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Original Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ASPIRIN, RAMIPRIL, HYDROCHLOROTHIAZIDE, SIMVASTATIN AND ATENOLOL FROM PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The present study was designed with an objective of a simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of Aspirin, Ramipril, Hydrochlorothiazide, Simvastatin and Atenolol from bulk drug and formulation.

Methods: The separation was achieved on a Hypersil Gold column (250 mm X 4.6 mm, 5 µ) as stationary phase with a mobile phase consisting of methanol: water in the ratio of 95:5% v/v at a flow rate of 1 mL/min and UV detection at 230 nm.

Results: The retention times were observed to be 1.983, 2.525, 3.108, 3.867 and 7.833 minutes for Aspirin, Ramipril, Hydrochlorothiazide, Simvastatin and Atenolol, respectively. The method was statistically validated for linearity, recovery, the limit of detection, limit of quantification, accuracy and precision.

Conclusion: Thus, proposed method was found sensitive, precise, accurate and specific and be used for quantitative estimation of Aspirin, Ramipril, hydrochlorothiazide, Simvastatin and Atenolol in commercial pharmaceutical dosage form.

Keywords: Aspirin, Atenolol, Ramipril, Hydrochlorothiazide, Simvastatin, RP-HPLC.

INTRODUCTION

Cardiovascular disease (also called heart disease) is a class of diseases that involve the heart, the blood vessels (arteries, capillaries, and veins) or both [1]. There are many risk factors associated with heart diseases. Hypertension is the single biggest risk factor nowadays. Hence, antihypertensive therapy considerably reduces the risk of developing cardiovascular complications that cause a high mortality rate; use of evidence-based multidrug regimens for patients at high risk for cardiovascular disease would be cost-effective in low-income and middle-income countries. For prevention of high risk for cardiovascular disease various combinations of antihypertensive drugs along with aspirin, a calcium-channel, an angiotensin-converting-enzyme inhibitor and a statin are available. Combinations of drugs are used in the treatment of hypertension to improve the tolerability and decrease toxicity profile of the therapy [2-3].

Aspirin is chemically, 2-(acetyloxy) benzoic acid and exerts its anti-inflammatory, analgesic and antipyretic actions. Aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) inhibit the activity of the enzyme called cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever [4].

Ramipril is (2*S*, 3aS, 6aS)-1[(*S*)-N-[(*S*)-1-carboxy-3-phenylpropyl] alanyl] octa hydro cyclohepta [*b*] pyrrole-2-carboxylic acid, 1-ethyl ester. Ramipril 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 is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events [5].

Hydrochlorothiazide is 6-Chloro-3, 4-dihydro-2H-l, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, a thiazide diuretic, by blocking the sodium-chloride symporter and effectively reduces the osmotic gradient and water reabsorption throughout the nephron [6]. Simvastatin is a prodrug and is hydrolyzed to its active β-hydroxyacid form, simvastatin acid, after administration. Simvastatin is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the biosynthetic pathway for cholesterol [7].

Atenolol is chemically 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide and belongs to a class of medications called beta-blockers. Atenolol is a beta-adrenergic (beta-1 selective) agent that blocks beta receptors on the heart [8].

A literature survey revealed that spectrophotometric, chromatographic methods have been reported for determination of ASP [9-14], RAM [15-19], HCTZ [20-27], SIM [28-30] and ATN [31-36] in single and multicomponent pharmaceutical formulations or from biological fluids. However, there were few HPLC methods for simultaneous estimation of ASP, RAM, HCTZ, SIM and ATN reported.

Analysis of ASP, RAM, HCTZ, SIM and ATN has been carried out by gradient HPLC with the flow rate of 1.5 mL/min [37] while the proposed method employs isocratic elution and hence is simpler. Another method reported for analysis of ASP, RAM, HCTZ, SIM and ATN required 13 min of HPLC runtime in comparison to the proposed method [38].

The proposed method is relatively better or comparable in terms of sensitivity, accuracy, and precision to the methods reported for analysis of ASP, RAM, HCTZ, SIM and ATN. Rapid simultaneous estimation of ASP, RAM, HCTZ, SIM and ATN using a simple isocratic HPLC system with high sensitivity of estimation indicates easy application for analysis. The structures of the drugs are shown in Fig.1.

