly sensitive, precise, accurate and easily applicable for routine analysis and stability studies due to good resolution between peaks.

Keywords: Aspirin, Ramipril, Simvastatin, HPLC

INTRODUCTION

Cardiovascular diseases (CVDs) are the disorders of the heart and blood vessels which primarily includes coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure.[1]

Aspirin

Aspirin (Fig I) is a salicylate drug, often used as an analgesic, an antipyretic and also as an anti-inflammatory medication.[6-8] The platelet aggregation-inhibiting effect of acetyl salicylic acid specifically involves the compound's ability to act as an acetyl donor to cyclooxygenase. Irreversibly acetylating renders cyclooxygenase inactive, thereby preventing the formation of the aggregating agent, thromboxanes A2 in platelets. Since, platelets lack the ability to synthesize new proteins; the effects persist for the life of the exposed platelets (7-10 days). Acetyl salicylic acid may also inhibit production of the platelet aggregation inhibitor, prostacyclin (prostaglandin I2), by blood vessel endothelial cells; however inhibition prostacyclin production is not permanent as endothelial cells can produce more cyclooxygenase to replace the non functional enzyme.

Aspirin is chemically 2-acetoxybenzoic acid.[2-5]

Fig. I: Aspirin

Ramipril

Ramipril (Fig II) is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat, the principle active metabolite of ramipril competes with AT-I for binding to ACE and inhibits and enzymatic proteolysis of AT-I to AT-II. Decreasing AT-II levels in the body decreases blood pressure by inhibiting the pressor effects of AT-II. Ramipril also causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by AT-II on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.[2-5]

Ramipril is chemically (2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxilic acid.

Fig. II: Ramipril

Simvastatin

Simvastatin (Fig.3) is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. [2-5] It is a member of the statin class of pharmaceuticals, and is a synthetic derivate of a fermentation product of *Aspergillus terreus*. The 6-membered lactone ring of simvastatin is hydrolyzed *in vivo* to generate the beta,delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA) that competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme thereby reduces the quantity of mevalonic acid, a precursor of cholesterol.

Simvastatin is chemically (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxo oxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl2,2-dimethylbutanoate.

Fig. III: Simvastatin

Many analytical methods have been reported for the estimation of ASP, RAM and SIM individually and in combination[9-17] in pharmaceutical formulations and bulk samples. As per our knowledge and thorough literature survey revealed that there is no isocratic, single run method available for simultaneous estimation of ASP, RAM and SIM. The combination we are selected is a Spanish polypill which was under phase II clinical trials. In the current study we employed simple mobile phase preparation. The proposed method is easy to perform and applicable for quality control analysis and also stability studies.

MATERIALS AND METHODS

Instrumentation

JASCO 2080 model chromatograph equipped with Lichrosphere 100 RP-18 (5 µm, 250 mm x 4.60 mm I.D.) was employed for the study. Sample injection was done with a Rheodyne 7725 injection valve via a 20 µl loop. Detection of the drug was done by using a UV-2075 detector (JASCO) and the output signal was monitored and integrated by JASCO -BORWIN software. Solubility of the compound was enhanced by sonication on an ultrasonicator. UV spectrum of aspirin, ramipril and simvastatin combination was taken using a JASCO V-550 UV-Vis spectrophotometer in order to select the working wavelength for detection of the drugs. All the weighing in the experiments was done with Digisum Electronic analytical balance (model DI 707).

Chemicals and reagents

The reference samples of ASP, RAM and SIM were obtained from Aurobindo Pharma, Dr. Reddy's Laboratories and Smilax Laboratories Limited respectively, Hyderabad, India.

Purified water was prepared by using 0.45 Millipore Milli-Q water purification systems. HPLC grade acetonitrile was used for preparing the mobile phase and the diluent. Triethylamine and orthophosphoric acid were of analytical grade obtained from Sigma Aldrich.

Preparation of standard and working stock solution

The stock solutions were prepared by dissolving the suitable quantity of ASP, RAM and SIM to get the concentrations of 2000µg/mL aspirin, 100µg/mL ramipril and 400µg/ml Simvastatin were prepared. Further dilutions were made from the working stock solution in the required concentration range in 10 ml volumetric flasks for the calibration curve.

