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Development and validation of LC–MS/MS method for the quantitative determination of Lacidipine in human plasma using solid-phase extraction

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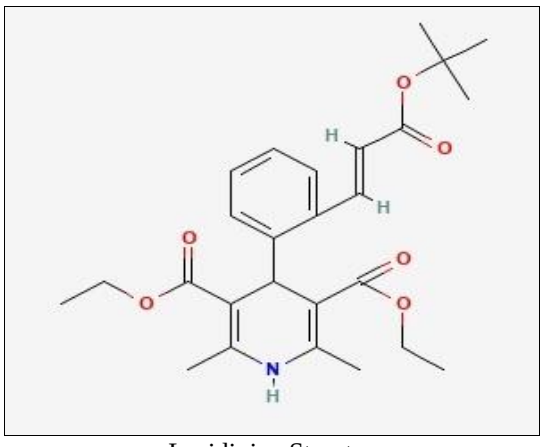
Abstract
A rapid, sensitive, and reproducible LC-MS method was developed and validated for the quantitative determination of lacidipine in human plasma using carbamazepine as the internal standard. Plasma samples were processed by solid-phase extraction using Oasis HLB cartridges and separated on a Zorbax Eclipse XDB-Phenyl column with acetonitrile and 5 mM ammonium acetate (80:20, v/v) at a flow rate of 1.0 mL/min. Detection was performed using positive electrospray ionization. The chromatographic run time was 2.5 min, enabling high-throughput analysis. The assay demonstrated linearity over the concentration range of 0.2052–12.5026 ng/mL with a correlation coefficient greater than 0.99 and an LLOQ of 0.2052 ng/mL. Validation studies confirmed satisfactory selectivity, precision, accuracy, recovery, matrix effect, dilution integrity, ruggedness, carry-over, and stability under all tested conditions. Mean extraction recovery of lacidipine was approximately 91.9%, and no significant endogenous interference or matrix effect was observed. The proposed method is appropriate for routine pharmacokinetic, bioavailability, bioequivalence, and therapeutic monitoring studies requiring rapid and reliable quantification of lacidipine.

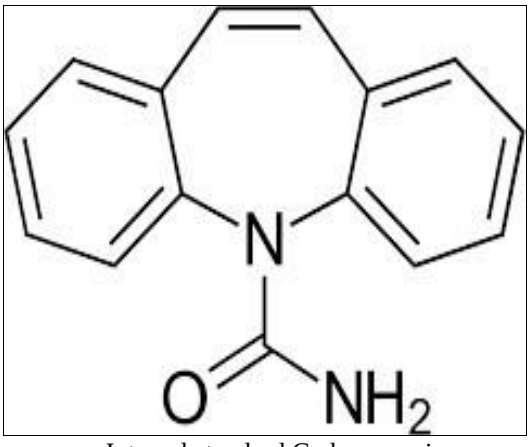
Keywords: Lacidipine, LC-MS/MS, Bioanalytical Method validation, Human Plasma, Solid-Phase Extraction, Pharmacokinetics, Bioequivalance

Introduction

Lacidipine is a highly lipophilic third-generation dihydropyridine calcium channel blocker used for the treatment of hypertension; however, its extensive first-pass metabolism results in very low plasma concentrations, making accurate bioanalysis analytically challenging. The present study describes the development and validation of a rapid, sensitive, and selective LC–MS/MS method employing solid-phase extraction and carbamazepine as the internal standard for the quantification of lacidipine in human plasma. The optimized method provides a short chromatographic run time, efficient sample clean-up, and reliable analytical performance across the validated concentration range. Comprehensive validation demonstrated acceptable selectivity, linearity, precision, accuracy, recovery, matrix effect, carry-over, and stability, supporting its suitability for routine pharmacokinetic, bioavailability, and bioequivalence studies.



Lacidipine Structure



Internal standard Carbamazepine

Materials and Methods

Chemicals and Reagents

Lacidipine reference standard and carbamazepine (internal standard, IS) were used for method development and validation. HPLC-grade acetonitrile, methanol, ammonium acetate, formic acid, and analytical-grade reagents were employed throughout the study. Human K_2EDTA plasma was used as the biological matrix for preparation of calibration standards and quality control (QC) samples. Ultrapure water was obtained using a laboratory purification system. All solvents and reagents were of LC–MS grade or equivalent analytical quality.

