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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LETROZOLE AND PALBOCICLIB BY USING RP-HPLC TECHNIQUE

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ABSTRACT: A simple, precise and economical RP-HPLC method have been developed for simultaneous estimation of Letrozole and Palbociclib in bulk form and pharmaceutical formulation. RP-HPLC was developed using Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.column, utilizing a mobile phase of Acetonitrile : Buffer (40:60) (pH-3.4 adjusted with orthophosphoricacid) (with a flow rate of 1.0ml/min) and U.V detection was carried out at 330nm. Optimization of this method was carried out as a function of mobile phase composition, pH and flow rate. RP-HPLC method shows linearity in the range of for 6-14µg/ml and 10-30µg/ml Letrozole and Palbociclib respectively. The LOD and LOQ values for Letrozolewere 0.09µg/ml & 0.29µg/ml and 0.1µg/ml & 0.3µg/ml for Palbociclibrespectively. The results of % purity were found to be 99.78(±0.48) /99.77(±0.12) for HPLC method for Letrozole and Palbociclib respectively. Results of descriptive statistics studies conclude that RP-HPLC method found to be more sensitive and convenient for the estimation of methodLetrozole and Palbociclib in bulk form and pharmaceutical formulation.

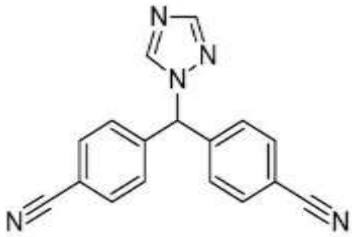
KEYWORDS: Letrozole and Palbociclib, RP-HPLC, Accuracy, Precision, ICH Guidelines.

INTRODUCTION: Combination therapy or poly therapy is therapy that uses more than one medication[1-3]. The Letrozole is indicated to treat postmenopausal women with hormone receptor (HR) positive early breast cancer,Palbociclib is indicated in combination with Letrozole as initial endocrine-based therapy for the treatment of human epidermal growth factor receptor type 2 (HER2)-negative and hormone receptor (HR)-positive tumors in adult patients with advanced/metastatic breast cancer. It is as well approved in combination with fulvestrant in patients with disease progression with prior endocrine therapy.This method was validated according to ICH guidelines for specificity, LOD, LOQ, Precision, Accuracy, and Linearity[4-7]. The method showed good reproducibility and recovery with %RSD less than 2. Hence we had made an attempt to develop a simple accurate and precise RP HPLC method for the simultaneous estimation of Tamoxifen and Palbociclib in bulk and in tablet dosage form[8-11].

DRUG PROFILE[12-15]:

Name: Letrozole:

Structure:

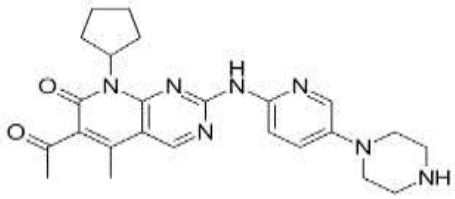


IUPAC Name:4-[(4-cyanophenyl)-(1, 2, 4-triazol-1-yl) methyl]benzonitrile.

Indication: Letrozole is indicated to treat postmenopausal women with hormone receptor (HR) positive early breast cancer.

Name: Palbociclib:

Structure:



IUPAC Name:6-acetyl-8-cyclopentyl-5-methyl-2-[(5-piperazin-1-ylpyridin-2-yl)amino]pyrido[2,3-d]pyrimidin-7-one.

Indication: Palbociclib is indicated in combination with Letrozole as initial endocrine-based therapy for the treatment of human epidermal growth factor receptor type 2 (HER2)-negative and hormone receptor (HR)-positive tumors in adult patients with advanced/metastatic breast cancer.

