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A Simple and Rapid Ultra-Performance Liquid Chromatographic Assay Method for the Simultaneous determination of Aspirin, Clopidogrel Bisulphate and Atorvastatin Calcium in Capsule Dosage Form

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Abstract: A simple, rapid, reliable and precise reversed phase ultra performance liquid chromatographic method has been developed and validated for the simultaneous estimation of aspirin (ASP), clopidogrel bisulphate (CLP) and atorvastatin calcium (ATV) from capsule dosage form. Chromatography was carried out at 25°C on a 50 × 2.1 mm i.d., 1.7 µm Acquity BEH C_{18} column with isocratic mobile phase 0.1% orthophosphoric acid and acetonitrile (55:45, v/v) at a flow rate of 0.35 mL/min. The detection was carried out at 230 nm. The retention times were about 0.59, 1.04 and 2.89 min for ASP, CLP and ATV, respectively. The total runtime was less than 4 min. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 12-48 µg/mL for ASP, 12-48 µg/mL for CLP and 3.2-12.8 µg/mL for ATV. Limit of detection obtained were 0.03 µg/mL for ASP, 0.06 µg/mL for CLP and 0.07 µg/mL for ATV. The proposed method can be used for the estimation of these drugs in combined dosage forms.
Keywords: Ultra performance liquid chromatography, Assay method, Isocratic, Capsule dosage forms, Aspirin, Clopidogrel bisulphate and Atorvastatin calcium.

1. Introduction

Aspirin (ASP) is 2-acetyloxybenzoic acid, often used as an analgesic, antipyretic, anti-inflammatry and an antiplatelet[1]. It suppresses the production of prosta-glandins and thromboxanes due to inactivation of the cyclooxygenas enzyme [2,3].

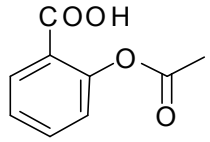
Clopidogrel bisulphate (CLP) is methyl (s)-2-chlorophenyl (4,5,6,7-tetrahydrothioeno-[3,2-C] pyridine -5-yl) acetate bisulphate, an ADP antagonist. It is used as an anti thrombic agent [4,5]. Clopidogrel bisulphate is not official in any pharmacopoeia.

Atorvastatin calcium (ATV) is [R-(R*,R*)]-2-(4-fluorophenyl)-β,ö-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, a synthetic lipid-lowering agent which is about a 100 times as potent as the other drugs in its class and at lower costs than most of the others [6]. ATV is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [7] (Figure 1).

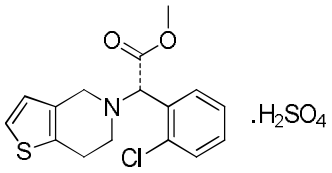
Many dosage forms of ASP, CLP and ATV as a single or as combination dosage form with others are available on the local market for effective therapy. Literature survey revealed several analytical methods such as spectrometry, stability indicating HPTLC, GC, simple and stability indicating HPLC, LC-MS-MS and LC-ESI-MS have been reported for the determination of

ASP [8-15], CLP [16-23] and ATV [24-34] in pharmaceutical dosage forms and biological samples.

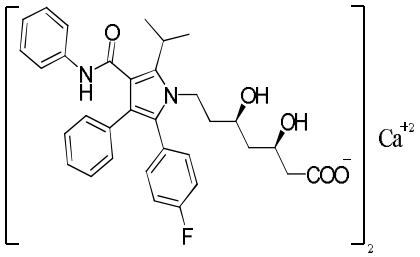
To our present knowledge, there is no method reported for the estimation of ASP, CLP and ATV in combination formulation. Hence, the aim was to develop a rapid, sensitive, simple and accurate UPLC method which can estimate the three components simultaneously. The present investigation describes a simple, sensitive, rapid and precise LC method for the simultaneous estimation of ASP, CLP and ATV in marketed pharmaceutical dosage form. With the developed method, only this mobile phase is sufficient for quantification of ASP, CLP and ATV either in combination (i.e., ASP + CLP, ASP + ATV, ATV + CLP) or in single dosage form as per availability of formulation. Many pharmaceutical industries manufacture their formulation of all mentioned drugs either in combination or in single dosage form. Most of the pharmaceutical industries use time consuming LC method and different mobile phases for different dosage form of drugs. But with the proposed method developed, time and cost required for changing different mobile phases could be saved, because only one mobile phase can be used for all the drugs and their combinations.