Instrumentation

Chromatographic analysis was performed using a high-performance liquid chromatography system coupled to a

triple quadrupole tandem mass spectrometer equipped with an electrospray ionization (ESI) source. Data acquisition and quantitative analysis were carried out using the instrument software supplied by the manufacturer. The optimized analytical conditions provided high sensitivity and reproducibility for lacidipine determination in human plasma.

Chromatographic Conditions

Chromatographic separation was achieved on a Zorbax Eclipse XDB-Phenyl analytical column using a mobile phase consisting of acetonitrile and 5mM ammonium acetate (80:20, v/v) delivered under isocratic conditions at a flow rate of 1.0mL min^{-1}. The total chromatographic run time was 2.5 min, allowing rapid sample analysis while maintaining satisfactory peak symmetry and resolution.

Mass Spectrometric Conditions

Detection was performed using positive electrospray ionization in multiple reaction monitoring (MRM) mode. The precursor-to-product ion transitions selected for quantification were m/z 456.4 → 354.3 for lacidipine and m/z 237.1 → 194.1 for carbamazepine. Compound-dependent and source parameters were optimized to maximize signal intensity and analytical sensitivity.

Preparation of Standard

Primary stock solutions of lacidipine and carbamazepine were prepared in a suitable organic solvent and subsequently diluted to obtain working standard solutions. Calibration standards were prepared by spiking blank human plasma over the validated concentration range of 0.2052–12.5026 ng mL^{-1}. Independent low, medium, and high quality control samples, together with the lower limit of quantification (LLOQ) QC sample, were similarly prepared for method validation.

Plasma Sample Preparation

Plasma samples were processed using solid-phase extraction (SPE) with Oasis HLB cartridges. Aliquots of plasma containing calibration standards, QC samples, or study samples were mixed with the internal standard solution before extraction. After conditioning and equilibration of the SPE cartridges, samples were loaded, washed to remove endogenous matrix components, and eluted with a suitable organic solvent. The eluates were evaporated under a gentle stream of nitrogen, reconstituted in the mobile phase, and transferred to autosampler vials for LC–MS/MS analysis. The SPE procedure produced clean extracts with high and reproducible recovery.

Method Development

Method development focused on achieving rapid chromatographic separation, high analytical sensitivity, and minimal matrix interference. Different stationary phases, mobile-phase compositions, and flow rates were evaluated to optimize peak shape, retention time, and ionization efficiency. A phenyl stationary phase combined with acetonitrile and 5 mM ammonium acetate (80:20, v/v) produced the most satisfactory chromatographic performance. Carbamazepine was selected as the internal standard because of its suitable extraction behavior, chromatographic compatibility, and stable ionization response. Solid-phase extraction was chosen over other extraction approaches to improve analyte recovery and reduce matrix effects, resulting in enhanced assay reproducibility.

Table 1: chromatographic conditions

Chromatographic conditions	
Column	ZorbaxEclipseXDB-Phenyl,4.6×75mm,5µm particle size (Manufacturer: Agilent)
Mobile Phase	Acetonitrile and 5mM ammonium acetate in an 80:20 (v/v) ratio
Rinsing Solution	Acetonitrile and Milli-Qwaterina50:50(v/v) ratio
Flow Rate	1mL/minute
Sample Cooler Temperature	10°C
Injection Volume	20µL
Rinse Dip Duration	0Sec
Needle Rinse Volume	500µL
Analyte Retention Times	1.30-0.30minutes (lacidipine) 0.95-0.30minutes (Carbamazepine)
Run Time	2.50 minutes