MATERIALS AND METHODS[16-20]:

Drug samples:

Pharmaceutically pure sample ofLetrozole and Palbociclib drug was obtained fromSd fine-Chem ltd; Mumbai. Commercial tablet of Letrozole and Palbociclib (100mg) was procured from the local drug market. Acetonitrile and water, methanol, orthophosphoricacid acid, KH_2PO_4, K_2HPO_4 were HPLC from merckKGaA, 64271 Darmstadt, Germany.

Instruments:

Analytical HPLC –auto sampler-UV detector separation model 2695, UV detector 2487 Empower- software version-2, UV double beam spectrometer UV 3000+, Digital weighing balance(sensitivity 5 mg), PH meter, Sonicator.

Preparation of the Letrozole and Palbociclib standard solution:

Preparation of standard solution of Letrozole:

Weighed accurately 10mg of standard Letrozole and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve in 100ml of volumetric flask. The final volume was made up to the mark with same solvent. The final solution contained about 100 µg/ml of Letrozole

Preparation of standard solution of Palbociclib:

First 10 mg of Palbociclib was weighed accurately and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve it in mobile phase. The final volume was made up to the mark with same solvent. The final solution contained about 100 µg/ml of Palbociclib.

Initialization of the instrument:

The HPLC instrument was switched on. First the column was washed with the HPLC grade water for 45 minutes. After washing the column that the column is saturated with the mobile phase in 45 minutes. The mobile phase was run to find the peaks or identification of peaks. After 20 minutes the standard drug solution was prepared and injected in HPLC system.

Optimized Chromatographic conditions:

Mobile phase	Acetonitrile : Buffer pH-3.4 with OPA (40:60)
Wavelength	330 nm
Flow rate	1.0 ml/ min.
Auto Sampler Temperature	Ambient
Injection Volume	20µl
Run time	6 min.
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm
Column Temperature	Ambient

Chromatographic trial for simultaneous estimation of Letrozole and Palbociclibby RP-HPLC

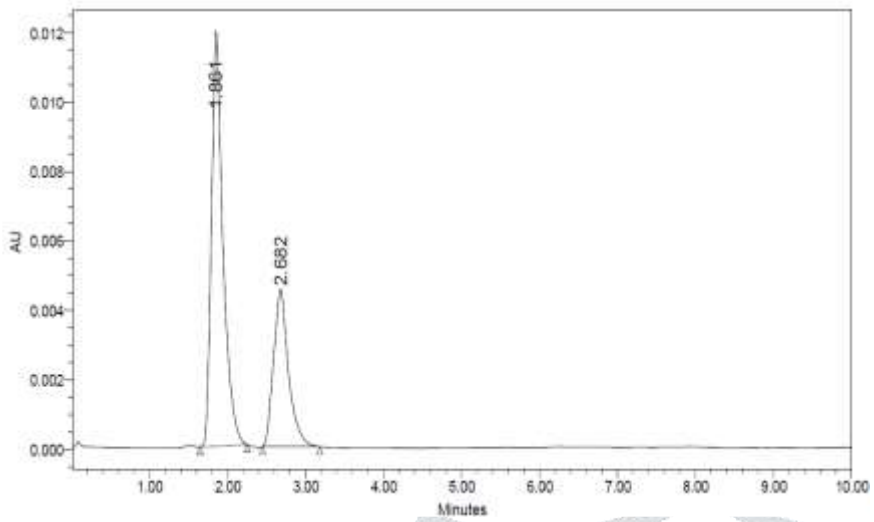


Fig. No.1:The chromatogram obtained after optimized condition Letrozole (1.861 min) and Palbociclib (2.682 min)

RESULTS AND DISCUSSION:

Method Development:

Determination of wavelength of maximum absorbance for of Letrozole:

Standard of Letrozolesolution (1ml) was transferred to separate 10 ml volumetric flask. The final volume was adjusted to 10 ml with the same mobile phase. The absorbance of the final resulted solution was scanned in the range 400 to 220 nm against mobile phase as blank.

