Scientific Research Publishing

American Journal of Analytical Chemistry, 2016, 7, 918-926
http://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Development and Validation of Amlodipine Impurities in Amlodipine Tablets Using Design Space Computer Modeling

Chamarthi R. P. Kishore*, G. V. Krishna Mohan

Department of Chemistry, K.L. University, Vaddeswaram, India
Email: *phanikishore82@gmail.com

How to cite this paper: Kishore, C.R.P. and Mohan, G.V.K. (2016) Development and Validation of Amlodipine Impurities in Amlodipine Tablets Using Design Space Computer Modeling. *American Journal of Analytical Chemistry*, **7**, 918-926.
http://dx.doi.org/10.4236/ajac.2016.712078

Received: July 6, 2016
Accepted: December 25, 2016
Published: December 28, 2016

Abstract

A rapid, sensitive, robust, rugged and linear HPLC method is developed using QbD approach and validated as per ICH for the estimation of amlodipine impurities in tablet dosage form. Phosphate buffer with triethyl amine adjusted to pH to 2.8 is used as the mobile phase and 3 µ particle size C18 column of 150 mm length and 4.6 mm internal diameter is used. Using photo diode array (PDA) detector, the compounds are monitored at 340 nm. All impurities are well separated and flow Gradient has been optimized to obtain the acceptable resolution between impurities and amlodipine. Diluent was chosen, based on the impurity peak shapes and recoveries. Test concentration and injection volume have been optimized to obtain limit of quantification (LOQ) values below the reporting threshold.

Keywords

HPLC, LOQ, QbD, Reporting Threshold

1. Introduction

Amlodipine (chemically known as (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate with molecular formulae $C_{20}H_{25}ClN_2O_5$ and molecular weight of 408.9 g/mol) is used to treat the high blood pressure by allowing the free flowing of blood through blood cells. Amlodipine belongs to calcium channel blocker group. Many pharma companies are formulating and marketing amlodipine as single or in combination with other active ingredients under different brand names like Asomex, Istin, Norvasc, Caduet and Twynsta. Stability studies provide us with information on the quality of the

drug product. The studies must include the tests, which can monitor the quality of the drug product [1]. Impurities can be generated by drug excipient interactions, storage conditions, hydrolysis etc. A sensitive, reproducible method is to be developed and validated to monitor the impurities in drug product.

Literature survey reveals that some analytical methods are available for the estimation of amlodipine alone or in combination with other drugs using HPLC, HPTLC, and LC-MS [2]-[13]. Ph. Eur monograph method is also reported to estimate impurities in amlodipine besylate raw material. However, none of the analytical methods reported the estimation of all known and unknown impurities for amlodipine besylate. This paper describes the quantification of all impurities (IMP-A, IMP-B, IMP-D, IMP-E, IMP-F, IMP-G and IMP-H) of amlodipine besylate in amlodipine tablets.

2. Materials and Methods

2.1. Chemicals and Reagents

Amlodipine besylate tablets and all impurities were synthesized and supplied by Dr. Reddy's Laboratories Ltd. Potassium dihydrogen phosphate, triethyl amine, orthophosphoric acid of AR grade and methanol and acetonitrile of gradient grade were purchased from Merck Chemicals Ltd.

2.2. Chromatographic Conditions

The mixed buffer was prepared by adding 7 ml of triethyl amine to 1000 ml of 50 mm monobasic phosphate buffer and then the pH was adjusted to 2.8 with orthophosphoric acid. Mobile phase-A was prepared by mixing the buffer with methanol in the ration of 60:40 (v/v) while Mobile phase-B was prepared by mixing the buffer with methanol and acetonitrile in the ratio of 20:40:40 (v/v/v). Mobile phase-A contains more buffer concentration to separate all impurities. Mobile phase-B contains more organic concentration to elute all the impurities.