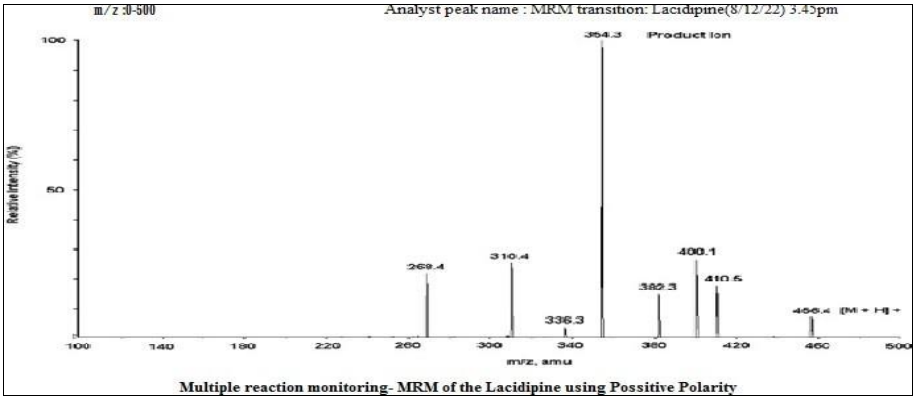


Fig 1: Mass Spectra of Lacidipine

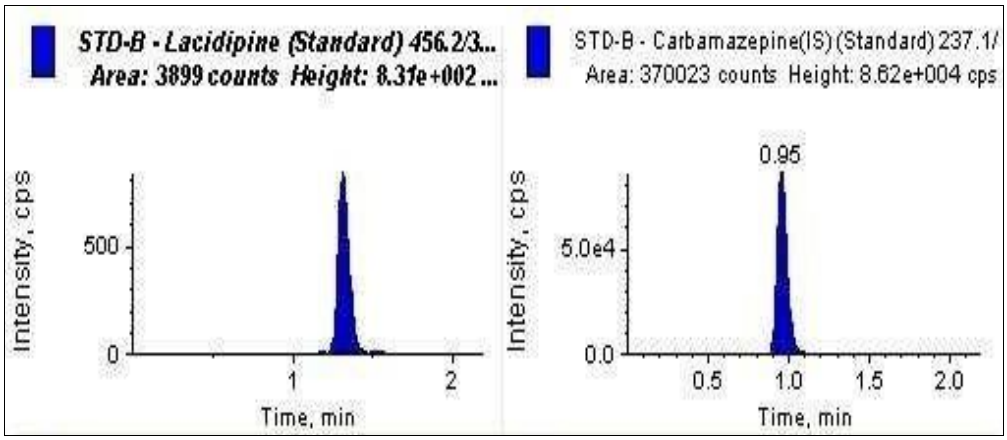


Fig 2: Representative Chromatogram of Blank Plasma Internal Standard Sample of Lacidipine for Selectivity

Table 1.1: m/z values of Parent ions

Compound	Molecular weight(g/mol)	m/z value (amu) of Parent ion
Lacidipine	455.5	456.4
Carbamazepine	236.26	237.10

Representative Chromatogram of Blank Plasma and Internal Standard Sample of Lacidipine and carbamazepine

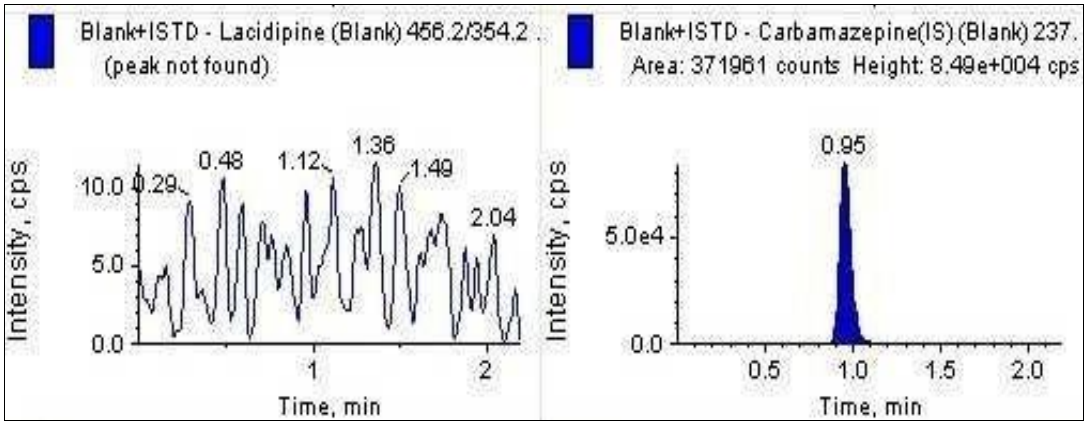


Fig 3: Representative Chromatogram of an Aqueous Standard and internal Standard Mixture of Lacidipine

Method Validation

The developed LC–MS/MS method was validated according to accepted bioanalytical method validation requirements. Validation parameters included selectivity, carry-over, sensitivity, calibration curve linearity, intra- and inter-batch precision, accuracy, extraction recovery, matrix effect, dilution integrity, ruggedness, and stability under various storage and processing conditions. Acceptance criteria for each parameter were evaluated before the method was considered suitable for routine bioanalytical application.

System suitability test for Lacidipine and Carbamazepine

System Suitability Test

Six sequential Injections were prepared using lacidipine at the midpoint concentration of the calibration curve (12.0092ng/mL) and carbamazepine at its working concentration (0.8060 ng/ml) were used to test the system's applicability.

