CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.10029836

https://www.iajps.com/volumes/volume10-september-2023/44-issue-09-september-23

Available online at: http://www.iajps.com ***Research Article***

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IFOSFAMIDE IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM

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Article Received: July 2023 Accepted: August 2023 Published: September 2023

Abstract:

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Ifosfamide in pure form and pharmaceutical dosage form. Separation of Ifosfamide was successfully achieved on a Symmetry ODS C18 (4.6 x 150mm, 5µm) column in an isocratic mode utilizing ACN: Methanol in the ratio of 60:40% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 251nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 10-50mcg/mL for Ifosfamide. The correlation coefficient was found to be 0.999 for Ifosfamide. The LOD and LOQ for Ifosfamide were found to be 1.3µg/mL and 4.0µg/mL respectively. The proposed method was found to be good percentage recovery for Ifosfamide, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: *Ifosfamide, RP-HPLC, Accuracy, ICH Guidelines.*

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QR code

Please cite this article in press ***Dhobba. Prathima*** *et al,* ***Rp-Hplc Method Development And Validation For The Estimation Of Ifosfamide In Pure Form And Pharmaceutical Dosage Form,*** *Indo Am. J. P. Sci, 2023; 10 (09).*

INTRODUCTION:

Analytical chemistry is a branch of chemistry that determines the nature and identity of a substance and its composition. In the early twentieth century there were only four accepted branches of chemistry, organic chemistry, inorganic chemistry, physical chemistry, biochemistry. At that time, analysis was considered to be a service to the other four branches. Its importance grew and in the process, absorbed techniques and skills from all other four branches. So by the 1950's, analytical chemistry was finally accepted as a branch of chemistry in its own right. There are basically two types of analysis, qualitative analysis and quantitative analysis. The former identifies the nature of substance and if it is mixture, the nature of the components present, where as the latter determines the elemental composition of the substance and/ or the quantitative distribution of each component.

Pharmaceutical analysis deals with the analysis of a pharmaceutical(s) substance. It is generally known that pharmaceutical is a chemical entity of therapeutic interest. A more appropriate term for pharmaceutical is active pharmaceutical ingredient (API) or active ingredient.

Pharmaceutical analysts in research and development(R&D) of Pharma industry plays a very comprehensive role in new drug development and follow up activities to assure that, a new drug product meets the established standards, its stability and continued to meet the purported quality throughout its shelf-life.

The different activity of R&D includes drug development (synthesis and manufacture) formulation, clinical trials, evaluation and finally launching i.e. finished products. Closely associated with these processes are regulatory and quality assurance functions.

Before submitting the drug product for approval to the regulatory authorities, assuring that all batches of drug products comply with specific standards, utilization of approved ingredients and production methods. It becomes the responsibility of pharmaceutical analysts in quality control (QC), quality assurance (QA) department. The methods are generally developed in an analytical R & D and transferred to QC or other department.

Quality Assurance and Quality Control plays a central role in determining the safety and efficacy of medicines. A highly specific and sensitive analytical technique holds the key to design, development, standardization and quality control of medicinal products. [1,2]

Modern pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. There are physicochemical properties of pharmaceutical compounds through the use of advanced instrumental methods. There is a need for quality assurance of pharmaceutical products throughout their shelf life. This requires that interactions of the drug substances with the excipients in the presence of residual solvents, as well as other potential degradation reactions that may occur in the formulated product.

The pharmaceutical industry is under increased scrutiny from the government and public interest groups to contain costs and yet consistently deliver to market safe, efficacious products that fulfill unmet medical needs. The industry has streamlined its operations with respect to drug discovery, development and manufacturing.

Traditionally viewed as a service organization, the analytical department has become a significant partner in the drug development process. Analytical data has become a critical path activity for the selection of candidate molecules for full development. Working under sample-limited conditions and in full compliances of current good manufacturing practice (cGMP), Pharmaceutical analysts are called on to generate accurate and precise data – almost on demand.

