International Journal of Multidisciplinary Research and Growth Evaluation.

RP-HPLC method development and validation for the estimation of Avapritinib in bulk form and marketed pharmaceutical dosage form

R. Sridhar Babu [1], Syed Hafeez Ahmed [2*], Shaik Mohd Khasim [3], Syeda Nusrath Fatima [4], Qayam Ali Khan [5], Lubna Hashmi [6], Hajira Samreen [7], Muneeza Fatima [8]

[1, 4-8] Department of Pharmaceutics, Shadan College of Pharmacy, Peerancheru, Hyderabad, Telangana, India

[2] Department of Pharmaceutical Analysis, Shadan College of Pharmacy, Peerancheru, Hyderabad, Telangana, India

[3] Director & Professor Department of Pharmacology, Shadan College of Pharmacy, Peerancheru, Hyderabad, Telangana, India

* Corresponding Author: **Syed Hafeez Ahmed**

Article Info

ISSN (online): 2582-7138
Impact Factor: 5.307 (SJIF)
Volume: 05
Issue: 01
January-February 2024
Received: 23-10-2023;
Accepted: 26-11-2023
Page No: 161-167

Abstract

The objective of the present study was to develop and validate a novel RP-HPLC method for the determination of Avapritinib in the pharmaceutical dosage form. Chromatographic separation was conducted on HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters), Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm and equipped with ultra-violet detector. The mobile phase consisted of Acetonitrile, Methanol and 0.1% Ortho Phosphoric acid mixed in the ratio of 60:30:10 v/v, was used at a flow rate of 1.0 ml/min, and the detection wavelength was set at 235 nm. The retention time for Avapritinib was found to be 2.597 min. The calibration was linear (r2 = 0.999) in the concentration range of 6-14 µg/ml. The limit of detection and the limit of quantitation were found to be 0.8µg/ml and 0.24µg/ml, respectively. The % Recovery of Avapritinib in tablet formulation was observed in the range of 98-102%. Percentage assay of Avapritinib (Ayvakit) was found to be 99.89% w/w. Thus the novel proposed method for Avapritinib was found to be feasible for the estimation of Avapritinib in bulk as well as the marketed pharmaceutical dosage form.

Keywords: Avapritinib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines

Introduction

Avapritinib is an orally bioavailable inhibitor of specific mutated forms of platelet-derived growth factor receptor alpha (PDGFR alpha; PDGFRa) and mast/stem cell factor receptor c-Kit (SCFR), with potential antineoplastic activity. Upon oral administration, Avapritinib [1] specifically binds to and inhibits specific mutant forms of PDGFRa and c-Kit, including the PDGFRa D842V mutant and various KIT exon 17 mutants. This results in the inhibition of PDGFRa- and c-Kit-mediated signal transduction pathways and the inhibition of proliferation in tumor cells that express these PDGFRa and c-Kit mutants. PDGFRa and c-Kit, protein tyrosine kinases and tumor-associated antigens (TAAs), are mutated in various tumor cell types; they play key roles in the regulation of cellular proliferation. Avapritinib, or BLU-285, is a selective tyrosine kinase inhibitor of KIT and platelet derived growth factor receptor alpha indicated for the treatment of unresectable, metastatic gastrointestinal stromal tumors. It is one of the first medications available for the treatment of multidrug resistant cancers. Avapritinib shares a similar mechanism with [Ripretinib]. Avapritinib [2] was granted FDA approval on 9 January 2020.Avapritinib is a Kinase Inhibitor. The mechanism of action of Avapritinib is as a Tyrosine Kinase Inhibitor, and Cytochrome P450 2C9 Inhibitor, and P-Glycoprotein Inhibitor, and Breast Cancer Resistance Protein Inhibitor, and Multidrug and Toxin Extrusion Transporter 1 Inhibitor, and Multidrug and Toxin Extrusion Transporter 2 K Inhibitor, and Bile Salt Export Pump Inhibitor. The IUPAC Name of Avapritinib [3] is (1S)-1-(4-fluorophenyl)-1-[2-[4-[6-(1-methylpyrazol-4-yl)pyrrolo[2,1-f][1,2,4]triazin-4-yl]piperazin-1-yl]pyrimidin-5-yl]ethanamine. The Chemical Structure of Avapritinib is as following