Sensitivity

LLOQ was used to determine the sensitivity. Using the identical stock solutions, six LLOQ and calibration curve samples were created. Using the calibration curve data, the LLOQ concentrations were recalculated. For lacidipine in human plasma, the LLOQ value of 0.2052ng/ml was determined to be the lowest limit of accurate quantification. Tables 21a and 21b showed the results. For lacidipine at this concentration, the precision and accuracy were 2.34percent and 92.93%, respectively. LLOQs 1 through 6 have S/N ratios of 31.8, 31.9, 35.9, 42.1, 37.7, and 35.8, in that order.

Matrix Effect

Several batches of blank matrices were used to create six sets of LQC (0.6096ng/mL) and HQC (10.7624 ng/mL) samples in triplicate. A fresh spiked calibration curve standard was used for analysis of all the Q.C. samples. Utilising data from the calibration curve, the Q.C. concentrations were computed

The relationship between lacidipine concentration and detector response in human plasma was best described by regression equation using a weighting factor of 1divided by the square of the drug-to-internal standard concentration ratio.

Precision and Accuracy

In order to evaluate both accuracy and precision of the

devised method of analysis five distinct quality control (QC) concentrations spanning the calibration curve (CC) range were prepared, processed, and evaluated using six replications per Q.C concentration.

Within-batch Precision and Accuracy At every QC level of a bio analytical batch, the C.V. percentage and the % Nominal were calculated to determine the batch's precision and accuracy. The range of within-batch accuracy for LLOQ QC, LOQC, MQC 1, MQC 2, and HQC was 88.81% to 99.84%,92.40% to 98.93%,93.98% to102.22%,98.72% to100.72%,and 93.39% to 105.36%, respectively.

Intra-day Precision and Accuracy: At each QC level, Intra-day precision and accuracy were evaluated by calculating the coefficient of variation (C.V.%) and the percentage of the nominal concentration (% Nominal), respectively for all bio analytical batches run in a day.

Recovery

24 blank plasma samples have been processed. Six sets of recovery comparison samples were prepared by reconstituting each set with a volume of 0.2500 mL from the final recovery dilutions of quality control samples at LQC, MQC 2, and HQC levels, along with the internal standard, was used to represent 100% extraction and direct injection. These lacidipine recovery reference samples were evaluated against the extracted QC samples from PA Batch V at the corresponding LQC, MQC 2, and HQC levels. Additionally, the internal standard response at the MQC 2 level (within the range of 25–30) was assessed by comparing it with the internal standard responses from the recovery reference samples.

Dilution Integrity

A 1.7-fold increase in the standard concentration (21.2545ng/mL) was used to create twelve sets of dilution integrity samples. After being diluted twice and four times with screened blank plasma, six sets of dilution integrity samples were processed. The samples were processed along with the same set of calibration curve standards. The unaltered concentration range utilized to assess precision and accuracy in PA Batch II was examined using these quality control samples. Appropriate dilution factors were applied to determine the concentrations of the quality control samples. The outcomes were shown in Tables 3.18a and 3.18b. Results for lacidipine show adequate dilution integrity at two and four times dilution. The precision and accuracy of lacidipine were 1.05% and 102.42%, respectively, for a dilution factor of

2. Likewise, lacidipine accuracy and precision were 0.45% and 98.66%, respectively, for a dilution factor of 4.

Ruggedness

One precision and accuracy batch (PA BATCH - V) was processed and analyzed by multiple columns of same make and different solutions. The batch precision values for LLOQ QC, LQC, MQC 1, MQC 2, and HQC were found to be 5.48%, 3.38%, 2.20%, 5.94%, and 3.92%, respectively. LLOQQC,LQC,MQC1,MQC 2,and HQC had respective within-batch accuracy of 96.76%, 93.91%, 94.94%, 101.95%, and 98.02%.

Hemolysis and Lipemic effect

Three LQC (0.6096ng/ml) and HQC (10.7634ng/ml) samples were prepared in haemolytic and lipemic plasma, processed, and paired with newly spiked calibration curve samples. Table 3.20 a, 3.20 b, and 3.20.c displayed the findings. The results for lacidipine indicated that neither lipemic nor haemolytic plasma had any significant impact. At both low-quality control (LQC) and high-quality control (HQC) levels, the percentage of nominal values observed in haemolysed samples ranged from 96.45% to 100.88%, with precision values falling between 1.61% and 2.3%. The precision ranged from 1.23% to 2.68%, while the nominal percent for lipemic impact at the LQC and HQC levels varied from 96.20% to 107.39%.

