International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.13 | Issue 2 | Apr - Jun -2024

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v13.iss2.2024.116-125

Research

A Study On Method Development And Validation For The Estimation Of Anti T.B Drugs In Oral Dosage Form By Rp- Hplc1

Mekala Sunil[1], Ch.Anusha[2], Ch.Teja Bhagyaraju[2], G.Triveni[2], I.Anusha[2], K.Thiruala[2] Prasanna Reddy[2], M.Neelima[2]

[1]Professor, Vishwa Bharathi college of Pharmaceutical Sciences, Perecherla, Guntur, Andhra Pradesh, India.
[2]Scholar, Vishwa Bharathi college of Pharmaceutical Sciences, Perecherla, Guntur, Andhra Pradesh, India.

*Author for Correspondence: Dr. Mekala Sunil
Email: drsunilmekala@gmail.com

Published on: 23 Feb 2024

Published by:
DrSriram Publications

Abstract

The pharmaceutical analysis defined as "the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of a given sample of a medicine, the detection and estimation of impurities, which may be present in drug substance (or) given sample of medicine". Chronological order of the events that are the most notable in the development of the present state of the field. Since the various types of chromatography (liquid, gas, paper, thin-layer, ion exchange, supercritical fluid, and electrophoresis) have many features in common, they must all be considered in development of the field. Analytical method development and method validation was performed for RP-HPLC method for the Isoniazid and Rifampicin in tablet formulation as per ICH norms for the following parameters: system suitability, linearity and precision (repeatability), intermediate precision (ruggedness), specificity and accuracy. From the results obtained, it was observed that the developed method was proven to be specific, precise, linear, accurate, rugged and robust and is suitable for its intended purpose.

Keywords: RP-HPLC, isoniazid, pyrazinamide, rifampicin, Determination, Drug substance, Formulation.

INTRODUCTION

The pharmaceutical analysis defined as "the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of a given sample of a medicine, the detection and estimation of impurities, which may be present in drug substance (or) given sample of medicine". [1]The substance may be a single compound or a mixture of compounds and may be in the form a tablet, pill, capsule, ampoule, liquid, mixture or an ointment.[2] The quality control tests involve methods which embrace chemicals, physio – chemical, instrumental, microbiological (or) biological procedures. The pharmaceutical analysis deals with the subject of determining the composition of material in terms of the elements or compound (drug) present in the system.[3] Any type of analysis involves two steps. 1. Identification (qualitative) Estimation (quantitative). 2. In

qualitative analysis, a reaction is performed in such a way as to indicate the formation of a precipitate, a change of a colour, the dissolution of a precipitate complex formation and the evaluation of a gas. Quantitative analysis is performed ordinarily through five steps. They are sampling, dissolution, precipitation, measurement and calculation.[5]

A) Specificity and selectivity[6]

i. Selectivityistheabilitytomeasureaccuratelyandspecificallytheanalyteinthep resence of components that may be expected to be present in the sample matrix.
ii. Specificity for an assay ensures that the signal measured comes from the substance of interest and that there is no interference from excipients and/or degradation products and/or impurities.
iii. Determination of this can be carried out by assessing the peak identityand purity.
iv. Diodearray detectors can facilitate the development and validation of HPLC assays. Spectra] data obtained from diode array detectors, effectively supplement the retention time data for peak identification, also spectral manipulation often provides information about the peak purity. The table below lists several of the techniques available for assessing peak identity and purity.
v. The purity index is a measure of the peak's relative purity, measured using a full comparis on of spectral data for the leading and training edge of the peak. A value of 1.5 is commonly accepted to indicate a purepeak. But>1.5wouldindicatethepresence of an impurity.

B) Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed at the variance, standard deviation or coefficient of variation of a series of measurements. Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.[7]

C) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found[8].