Method Development

A number of eluting systems were examined for the optimization of the mobile phase for separation of the drugs. We tried phosphate buffer of same pH range but the preparation of water containing orthophosphoric acid is easy to prepare and when we use phosphate buffer of pH 3 with same mobile phase composition the RT of SIM is around 60 min. But it will come down when we use the buffer in the current method. Mixtures containing buffer and acetonitrile were used as eluting systems in different proportions like 50:50, 40:60 and 30:70 v/v. A mixture of buffer and acetonitrile in the ratio of 30:70 v/v provided an efficient separation of the drugs with good peak shapes. A flow rate of 1.0 ml/min was found to be optimum in the range of 0.8-1.0 ml/min which gave retention times of 4.075 min for ASP, 6.850 min for RAM and 17.400 min for SIM with baseline stability.

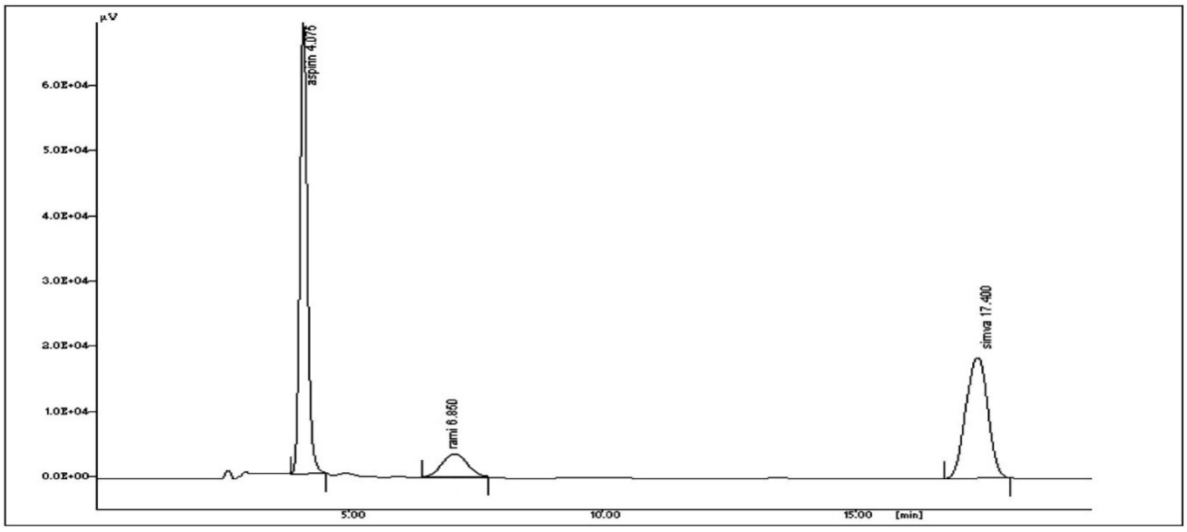

Fig. IV: Chromatogram of standard solution of ASP, RAM and SIM

Quantitative Aspects

Method validation

As per the International Conference on Harmonization (ICH) guidelines [18-20], the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

System suitability

For system suitability, six replicates of the working standard sample were injected and the parameters like plate number (N), HETP and peak asymmetry of samples were calculated and the results are listed in Table-1.

Linearity

To establish linearity, a stock solution containing 2000 µg/ml ASP, 100 µg/ml RAM and 400 µg/ml SIM were prepared using diluent (mixture containing buffer and acetonitrile in the ratio of 80:20 v/v) and was further diluted to yield solutions in the concentration range of 100-2000 µg/ml, 5-100 µg/ml and 20-400 µg/ml of ASP, RAM and SIM respectively.

The solutions were prepared and analyzed in triplicate. The experiment was repeated three times by preparing different solutions and analyzed by injecting 20 µl in HPLC. Linearity data for ASP, RAM and SIM are given in Table-2

Table 1: system suitability parameters for the proposed method

Parameter	Result		
	ASP	RAM	SIM
Theoretical plates	5983.84	4305.23	8030.93
HETP	$4.18x10^{-5}$	$5.81x10^{-5}$	$3.11x10^{-5}$
Asymmetry	1.18	0.86	0.97
LOD(µg/ml)	6.602	0.685	2.324
LOQ(µg/ml)	20.005	2.076	7.043

*ASP= Aspirin, RAM= Ramipril, SIM= Simvastatin

Table 2: linearity parameters for calibration curve (n=6)

Name of the compound	Conc. (µg/ml)	Equation of regression line	R^2
ASP	100-2000	y= 13548x + 5412	0.999
RAM	5-100	y = 933.02x - 180.08	0.999
SIM	20-400	y = 14880x + 1505.3	0.999

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is defined as the smallest level of analyte that gives a measurable response. LOD is based on S/N ratio (signal/noise) typically for HPLC methods. Six replicates of the analyte were measured. The LOQ is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the precision expressed by relative standard deviation (RSD) is less than 2% and accuracy expressed by relative difference in the measured value and true value is also less than 2%. In other words, the analyte response is 10 times greater than the noise response. Six replicates of the analyte were analyzed and quantified. The LOD and LOQ of ASP, RAM and SIM are 6.602µg/ml and 20.005µg/ml, 0.685µg/ml and 2.076µg/ml, and 2.324µg/ml and 7.043µg/ml respectively.