(a)



(b)



(c)

Figure 1 Molecular structure of (a) ASP (b) CLP and (c) ATV.

2. Experimental

2.1 Materials and reagents

ASP, CLP and ATV working standards with 99.95, 99.98 and 99.93 % purity, respectively, were provided as a gift sample by Torrent Research Centre (Ahmedabad, India). Capsule dosage form (Ecosprin GOLD 20; 75 mg ASP, 75 mg CLP and 20 mg ATV per capsule) of USV Ltd. (Mumbai, India) was purchased from local market. Methanol and acetonitrile (HPLC grade) were obtained from Finar Chemicals (Ahmedabad, India). HPLC grade orthophosphoric acid (88 %) was from Spectrochem (Mumbai, India). Distilled water was prepared using a Milli-Q system, Millipore (Milford, MA, USA). Nylon syringe filters (0.22 µm) were from Millipore (Mumbai, India).

2.2 Instrumentation

The chromatographic separation was carried out using a Waters Acquity system (Waters, Milford, MA, USA), consisted of a Binary solvent manager, a sample manager and a PDA detector. The output signal was monitored and processed by Empower software, A Sartorius CPA2P analytical micro-balance (Gottingen, Germany), an ultra sonic cleaner SONICA from Spincotech Pvt. Ltd. (Mumbai, India) and pH meter LI 610 ELICO (Mumbai, India) were also used.

2.3 Chromatographic Conditions

The separation was achieved on Acquity UPLC BEH C_{18} (2.1 × 50 mm i.d., 1.7 µm particle size), the mobile phase was 0.1 % orthophosphoric acid and acetonitrile (55:45, v/v). The flow rate of mobile phase was 0.35 mL/min and the detection was monitored at a wavelength of 230 nm. The mobile phase was filtered through a nylon 0.22 µm membrane filter and was degassed before use. The column temperature was maintained at 25°C and injection volume was 5 µL.

2.4 Preparation of Standard Stock Solution

Accurately weighed ASP (75 mg), CLP (75 mg) and ATV (20 mg) were transferred to 100 mL volumetric flasks, dissolved and diluted to the mark with methanol to obtain a standard stock solution of ASP (750 µg/mL), CLP (750 µg/mL) and ATV (200 µg/mL). An aliquot of the stock solution (1 mL) was transferred to a 25 mL volumetric flask, and diluted to the mark with mobile phase to obtain a mixed working standard solution of ASP (30 µg/mL), CLP (30 µg/mL) and ATV (8 µg/mL).

2.5 Preparation of Sample Solution

Powder of 20 capsules (Ecosprin GOLD 20), each containing 75 mg ASP, 75 mg CLP and 20 mg ATV, were weighed and analysed: a quantity of powder equivalent to one capsule was weighed and transferred to a 100 mL volumetric flask containing 50 mL

methanol and sonicated for 15 min. The flask was allowed to cool down to room temperature and the volume was made up to the mark with methanol to obtain sample stock solution of ASP (750 µg/mL), CLP (750 µg/mL) and ATV (200 µg/mL). The solution was filtered using a nylon 0.22 µm membrane filter. An aliquot of the sample stock solution (1 mL) was transferred to a 25 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of ASP (30 µg/mL), CLP (30 µg/mL) and ATV (8 µg/mL).

2.6 Method Validation

The developed LC method was validated to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2 (R1) [35]. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

2.6.1 Specificity study

The specificity of the RP-UPLC method was checked by comparison of chromatograms obtained from standard, sample and the corresponding placebo.