MATERIALS AND METHODS

Chemicals and reagents

Aspirin (ASP), Hydrochlorothiazide (HCTZ), Ramipril (RAM), Simvastatin (SIM) and Atenolol (ATN), were kindly supplied by Wockhardt Pharmaceuticals Ltd., Aurangabad, Emcure Pharmaceuticals Ltd, Pune and Smilax Laboratories Limited,

Hyderabad, India. Marketed sample of ASP, RAM, HCTZ, SIM and ATN (Polycap) in their combined capsule dosage form of Cadila Pharmaceuticals Ltd. India, was used for analysis. Each capsule contained 50mg of ATN, 100mg of ASP, 12.5mg of HCTZ, 5mg RAM and 20mg of SIM. For HPLC work, double distilled water was prepared in the laboratory. Methanol used was of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The separation of five drugs was carried out on an isocratic JASCO RP-HPLC system using Hypersil ODS C18 column (250× 4.6 mm i. d., particle size 5 mm) as a stationary phase and methanol: water (95:5% v/v) as mobile phase. The HPLC system consisted of intelligent HPLC pump model (Jasco PU 1580). The solutions were injected into the chromatograph through a Rheodyne valve, with a 20 µL loop with auto sampler (AS 1555).

The detector consisted of a UV/ VIS (Jasco UV 1575). Data were integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase flow rate was 1 mL/min and a detection wavelength of 230 nm was selected for analysis (Fig.2). An ultrasonic bath was used to remove the air from the mobile phases, operating at ambient temperature.

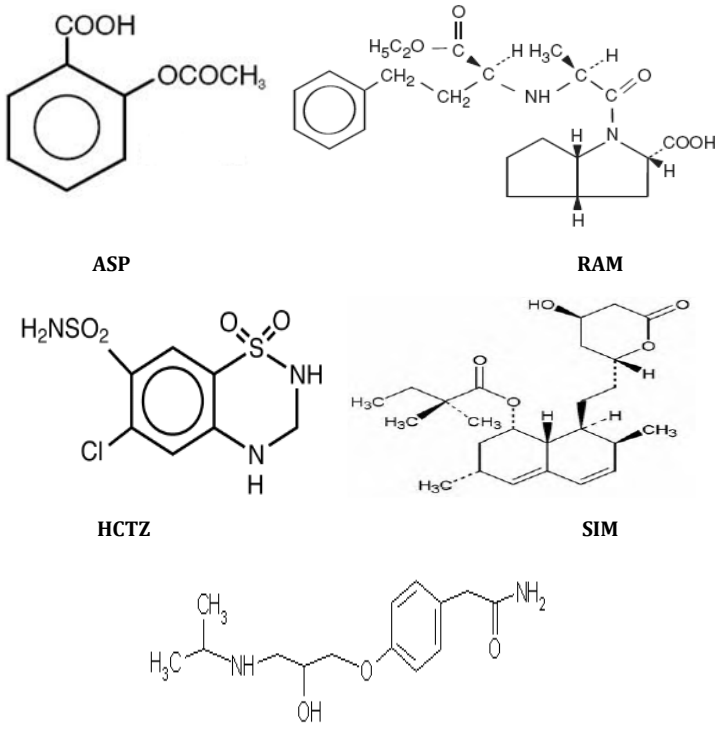

Fig. 1: Chemical Structures of ASP, RAM, HCTZ, SIM, and ATN.

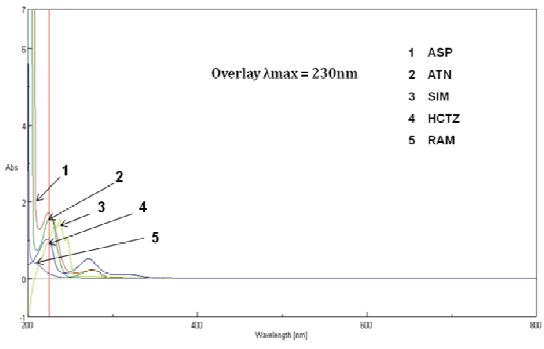

Fig. 2: Overlay Spectrum for ASP, RAM, HCTZ, SIM and ATN (λmax = 230 nm)

Preparation of Standard and Sample solutions

Accurately weighed 50mg of ATN, 100mg of ASP, 12.5mg of HCTZ, 5mg RAM and 20mg of SIM were dissolved in and diluted with methanol up to 50 ml to obtain a standard stock solution of ATN (1000 µg/ml), ASP (2000 µg/ml), HCTZ (250 µg/ml), RAM (100 µg/ml) and SIM (400 µg/ml).