Precision

The intra- and inter-day precision were determined by analyzing 1000 µg/ml ASP, 50 µg/ml RAM, 200 µg/ml SIM on same day and consecutive days, respectively. The intermediate precision was determined by changing column brand and also whole experiment was conducted by different analyst on different instrument. The results of intraday and interday precisions are represented in the Table-3.

Accuracy

The accuracy of the method was determined by spiking a known mixture of the drugs corresponding to ASP (400 µg/ml, 500 µg/ml, 600 µg/ml), RAM (20 µg/ml, 25 µg/ml, 30 µg/ml) and SIM (80 µg/ml, 100 µg/ml, 120 µg/ml) in triplicate and then determining the percent recovery by calculating differences between the peak areas obtained for fortified and unfortified solution. The results are incorporated in Table-4.

Robustness

The robustness of the method was determined as per USP guidelines under a variety of conditions including change in flow rate, pH of buffer, and buffer concentration. The results obtained by deliberately variation in method parameters and data are summarized in Table-5.

Table 3: Results of precision (n=6)

	% Assay					
	ASP	%RSD	RAM	%RSD	SIM	%RSD
Day 1	1001.91	0.30	50.10	0.64	202.87	0.22
Day 2	1002.92	0.16	49.86	0.80	202.02	0.67
Mean	1002.42		49.98		202.45	
% RSD	0.071		0.340		0.297	

Table 4: Accuracy and Recovery studies

Drug	Added concentration(µg/ml)	Measured concentration(µg/ml)	% Recovery	Mean % recovery±RSD
	400	400.103	100.026	
ASP	500	500.643	100.129	100.011±0.126
	600	599.267	99.878	
	20	20.310	101.550	
RAM	25	24.883	99.532	100.783±1.085
	30	30.380	101.267	
	80	79.747	99.683	
SIM	100	98.750	98.750	99.419±0.546
	120	119.79	99.825	

Table 5: Results of robustness study

	ASP			RAM			SIM		
	R.T	**A.F**	**T.P**	**R.T**	**A.F**	**T.P**	**R.T**	**A.F**	**T.P**
Flow 0.7ml	4.23	1.17	50492	6.98	1.10	56800	17.45	0.97	876540
Flow 0.9ml	3.90	1.18	59854	6.64	1.16	43052	17.19	1.02	803076
pH 2.4	4.13	1.17	64348	6.95	0.76	65786	17.89	0.76	765479
pH 2.6	4.07	1.17	68663	6.85	0.67	45798	17.56	0.98	776443
Buffer conc. 0.05	4.02	1.16	88457	6.56	1.04	75798	17.54	0.24	787378
Buffer conc. 0.20	4.08	1.18	68443	6.98	0.34	74567	17.98	0.82	786548

*R.T = Retention Time, A.F= Assymetric Factor, T.P= Theoretical Plates

RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of buffer with acetonitrile were tested. Mobile phase containing a mixture of acetonitrile and buffer in the ratio of 70:30 v/v resulted in peaks with good shape and resolution. A flow rate of 1.0 ml/min was found to be optimum in the 0.8-1.0 ml/min range resulting in the short retention time, baseline stability and minimum noise.

By applying the proposed method, the retention times of ASP, RAM and SIM were found to be 4.075 min, 6.850 min and 17.400 min respectively. Quantitative linearity was obeyed in the concentration range of 100-2000 µg/ml, 5-100 µg/ml and 20-400 µg/ml of ASP, RAM and SIM respectively. The regression equations of concentration over their peak areas were found to be y= 13548x+5412 (R^2=0.9992), y= 933.02-180.08 (R^2=0.9991) and y= 14880x+1505.3 (R^2=0.9993) for ASP, RAM and SIM respectively where y is the peak area and x is concentration in µg/ml. The number of theoretical plates obtained was 5983.84, 4305.23 and 8030.93 respectively which indicates the efficient performance of the column. The limit of detection and limit of quantitation were found to be 6.602 and 20.005 µg/ml, 0.685 and 2.076 µg/ml, and 2.324 and 7.043 µg/ml respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

Thus the proposed isocratic RP-HPLC method was found be simple, precise, and accurate and involved simple steps for preparing mobile phase and resolution is also good between peaks and the method could be easily applicable for routine drug analysis in laboratories and pharmaceutical companies.

