INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally Indexed Journal

Indexed in Chemical Abstract Services(USA),Index Coppernicus ,Ulrichs Directory of Periodicals,Google scholar ,Cabi ,DOAJ ,PSOAR, EBSCO ,SCOPUS, EMBASE etc.

Rapid Publishing

The International Journal of Pharma and Bio Sciences (IJPBS) is an international journal published quarterly. The Aim of IJPBS is to publish peer reviewed research and review articles in less time without much delay in the developing field of Pharmaceutical and Biological sciences. One week from the date of manuscript submission author gets the decision of acceptance and if accepted the manuscript will be processed within 3 weeks (approx.) for publication.

Pharmaceutical Sciences

Branches

- Pharmaceutics
- Novel drug delivery sys
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

Branches

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Allied Sciences
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

ELSEVIER

Impact Factor 0.47*

**Refer Instruction to authors available at www.ijpbs.net*

Indexed In Elsevier Bibliographic Database (SCOPUS, EMBASE and Sciverse)

Journal Home page
www.ijpbs.net

FOR INSTRUCTION TO AUTHORS VISIT
www.ijpbs.net
FOR ANY QUERIES EMAIL TO

- editorofijpbs@yahoo.com
- editorijpbs@rediffmail.com
- prasmol@rediffmail.com

Int J Pharm Bio Sci 2012 July; 3(3): (P) 577 - 585

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND ASPIRIN IN CAPSULE DOSAGE FORM

CHIRAGSINH SOLANKI* AND NISHITKUMAR PATEL

Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Anand, Gujarat, India

ABSTRACT

A simple, specific, precise and accurate reversed-phase HPLC method was developed and validated for simultaneous estimation of rosuvastatin calcium and aspirin in capsule dosage forms. The separation was achieved by HyperChrom ODS-BP C_{18} column (200 mm x 4.6 mm, 5.0µm) using acetonitrile and 0.050 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 with ortho phosphoric acid (55:45, v/v) as eluent, at a flow rate of 1 ml/min. Detection was carried out at wavelength 241 nm. The retention times of rosvastatin calcium and aspirin were 5.33 min and 3.56 min, respectively. The linearity was established over the concentration range of 0.5 - 6 µg/ml and 2.5 – 30 µg/ml with correlation coefficients (r^2) 0.9998 and 0.9995 for rosuvastatin calcium and aspirin, respectively. The mean recoveries were found to be in the range of 99.58% – 101.0% and 99.83%–100.11% for rosvastatin calcium and aspirin, respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of rosuvastatin calcium and aspirin in their combined capsule dosage forms.

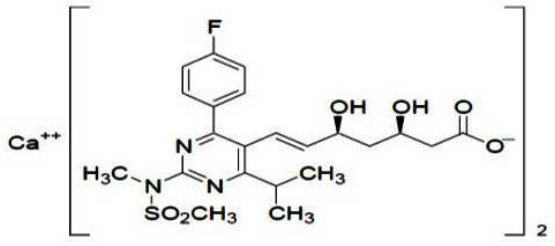
KEYWORDS: **Rosuvastatin calcium, Aspirin, RP-HPLC, Method validation, Capsule**

CHIRAGSINH SOLANKI
Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Anand, Gujarat, India

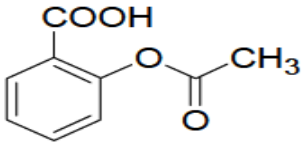
INTRODUCTION

Rosuvastatin calcium (ROS) is chemically (E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl (methyl-sulphonyl) amino] pyrimidin-5-yl}-3,5-dihydroxyhept-6-enoic acid calcium (Figure 1a). ROS is in a group of drugs called hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors, or "statins." It reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood, while increasing levels of high-density lipoprotein in the management of hyperlipidaemias[1]. Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1b). It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infraction[1-3]. ROS and ASP in combined dosage form are used for the treatment of dyslipidemia associated with arthersclerotic arterial disease with risk of Myocardial infarction, stroke or peripheral vascular disease.