D) Linearity andrange

Linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. For example, calculation of a regression line by the method of least square. Therefore data from regression line itself may be helpful to provide mathematical estimates of the degree of linearity.[9]

E) Limit of Detection

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

F) Limit of Quantification

The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision

G) Robustness

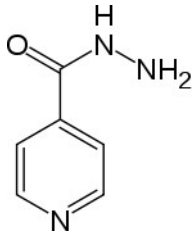
The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.[10]

F) Ruggedness

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.[11]

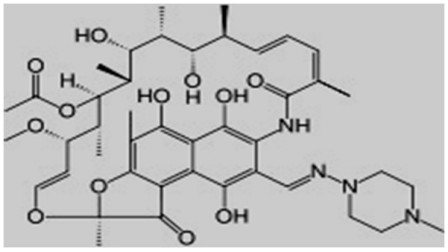
2. DRUGPROFILE

2.1 Isoniazid[12] Structure



Nomenclature : Isonicotinohydrazide
Molecular Formula : C6H7N3O
Molecular Weight : 137.1g/mol
Appearance : A white or almost white, crystalline powder or Colorless crystals
Solubility : freely soluble in water, sparingly soluble in Alcohol
Category : Anti–Tuberculosis

2.2 Rifampicin [13] Structure



Nomenclature : (7*S*,9*E*,11*S*,12*R*,13*S*,14*R*,15*R*,16*R*,17*S*,18*S*,19*E*,21*Z*)-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-{(E)-[(4-methylpiperazin-1- yl)imino]methyl}-6,23-dioxo-8,30-dioxa-24-azatetracyclo[23.3.1.1^{4,7}.0^{5,28}] triaconta- 1(28),2,4,9,19,21,25(29),26-octaen-13-yl acetate.
Molecular Formula : C43H58N4O12
Molecular Weight : 823g/mol
Appearance Category : Anti–Tubercul

Aim and scope

Isoniazid and Rifampicin is official in I.P, B.P, and U.S.P. From the literature survey, it was found that there were only few RP-HPLC methods reported for the estimation of Isoniazid and Rifampicin in pharmaceutical dosage forms. Hence, the aim of present work is to develop RP-HPLC method for estimation of Isoniazid and Rifampicin from the tablet dosage form and to validate the developed RP- HPLC method by validation parameters as per ICH guidelines.

MATERIALS AND INSTRUMENTSUSED

S.No.	NAME	MODEL	MANFACTURER/SUPPLIER
1.	Weighing balance	AUM220D	Shimadzu
2.	Sonicator	Sonorex	Sonorex dg 10p
3.	pH Meter	9087	ELICO pH METER
4.	HPLC-UV,PDA	Waters e 2116	Waters
5.	Colum	Zodiac C18	Zodiac

Active Ingredients used

S.No.	NAME	SPECIFICATION
1.	Isoniazid	As Reference standard
2.	Rifampicin	As Reference standard

Chemicals used

S.No.	NAME	MODEL	MANFACTURER/SUPPLIER
	Pottasiumdihydrogen		
1.	orthophosphate	HPLC	Rankem Chemicals
2.	Methanol	HPLC	Rankem Chemicals
3.	Phosphoric acid	HPLC	Merck Chemicals
4.	Milli-Q Water	HPLC	In House production

RP-HPLC METHOD DEVELOPMENT

In case of analytical method development and for drugs analysts should decide whether the given analytical method is suitable for the assay of the drug. The method development of new improved method usually trailers existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. In the development stage, decision regarding choice of column, mobile phase, detectors and method of quantitation must be addressed. In this way, development considers all the parameters pertaining to any methods.[12]

.

Selection of stationaryphase

Proper selection of the stationary phase depends up on the nature of the sample andchemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C18columns could be appropriately used for the separation ofIsoniazid and Rifampicin.

Selection of wavelength

The sensitivity of the HPLC depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for both drugs to be detected. The wavelength for measurement was selected as 263 nm from the absorption spectrum.

Selection of mobile phase

The mobile phase was selected and chromatograms were recorded, trails were done on Isoniazid and Rifampicin.