A column with 150 mm length, 4.6 mm internal diameter and 3 μ particle sizes, C18 as stationary phase was used to separate all the impurities. Column temperature was maintained at 35˚C. All impurities were monitored at 340 nm except impurity-D. Impurity-D was monitored at 270 nm. Gradient mode flow was used to separate the impurities. Flow rate of the mobile phase was kept at 1.0 mL/min and 100 μL samples were injected into HPLC. Analysis was performed on Waters HPLC system with PDA detector. The diluent for extraction of impurities and amlodipine from formulation matrix was prepared by admixing Buffer, Methanol and acetonitrile in the ratio of 70:15:15 (v/v/v).

2.3. Solution Preparations

2.3.1. Standard Stock Preparation

70 mg of amlodipine besylate standard (potency-72%) was weighed and transferred into 250 mL of volumetric flask and it was dissolved and diluted to volume with methanol. 5 mL of this solution was diluted to 100 mL with methanol.

2.3.2. Final Standard Stock Preparation

5 mL of the standard stock solution was diluted to 100 mL with diluent.

2.3.3. Sample Preparation

20 tablets were crushed into fine powder and then the powder equivalent to 25 mg of amlodipine was transferred into 100 ml volumetric flask and then 70 ml of diluent was added. The resulting solution was sonicated for 30 minutes with intermittent shaking and the temperature of the sonication was maintained below 25˚C. Then the solution was diluted to the volume with the diluent and was centrifuged the sample at 4000 RPM for 15 mins and then few ml of the supernatant solution was filtered through 0.45 µ membrane filter.

2.3.4. Impurity Stock Solution Preparation

2 mg of each impurity was weighed, transferred to 20 ml volumetric flask and then dissolved in methanol and the resulting solution was diluted to the mark.

2.3.5. Spiked Sample Preparation

Tablet powder equivalent to 25 mg of amlodipine was weighed and transferred into 100 mL volumetric flask. 70 ml of the diluent was added. Then, 1.25 mL of impurity stock solution was added. The resulting solution was sonicated for 30 mins and then diluted to volume with the diluent. Then the solution was centrifuged at 4000 RPM for 15 mins and filtered the few ml of the supernatant solution through 0.45 µ membrane filter.

2.4. Analytical Method Validation

The developed method was validated as per ICH guidelines for specificity, linearity, precision, ruggedness and robustness.

3. Results and Discussion

3.1. Optimization of Chromatographic Conditions

Different trials were taken with mobile phases containing ammonium salts, phosphate and sodium perchlorate buffers to obtain optimum resolution between impurities. Final chromatographic conditions were finalized based on the DOE. Fractional design was used to perform DOE by considering the flow rate, pH of the buffer, % methanol and % acetonitrile in mobile phase-B as factors and resolution between the close eluting impurities (Impurity-B & H) as responses. A text plan was shown in Table 1 using 10 combinations of the factors used for evaluation study.

The obtained results were transcribed back into Minitab software for modelling purposes. The effects of factors on resolutions were evaluated using Mini tab software generated three-dimensional plots and Pareto chart. Figure 1 represents main effects plot for the resolution between Imp-B&H. Figure 2 represents interaction plot, Figure 3 represents pareto chart for standardized effects and Figure 4 represents contour plot for resolution. Flow rate and % acetonitrile play major role in separation of impurities.

Table 1. Design of experiments.

Std Order	Run Order	Center Pt	Flow	pH	% MeOH	% ACN	Resolution b/w B & H
1	1	1	0.8	2.6	35	35	1.765
2	2	1	1.2	2.6	35	45	2.951
3	3	1	0.8	3.0	35	45	1.774
4	4	1	1.2	3.0	35	35	2.613
5	5	1	0.8	2.6	45	45	1.806
6	6	1	1.2	2.6	45	35	2.706
7	7	1	0.8	3.0	45	35	1.611
8	8	1	1.2	3.0	45	45	2.813
9	9	0	1.0	2.8	40	40	2.232
10	10	0	1.0	2.8	40	40	2.212