The review of literature revealed that spectrophotometry[4,5], HPLC[6,7], HPTLC[7-9], LC-MS-MS[10], LC-electrospray tandem mass spectrometry[11,12] have been reported for ROS in single form and in combination with other drugs. Several methods have been reported for ASP in single form and in combination with other drugs including spectrophotometry[13-15], HPLC[16-20] and HPTLC[21,22]. The present work describes the development of a simple, precise and accurate RP-HPLC method for the simultaneous estimation of ROS and ASP in capsule dosage forms. The developed method was validated in accordance with ICH Guidelines[23] and successfully employed for the assay of ROS and ASP combine capsule.



(a)



(b)

Figure 1
Chemical structure of ROS (a) and ASP (b)

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure ROS and ASP were kindly provided by Relax Pharmaceuticals, Vadodara, Gujarat, India and Baroque pharmaceuticals, Khambhat, Gujarat, India respectively as gift samples. HPLC grade acetonitrile water were purchased from RFCL limited, New Delhi, India, while Analytical grade ortho phosphoric acid and potassium dihydrogen phosphate were purchased from S. D. Fines Chemicals, Mumbai, India. Capsules

of ROS and ASP in combine dosage form, UNISTAR*, with a 10 mg ROS and 75 mg ASP label claim, manufactured by Unichem Laboratories Ltd, India were procured from a local pharmacy.

Instrumentation

An isocratic HPLC system (Analytical technologies limited) consisted of P2230 plus HPLC pump, variable wavelength programmable UV 2230 plus detector system, Rhenodyne valve with 20µl fixed loop and Analchrom 2006 as operating software. The chromatographic column used was HyperChrom ODS-BP C_{18} column (200 mm×4.6mm i.d, particle size 5µm). Analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.

Chromatoghraphic condition

A mixture of acetonitrile and 0.050 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 with ortho phosphoric acid (55:45, v/v) was used as mobile phase and was filtered through 0.45 µ membrane filter prior to use. The flow rate of mobile phase was maintained at 1 ml/min. Detection was carried out at 241 nm at the ambient temperature. The total run time 10 min was used with injection volume of 20 µl.

Preparation of mobile phase and Standard stock solutions

Accurately weighed Potassium dihydrogen phosphate (3.062 g) was dissolved in 450 ml of water. This solution was mixed with 550 ml of Acetonitrile. Finally the pH was adjusted to 3.0 with ortho phosphoric acid. The solution was sonicated for 10 minutes and filtered through 0.45 µ membrane filter. 100 mg of standard ROS and ASP were accurately weighed and transferred separately to a 100 ml volumetric flask and dissolved in 50 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000 µg/ml ROS and ASP, respectively. Appropriate volume of aliquot from ROS and ASP standard stock solution was further diluted with mobile phase to obtain final concentration of 50 µg/ ml and 250 µg/ ml, respectively.

Preparation of sample solution from combined dosage form

The content of 20 capsule were taken and weighed. Powder equivalent to 20 mg ROS and 150 mg was accurately weighed and transferred to 100 ml volumetric flask. 50 ml of mobile phase was added to same volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with same mobile phase. The above solution was filtered through whatman filter paper (0.45µm). 10 ml of aliquot was taken and transferred to volumetric flask of 50 ml capacity and volume was made up to the mark with the mobile phase. Further 0.5 ml of this solution was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with the mobile phase to give a solution containing 2 µg/ml ROS and 15 µg/ml ASP. This solution was sonicated for 5 min. and filtered through 0.45µm whatman filter. This solution was used for the estimation of both drugs.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and system suitability. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% relative standard deviation- %RSD) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium

stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Linearity

Appropriate volume of aliquot from ROS and ASP standard stock solution was transferred to same volumetric flask of 10 ml capacity. The volume was adjusted to the mark with mobile phase to give a solution containing 0.5, 1, 2, 3, 4, 5 and 6 µg/ml ROS and 2.5, 5, 10, 15, 20, 25 and 30 µg/ml ASP. The mixed standard solution was chromatographed using above chromatoghraphic condition (n=6). All solutions were filtered through 0.45 µm filter prior to use. Calibration curves were constructed by plotting average peak area versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to pre-analyzed test sample preparation at 3 different concentration levels 80, 100 and 120 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was chromatographed 3 times and average recoveries were measured.