ASSAY METHOD DEVELOPMENT

The objective of this experiment was to optimize the assay method for simultaneous estimation of Isoniazid and Rifampicin based on the literature survey made and the methods given in pharmacopoeia. Trials done for optimization were as follows:

Trials

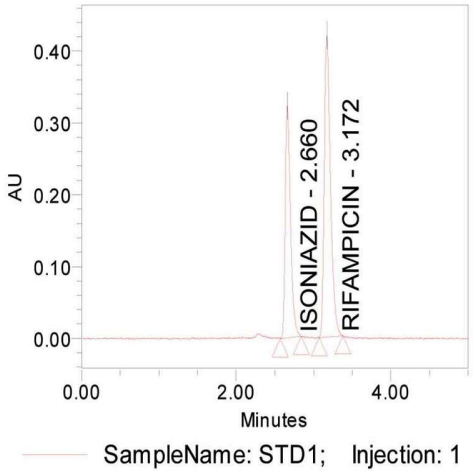
Buffer preparation: 17.418 gm of Potassium Hydrogen Orthophosphate was mixed with 1000 ml of Mille-Q water and shaked for 15min and degassed.

Trial 1:

Mobile phase A : Potassium Hydrogen Orthophosphate
Mobile phase B : Methanol

Chromatographic conditions

Column : Agilent Zorbax Sb-C18, (4.6 × 250 mm, 5µ)
Detectorwavelength : 263 and 274 nm
Columntemperature : 30^{o}C
InjectionVolume : 10µl
Flow rate : 1.0 ml/min
Runtime : 15 min



Tailing was not satisfactory for both Rifampcin and Isoniazidand the retention time of Rifampicin and Isoniazid were found to be 3.17 and 2.6 minutes respectively.

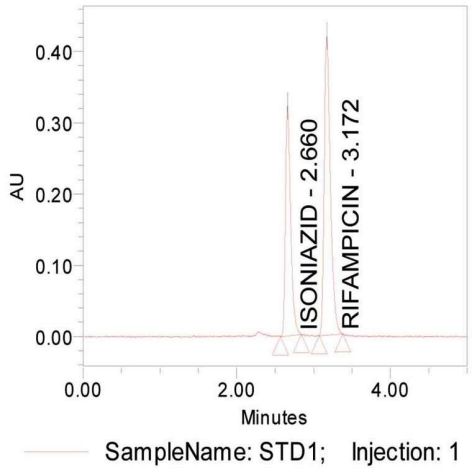
Trial 2: (OPTIMIZED METHOD)

Buffer Preparation: 17.418 gm of Potassium Hydrogen Orthophosphate was mixed with 1000 ml of Mille-Q water and shaked for 15min and degassed.

MobilephaseA : Potassium HydrogenOrthophosphate
Mobilephase B : Methanol

Chromatographic Condition:

Column : AgilentZorbax Sb-C18, (4.6 × 250 mm, 5µ)
Columntemperature :30^0C
Inj.Volume : 10µl
Flow rate : 1.0 ml/min
λmax : 263nm
Runtime : 15 min



Tailing was satisfactory for both Rifampcin and Isoniazid and the retention time of Rifampicin and Isoniazid were found to be 3.17 and 2.6 minutes respectively.

Sample preparation

Preparation of sample solution of Isoniazid and Rifampicin for trials

10 tablets were weighed accurately and finely powdered. Tablet powder of902.55 mg equivalent to 450 mg of Rifampicin and 300 mg of Isoniazid was weighed and transferred into a 50 ml standard volumetric flask. After this 25 ml of HPLC Water (diluent) was added and sonicated for 30 minutes with intermittent shaking and

cooled to room temperature. Volume was made with HPLC Water (diluent) and mixed well.
5ml of stock was pipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µ g of Isoniazid per ml respectively.

Standard solution preparation

450 mg of Rifampicin (RIF) and 300 mg of Isoniazid (ISN) was accurately weighed and transferred into a 50 ml standard volumetric flask. After this 5 ml of HPLC water was added and sonicated for 30 minutes with intermittent shaking and cooled to room temperature. Volume was made with diluent and mixed well. 5ml of stock was pipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µ g of Isoniazid per ml respectively.

METHOD VALIDATION

VALIDATION

According to ICH guidelines method validation can be defined as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".Such validated analytical method for qualitative and quantitative testing of the drug molecule assume greater importance when they are employed to generate quality and safety compliance data during development, pre-formulation studies and post approval of drug products. The ICH of Technical Requirements for the Registration of Pharmaceutical for human use has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics

Parameters Used for Assay Validation

The validation of the assay procedure was carried out using the following parameters.