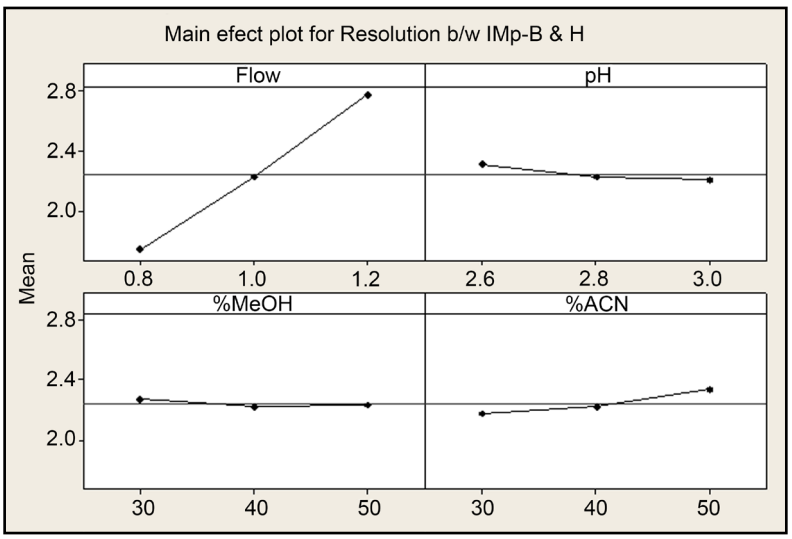

Figure 1. The main effect chart for the resolution between Imp-B & H.

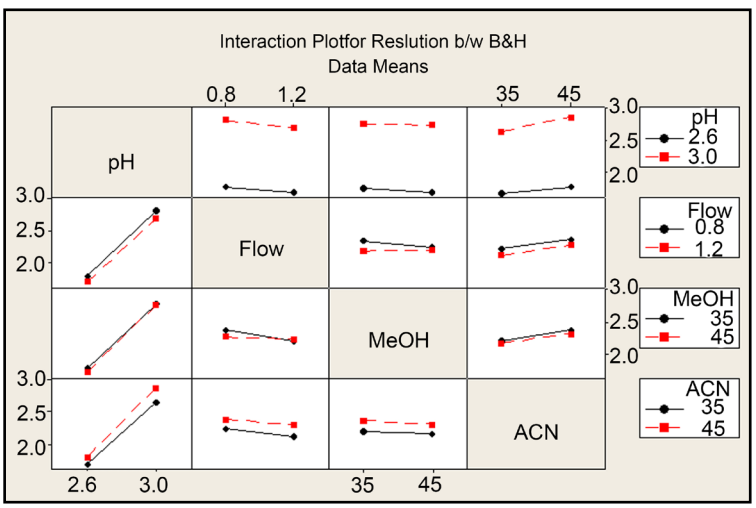

Figure 2. The interaction plot for the resolution between Imp-B & H.