Stability Studies

In order to test the stability of the stock solutions for lacidipine and Carbamazepine, used as the internal standard, was co-analyzed with six replicates of lacidipine stock dilutions, corresponding to the two intermediate quality control concentration levels and carbamazepine at working concentration were injected at room temperature (20 ± 5 0C) after nine hours. The mean area response of the zero-hour samples was compared to that of lacidipine and carbamazepine at nine hours. The findings were displayed in tables. The stability of lacidipine in a stock solution at room temperature (20 ± 5 0C) was determined to be 97.22% stable, with precision values of 1.40 % and 3.54% at 0 and 9 hours, respectively. At 0 and 9 hours, the accuracy of the carbamazepine stock solution stability at room temperature (20 ± 5 0C) was 0.86 and

5.85percent, respectively, and the stability percentage was found to be103.40%.

Results and Discussion

Table 1.2: System suitability test for Lacidipine and Carbamazepine

S.No	Lacidipine		Carbamazepine	
	Retention time	Area	Retention time	Area
1	1.31	359849	0.95	462781
2	1.32	378876	0.96	455282
3	1.32	367284	0.96	464376
4	1.31	373643	0.95	472986
5	1.31	369875	0.94	453234
6	1.31	381462	0.95	449823
Mean	1.313	371831	0.951	459747
SD	0.00516	7921.376	0.00752	8564.404
C.V.%	0.39	2.13	0.79	1.86
N	6	6	6	6

Table 1.3: Data for Linearity in terms of concentration response for Lacidipine

	Nominal Concentration (ng/mL)								Slope	Intercept	R	R^2
	STD-A	**STD-B**	**STD-C**	**STD-D**	**STD-E**	**STD-F**	**STD-G**	**STD-H**				
CC	**0.2052**	**0.4105**	**1.2828**	**2.5655**	**5.1311**	**7.6016**	**10.0021**	**12.5026**				
1	0.2067	0.4044	1.3474	2.2558	5.2033	7.3682	10.7164	12.7901	0.0947	0.0028	0.9978	0.9956
2	0.2018	0.4209	1.3988	2.1974	5.1312	7.4195	10.6008	12.6049	0.0938	0.0024	0.9968	0.9936
3	0.1960	0.4446	1.4038	2.1879	5.0820	7.4327	10.3987	12.5873	0.0974	0.0013	0.9961	0.9922
4	0.2013	0.4198	1.3648	2.5074	5.1704	7.4074	9.8439	12.3602	0.1150	0.0003	0.9994	0.9988
5	0.2059	0.4054	1.3263	2.4726	5.1434	7.4197	10.1035	12.7832	0.1150	0.0019	0.9996	0.9992
Mean	0.20234	0.41902	1.36822	2.32422	5.14606	7.40950	10.33266	12.62514				
S.D	0.004279	0.016261	0.033180	0.154045	0.045278	0.024761	0.358789	0.176231				
C.V.%	2.11	3.88	2.43	6.63	0.88	0.33	3.47	1.40				
% Nominal	98.61	102.08	106.66	90.60	100.29	97.47	103.30	100.98				

Table 1.4: Precision and Accuracy

Batch No		LLOQQC	LQC	MQC1	MQC2	HQC
	QC	0.2074	0.6101	1.9066	6.3553	10.7717
1.	1	0.1796	0.5636	1.8561	6.8620	11.6295
	2	0.2076	0.6403	1.8450	6.5324	10.7903
	3	0.1732	0.5716	1.9125	6.3068	11.1879
	4	0.1763	0.5750	1.8439	6.9111	11.5895
	5	0.1966	0.6011	1.8341	6.5313	10.7301
	6	0.1718	0.5776	1.8878	6.3448	10.9627
	Mean	0.18418	0.58820	1.86323	6.58140	11.14833
	S.D	0.014558	0.028454	0.030452	0.254454	0.391224
	C.V.%	7.90	4.84	1.63	3.87	3.51
	% Nominal	88.81	96.41	97.73	103.56	103.50