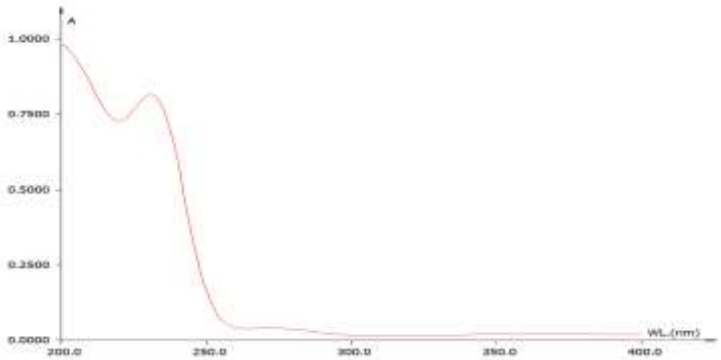


Fig. No.2:UV Spectrum ofLetrozole (292 nm)

Determination of Maximum wavelength for Palbociclib:

First of all take 1ml of standard Palbociclib solution from the above standard solution (1 ml) was transferred to separate clean and dry of 10 ml volumetric flask. The final volume was adjusted to 10ml with same mobile phase (Solvent). The absorbance of the final resulted solution was scanned in the range 400 to 220 nm against solvent mixture as blank.

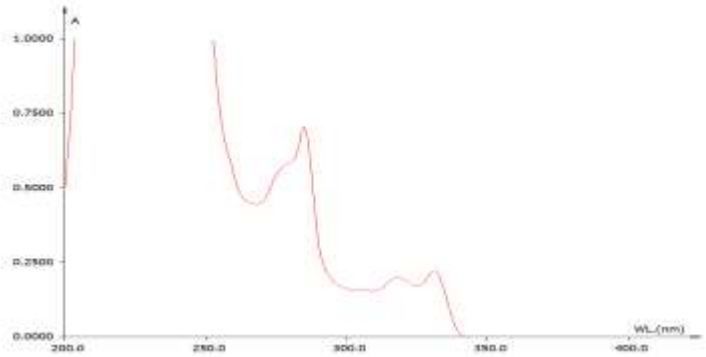


Fig. No.3:UV Spectrum of Palbociclib (342 nm)

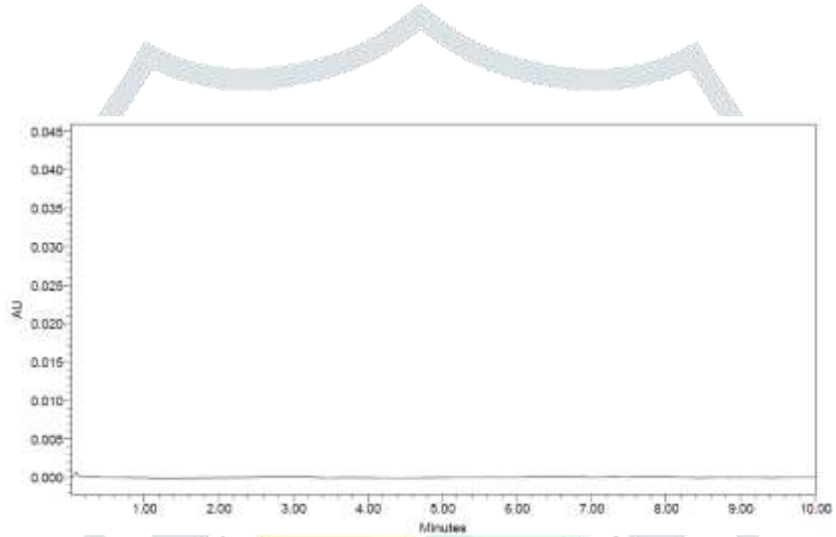


Fig. No.4: Chromatogram of baseline (Mobile Phase Preparation)