To determine the content of ASP, RAM, HCTZ, SIM and ATN simultaneously in capsule (label claim: 50mg of ATN, 100mg of ASP, 12.5mg of HCTZ, 5mg RAM and 20mg of SIM per tablet), twenty capsule were weighed, their mean weight determined and they were finely powdered and powder equivalent to 50mg of ATN, 100mg of ASP, 12.5mg of HCTZ, 5mg RAM and 20mg of SIM was weighed. Then equivalent weight of the drug was transferred into a 50 ml volumetric flask containing 20 ml methanol, sonicated for 10 min and diluted to 50 ml with methanol to obtain solution of ATN (1000 µg/ml), ASP (2000 µg/ml), HCTZ (250 µg/ml), RAM (100 µg/ml) and SIM (400 µg/ml). The mixture was filtered using whatmann filter.

Method validation

The method was validated in accordance with ICH guidelines [39].

System suitability

System suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their resolution (Rs), retention time, theoretical plates number (N) and tailing factors (T).

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved all the drugs very

efficiently, as showed in Fig. 3. The identities of the peak for ASP, RAM, HCTZ, SIM and ATN were confirmed by comparing the Rt with those of standards.

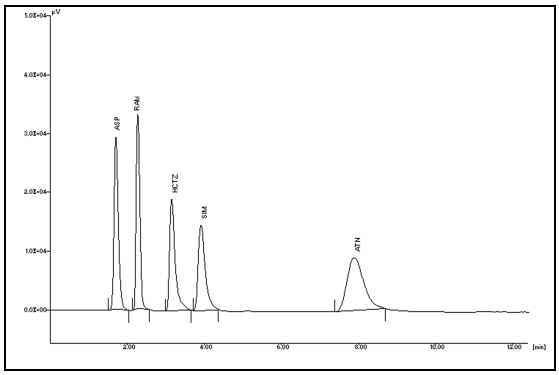

Fig.3: Chromatogram of ASP, RAM, HCTZ, SIM and ATN

Linearity

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 20-120 µg/mL for ASP, 1-6 µg/mL for RAM, 2.5-15 µg/mL for HCTZ, 4-24 µg/mL for SIM and 10–60 µg/mL for ATN, respectively. A 20 µL of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. Calibration plots were constructed by plotting peak area against the corresponding amount of each drug.

Limit of detection and limit of quantitation

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision

The precision of the proposed method was assessed as repeatability and intermediate precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated 3 different days over a period of a week to evaluate day-to-day variability (intermediate precision).

Accuracy

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized for simultaneous determination of LOS, HCTZ and AMLO. Good resolution of both the components was obtained with methanol: water at ratio 95: 5 v/v. The flow rate of 1 mL/min was optimum. UV detection was made at 230 nm. At this wavelength ASP, RAM, HCTZ, SIM and ATN can be quantified. Hence, 230 nm determined empirically has been found to be optimum. The average retention times for ASP, RAM, HCTZ, SIM and ATN was found to be 1.983, 2.525, 3.108, 3.867 and 7.833 min, respectively.

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System Suitability Parameters of RP-HPLC for capsule Analysis

Parameter	ASP	RAM	HCTZ	SIM	ATN
Resolution (Rs)	0.00	2.446	3.146	4.460	8.235
Retention Time in min	1.983	2.525	3.108	3.867	7.833
Theoretical plates number (N)	3423	5528	6782	4152	4567
Tailing Factor	1.02	1.20	1.16	1.12	1.32