Precision

The repeatability was evaluated by assaying 6 times of test samples prepared for assay determination. The intraday and interday precision study of ROS and ASP was carried out by estimating different concentrations of ROS (1, 3, 5 µg/ml) and ASP (5, 15, 25 µg/ml), 3 times on the same day and on 3 different days and the results are reported in terms of %RSD.

Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10σ/S criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. For the proposed method it was done by changing the mobile phase composition (acetonitrile-buffer, 53:47 and 57:43, v/v), by changing the pH (±0.2 unit), by changing flow rate ((±0.1 unit) and by observing the stability of the drugs for 24 h in the mobile phase.

System suitability

The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Determination of ROS and ASP from combined dosage form

Sample solution was injected 6 times at above chromatographic conditions. An average peak area was measured from chromatograms. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

Optimizations of chromatographic conditions were performed to obtain the best resolution and peak parameter (asymmetry, theoretical plates). For the selection of mobile phase initially methanol-water and acetonitrile-water have been tried in different ratio which gave poor peak shape. Then acetonitrile-0.050 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 in different ratio have been tried. Finally, acetonitrile and 0.050 M

potassium dihydrogen phosphate buffer adjusted to pH 3.0 with orthophosphoric acid. (55:45 v/v) was found to be satisfactory and gave two symmetrical peaks with good resolution (10.73) for ROS and ASP at flow rate of 1 ml/min. The average retention time for ROS and ASP were 5.33 ± 0.09 and 3.56 ± 0.04 minutes, respectively (Figure 2). The asymmetric factors for ROS and ASP were 1.20 and 1.29, respectively. For the selection of detection wavelength overlain UV spectrum of ROS and ASP was taken which revealed that at 239 nm both the drugs possess significant absorbance (Figure 3). With the consideration of lower amount of ROS and to enhance ROS quantification, same concentration mixture of both drugs was chromatographed with detection wavelength 239, 240, 241 and 242 nm and compare for area and peak height and then 241 nm was found to be satisfactory wavelength for considerable quantification of both drugs in a mixture.

Summary of validation parameters for proposed method was given in Table 1.

Table 1
Summary of Validation Parameters of RP-HPLC method

Parameters	ROS	ASP
Recovery %	99.58% – 101%	99.83% –100.11%
Repeatability(%RSD, n=6)	0.78757	0.51402
Precision((%RSD)		
Intra-day (n=3)	0.169 – 0.217	0.239 – 0.655
Inter-day (n=3)	0.301 – 0.761	0.196 – 0.529
LOD (µg/ml)	0.07946	0.60116
LOQ (µg/ml)	0.24077	1.82169
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hrs.	Suitable for 24 hrs.

The specificity of the HPLC method is illustrated in Figure 4 where complete separation of ROS and ASP were noticed in presence of commonly used excipients. The average retention time ± standard deviation for ROS and ASP were found to be 5.35 ± 0.078 and 3.54 ± 0.042 min, respectively, for 3 replicates. The peaks obtained were sharp and have clear baseline separation.

Linearity was assessed for ROS and ASP by plotting calibration curves of the peak area versus the concentration over the concentration range 0.5-6 µg/ml and 2.5-30 µg/ml, respectively. The correlation coefficients (r^2) for ROS and ASP were found to be 0.9998 and 0.9995, respectively (Table 2).