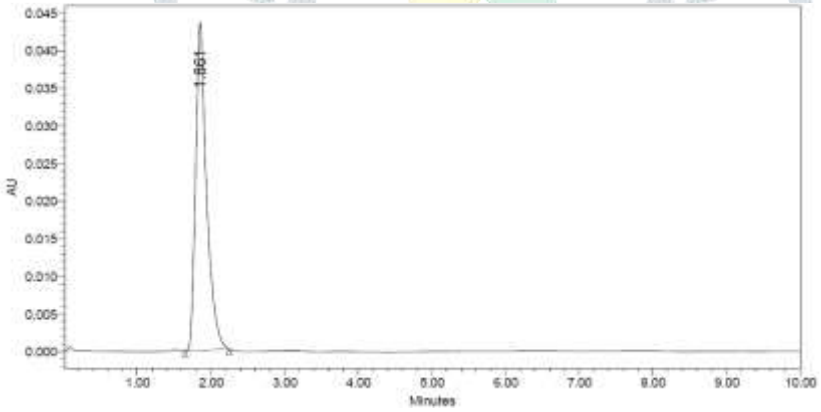


Fig. No.5: Chromatogram showing standard injectionLetrozole

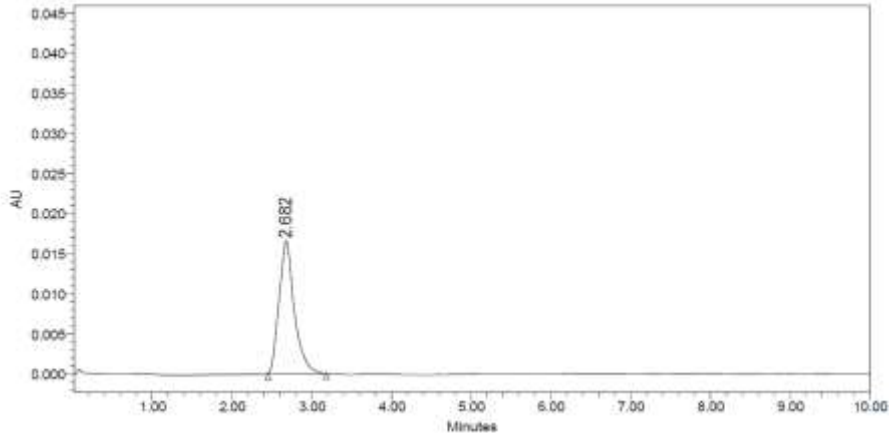


Fig. No.6: Chromatogram showing standard injectionPalbociclib

Accuracy:

Table No.1.Showing Accuracy Results for Letrozole:

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.039	348673	100.487	Mean= 100.633% S.D. = 0.182066 % R.S.D.= 0.180921
S_2 : 80 %	8	8.046	348945	100.575	
S_3 : 80 %	8	8.067	349745	100.837	
S_1 : 100 %	10	9.862	419823	98.62	Mean= 99.95% S.D. = 1.340112% R.S.D.= 1.340782
S_2 : 100 %	10	9.993	424941	99.93	
S_3 : 100 %	10	10.130	430295	101.3	
S_7 : 120 %	12	12.115	507788	100.958	Mean= 100.9717% S.D. = 0.512637 % R.S.D.= 0.507703
S_8 : 120 %	12	12.179	510262	101.491	
S_9 : 120 %	12	12.056	505468	100.466	

Table No.2.ShowingAccuracy Results for Palbociclib:

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	16	15.991	989572	99.943	Mean= 100.1577% S.D. = 0.654939 % R.S.D.= 0.653908
S_2 : 80 %	16	16.143	998756	100.893	
S_3 : 80 %	16	15.942	986589	99.637	
S_4 : 100 %	20	19.995	1231734	99.975	Mean= 100.795% S.D. = 0.822511% R.S.D.= 0.816024
S_5 : 100 %	20	20.158	1241569	100.79	
S_6 : 100 %	20	20.325	1251694	101.62	
S_7 : 120 %	24	24.335	1494218	101.395	Mean= 100.805% S.D. = 0.613739 % R.S.D.= 0.608837
S_8 : 120 %	24	24.204	1486312	100.85	
S_9 : 120 %	24	24.041	1476398	100.170	

The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision:

Repeatability:

The precision of each method was achieved separately from the peak areas obtained by actual estimation of 5 injections of fixed homogenous sample concentrations of Letrozole and Palbociclib. The % relative standard deviation for the Letrozole and Palbociclib was calculated.