REFERENCES

1. Vijay Kumar, Bhagwat Prasad, Saranjit Singh. Pharmaceutical issues in the development of a polypill for the treatment of cardiovascular diseases. Drug Discovery Today: Therapeutic Strategies. 2008; 5(1):63-71.
2. John H. Block and John M. Beale Jr. Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Eleventh Edition. Philadelphia: Lippincott Williams & Wilkins, 2007.
3. Graham L. Patrick. An Introduction to Medicinal Chemistry. Fourth Edition. Oxford: Oxford University Press, 2009.
4. Gareth Thomas. Medicinal Chemistry An Introduction. Second Edition. Chichester: John Wiley & Sons, Ltd, 2007.
5. Richard B. Silverman. The Organic Chemistry of Drug Design and Drug Action. Second Edition. San Diego: Elsevier Academic Press, 2004.
6. Laurence L. Brunton, John S. Lazo, Keith L. Parker. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Eleventh edition. New York: McGraw-Hill Medical Publishing Division, 2001.
7. HP Rang, MM Dale, JM Ritter, RJ Flower. Rang and Dale's Pharmacology. Sixth edition. Edinburgh: Churchill Livingstone, 2008.
8. Drug Information 2009, American Society of Health System Pharmacists.
9. Mrunalini C Damle, Shangiresh Madhukar Patole, Laxman Venkatrao Patole and Amol Suresh Khodke, A validated HPLC method for analysis of Atorvastatin calcium, Ramipril and Aspirin as the bulk drug and in combined capsule dosage forms. Pharmacie Globale. 2010, (IJCP),1(09).
10. Purushotam K. Sinha, Mrinalini C. Damle 1 and K.G. Bothara, A Validated Stability Indicating HPTLC Method for Determination of Aspirin and Clopidogrel Bisulphate in Combined Dosage Form. Eurasian J. Anal. Chem. 2009, 4(2): 152-160, 2009
11. Ismail*, R Rajavel, M Ganesh, M Jagadeeswaran, K Srinivasan, J Valarmathi and T Sivakumar, RP-HPLC Method for the Simultaneous Determination of Aspirin, Atorvastatin and Pioglitazone in Capsule Dosage Form. Asian J. Research Chem, 2008, 1(1): July-Sept.
12. Bilal Yilmaz, Determination of Ramipril in pharmaceutical preparations by High-Performance Liquid Chromatography. International Journal of Pharmaceutical Sciences Review and Research, 2010, 1(1):39.
13. Lincy Joseph*, Mathew George and Venkata Ranga Rao B, Simultaneous estimation of Atorvastatin and Ramipril by RP-HPLC and Spectroscopy. Pak. J. Pharm. Sci., 2008, 21(3):282-284.
14. R. J. Chandra Bose 1*, G. Sivanseyal 2, K. K. Duraisamy 1, N. S.Surender 3, P.Ramasamy 3, Validated Rp-Hplc Method for the Simultaneous Estimation of Ramipril and Metoprolol Tartrate in Bulk and Tablet Dosage Form. Asian Journal of Biochemical and Pharmaceutical Research, 2011, 2(1):171.
15. Kuldeep Chouhan, Shubham Agrawal, Chanchal Raj, Samir Jain, A. Balasubramaniam and Navin R. Raj*, Validated RP-HPLC Method for simultaneous determination of Atorvastatin and Ramipril and its application in drug formulation. Der Pharmacia Lettre. 2011, 3(2): 194-202.
16. Nilesh Jain*1, Ruchi Jain 1, Hemant Swami 2 and Deepak Kumar Jain 3, RP-HPLC Method for Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and its Combined Dosage Form. Asian J. Research Chem. 2008, 1(1): 29, July-Sept.
17. Susheel J. V., Thengungal K. R., Quantitative Simultaneous Determination of Amlodipine, Valsartan and Hydrochlorthiazide in EXFORGE HCT using HPLC and HPTLC, Journal of Liquid Chromatography & Related Technologies, 2011, 34:12, 981-994.
18. ICH, Q2B- Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Nov, 1996.
19. International Conference on Harmonization, ICH Q1 A (R2); Stability Testing of New Drug Substances and Products 2003.
20. ICH Steering Committee (1996) International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use, validation of analytical procedure methodology, Geneva.