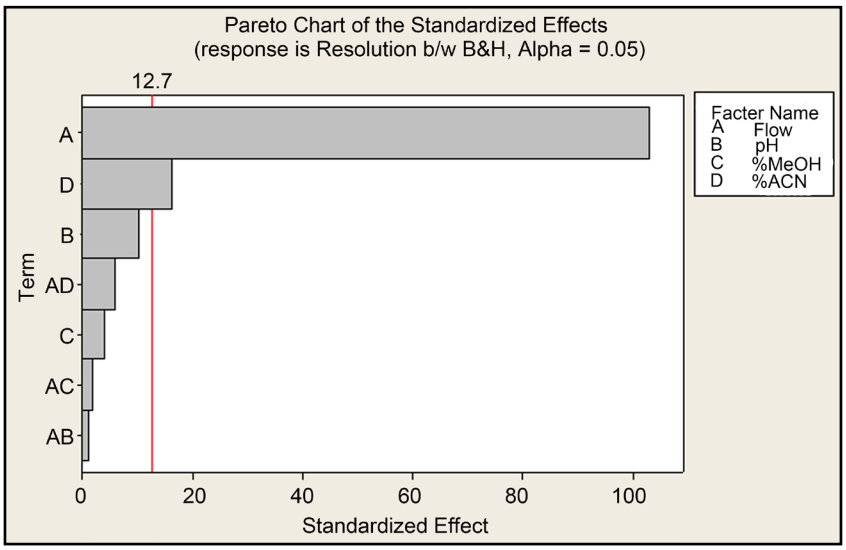

Figure 3. The Pareto chart for standardized effects on the resolution between Imp-B & H.

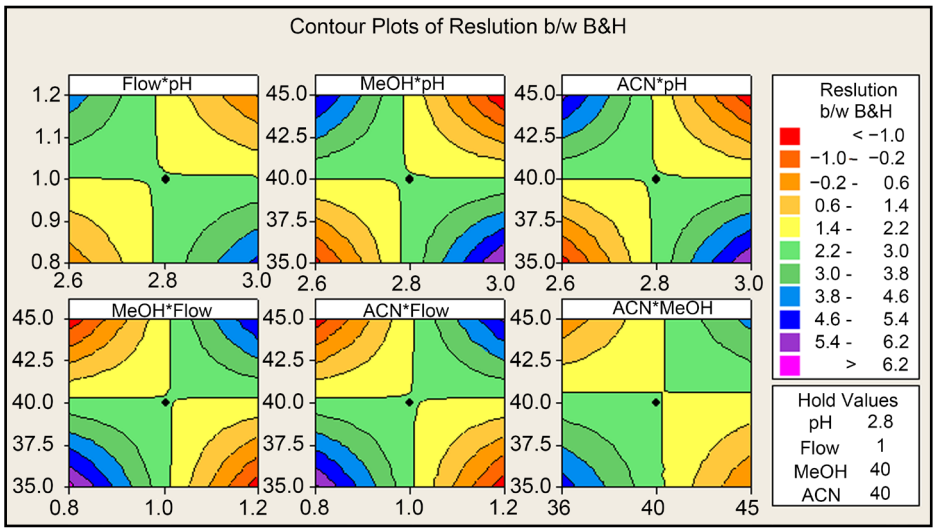

Figure 4. The contour plot for the resolution between Imp-B & H.

Design space was established and recommended parameters were near to the experimentally proposed values. The Proposed HPLC method was shown in **Table 2**.

Further, experimentally obtained data were used in setting the lower and upper bounds for each response variables. Using modelled data, visual inspections of interactive effects were performed from multiple overlay graphs plotting two parameters at a time.

Diluent was finalized based on recovery and peak shape. Injection volume and test concentrations were optimized to have LOQ value less than reporting threshold. Gradient was optimized to get optimal resolution between all impurities and main analyte. **Figure 5** represents the chromatogram for standard, **Figure 6** represents chromatogram for spiked sample at 340 nm and **Figure 7** represents chromatogram for spiked sample preparation at 270 nm.

Table 2. HPLC method conditions.

Column	Inertsil ODS-3, 150 × 4.6 mm 3 µ
Column temperature	35˚C
Wave length	270 nm for Imp-D and 340 nm for remaining impurities
Injection volume	100 µL
Run time	70 minutes
Gradient	90% A upto 8 min (isocratic) with 1.0 mL Linear gradient to 75% A at 20 min with 1.0 mL Linear gradient to 70% A at 45 min with 1.0 mL Linear gradient to 20% A at 50 min with 1.2 mL Linear gradient to 0% A at 52 min with 1.2 mL 0% A from 52 min to 60 min with 1.2 mL Linear gradient to 90% A at 62 min with 1.0 mL 90% A from 62 min to 70 min with 1.0 mL