Novel delivery systems pose special analytical challenges. The commonly used tests of pharmaceutical analysis generally entail compendial testing; these methods, method development, setting specifications, and method validation. The drug product remains within specifications established to ensure its identity, strength, quality and purity. It is necessary to conduct stability studies to predict, evaluate and ensure drug product safety.

Analytical data are the foundation and backbone for pharmaceutical development, leading to approval and production of new drugs for market. Potential areas in pharmaceutical analysis are highlighted, based on the successful demonstration made with analysis of proteins, peptides, DNA, and small molecules including chiral separations.

CHROMATOGRAPHY:

Russian botanist Michael Tswett invented chromatography as a separation technique. He described in detail the separation of pigments, the colored substances by percolation through the column, followed by development with pure solvents. The first paper of Tswett, was published in 1903, contains a study of more than 100 absorbents used in conjunction with several different solvents.

Types of Chromatography:

Chromatography characterized as a separation method based on the differential migration of solute through a system of two phases, one is mobile phase another one is stationary phase. Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption, desorption steps on the stationary phase.

Chromatography is mainly divided into two categories:

1. *Adsorption Chromatography:*

Separation is mainly due to the interaction between solute and surface on the adsorbent. In this, stationary phase is solid and mobile phase is liquid. e.g: TLC and HPTLC

2. *Partition Chromatography:*

Separation is based on the partition between two phases. In this mode, both stationary phase and mobile phase are liquids

e.g: HPLC, GLC, and PC.

High performance liquid chromatography (HPLC):

It is analytical chromatographic technique that is useful for separation of ions or molecules that are dissolved in a solvent.

TYPES OF HPLC:

Normal phase chromatography:

Normal phase chromatography is chromatographic technique that uses organic solvents for mobile phase and a polar stationary phase. Here, the less polar compound elutes faster than the more polar compound.

Reverse phase chromatography:

Reverse phase chromatography - a bonded phase chromatography technique, uses water as base solvent. Separation is based on solvent strength and selectivity. Separation is also affected by column temperature and pH. In general, the more polar compounds elute faster than the less polar compounds. UV detection is the most common detection technique used.

Mechanism:

The separation mechanism in reverse phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. Reverse phase chromatography is an adsorptive process by experimental design, which relies on a partitioning mechanism to effect separation. The solute molecules partition (i.e. an equilibrium is established) between the mobile phase and the stationary phase.

Stationary phase:

The most popular column is a octadecyl carbon chain (C18) bonded silica. This is followed by C8 bonded silica, pure silica, cyano bonded silica and phenyl bonded silica. C18, C8 and phenyl are dedicated reversed phase packing while cyano columns can be used in a reverse phase mode depending on analyte and mobile phase conditions.

INSTRUMENTATION:

The essential parts of apparatus for the High Performance Liquid Chromatography are:

- Solvent reservior
- Mobile phase
- Pump system
- Sample Injection System
- Column
- Detector

Solvent reservoir:

A modern HPLC apparatus is equipped with one or more glass or stainless steel reservoirs. The reservoir is often equipped with an online degasser which removes the dissolved gasses usually oxygen and nitrogen, which interfere by forming bubbles. Degasser may consist of vacuum pumping system, distillation system, system devices for heating, and solvent stirrer.

Mobile phase:

One of the greatest advantages of HPLC is versatility afforded by liquid mobile phase. Sufficient solubility of solute molecules in the mobile phase must be ensured in order to prevent precipitation.

For the mobile phase, first variable to be decided is whether an organic or aqueous eluent should be used. With RP-HPLC analysis, either an aqueous eluent or variety of organic solvents such as methanol or acetonitrile is tried first. If the k values are too large with an aqueous solvent, then the separation should be attempted by using mixture, in various proportions. Many simple analyses can be carried out with isocratic elution using an aqueous eluent to which an organic modifier is added. If the sample to be analyzed contains a very complex mixtures or mixture of compounds of diverse structure and retention behavior, then either a ternary mixture of solvents can be used isocratically or gradient elution may be necessary.