2.6.2 Linearity and Range

The linearity of the method was determined at seven concentration levels ranging from 12 - 48 µg/mL for ASP, CLP and 3.2 - 12.8 µg/mL for ATV. The calibration curves were constructed by plotting peak areas versus concentration of ASP, CLP and ATV. The slope, *Y*-intercept and correlation coefficient were calculated.

2.6.3 Accuracy (% Recovery)

The accuracy of the method was evaluated in triplicate at three concentration levels, 50, 100 and 150 % of the target test concentration (30 µg/mL of ASP and CLP, 8 µg/mL of ATV). The percentages of recoveries were calculated.

2.6.4 Precision

Precision was investigated using the sample preparation procedure for six real samples of commercial capsules (Ecosprin GOLD 20).

Method Precision (Intra-day): The precision of the method was evaluated by carrying out six independent assays of ASP, CLP and ATV (30 µg/mL of ASP and CLP, 8 µg/mL of ATV) test samples against qualified reference standard.

Intermediate Precision (Inter-day): A different analyst on a different day in the same laboratory evaluated the intermediate precision % RSD of the method. Six test samples were assayed against reference standard.

2.6.5 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using signal-to-noise ratio of 3:1 and 10:1 as per ICH guidelines.

2.6.6 Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions: Flow rate (±0.010), composition of mobile phase (53:47 and 57:43, v/v), column temperature (± 5°C) and wavelength of detection (± 2 nm).

2.6.7 System-Suitability Test (SST)

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (R_T), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated using five replicate injections of the drugs at a concentration of ASP (30 µg/mL), CLP (30 µg/mL) and ATV (8 µg/mL).

3. Results and Discussion

For successful method validation, preliminary tests were performed with the objective to select adequate and optimum condition. Parameters, such as choice of analytical column, pH of buffer, mobile phase composition and proportion, detection wavelength and other factors were exhaustively studied. Various reversed columns and isocratic mobile phase system were tried. When experiments were performed with methanol instead of acetonitrile as the organic modifier in the mobile phase, late elution of analyte with peak tailing and high column pressure were observed. Hence, the experiments were carried out with acetonitrile as an organic modifier. A satisfactory separation of the three drugs was achieved on a Acquity BEH column with a mobile phase of 0.1 % orthophosphoric acid and acetonitrile (55:45, v/v) at a flow rate of 0.35 mL/min. Quantification was achieved with PDA detection at 230 nm based on the peak area. Better resolution of the peaks with clear base line separation was found (Figure 2).