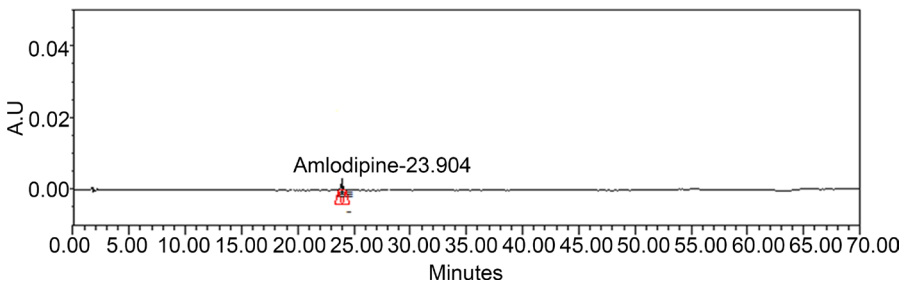

Figure 5. The chromatogram for standard preparation.

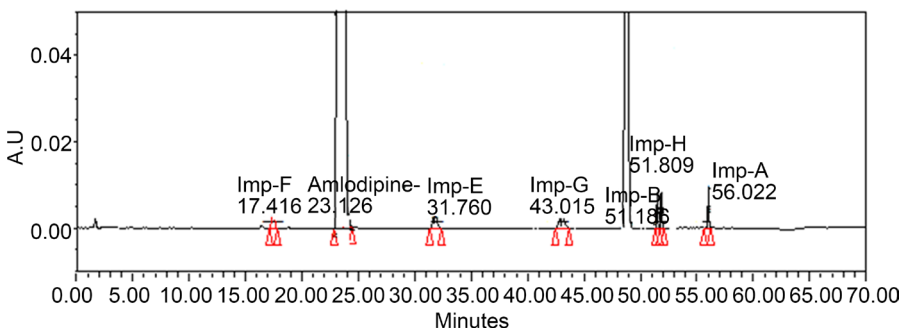

Figure 6. The chromatogram for sample preparation at 340 nm.

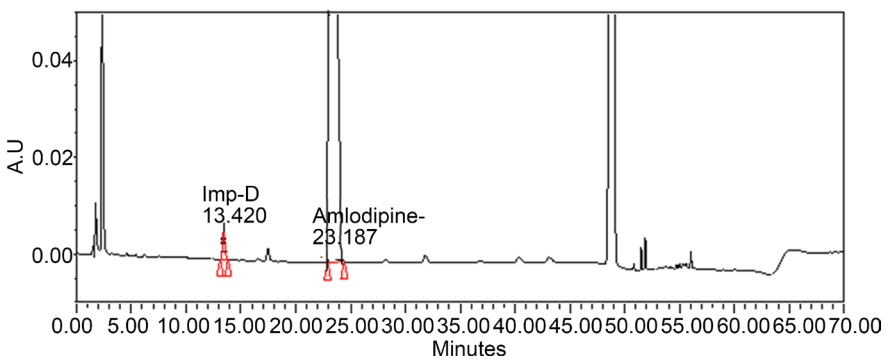

Figure 7. The chromatogram for sample preparation at 270 nm.

3.2 Method Validation

3.2.1. System Suitability

The authors prepared the standard at 0.2% level of the test concentration and injected it into HPLC system; and then calculated the % RSD for peak areas, USP plate count, tailing factor of amlodipine peak from replicate standard injections. % RSD from replicate injections was found to be 3.2. The tailing factor for main analyte is found to be 1.0 and plate count is found to be 43685. Results are tabulated in Table 3. Resolution between Impurity-B and Impurity-H was found to be 2.7.

3.2.2. Linearity and Range

Linearity was established over the range of 0.0001 mg/ml to 0.05 mg/ml for all the impurities and main analyte. Six different linearity solutions were prepared and injected into system. Results are tabulated in Table 4.