Pumping system [11]:

The function of the pump in HPLC is to pass mobile phase through the column at a controlled flow rate. Features of an ideal pumping system include:

- Generating pressure upto 6000 psi.
- Pulse free output.
- Flow rates ranging from 0.1 to 10 ml/min.
- Flow control and reproducibility of 0.5% relative or better.
- Corrosion resistant components.

There are three types of pumps commonly used

- Reciprocating pumps
- Displacement pumps
- Pneumatic pumps

Sample injection system:

The limiting factor in the precision of LC measurements lie in reproducibility with which samples are introduced into the column packing. The earliest and simple means of sample introduction was syringe injection through a self-sealing elastomeric septum. In stop flow injections, the flow of solvent is stopped momentarily, and fitting at column head is removed and the sample is injected directly into the head of column packing. After replacing the fitting the system is again pressurized.

MATERIALS AND METHODS:

Ifosfamide provided by Sura labs, Water and Methanol for HPLC LICHROSOLV (MERCK)m, Acetonitrile for HPLC Merck

Hplc method development:

TRAILS:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Ifosfamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of the above Ifosfamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Methanol (60:40% v/v) respectively.

Optimization of Column: The method was performed with various C18 columns like Symmetry, Zodiac, Xterra. Symmetry ODS C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA 996 detector model.

Temperature	:	Ambient
Column	:	Symmetry ODS C18 (4.6 x 150mm, 5µm)
Mobile phase	:	ACN: Methanol (60:40% v/v)
Flow rate	:	1.0mL/min
Wavelength	:	240 nm
Injection volume	:	10 µl
Run time	:	8 minutes

Method validation

Preparation of mobile phase:

Preparation of mobile phase: Accurately measured 600 ml (60%) of HPLC Acetonitrile and 400 ml of Methanol (40%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Standard

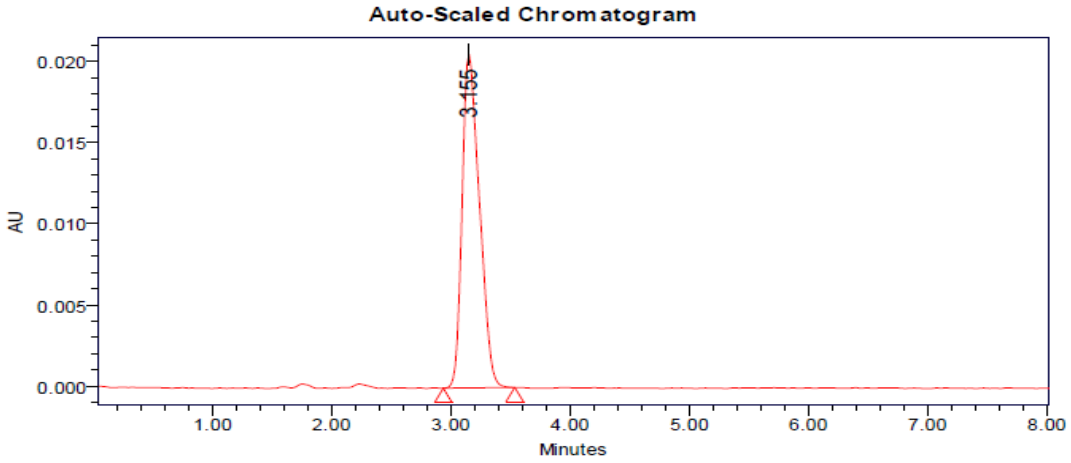

Figure: Optimized Chromatogram

Table: Peak results for Optimized Chromatogram

S.No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Ifosfamide	3.155	225645	20523	1.36	6125

Observation:

This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

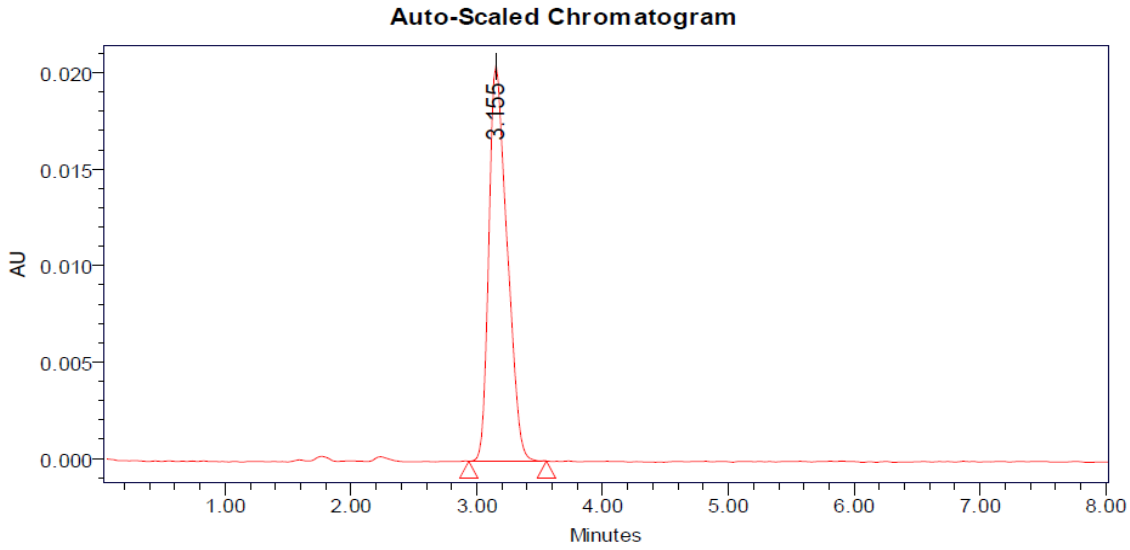

Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.No	Name	Retention time (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Ifosfamide	3.155	225654	20598	1.34	6098

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.

Assay (Standard):

Table: Results of Assay (Standard) for Ifosfamide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ifosfamide	3.146	225685	20568	6125	1.36
2	Ifosfamide	3.123	226584	20653	6132	1.38
3	Ifosfamide	3.192	225687	20548	6129	1.34
4	Ifosfamide	3.164	226548	20698	6187	1.35
5	Ifosfamide	3.181	226874	20548	6159	1.35
Mean			226275.6			
Std. Dev.			552.8682			
% RSD			0.244334			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table : Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ifosfamide	3.170	224596	20469	1.35	6098	1
2	Ifosfamide	3.174	224658	20489	1.34	6108	2
3	Ifosfamide	3.170	224585	20458	1.35	6107	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of sample}}{\text{Label claim}} \times 100$$

The % purity of Ifosfamide in pharmaceutical dosage form was found to be 100.02%.

LINEARITY

Table: Data for Linearity

Concentration µg/ml	Average Peak Area
10	78683
20	146545
30	213584
40	279895
50	346568