Table 1.5: Recovery results of Lacidipine

	LQC Response		MQC2 Response		HQC Response	
	Extracted QC	**Un extracted QC**	**Extracted QC**	**Un extracted QC**	**Extracted QC**	**Un extracted QC**
RECQC	**LQC (25-30)**	**LQC (1-6)**	**MQC2 (25-30)**	**MQC2 (1-6)**	**HQC (25-30)**	**HQC(1-6)**
1	19918	22286	203085	230395	340221	383760
2	19500	22247	202068	228547	340295	382581
3	20431	21781	200707	222361	352407	384100
4	19286	22506	213319	230378	345645	383086
5	21335	22128	233685	230795	370148	383120
6	21514	22460	211419	227107	368818	385848
Mean	20330.7	22234.7	210713.8	228263.8	352922.3	383749.2
S.D	934.95	262.44	12393.91	3214.97	13589.66	1160.05
C.V.%	4.60	1.18	5.88	1.41	3.85	0.30
N	6	6	6	6	6	6
% Recovery	91.44		92.31		91.97	

Stability Studies

Table 1.6: Room Temperature Stock Solution Stability of Lacidipine (0,9 Hours)

S.No.	Stock Solution Stability of Lacidipine	
	Area	
	0 hr	**9 hr**
1	268578	239215
2	264523	259754
3	267972	261153
4	266245	262786
5	264985	261266
6	258223	262191
Mean	265087.7	257727.5
S.D	3722.75	9127.82
C.V.%	1.40	3.54
%Stability	97.22	

Conclusion

Based on the results obtained in this study, it is concluded that the present validated method is effectively applicable for the quantification of Lacidipine in human plasma , using carbamazepine as the internal
standard, across the specified
concentration range of 80 to 3200ng/m of Eprosartan, 8.5 to 340ng/ml of Hydrochlorothiazide. Selectivity, precision, accuracy, linearity, and recovery were all met by the LC MS/MS method for determining the presence of Eprosartan and hydrochlorothiazide in human plasma.

Stability assessments were performed in K2EDTA-treated human plasma and for the stock solutions, and stock dilutions satisfied the requirements for acceptance, showing negligible degradation of lacidipine under the given storage conditions and times.

References

1. Bentley and Driver's Text-book of Pharmaceutical Chemistry. 8th ed. New Delhi, India: Oxford University Press, 2008, 9-103.

2. Chaithanya Sudha PD. Pharmaceutical analysis. Noida: Pearson Education in South Asia, 2013, 3-4.
3. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. Vol. I and II. 4th ed. New Delhi, India: CBS Publishers & Distributors, 2005.
4. Jerichorayel. What is the difference between classical and instrumental methods of analytical chemistry? e-Notes, 2016 Jan 9 [cited 2017 Nov 2]. Available from: https://www.enotes.com/homework-help/what-difference-between-classical-instrumental-563161
5. David H. Textbook of modern analytical chemistry. 1st ed. New Delhi: James M. Smith Publisher, 2000, 2.
6. Kenneth A, Connors A. Textbook of pharmaceutical analysis. 3rd ed. New Delhi: John Wiley & Sons, 2009, 109.
7. Guredeep RC, Shyam KA. Textbook of instrumental methods of chemical analysis. 5th ed. New Delhi: Himalaya Publishing House, 2007, 2-567.
8. Ashutosh Kar. Pharmaceutical drug analysis. 3rd rev ed. New Delhi: New Age International (P) Limited Publishers, 2012, 618.
9. Vibha G. Development and validation of HPLC method: a review. Int Res J Pharm App. [Details not provided].
10. Willard HH, Merritt LL, Dean JA. Instrumental methods of analysis. 7th ed. New Delhi: CBS Publishers, 1998, 580-610.
11. Sharma BK. Instrumental methods of chemical analysis. 20th ed. New Delhi: Goel Publishing House, 2001, 54-83.
12. Meyer VR. Practical high-performance liquid chromatography. 2nd ed. London: John Wiley & Sons, 1993, 26-58.
13. Jeffery GH, Bassett J. Vogel's textbook of quantitative chemical analysis. 5th ed. South Asia: Dorling Kindersley Pvt. Ltd, 1991, 217-235.
14. Kazakevich YV. HPLC for pharmaceutical scientists. New York, USA: John Wiley & Sons Inc, 2007, 75-139.
15. Ranjit S. HPLC method development and validation: an overview. J Pharm Educ Res,2013:4(1):26-33.
16. Mohammad TZ. HPLC method development and validation for pharmaceutical analysis: a review. Int Pharm Scien,2012:2(3):1.
17. Snyder RL, Kirkland JJ, Joseph LG. Practical HPLC method development. 2nd ed. USA: John Wiley & Sons Inc, 2011.