Table 2: Precision studies of proposed HPLC method

Conc.	Intra-day precision			Inter-day precision		
(µg/mL)	Measured Conc. ± SD	(%) RSD	Recovery[a](%)	Measured Conc. ± SD	(%) RSD	Recovery[a](%)
Aspirin						
40	39.90 ± 0.18	0.45	99.75	39.87 ± 0.35	0.88	99.67
80	79.92 ± 0.31	0.39	99.90	79.80 ± 0.64	0.80	99.75
120	119.96 ± 0.84	0.70	99.97	119.82 ± 0.95	0.79	99.85
Ramipril						
2	1.98 ± 0.012	0.60	99.00	1.97 ± 0.020	1.01	98.50
4	3.98 ± 0.051	1.28	99.50	3.95 ± 0.039	0.98	98.75
6	5.98 ± 0.034	0.56	99.67	5.95± 0.061	1.02	99.17
Hydrochlorothiazide						
5	4.96 ± 0.058	1.17	99.20	4.94 ± 0.064	1.29	98.80
10	9.96 ± 0.091	0.91	99.60	9.94 ± 0.025	0.25	99.40
15	14.89 ± 0.068	0.46	99.27	14.86 ± 0.017	0.11	99.07
Simvastatin						
8	7.98 ± 0.090	1.13	99.75	7.95 ± 0.080	1.00	99.38
16	15.97 ± 0.062	0.39	99.81	15.93 ± 0.050	0.31	99.56
24	23.95 ± 0.078	0.33	99.79	23.93 ± 0.024	0.10	99.71
Atenolol						
20	19.82 ± 0.092	0.46	99.10	19.87 ± 0.080	0.40	99.35
40	39.77 ± 0.082	0.20	99.43	39.86 ± 0.35	0.88	99.65
60	59.85 ± 0.192	0.32	99.75	59.55 ± 0.21	0.35	99.25