Table 2
Statistical data for ROS and ASP by RP- HPLC method

Parameter	ROS	ASP
Linear Range(µg/ml)	0.5-6.0	2.5-30
Slope	47.86598	26.76228
Intercept	19.59521	56.72639
Standard deviation of slope	0.31920	0.27006
Standard deviation of intercept	1.15248	4.87526

The recovery was found to be in the range of 99.58% – 101% for ROS and 99.83% – 100.11% for ASP (Table 1). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the %RSD (Table 1), which indicate good precision.

The Limit of detection for ROS and ASP were found to be 0.07946 µg/ml and 0.60116 µg/ml respectively. Limit of quantification for ROS and ASP were found to be 0.24077 µg/ml and 1.82169 µg/ml respectively (Table 1). The robustness of the method was assessed by assaying sample solutions under different analytical conditions with small but deliberate changes from the original conditions. The results obtained were not affected by these changes in terms of % assay, retention time and asymmetry (Table 3). The system suitability parameters for method were found to be satisfactory (Table 4). The RP-HPLC method was successfully applied to ROS and ASP combined capsule dosage form. The results are shown in Table 5.

Table 3
Robustness study ROS (2 µg/ml) and ASP (15 µg/ml)

Condition varied	Changed condition	R_T		A_s		% Assay	
		ROS	ASP	ROS	ASP	ROS	ASP
Change in Mobile phase ratio (v/v)	53:47	5.36	3.58	1.20	1.30	101.37	100.74
	57:43	5.31	3.53	1.18	1.28	99.80	99.58
Change in pH	3.2	5.30	3.57	1.21	1.32	98.47	101.27
	2.8	5.35	3.54	1.19	1.30	99.39	99.05
Change in flow rate (ml/min)	1.1	5.29	3.52	1.17	1.26	98.36	99.21
	0.9	5.36	3.60	1.22	1.32	101.75	101.14

Table 4
System Suitability Test Parameter

System Suitability Parameters	Proposed Method	
	ROS	ASP
Retention times (R_T) (min)	5.33 ± 0.09	3.56 ± 0.04
No. of Theoretical plates (N)	11229	12000
Resolution (R_S)	10.73	
Asymmetry factor (A_S)	1.20	1.29

Table 5
Assay Results of Marketed Formulation

Formulation	Actual concentration µg/ml		Amount obtained µg/ml		% ROS±S.D.	% ASP±S.D.
	ROS	ASP	ROS ± S.D.	ASP ± S.D.		
Capsule	2	15	2.02 ± 0.01	15.02 ± 0.51	101.00 ± 0.50	100.11 ± 0.51

n=3 determination

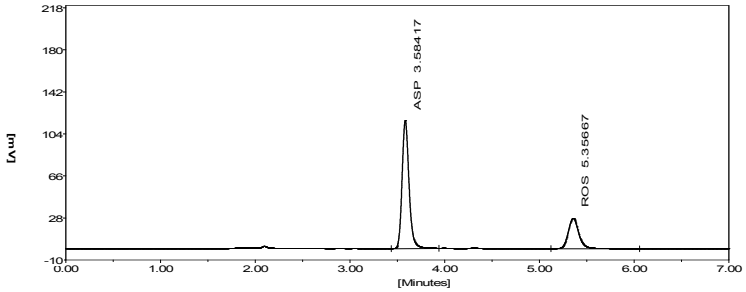


Figure 2
Chromatogram of mixed standard solution containing 4 µg/ml ROS and 20 µg/ml ASP