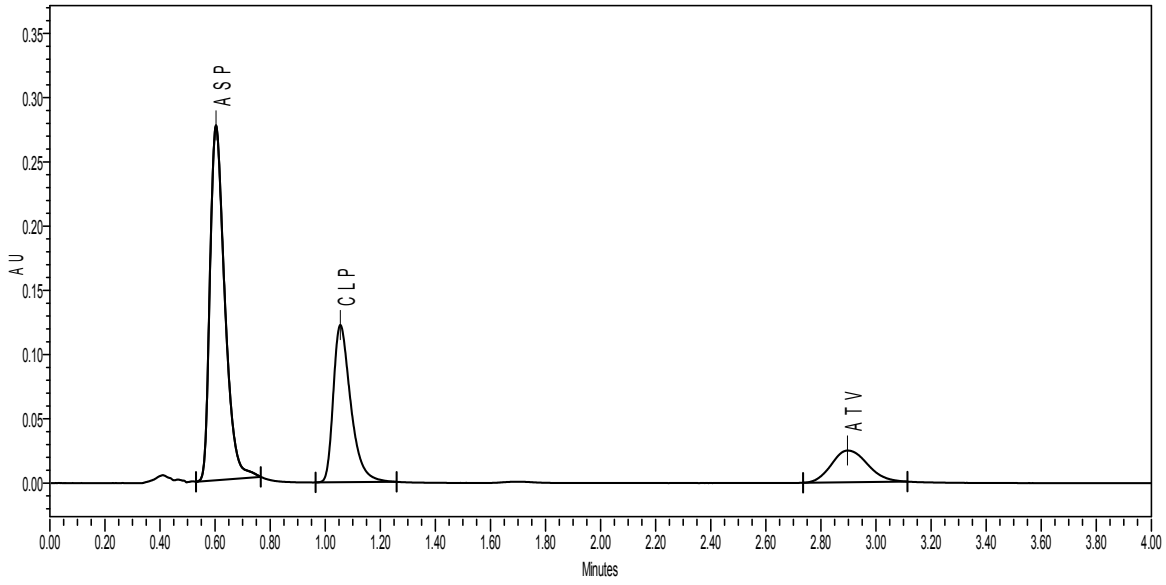


Figure 2 Chromatographic separation of ASP (0.59 min), CLP (1.04 min) and ATV (2.89 min) from their formulation

3.1.1 Specificity (Selectivity)

The selectivity of the RP-UPLC method was checked by comparison of chromatograms obtained from samples and the corresponding placebo. Additives in capsules are practically insoluble in methanol or the mobile phase whereas the active constituents are freely soluble. No interference from additives of the capsules was obtained.

3.1.2 Linearity

Linear correlation was obtained between peak area and concentration in the range of 12-48 µg/mL for ASP, 12-48 µg/mL for CLP and 3.2-12.8 µg/mL for ATV. The linearity of the calibration curves were validated by the value of correlation coefficient of the regression (r). The regression analysis of the calibration curves is shown in Table 1.

3.1.3 Accuracy

The recovery experiments were carried out by the standard addition method. The percentages of the recoveries obtained were 99.83 ± 0.21, 99.44 ± 0.59 and 99.11 ± 0.72 for ASP, CLP and ATV, respectively (Table 2). The recovery of the method was good.

3.1.4 Precision

The percentage RSD values for the precision study were 0.51, 0.48, 0.59 % (inter-day precision) and 0.56, 0.54, 0.65 % (intra-day precision) for ASP, CLP and ATV confirming a good precision (Table 2).

3.1.5 Limit of detection and limit of quantification

The LODs for ASP, CLP and ATV were found to be 0.03, 0.06 and 0.07 µg/mL, while the LOQs were 0.08, 0.15 and 0.18 µg/mL respectively (Table 2).

Table 1. Results from regression analysis of the calibration curves

Parameters	ASP	CLP	ATV
Slope	32727.9524	17965.3095	28648.7946
Intercept	6072.5714	-1066.2857	1739.9286
Correlation coefficient (r)	0.9995	0.9998	0.9996

Table 2. Summary of validation parameters for the proposed method

Parameters	ASP	CLP	ATV
LOD (µg/mL)	0.03	0.06	0.07
LOQ (µg/mL)	0.08	0.15	0.18
Accuracy (%) ± % RSD	99.83 ± 0.21	99.44 ± 0.59	99.11 ± 0.72
Precision (% assay ± % RSD)			
Intraday (n = 6)	100.22 ± 0.51	100.06 ± 0.48	100.50 ± 0.59
Interday (n = 6)	100.02 ± 0.56	99.97 ± 0.54	100.19 ± 0.65
Repeatability (% RSD)	0.095	0.121	0.262

LOD = Limit of detection
LOQ = Limit of quantification
RSD = Relative standard deviation
n = Number of determination

Table 3. Summary of system suitability parameters

Parameters	ASP	CLP	ATV
Retention time (min) ± % RSD	0.59 ± 0.09	1.04 ± 0.05	2.89 ± 0.04
Theoretical plates ± % RSD	2427.94 ± 0.60	4079.71 ± 0.48	6453.73 ± 0.25
Asymmetry ± % RSD	1.25 ± 0.12	1.18 ± 0.08	1.09 ± 0.13

RSD = Relative standard deviation

3.1.6 Robustness

The method was found to be robust, although small deliberate changes in method conditions did have a negligible effect on the chromatographic behavior of the solute. The results indicate that changing the mobile phase composition, column temperature and flow rate had no large effect on the chromatographic behavior of ASP, CLP and ATV. Even a small change of mobile phase composition and column temperature did not cause a notable change in the retention time of the used drugs for this method. A minor increase or decrease of the flow rate did also not cause any change in the tailing of peak of each drug. Alteration of the detection wavelength (±2 nm) caused no variation of peak areas.