[a]Mean from three analysis

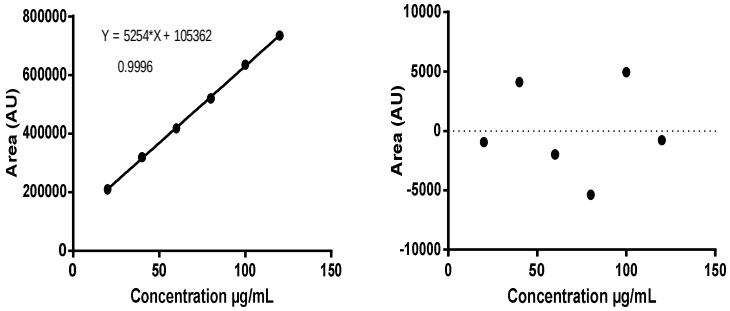

Fig. 4: Linear regression and residual plot for ASP

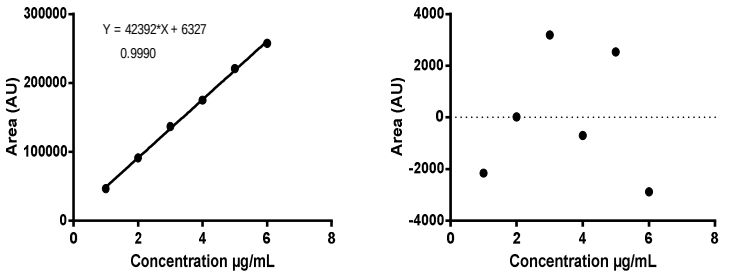

Fig. 5: Linear regression and residual plot for RAM

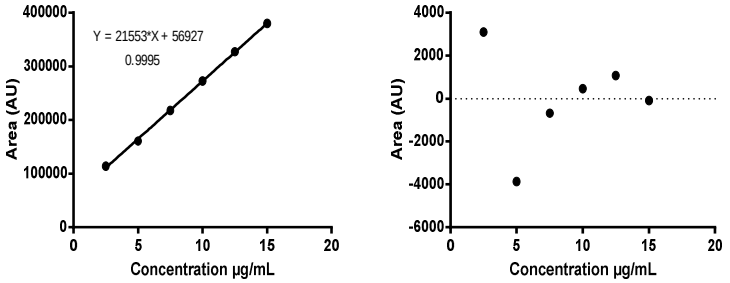

Fig. 6: Linear regression and residual plot for HCTZ

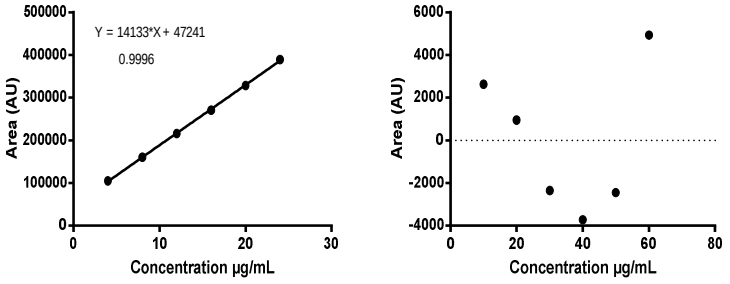

Fig. 7: Linear regression and residual plot for SIM

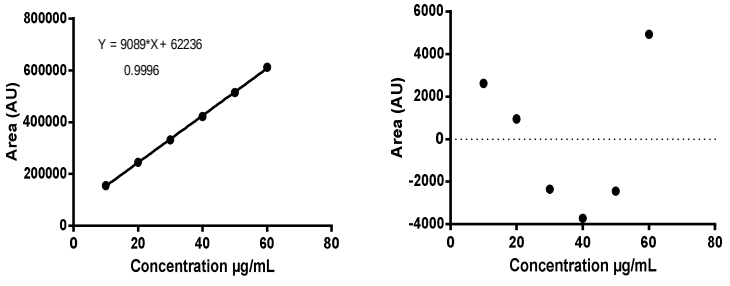

Fig. 8: Linear regression and residual plot for ATN

Linearity

Linearity was accessed by visualizing the calibration graph and plot of the residuals. The points distributed equally above and below the trend line showed linearity.

The linear regression equations were Y=5254X + 105362 (r^2= 0.9996) for ASP, Y= 42392X + 6327 (r^2= 0.9990) for RAM, Y= 21553X + 56927 (r2= 0.9995) for HCTZ, Y= 14133X + 47241 (r2= 0.9996) for SIM and Y= 9089X + 62236 (r2= 0.9996) for ATN.

The plots obtained from linear regression and residuals analysis are given in Figures 4, 5, 6, 7 and 8 for ASP, RAM, HCTZ, SIM and ATN, respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD for ASP, RAM, HCTZ, SIM and ATN were found to be 6.60, 0.2, 0.23, 1.32 and 1.98 µg/mL, respectively.

The LOQ for ASP, RAM, HCTZ, SIM and ATN were found to be 14, 0.5, 0.6, 3.97 and 5 µg/mL, respectively.

Precision

Precision was determined by analysis of standard solutions containing concentrations of ASP, RAM, HCTZ, SIM and ATN covering the entire calibration range. The precision of the method, as intra-day variation (RSD %) was determined by analysis of these solutions three times on the same day. Inter-day precision (RSD %) was assessed by analysis of these solutions on three different days over a period of one week. The results of the precision studies are as shown in Table 2.

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of an analytical method. Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate. The results are presented in Table 3.

Robustness

The results of robustness in the present method showed no significant changes occurring over changes which are summarized in Table 4. As the changes are not significant, we can say that the method is robust.

Table 3: Standard edition techniques for determination of ASP, RAM, HCTZ, SIM and ATN (n= 3)

Drug	Label claim (mg/capsule)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)
ASP	100	80	180	178.5	99.17
		100	200	199.8	99.90
		120	220	219.4	99.72
RAM	5	80	9	8.95	99.44
		100	10	9.95	99.50
		120	11	10.92	99.27
HCTZ	12.5	80	22.5	22.30	99.11
		100	25	24.80	99.20
		120	27.5	27.25	99.09
SIM	20	80	36	35.85	99.58
		100	40	39.75	99.37
		120	44	43.75	99.43
ATN	50	80	90	89.52	99.47
		100	100	99.82	99.82
		120	110	109.27	99.34

Table 4: Robustness evaluation of ASP, RAM, HCTZ, SIM and ATN

Chromatographic factors	Level	Chromatographic changes in t_R[a]				
		ASP	RAM	HCTZ	SIM	ATN
A: Flow rate mL/min.						
0.9	-0.1	2.021	2.560	3.152	3.890	7.875
1	0.0	1.983	2.525	3.108	3.867	7.833
1.1	+0.1	1.981	2.500	3.008	3.821	7.801
Mean ± SD		1.995 ± 0.023	2.528 ± 0.030	3.089 ± 0.074	3.859 ± 0.035	7.836 ± 0.037
B: % of methanol in the mobile phase (± 5%)						
90	-5.0	2.024	2.562	3.154	3.891	7.876
95	0.0	1.983	2.525	3.108	3.867	7.833
100	+5.0	1.982	2.501	3.007	3.822	7.803
Mean ± SD		1.996 ± 0.024	2.529 ± 0.031	3.089 ± 0.075	3.860 ± 0.035	7.837 ± 0.037

[a]Mean from three estimates

Analysis of marketed formulation

The proposed method was used to determine the potency of commercially available capsule (Polycap) containing 50mg of ATN, 100mg of ASP, 12.5mg of HCTZ, 5mg RAM and 20mg of SIM. Three replicate determinations (n=3) were carried out and Polycap Capsule were analysed, sharp and well defined peaks for ASP, RAM, HCTZ, SIM and ATN were obtained at Rt 1.983, 2.525, 3.108, 3.867 and 7.833 min, respectively, when scanned at 230 nm.