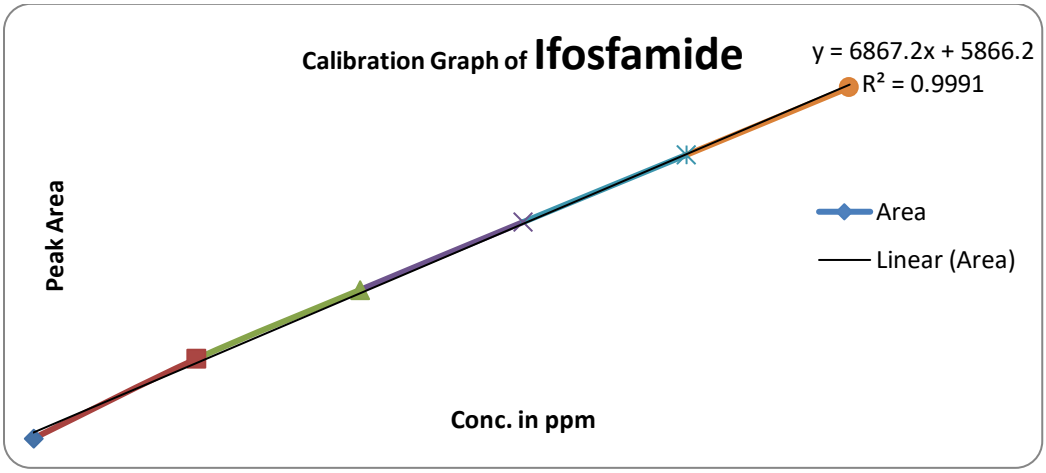

Fig: Calibration Curve of Ifosfamide

REPEATABILITY

Table-19: Results of method precision for Ifosfamide:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ifosfamide	3.165	225645	20562	6125	1.36
2	Ifosfamide	3.163	225847	20645	6129	1.36
3	Ifosfamide	3.158	226542	20534	6135	1.35
4	Ifosfamide	3.167	226598	20564	6189	1.36
5	Ifosfamide	3.171	226584	20549	6138	1.35
Mean			226243.2			
Std.dev			459.9258			
%RSD			0.203288			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table-20: Results of ruggedness for Ifosfamide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ifosfamide	3.165	226534	20653	6235	1.35
2	Ifosfamide	3.163	226542	20598	6198	1.36
3	Ifosfamide	30158	225989	20653	6254	1.36
4	Ifosfamide	3.167	226512	20548	6281	1.35
5	Ifosfamide	3.171	226531	20653	6199	1.36
6	Ifosfamide	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std. Dev.			304.2622			
% RSD			0.13443			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table-21: Results of Intermediate precision Analyst 2 for Ifosfamide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ifosfamide	3.173	225487	20542	6253	1.35
2	Ifosfamide	3.134	225484	20532	6098	1.36
3	Ifosfamide	3.161	225364	20541	6254	1.35
4	Ifosfamide	3.174	226513	20534	6235	1.36
5	Ifosfamide	3.199	225487	20549	6199	1.36
6	Ifosfamide	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

ACCURACY:

Table: The accuracy results for Ifosfamide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40%	100.42%
100%	212732	30	30.124	100.413%	
150%	316263.3	45	45.201	100.446%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ifosfamide in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Ifosfamide was Very poorly Soluble in water, Very soluble in methanol, ethanol, and acetonitrile. Practically insoluble in soybean oil, mineral oil.

ACN: Methanol (60:40 v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Ifosfamide in bulk drug and in Pharmaceutical dosage forms.

Acknowledgement:

The Authors are thankful to the Management and Principal, Department of Pharmacy, Sree Dattha Institute of Pharmacy, Ibrahimpatnam, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY:

1. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004,P12-23.
2. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
3. John Adamovies, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nd ed, P.74, 5-15.
4. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570
5. D. A. Skoog. J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
6. Skoog, Holler, Nieman. Principals of instrumental analysis 5th ed, Harcourt publishers international company, 2001, P.543-554.
7. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330
8. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
9. Michael E, Schartz IS, Krull. Analytical method development and validation. 2004, P. 25-46.

10. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nd ed, A Wiley international publication, 1997, P.235,266-268,351-353.653-600.686-695.
11. Basic education in analytical chemistry. Analytical science, 2001:17(1).
12. Method validation guidelines international onference on harmonization; GENEVA; 1996
13. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28
14. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.
15. Klaus Florey, Analysis profile of drugs substances, Academic press, New York, 2005, P.406-435.
16. P.N. Arora, P.K. Malhan. Biostatistics, Himalaya publishers house, India, P.113,139-140,154.
17. Doserge, Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry, 8th ed, Lippincott Company, 1982, P.183-197.