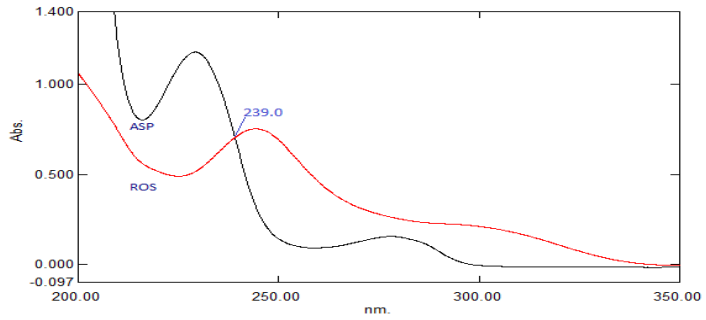


Figure 3
Overlain spectrum of 10 µg/ml ROS and 30 µg/ml ASP in Mobile phase

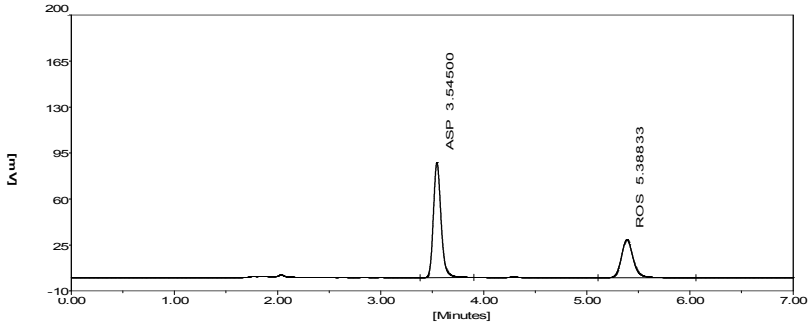


Figure 4
Chromatogram of ROS (3 µg/ml) and ASP (15 µg/ml) in the presence of excipients.

CONCLUSION

The proposed RP-HPLC provides simple, specific, precise and accurate quantitative analysis for simultaneous determination of ROS and ASP in combined capsule dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and system suitability. The method gives good separation with short analysis time (< 6 min) and can be used for routine analysis of ROS and ASP in combined dosage form.

ACKNOWLEDGEMENT

Authors are thankful to Relax Pharmaceuticals (Vadodara, India) and Baroque Pharmaceuticals (Khambhat, India) for

providing gratis sample with the great pleasure. The authors also thankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for providing the necessary facilities for research work.

REFERENCES

1. Martindale-The Complete Drug Reference, 34th edn, Pharmaceutical Press. London: 15-19, 966, (2005).
2. Merk index, An encyclopedia of chemicals, drugs and biological, 14th edn, Merck Research Laboratories, USA: 17, (2006).
3. Antithrombotic Trialists' (ATT) Collaboration, Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet, 373: 1849–1860, (2009).
4. Amr M. Badawy, Nadia M. Mostafa, Abd El-Aziz B, Abd El-Aleem, Nesrine T. Lamie, Stability indicating spectrophotometric methods for determination of rosuvastatin in the presence of its acid degradation products by derivative spectrophotometric tecniques. Journal of Advanced Pharmaceutical Research, 2(1): 44-55, (2011).
5. Chirag B Pandya, Channabasavaraj K. P. and Shridhara H. S., Simultaneous estimation of rosuvastatin calcium and ezetimibe in bulk and tablet dosage form by simultaneous equation. International Journal of ChemTech Research, 2: 2140-2144, (2010).
6. H. O. Kaila, M. A. Ambasana, R. S. Thakkar, H. T. Saravaia, A. K. Shah, A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. Indian Journal of Pharmaceutical Sciences, 72(5): 592-598, (2010).
7. John Varghese, Susheel, Kochupappy Ravi, Thengungal, Determination of rosuvastatin and ezetimibe in a combined tablet dosage form using high-performance column liquid chromatography and high-performance thin-layer chromatography. Journal of AOAC International, 93(4): 1222-1227, (2010).
8. S. Uma Devi, E. Pushpa Latha, C. V. Nagendra Kumar Guptha and Mr.P. Ramalingam, Development and validation of HPTLC method for estimation of rosuvastatin calcium in bulk and pharmaceutical dosage forms. International Journal of Pharma and Bio Sciences, 2(2): 134-140, (2011).
9. B. G. Chaudhari, N. M. Patel, P. B. Shah, Determination of simvastatin, pravastatin sodium and rosuvastatin calcium in tablet dosage forms by HPTLC. Indian J. Pharm. Sci., 69(1): 130-132, (2007).
10. C.K. Hull, A.D. Penman, C.K. Smith, P.D. Martin, Quantification of rosuvastatin in human plasma by automated solid phase extraction using tandem mass spectrometric detection. Journal of Chromatography B, 772: 219–228, (2002).
11. Ravi Kumar Trivedi, Raja Reddy Kallem, Ramesh Mullangi, Nuggehally R. Srinivas, Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC–MS/MS with electrospray ionization: Assay development, validation and application to a clinical study. Journal of Pharmaceutical and Biomedical Analysis, 39: 661–669, (2005).
12. Jun Gao, Dafang Zhong, Xiaotao Duan, Xiaoyan Chen, Liquid chromatography/negative ion electrospray tandem mass spectrometry method for the quantification of rosuvastatin in human plasma: Application to a pharmacokinetic study. Journal of Chromatography B, 856: 35–40, (2007).
13. Madhuri D. Game, K. B. Gabhane, D. M. Sakarkar, Quantitative analysis of clopidogrel bisulphate and aspirin by first derivative spectrophotometric method in tablet. Indian J. Pharm. Sci., 72(3): 365-368, (2010).