3.2.3. Specificity

Test samples were subjected to different stress conditions like acid, base, water hydrolysis, peroxide oxidation, thermal degradation, sun light/UV degradation and Humidity degradation. Sample was exposed to acidic (0.1 N HCl/5 mL/30 min reflux), alkaline (0.1 N NaOH/5 mL/60 min reflux), Oxidation (% 5H_2O_2/10 mL/30 min reflux), thermal (105˚C/24 hrs), water (10 mL/60˚C/30 min reflux) water conditions, sunlight (1.2 million lux hrs), UV light (200 watt hours) and Humidity (90% RH for 7 days). All the samples were injected into HPLC system with PDA detector to identify the purity of the known and main analyte peaks. Purity angles were less than purity threshold for all the known impurities and amlodipine peak. The results were tabulated in Table 5. On

Table 3. System suitability parameters.

System suitability parameters	Observation	Acceptance criteria
Tailing factor for amlodipine peak in diluted standard preparation	1.0	Not more than 2.0
Plate count for amlodipine peak	43,685	Not less than 2000
% RSD for areas of amlodipine	3.2	Not more than 3.0
Resolution between Imp-B and Imp-H	2.7	Not less than 2.0

Table 4. LOD, LOQ, linearity, precision and recovery values.

Parameters	Imp-A	Imp-B	Imp-D	Imp-E	Imp-F	Imp-G	Imp-H
LOQ (ppm)	0.095	0.11	0.09	0.15	0.10	0.15	0.12
LOD (ppm)	0.031	0.06	0.03	0.06	0.04	0.06	0.04
Precision	92.3 - 104.7	93.7 - 105.9	97.1 - 101.4	98.1 - 105.4	93.7 - 107.8	94.9 - 106.0	93.6 - 102.8
Accuracy							
LOQ	89.5 - 107.4	95.3 - 111.6	98.3 - 101.6	88.3 - 95.0	95.0 - 100.0	90.0 - 93.4	91.7 - 112.5
50%	97.4	100.3	100.7	105.6	107.1	99.4	101.8
100%	87.0	101.5	92.6	96.7	107.9	109.7	99.6
150%	89.6	99.8	96.4	97.2	108.7	109.9	107.5

Table 5. Summary of peak purity and degradation data for amlodipine in stress study.

Stress condition	Purity angle	Purity threshold	% Degradation
Acid degradation (0.1 N HCl/1 hr reflux)	0.080	0.694	3.79
Base degradation (0.1 N NaOH/20 min reflux)	0.088	0.610	3.62
Peroxide degradation (10% H_2O_2/20 min reflux)	0.109	0.663	0.79
Thermal (105˚C/72 hrs/solid)	0.090	0.684	0.56
Water	0.114	0.617	3.87
Sun light/UV light	0.102	0.626	0.73
Humidity	0.108	0.598	0.41

perusal of the results, it may be concluded that all the unknown impurities generated in the degradation are well separated from the known and amlodipine peaks. Hence, the developed method is specific.

3.2.4. Precision and Accuracy

Recovery studies were performed for all the impurities from 0.000125 mg/ml to 0.001875 mg/ml and values were found to be between 85% - 110%. Precision was performed by preparing six samples by spiking the impurities at 0.5% of the target test concentration. Results were tabulated in Table 4. The resultant % RSD values for the % impurities were found the below 5.0 (n = 6). Hence, it may be concluded that the method is precise and accurate.

3.2.5. LOD and LOQ

LOQ and LOD were established for the impurities and amlodipine using slope method. LOQ values were found to be 0.01. The concentration with signal to noise ratio about three was taken as LOD and ten was taken as LOQ. Values were presented in Table 4.

3.2.6. Mobile Phase and Solution Stability

Mobile phase and solution stability were established over a period of 24 hours on bench top. System suitability parameters were evaluated and % impurities were calculated against fresh standard. From the results, it may be concluded that solution and mobile phase are stable up to 24 hours on bench top.