3.1.7 System suitability test

The percentage of relative standard deviation (% RSD) for ASP, CLP and ATV were found to be 0.095, 0.121 and 0.262 respectively using this method (Table 2, 3). All the results were within the acceptable range.

Conclusions

The validated RP-UPLC method employed here proved to be simple, rapid, specific, accurate, precise, sensitive and robust. It can be successfully used for routine analysis of ASP, CLP and ATV in combined dosage form without any interference from common excipients and impurity.

With the developed method, only this mobile phase is sufficient for quantification of ASP, CLP and ATV either in combination or in single dosage form as per availability of formulation for many pharmaceutical industries. Hence, the proposed method can save labour cost and analysis time for changing mobile phase. This makes the method suitable for routine analysis in quality control laboratories.

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References

1. Hervey P.S., and Goa K.L., Extended-Release Dipyridamole-Aspirin**,** Drugs, 1999, 58, 469-475.
2. Tohgi H., Konno S., Tamura K., Kimura B., and Kawano K., Stroke, 1992, 23, 1400-1403.
3. Tripathi K.D., Essential of medicinal pharmacology, 5th Edn., Jaypee Brothers Medical publisher ltd., New Delhi, 2004, 560.
4. Satoskar R.S., Bhandarkar S.D., and Ainepure S.S., Pharmacology and pharmacotherapeutics, 15th Edn., Popular Prakashan, Mumbai, 1997, 462.
5. Budavari S., The Merck Index: Merck and Co. Inc., NJ, 12th Edn., 1996, 406.
6. Malhotra H.S., and Goa K.L., Atorvastatin: an updated review of its pharmacological properties and use in dyslipidaemia, Drugs, 2001, 61, 1835-1881.
7. Martindale-The complete drug reference, 35th Edn., Pharmaceutical Press, London, 2007, 866.
8. Ahmed M., Biswas H.U., and Sadik G., Development of a spectrometric method for the determination of aspirin in blood samples, J. Med. Sci. 2001, 1, 61-62.
9. Umapathi P., Parimoo P., Thomas S.K., and Agrawal V., Spectrofluorometric estimation of aspirin and dipyridamole in pure admixtures and in dosage forms, J. Pharm. Biomed. Anal., 1997, 15, 1703-1708.
10. Vora D.N., and Kadav A.A., Validated Ultra HPLC Method for the Simultaneous Determination of Atorvastatin, Aspirin, and their Degradation Products in Capsules, J. Liquid Chromatogr and Relate Tech., 2008, 31, 2821-2837.
11. Gandhimathi M., Ravi T.K., Abraham A., and 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, 1145-1148.
12. Sawyer M., and Kumar V., A Rapid High-Performance Liquid Chromatographic Method for the Simultaneous Quantitation of Aspirin, Salicylic Acid, and Caffeine in Effervescent Tablets, J. Chromatogr. Sci., 2003, 41, 393-397.
13. Chao W., Vickers T.J., and Mann C.K., Direct assay and shelf-life monitoring of aspirin tablets using Raman spectroscopy, J. Pharm. Biomed. Anal., 1997, 16, 87-94.
14. Abu-Qare A.W., and Abou-Donia M.B., A validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, acetylsalicylic acid and caffeine in rat plasma and urine, J. Pharm. Biomed. Anal., 2001, 26, 939-947.
15. Shah D.A., Bhatt K.K., Mehta R.S., Shankar M.B., Baldania S.L., and Gandhi T.R., Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form, Indian J. Pharm. Sci., 2007, 69, 546-549.
16. Mishra P., and Dolly A., Spectrophotometric Methods For Determination Of Clopidogrel In Tablets, Indian J. Pharm. Sci., 2005, 67, 491-493.
17. Mitakos A., and Panderi I., A validated LC method for the determination of clopidogrel in pharmaceutical preparations, J. Pharm. Biomed. Anal., 2002, 28, 431-438.
18. Kample N.S., and Venkalachalam A., Gas Chromatographic Determination Of Clopidogrel From Tablet Dosage Forms, Indian J. Pharm. Sci., 2005, 67, 128-129.
19. Patel R.B., Shankar M.B., Patel M.R., and Bhatt K.K., Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate in pure powder and tablet formulations by high-performance column liquid chromatography and high-performance thin-layer chromatography, J. AOAC Int., 2008, 91, 750-755.
20. Panchal H.J., Suhagia B.N., Patel N.J., Rathod I.S., and Patel B.H., Simultaneous Estimation of Atorvastatin Calcium, Ramipril and Aspirin in Capsule Dosage Form by RP-LC, Chromatographia 2009, 69, 91-95.
21. Sultana N., Arayne M.S., Nawaz M., Ali K.A., Development and validation of a liquid chromatographic method for thdetermination of clopidogrel from pharmaceutical dosage form, J. Indian Chem. Soc., 2009, 86, 406-409.
22. Gandhimathi M., and Ravi T.K., High performance liquid chromatographic determination of aspirin and clopidogrel in tablets, Indian J. Pharm. Sci., 2007, 69, 123-125.
23. Kachhadia P.K., Doshi A.S., and Joshi, H.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, J. AOAC Int., 2009, 92, 152-157.