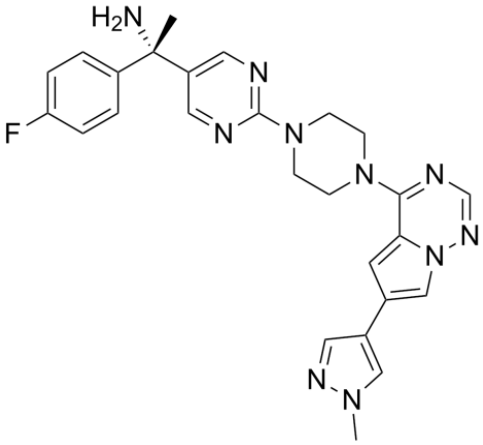

Fig 1: Chemical Structure of Avapritinib

As per the literature review [31-32], Avapritinib was estimated individually by few methods like simple HPLC, Ultra HPLC and HPLC-MS method validation of Avapritinib. The objective of the work is to develop RP-HPLC method for estimation of Avapritinib in tablet dosage form with simple, rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines [30].

Materials and Methods

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table 2: List of Chemicals used

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
8.	Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

Method development and its validation for avapritinib By RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis [4]. Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Avapritinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Avapritinib. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Avapritinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions: The chromatographic conditions[5] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 40 : 60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 55 : 45	1.0ml/min	235nm	Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile : Water = 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Preparation of Mobile Phase

600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration [6].

Results and Discussion

Method Development

Selection of Wavelength

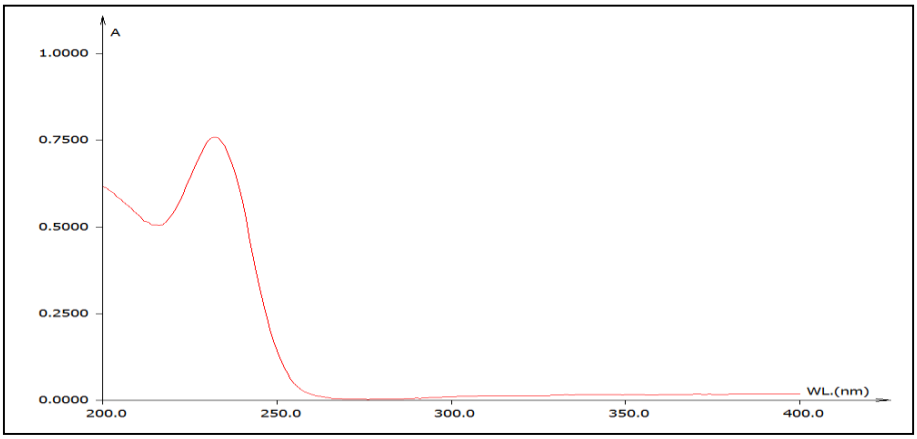

Fig 2: UV spectrum for Avapritinib

Observation: While scanning the Avapritinib solution we observed the maxima at 235nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table 4: Summary of optimised Chromatographic conditions

Mobile phase	ACN : Methanol: 0.1% OPA = 60:30:10
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	2.597 minutes