1) Parameters:

1.1 System suitability
1.2 Specificity
1.3 Method Precision
1.4 Linearity & range
1.5 Accuracy / Recovery studies
1.6 Robustness

PREPARATION OF STANDARD AND SAMPLE SOLUTION
STANDARD PREPARATION

Buffer Preparation: 17.418 gm of Potassium Hydrogen Orthophosphate was mixed with 1000 ml of Mille-Q water and shaked for 15min and degassed.
MobilephaseA : Potassium HydrogenOrthophosphate
MobilephaseB :Methanol

Chromatographic Condition:

Column	: Agilent Zorbax Sb-C18, (4.6 × 250 mm, 5µ)
Columntemperature	: 30ºC
Inj.Volume	: 10µl
Flowrate	: 1.0 ml/min
λmax	: 263nm
Runtime	: 15

Sample preparation

Preparation of sample solution of Isoniazid and Rifampicin for trials:

10 tablets were weighed accurately and finely powdered. Tablet powder of 902.55 mg equivalent to 450 mg of Rifampicin and 300 mg of Isoniazid was weighed and transferred into a 50 ml standard volumetric flask. After this 25 ml of HPLC Water (diluent) was added and sonicated for 30 minutes with intermittent shaking and cooled to room temperature. Volume was made with HPLC Water (diluent) and mixedwell. 5ml of stock waspipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µg of Isoniazid per ml respectively.

Standard preparation Standard solutionpreparation

450 mg of Rifampicin (RIF) and 300 mg of Isoniazid (ISN) was accurately weighed and transferred into a 50 ml standard volumetric flask. After this 5 ml of HPLC water was added and sonicated for 30 minutes with intermittent shaking and cooled to room temperature. Volume was made with diluent and mixed well. 5ml of stock waspipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µg of Isoniazid per ml respectively.

SYSTEM SUITABILITY

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, HPLC method and the procedure should be capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

SPECIFICITY

Specificity is the ability to assess unequivocally of an analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Precision

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as %RSD for the statistically significant number of samples.

Method Precision

Six sample preparations were prepared individually using single batch of Isoniazid and Rifampicin tablets (1/32 mg) as per test method and injected each solution. Resulted chromatogram was shown in the fig. no. 6. And data was shown in below table10.

LINEARITY AND RANGE

Linearity

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of an analyte in the sample.

Range

Range of an analytical procedure was the interval between the upper and lower concentration (amount) of an analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has suitable level of precision, accuracy and linearity.

METHOD ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the values which is accepted either as a conventional true value or an accepted reference value for the observed value.

Table 1: Results of Accuracy study (ISONIAZID)

ISONIAZID						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**%`Recovery**	**%Mean**
50%	451.28	756728	594.007	594.91	100	100
50%	451.28	756907	594.007	594.05	100	
50%	451.28	756975	594.007	594.10	100	
50%	451.28	756326	594.007	594.59	100	
50%	451.28	756274	594.007	594.55	100	
100%	902.55	1512475	1188.000	1189.04	100	100
100%	902.55	1518251	1188.000	1193.58	100	
100%	902.55	1512296	1188.000	1188.90	100	
150%	1353.83	2260654	1782.007	1777.23	100	100
150%	1353.83	2262479	1782.007	1778.66	100	
150%	1353.83	2260325	1782.007	1776.97	100	

150%	1353.83	2262513	1782.007	1778.69	100
150%	1353.83	2263234	1782.007	1779.26	100
150%	1353.83	2266385	1782.007	1781.74	100

Table 2: Results of Accuracy study (RIFAMPICIN)

RIFAMPICIN				
Sample Area	**µg/ml added**	**µg/ml found**	**% Recove ry**	**% Mean**
1265170	891.010	899.73	101	
1262461	891.010	897.81	101	
1261719	891.010	897.28	101	
1263056	891.010	898.23	101	101
1268196	891.010	901.89	101	
1262096	891.010	897.55	101	
2523741.00	1782.000	1795.78	101	101
2525279.00	1782.000	1795.87	101	
2528251.00	1782.000	1797.98	101	
3782160	2673.010	2689.71	101	
3785672	2673.010	2692.21	101	
3782793	2673.010	269016	101	101
3785575	2673.010	2692.14	101	
3788145	2673.010	2693.97	101	
3789803	2673.010	2695.15	101	

ROBUSTNESS

The robustness is a measure of method capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of method reliability during normal use. Standard was prepared and injected into the chromatographic system as per the conditions specified in the method. The same standard was reinjected by altering one parameter at a time, keeping other parameters constant. A set of system suitability data was calculated for standards injected under altered method conditions and compared against the values generated under normal method conditions. The results were tabulated in below table.