4. Conclusion

A novel HPLC method has been developed for the estimation of amlodipine impurities in amlodipine tablets formulation. Placebo interference was not observed at known and unknown impurities. Method is found to be linear, precise, accurate, robust and rugged. Hence, this method can be used for the estimation of amlodipine impurities in regular as well as stability sample analysis.

Acknowledgements

The author thanks the K.L. University, Department of Chemistry for providing neces-

sary support in completing the work.

References

[1] Stability Testing of New Drug Products and Substances Q1A(R2).

[2] Agrekar, A.P. and Powar, S.G. (2000) Simultaneous Determination of Atenolol and Amlodipine in Tablets by High-Performance Thin-Layer Chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, **21**, 1137-1142. https://doi.org/10.1016/S0731-7085(99)00210-1

[3] Chitlange, S.S., Bagri, K. and Sakarkar, D.M. (2008) Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. *Asian Journal of Research in Chemistry*, **7**, 15-18.

[4] Chaudhri, B.G., Patel, N.M. and Shah, P.B. (2007) Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Amlodipine from Their Combination Drug Products. *Chemical and Pharmaceutical Bulletin*, **55**, 241-246. https://doi.org/10.1248/cpb.55.241

[5] Bahrami, G. and Mirzaeei, S.H. (2004) Simple and Rapid HPLC Method for Determination of Amlodipine in Human Serum with Fluorescence Detection and Its Use in Pharmacokinetic Studies. *Journal of Pharmaceutical and Biomedical Analysis*, **36**, 163-168. https://doi.org/10.1016/j.jpba.2004.05.016

[6] http://en.wikipedia.org/wiki/Amlodipine

[7] Khan, M.R. and Jain, D. (2006) Simultaneous Spectrophotometric Determination of Atorvastatin Calcium and Amlodipine Besylate in Tablet. *Indian Journal of Pharmaceutical Sciences*, **68**, 546-548. https://doi.org/10.4103/0250-474X.27846

[8] Malesuik, M.D., Cardoso, S.G., Bajerski, L. and Lanzanova, F.A. (2006) Determination of AML in Pharmaceutical Dosage Forms by Liquid Chromatography and Ultraviolet Spectrophotometry. *Journal of AOAC International*, **89**, 359-364.

[9] Rajeswari, K.R., Sankar, G.G., Rao, A.L. and Sheshagirirao, J.V. (2006) RP-HPLC Method for Simultaneous Determination of Atorvastatin Calcium and Amlodipine in Tablet. *Indian Journal of Pharmaceutical Sciences*, **68**, 275-257. https://doi.org/10.4103/0250-474X.25738

[10] Sahu, R. and Patel, V.B. (2007) Simultaneous Spectrophotometric Determination of Amlodipine Besylate and Atorvastatin Calcium in Binary Mixture. *Indian Journal of Pharmaceutical Sciences*, **69**, 110-111. https://doi.org/10.4103/0250-474X.32119

[11] Sivakumar, T., Manavalan, R., Muralidharan, C. and Valiappan, K. (2007) An Improved HPLC Method with the Aid of a Chemometric Protocol: Simultaneous Analysis of Amlodipine and Atorvastatin in Pharmaceutical Formulations. *Journal of Separation Science*, **30**, 3143-3153. https://doi.org/10.1002/jssc.200700148

[12] Meyyanthan, S.N. and Suresh, B. (2005) HPTLC Method for the Simultaneous Determination of AML and Benazepril in Their Formulations. *Journal of Chromatographic Science*, **43**, 73-75. https://doi.org/10.1093/chromsci/43.2.73

[13] Feng, Y., Zhang, L., Shen, Z., Pan, F. and Zhang, Z. (2002) Analysis of AML in Human Plasma by Liquid Chromatography-Mass Spectrometry. *Journal of Chromatographic Science*, **40**, 49-53.