The amount of the label claim measured were 99.92 ± 1.03 % for ASP, 99.60 ± 1.14 % for RAM, 99.23 ±1.22% HCTZ, 99.65 ±1.40% and 99.82 ±1.37% for ATN.

CONCLUSION

A simple isocratic RP-HPLC method was developed and validated for the simultaneous quantitative assay of ASP, RAM, HCTZ, SIM and ATN in capsule dosage form. The validation results reveal that, method is precise, linear, robust and accurate, which proves the reliability of the proposed method. The short runtime and low solvent consumption are helpful for applying routine quality control analysis.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. http: //en.wikipedia.org/wiki/Cardiovascular_disease.
2. Gaziano TA, H Opie L, Weinstein MC, Cardiovascular disease prevention with a multidrug regimen in the developing world: a cost effectiveness analysis. The Lancet 2006;368:679–86.
3. Bhatia NM, Gurav SB, Jadhav SD, Bhatia MS, RP-HPLC method for simultaneous estimation of atorvastatin calcium, losartan potassium, atenolol and aspirin from tablet dosage form and plasma. J Liquid Chromatography Related Technologies 2012;35:428–43.
4. Vane JR, Botting RM. Review article: the mechanism of action of aspirin. Thrombosis Res 2003;110:255–8.
5. http: //www.drugbank.ca/drugs/DB00178.
6. http: //www.drugbank.ca/drugs/DB00999.
7. http: //www.rxlist.com/zocor-drug/clinical-pharmacology. htm.
8. http: //www.medicalfaq.net/mechanism of action/ta-28508.
9. Patel GF, Vekariya NR, Dholakiya RB. Estimation of aspirin and atorvastatin calcium in combined dosage form using derivative spectrophotometric method. Int J Pharm Res 2010;2 (1):62–6.
10. Ismail RR, Ganesh M, Jagadeeswaran M, Srinivasan, K, Valarmathi J, Sivakumar T. RP-HPLC method for the simultaneous determination of aspirin, atorvastatin and pioglitazone in capsuled form. Asian J Res Chem 2008;1(1):40–2.
11. Gandhimathi M, Ravi T K, Abraham A, Thomas R. Simultaneous determination of aspirin and isosorbide 5-mononitrate in formulation by reversed phase high pressure liquid chromatography. J Pharm Biomed Anal 2003;32:1145–8.
12. Yamamoto E, Takakuwa S, Kato T, Asakawa N. Sensitive determination of aspirin and its metabolites in plasma by lc-uv using online solid phase extraction with methyl cellulose immobilized anion–exchange restricted access media. J Chromatogr B 2007;846 (1–2): 132–138.
13. Walter LJ, Biggs DF, Coutts RT. Simultaneous glc estimation of salicylic acid and aspirin in plasma. J Pharm Sci 1974;63(11):1754–8.
14. Cesar J, Ronei LA, Poppi J. Simultaneous determination of acetylsalicylic acid, paracetamol and caffeine using solid-phase molecular fluorescence and parallel factor analysis. Anal Chim Acta 2009;642:212–6.
15. Kalyana B, Ramu K, Raghubabu. Development and validation of ramipril estimation from capsules using visible spectrophotometric method. Int J Chem Res 2011;2(2):16-9.
16. Kurade VP, Pai MG, Gude R. RP-HPLC estimation of ramipril and telmisartan in tablets. Indian J Pharm Sci 2009;71(2):148–51.
17. Aboul-Enein HY, Thiffault C, Determination of ramipril and its precursors by reversed phase high performance liquid chromatography. Anal Lett 1991;24:2217–24.
18. Motofumi I, Takeo K, Junichi G, Toshio N, Separation of ramipril optical isomers by high-performance liquid chromatography. J Liq Chromatogr 1990;13:991–1000.
19. Wayadande JA, Dubey R, Bhusari VK, Dhaneshwar SR. Validated HPTLC method for simultaneous estimation of ramipril and metolazone in bulk drug and formulation. Der Pharm Sinica 2011;2(5):273-81.
20. Sahu R, Patel VB, Simultaneous spectrophotometric estimation of hydrochlorothiazide and bisoprolol fumarate in combined dosage forms. Indian J Pharm Sci 2006;68(6):764-7.