Table No.3.Showing % RSD Results for Letrozole and Palbociclib:

Concentration of Letrozoleand Palbociclib in ppm	Rt of Letrozole	Peak area of Letrozole	Rt of Palbociclib	Peak area of Palbociclib
10 +10	2.264	3303800	3.132	951802
10 +10	2.246	3349883	3.132	958267
10 +10	2.264	3353514	3.129	954481
10 +10	2.246	3384162	3.113	952151
10 +10	2.280	3390496	3.113	952308
AVG	2.26	3356371	3.1238	953801.8
S.D.	0.014353	34463.10324	0.009935	2709.017
% RSD		**1.026796598**		**0.284023**

The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Letrozole and 10 µg/ml for Palbociclib (n =5) showed a %RSD of 1.026796598 for Letrozoleand 0.284023 forPalbociclib. It was concluded that the analytical technique showed good repeatability.

Linearity and Range:

Linearity range was found to be 0-14 µg/ml for Letrozole and 0-30 µg/ml for Palbociclib. The correlation coefficients were found to be 0.999 & 0.999, the slopes were found to be 39036 & 60481 and intercept were found to be 34828 & 22371 for Letrozoleand Palbociclib respectively.

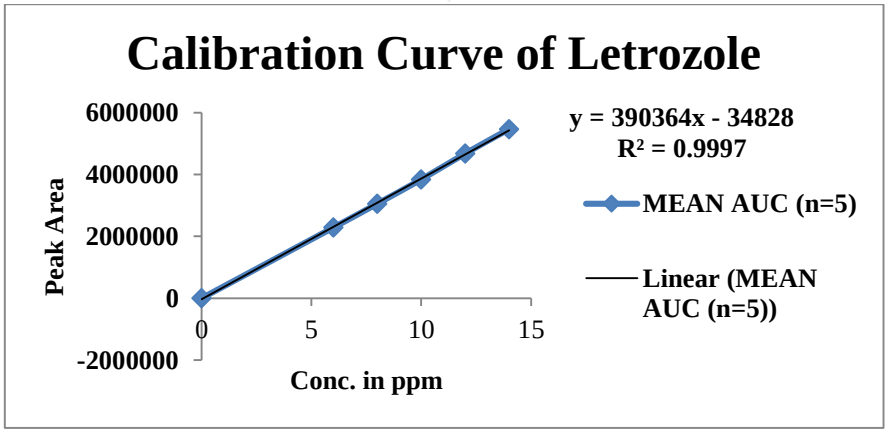


Fig. No.7: Standard curve for Letrozole

Table No.4.Showing Standard curve for Letrozole:

CONC.(µg/ml)	MEAN AUC (n=5)
0	0
6	2281962
8	3053421
10	3837632
12	4673649
14	5462556