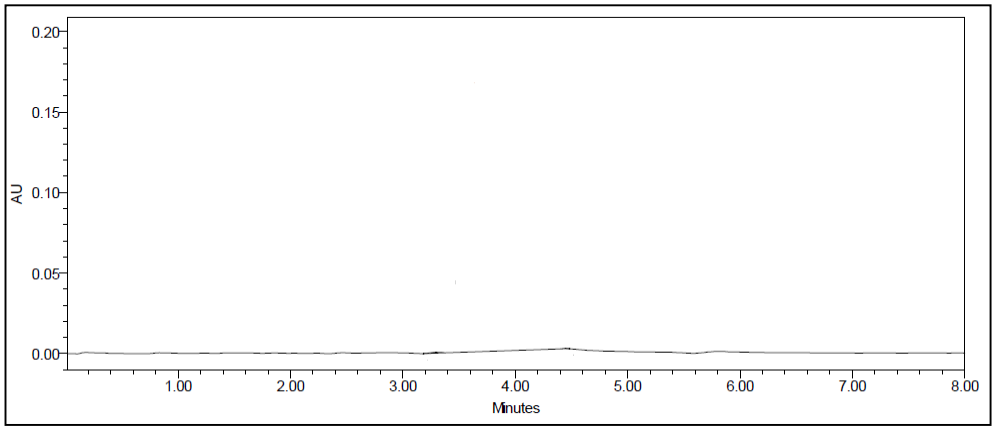

Fig 3: Chromatogram for Blank Solution

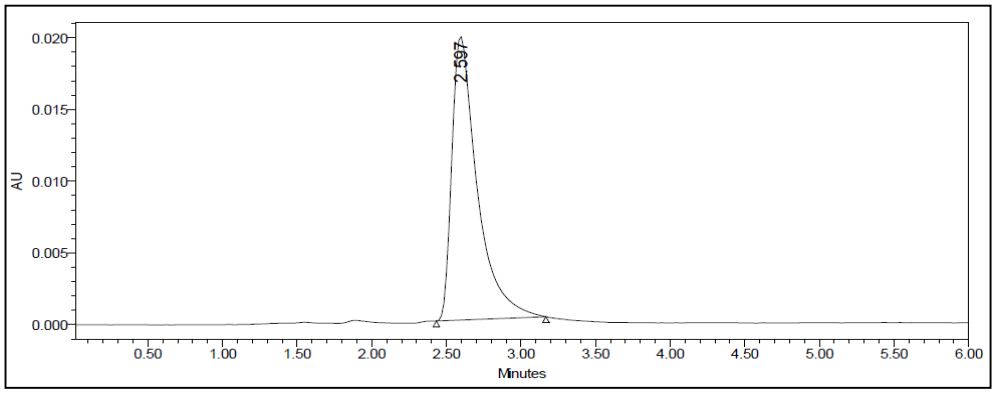

Fig 4: Chromatogram of Avapritinib in Optimized Condition

Final Result & Discussion: The selected and optimized mobile phase[7] was ACN: Methanol: 0.1% OPA = 60:30:10 and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 06 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry [8]. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Validation of Analytical Method

1. Accuracy

Recovery study

To decide the exactness [9] of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication of AVAPRITINIB were taken and added to the pre-broke down plan of fixation 10µg/ml. From that rate recuperation[10] esteems were computed. The outcomes were appeared in table-5.

Table 5: Readings of Accuracy

Conc. In ppm	Conc. Found	Peak Area		% Recovery
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S.D	0.775
			%RSD	0.767046
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S.D	0.628835
			%RSD	0.628688
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S.D	0.987021
			%RSD	0.979894

2. Precision

2.1. Repeatability

The precision [11] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Avapritinib (API). The percent relative standard deviation [12] was calculated for Avapritinib are presented in the table-6.

Table 6: Readings of Repeatability

HPLC Injection Replicates of Avapritinib	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

2.2. Intermediate Precision

2.2.1. Intra-assay & inter-assay

The intra & inter day variation[13] of the method was carried out & the high values of mean assay & low values of standard deviation[14]& % RSD (% RSD < 2%) within a day & day to day variations for Avapritinib revealed that the proposed method is precise.