21. Stolarczyk M, Masalanka A, Krzek J, Milczarek J. Application of derivative spectrophotometry for determination of enalapril, hydrochlorothiazide and valsartan in complex pharmaceutical preparations. Acta Poloniae Pharm Drug Res 2008;65(3):275-81.
22. Bhusari KP, Khedekar PB, Dhole S, Banode VS. Derivative and Q-analysis spectrophotometric methods for estimation of hydrochlorothiazide and olmesartan medoxomil in tablets. Indian J Pharm Sci 2009;71(5):505–8.
23. Tian DF, Tian XL, Tian T, Wang ZY, Mo FK. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by RP-HPLC. Indian J Pharm Sci 2008;70(3):372–4.
24. Hao Li, Yingwu Wang, Yao Jiang, Yunbiao Tang, Jiang Wang, Limei Zhao, *et al*. A liquid chromatography/ tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma. J Chromatogr B 2007;852(1-2):436-42.
25. Bhat LR, Godge RK, Vora AT, Damle MC. Validated RP-HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical formulation. J Liquid Chromatography Rel Technol 2007;30(20):3059-67.
26. Patel LJ, Suhagia BN, Shah PB, Shah RR. Simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form by RP-HPLC method. Indian J Pharm Sci 2006;68(5): 635-8.
27. Daniels SL, Vanderwielen AJ. Stability–indicating assay for hydrochlorothiazide. J Pharm Sci 1981;70(2):211-5.
28. Naga Raju K, Sudheer Babu I, Jyothi R, Prathyusha S. Spectrophotometric methods for estimation of simvastatin in bulk drug and its dosage form. Int J Pharm Chem Biol Sci 2012;2(1):124-9.
29. Jain N, Jain R, Swami H, Jain DK. RP-HPLC method for simultaneous estimation of simvastatin and ezetimibe in bulk drug and its combined dosage form. Asian J Res Chem 2008;1(1):29-31.
30. Shrestha B, Stephenrathinaraj B, Patel SS, Verma NK, Mazumder R. Simultaneous HPTLC estimation of simvastatin and ezetimibe in tablet dosage form. J Chem 2010;7(4):1206-11.
31. Kasture AV, Ramteke M, Simultaneous UV-spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. Indian J Pharm Sci 2006;68(3):394-6.
32. Al-Ghannam SM, A simple spectrophotometric method for the determination of β-blockers in dosage forms. J Pharm Biomed Anal 2006;40(1):151-6.
33. Barman R, Islam MA, Ahmed M, Wahed M I I, Islam R, Khan A, Hossain M B, *et al*. Simultaneous High-Performance liquid chromatographic determination of atenolol and amlodipine in pharmaceutical dosage form. Pakistan J Pharm Sci 2007;20(4):274–9.
34. Sathe SR, Bari SB. Simultaneous analysis of losartan potassium, atenolol and hydrochlorothiazide in bulk and in tablets by high-performance thin-layer chromatography with uv absorption densitometry. Acta Chromatogr 2007;19:270–8.
35. Rao DD, Satyanarayana NV, Sait SS, Reddy YR, Mukkanti K. Simultaneous determination of losartan potassium, atenolol and hydrochlorothiazide in pharmaceutical preparations by stability-indicating UPLC. Chromatographia 2009;70(3–4):647-51.
36. Lamprecht G, Kraushofer T, Stoschitzky K, Lidner W. Enantioselective analaysis of (R)-and (S)-atenolol in urine samples by high-performance liquid chromatography column switching set-up. J Chromatogr B Biomed Sci Appl 2000;740(2):219-26.
37. Prajapati JP, Patel AI, Patel MB, Prajapati RJ. Simultaneous determination of ramipril, atenolol, hydrochlorothiazide, simvastatin and aspirin in ramipril, atenolol, hydrochlorothiazide, simvastatin as tablets and aspirin as enteric coated tablets in capsules by RP-HPLC. Int J Pharm 2012;1(2):95-100.
38. Murthy RS, Ravi Kumar M, Useni Reddy Mallu, Hanimi Reddy Bapatu. A Simple RP-HPLC method for simultaneous analysis of aspirin, atenolol, hydrochlorothiazide, ramipril and simvastatin in pharmaceutical solid dosage forms. Int J Sci Innovations Discoveries 2012;2(1):137-51.
39. ICH, Q2B. Validation of Analytical Procedure: Methodology. International Conference on Harmonisation, IFPMA, Geneva; 2005.