24. Manoj K., Shanmugapandiyan P., and Anbazhagan S., RP HPLC method for simultaneous estimation of atorvastatin and aspirin from capsule formulation, Indian Drugs, 2004, 41, 284-288.
25. Raja R.K., Sankar G.G., Rao A.L., and Seshagirirao J.V.L.N., RP-HPLC method for the simultaneous determination of Atorvastatin and Amlodipine in tablet dosage form, Indian J. Pharm. Sci., 2006, 68, 275-277.
26. Yadav S.S., Mhaske D.V., Kakad A.B., Patil B.D., Kadam S.S., and Dhaneshwar S.R., A Simple And Sensitive HPTLC For The Determination Of Content Uniformity Of Atorvastatin Calcium Tablets, Indian J. Pharm. Sci., 2005, 67, 182-186.
27. Chaudhri B.G., Patel N.M., Shah P.B., and Modi K.P., Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe, Indian J. Pharm. Sci., 2006, 68, 793-796.
28. Jamshidi A., and Nateghi A.R., HPTLC Determination of Atorvastatin in Plasma, Chromatographia, 2007, 65, 763-766.
29. Bahrami G., Mohammadi B., Mirzaeei S., and Kiani, A., Determination of atorvastatin in human serum by reversed-phase high-performance liquid chromatography with UV detection, J. Chromatogr. B, 2005, 826, 41-45.
30. Erturk S., Seninc A.E., Ersoy L., and Ficicioglu, S., An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets, J. Pharm. Biomed. Anal., 2003, 33, 1017-1023.
31. Mohammadi A., Rezanour N., Ansari D.M., Ghorbani B.F., Hashem M., and Walker R.B., A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets, J. Chromatogr. B, 2007, 846, 215-221.
32. Alla K., A Stability-Indicating LC Method for the Simultaneous Determination of Metoprolol, Atorvastatin and Ramipril in Combined Pharmaceutical Dosage FormJ. AOAC Int., 2007, 90, 1547-1553.
33. Ma L., Dong J., Chen X.J., and Wang G.J., Development and Validation of Atorvastatin by LC–ESI–MS and Application in Bioequivalence Research in Healthy Chinese Volunteers, Chromatographia, 2007, 65, 737-741.
34. Kadav A.A., and Vora D.N., Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets, J. Pharm. Biomed. Anal., 2008, 48, 120-126.
35. ICH. Q2R1, Validation of analytical procedures and methodology, In Proceeding of the International Conference on Harmonization, Geneva, Switzerland, 2005.

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