Table 7: Results of Intra-Assay & Inter-Assay

Conc. Of Avapritinib(API) (µg/ml)	Observed Conc. of Avapritinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.46	0.62	8.05	0.96
10	10.87	0.85	9.43	0.71
12	11.81	0.92	12.04	0.65

3. Linearity & Range

The calibration curve [15] showed good linearity [16] in the range of 6 – 14 µg/ml, for Avapritinib (API) with correlation coefficient [17] (r^2) of 0.999 (Fig-5). A typical calibration curve has the regression equation [18] of y = 19423x + 5444 for Avapritinib.

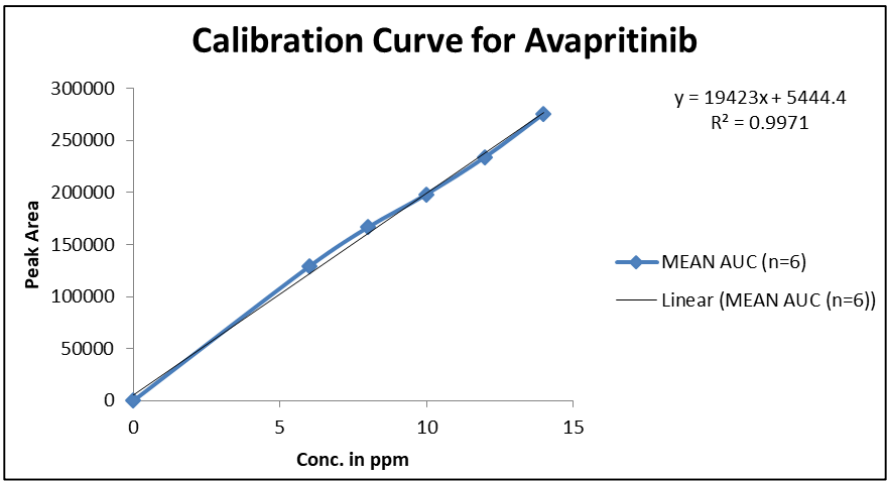

Fig 5: Calibration Curve of Avapritinib (API)

Table 8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

4. Method Robustness: Influence of small changes in chromatographic conditions [19] such as change in flow rate [20] (± 0.1ml/min), Wavelength of detection (±2nm) &organic phase in mobile phase (±5%) studied to determine the robustness [21] of the method are also in favour of (Table-9, % RSD < 2%) the developed RP-HPLC method [22] for the analysis of Avapritinib (API).

Table 9: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
More Organic	0.54
Less Organic	0.63
Wavelength of Detection (237 nm)	0.91
Wavelength of detection (233 nm)	0.93

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected [23] (LOD) & quantified [24] (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter

Framework appropriateness [25] testing is an essential piece of numerous scientific techniques. The tests depend on the idea that the gear, hardware, explanatory activities and tests to be broke down establish a vital framework that can be assessed all things considered. Following framework appropriateness test parameters [26] were built up. The information is appeared in Table-10.

Table 10: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.47
2	Asymmetry	T ≤ 2	Avapritinib=0.23
3	Theoretical plate	N > 2000	Avapritinib=2987
4	Tailing Factor	T<2	Avapritinib=1.17

7. Estimation of Avapritinib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume [27] was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was additionally infused into the HPLC framework [28] and the peak regions were recorded. The information is appeared in Table-11.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of medication acquired with test arrangement

AS = Peak Area of medication acquired with standard arrangement

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table 11: Recovery Data for estimation Avapritinib in Ayvakit Tablets

Brand Name of Avapritinib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
AyvakitTablets (Blueprint Medicines)	100mg	99.885 (±0.875)	99.89 (±0.452)

Result & Discussion

The amount of drug in Ayvakit Tablets was found to be 99.885 (±0.875)mg/tab for Avapritinib & % assay [29] was 99.89 %.

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Avapritinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mmx4.6mm i.d. Column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be soluble in water and DMSO, very soluble in acetonitrile and methanol. Utilizing these solvents with suitable arrangement more current techniques can be created and approved. Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Avapritinib it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Avapritinib in various details.