14. Zenon Kokot and Kinga Burda, Simultaneous determination of salicylic acid and acetylsalicylic acid in aspirin delayed-release tablet formulations by second-derivative UV spectrophotometry. Journal of Pharmaceutical and Biomedical Analysis, 18: 871–875, (1998).
15. Gopal Garg, Shallendra Saraf and Swarnlata Saraf, "Spectrophotometric determination of the Aspirin and Atenolol in combined dosage forms", Indian Journal of Pharmaceutical Education and Research, 42(1): 74-77, (2008).
16. D. A. Shah, K. K. Bhatt, R. S. Mehta, M. B. Shankar, S. L. Baldania and T. R. Gandhi, Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in capsule dosage form. Indian J. Pharm. Sci., 69(4): 546-549, (2007).
17. Eda Ross Montgomery, Suzanne Taylor, James Segretario, Elizabeth Engler, Dolores Sebastian, Development and validation of a reversed-phase liquid chromatographic method for analysis of aspirin and warfarin in a combination tablet formulation. Journal of Pharmaceutical and Biomedical Analysis, 15: 73-82, (1996).
18. Kachhadia, Pankaj K., Doshi, Ashish S., Joshi, Hitendra S., Validated column high-performance liquid chromatographic method for determination of aspirin and clopidogrel in combined tablets in the presence of degradation products formed under ICH recommended stress conditions. Journal of AOAC International, 92(1): 152-157, (2008).
19. M. Gandhimathi, T. K. Ravi, Annie Abraham, Renu Thomas, Simultaneous determination of aspirin and isosorbide 5-mononitrate in formulation by reversed phase high pressure liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis, 32: 1145-1148, (2003).
20. E. Deconinck, P. Y. Sacre, S. Baudewyns, P. Courselle, J. De Beer, A fast ultra high pressure liquid chromatographic method for qualification and quantification of pharmaceutical combination preparations containing paracetamol, acetyl salicylic acid and/or antihistaminics. Journal of Pharmaceutical and Biomedical Analysis, 56: 200-209, (2011).
21. S. V. Londhe, S. V. Mulgund, R. S. Deshmukh, K. S. Jain, Simultaneous HPTLC analysis of aspirin, atorvastatin calcium and clopidogrel bisulphate in the bulk drug and in capsules. Acta Chromatographica, 22: 297-305, (2010).
22. Hiral J. Panchal , Bhanubhai N. Suhagia, Natvarlal J. Patel, Simultaneous HPTLC analysis of atorvastatin calcium, ramipril, and aspirin in a capsule dosage form. Journal of Planar Chromatography, 22: 165-271, (2009).
23. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Geneva, (2005).