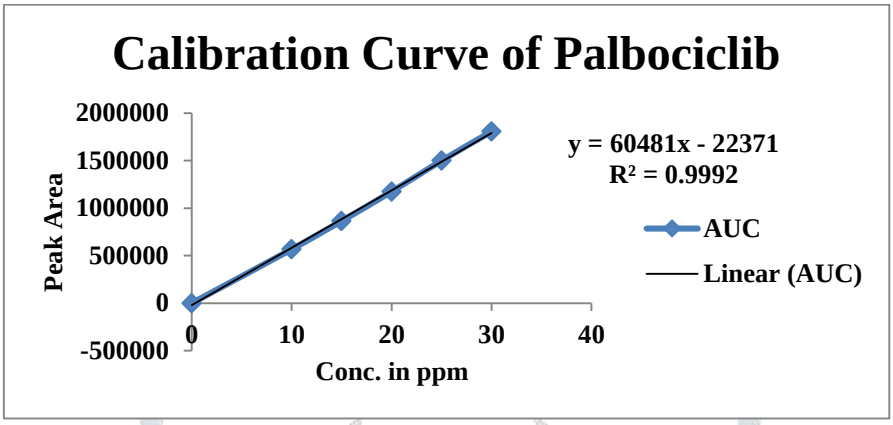


Fig. No.8: Standard curve for Palbociclib

Table No.5.Showing Standard curve for Palbociclib:

CONC.(µg/ml)	AUC MEAN AUC (n=5)
0	0
10	567458
15	865310
20	1174123
25	1500209
30	1806775

Limit of detection (LOD) & Limit of quantification (LOQ):

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The Minimum concentration level at which ananalyse can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.29 µg/ml respectively for Letrozole.

The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Palbociclib which represents that sensitivity of the method is high.

Assay:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where;

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

Table No.6.ShowingAssay of LetrozoleandPalbociclibTablets

Brand name of tablets	Labelled amount of Drug (mg) Letrozole&Palbociclib	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
LOTENSYL-AT tab	10/50	9.78 (±0.08) /49.22 (±0.05)	99.78(±0.48) /99.77(±0.12)

The assay of Lotensyl-Attablets containing Letrozole was found to be 9.78 (±0.08) and Palbociclib was found to be 49.22 (±0.05) and the % purity of the LetrozoleandPalbociclib was found to be 99.78(±0.48) /99.77(±0.12).

SUMMARY AND CONCLUSION:

The developed method was successfully applied for simultaneous estimation of Letrozoleand Palbociclib in compound tablet formulation.The proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Letrozole and PalbociclibAPI.

ACKNOWLEDGEMENT:

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REFERENCES:

1.Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
2.Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
3.Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.

4.Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology (2003), 5, PP 110-114.
5.Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
6.ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).
7.Skoog D.A., Holler F.J., Nieman T.A.: Principles of Instrumental Analysis 5th edition (2005), PP 733-738.
8.Beckett HA and Stenlake BJ: Practical Pharmaceutical chemistry. CBS Publishers. New Delhi; 4th edition (2003), PP 168-9.
9.British Pharmacopoeia: Convention Inc., Rockville, 2008.Vol.I; 965-966.
10. Sharma BK: Instrumental methods of chemical analysis. Goel Publishers; Meerut, 3rd edition (2005), PP 861-76.
11. Gennaro, A.R., Remington. The science and practice of pharmacy, 28th edition, Luppincott, Williams and Wilkins, Baltimore, Maryland, USA (2000),PP 534 - 549.
12. Ghulam A. Shabir: HPLC Method Development and Validation for Pharmaceutical Analysis (2004), PP 25-27.
13. International Conference on Harmonization: ICH Q 2 (R1) Validation of Analytical Procedure, Text and Methodology 1995.
14. L. R. Snyder, J.J. Kirkland, and J. L. Glajch, Practical HPLC Method Development, John Wiley & Sons, New York, 1997.
15. S.Ahuja and H. T. Rasmussen (ed), HPLC Method Development for Pharmaceuticals, Academic Press, 2007.
16. S.Ahuja and M.W. Dong (ed), Handbook of Pharmaceutical Analysis by HPLC, Elsevier/Academic Press, 2005.
17. Y. V. Kazakevich and R. Lo Brutto (ed.), HPLC for Pharmaceutical Scientists, Wiley, 2007.
18. U. D. Neue, HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, New York, 1997.
19. M. C. McMaster, HPLC, a practical user's guide, Wiley, 2007.
20. Michael, D.R., Stephen, P.H.A., & David, A.K., Pharmacology, Pharmaceutical Press, London, Chicago 2009.