References

1. https://go.drugbank.com/drugs/DB15233
2. https://pubchem.ncbi.nlm.nih.gov/compound/Avapritinib

3. https://en.wikipedia.org/wiki/Avapritinib
4. Morgan, David J., "Fraction collector (post on Flickr)". Flickr. Retrieved, 2015.
5. Karger, Barry L."HPLC: Early and Recent Perspectives". Journal of Chemical Education. 74: 45. Bibcode: 1997JChEd...74...45K, 1997.
6. Henry Richard A. The Early Days of HPLC at Dupont". Chromatography Online. Avanstar Communications Inc., 2009.
7. Iler RK. The Chemistry of Silica. John Wiley & Sons. New York, 1979.
8. Karger BL, Berry LV. Rapid liquid-chromatographic separation of steroids on columns heavily loaded with stationary phase. Clin. Chem. 1971; 17(8):757-64.
9. Giddings J. Calvin, Dynamics of Chromatography, Part I. Principles and Theory. Marcel Dekker, Inc., New York, 1965, 281.
10. Ettre C. Milestones in Chromatography: The Birth of Partition Chromatography (PDF). LCGC. 2001-2016; 19(5):506-512.
11. Martin, A J P; Synge, R L M,"Separation of the higher monoamino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool". Biochemical Journal. 1941; 35(1-2):91-121.
12. Lindsay S, Kealey D. High performance liquid chromatography. Wiley. from review Hung LB, Parcher JF, Shores JC, Ward EH. Theoretical and experimental foundation for surface-coverage programming in gas–solid chromatography with an adsorbable carrier gas. J. Am. Chem. Soc. 1987-1988; 110(11):1090-1096.
13. Journal of Pharmaceutical and Biomedical Analysis. 1999; 21(2):371-382.
14. Tropical Journal of Pharmaceutical Research, © Pharmacotherapy Group. 2009; 8(5):449-454.
15. Rabi Sankar, Instrumental Method of Analysis, 18-6, 18-3.
16. Lloyd R. Snyder, Practical HPLC Method Development, 2nd edition, P-503.
17. Guidance for industry, Analytical Procedure and Method Validation, U.S. Department of Health and Human Services FDA, 2000.
18. Validation of analytical procedures, methodology, ICH harmonized tripartite guideline, 1996, 108.
19. BK Sharma. Instrumental Methods of Chemical Analysis, 75-78, 113-115.
20. Galen W. Ewing, Instrumental Methods of Chemical Analysis, Vth Ed., 1.
21. Takeru Higuchi, EinarBrochmann, HanffenHanssen, Pharmaceutical Analysis, Ist edition, 1-10.
22. A.H. Beckett, J.B. Stenlake, Practical Pharmaceutical Chemistry, IV edition, Volume II, Pg no: 275-298.
23. Quality Assurance, worth the effort, Inforum. 2003; 7(4).
24. PD. Sethi, Quantitative Analysis of drugs in Pharmaceutical formulation, IIIrd Ed., 1-21, 51-56.
25. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
26. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
27. Development and validation of HPLC method - A Review, Vibha Gupta *et al*, International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4):17-25.
28. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj **et al*. International Journal of Analytical and Bioanalytical Chemistry, accepted, 2015.
29. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
30. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
31. Thakur Gajender Singh, Jagadeesh Babu Vadlakonda, V Rama Mohan Gupta, Analytical Method Development and Validation of Avapritinib by RP-HPLC Method, World Journal of Pharmacy and Pharmaceutical Sciences. 9(10):1834-1840.
32. Gowri Kusuma Kumari, Dr. Kantipudi Rambabu, Bio-analytical Method Development and Validation for Avapritinib in Rat Plasma by LC-MS/MS, /J. Pharm. Sci. & Res. 2021; 13(3):134-137.