Table 3: Data for variation in temperature and flow rate (ISONIAZID)

	Sample Name	**Inj**	**Nam e**	**RT**	**Area**	**USP Resolution**	**U SP Tailing**	**USP Plate Count**
1	FLOW1	1	ISONIAZID	2 .673	14574		1 .421	7399
2	FLOW2	1	ISONIAZID	2 .675	15782		1 .398	7247
3	TEMP1	1	ISONIAZID	2 .677	15164		1 .393	7510
4	TEMP2	1	ISONIAZID	2 .685	15232		1 .411	7515

Table 4: Data for variation in temperature and flow rate (RIFAMPICIN)

	Sample Na me	**Inj**	**Nam e**	**RT**	**Area**	**U SP Resolution**	**U SP Tailing**	**USP PlateCount**
1	FLOW1	1	RIFAMPICIN	3 .173	2748228		1 .378	7386
2	FLOW2	1	RIFAMPICIN	3 .173	2352295		1 .410	7350
3	TEMP1	1	RIFAMPICIN	3 .177	2544191		1 .374	7233
4	TEMP2	1	RIFAMPICIN	3 .186	2551703		1 .364	7300

RESULTS AND DISCUSSION

Analytical method development and method validation was performed for RP-HPLC method for the Isoniazid and Rifampicin in tablet formulation as per ICH norms for the following parameters: system suitability, linearity and precision (repeatability), intermediate precision (ruggedness), specificity and accuracy. The summary of results obtained in analytical method development and validation were tabulated in table no.26.

VALIDATION SUMMARY REPORT

The observations and results obtained for each of the parameters like system suitability, linearity, precision (repeatability), specificity, accuracy and robustness lies well within the acceptance criteria. So the developed method was simple, specific, linear, precise, and accurate and robustness could be extensively used for the Isoniazid and Rifampicin in tablet formulation system.

Table 5: Validation parameters and acceptance criteria for INH and RIF

S. No	Validation parameters	Specification	Results	
		System suitability	**Isoniazid**	**Rifampicin**
1	Retention time	Not applicable	2.660	3.172
	Tailing	NMT 2	1.469	1.412
	Resolution	NLT 2		3.697
	Theoretical plates	NLT 2500	7755	7613
	Similarity factor	0-98 to 1.02	0.99	0.99
	%RSD	NMT 2.0%	0.5	0.3
2	Specificity	There is no peak in blank at the Rt of analyte	Nil	Nil
		There is no peak in placebo at the Rt of analyte	Nil	Nil
3	Precision	The value should be between 97% to 103%	100	100
			99	100
			99	100
			99	100
			99	100
			99	100
		The %RSD of six replicate assay results NMT 2.0%	0.23	0.13
4	Accuracy (50%)	The value should be between 97% to 103%	100	101
	Accuracy (100%)	The value should be between 97% to 103%	100	101
	Accuracy (150%)	The value should be between 97% to 103%	100	101
5	Linearity	Correlation coefficient NLT 0.999	0.998	0.997
6	LOD	Not applicable	2.88 µg/ml	2.77 µg/ml
7	LOQ	Not applicable	9.58 µg/ml	9.22 µg/ml
8	Range	Not applicable	600µg to 1800 µg/ml	900µ g to 2700 µg/ml
9	Robustness(Flow-1)			
	Tailing	NMT 2	1.421	1.378
	Resolution	NMT 2	Nil	3.596
	Theoretical plates	NLT 2500	7399	7386
	Robustness(Flow-2)			
	Tailing	NMT 2	1.398	1.410
	Resolution	NMT 2	Nil	3.578
	Theoretical plates	NLT 2500	7247	7350
	Robustness(Temp-1)			
	Tailing	NMT 2	1.393	1.374
	Resolution	NMT 2	Nil	3.590
	Theoretical plates	NLT 2500	7510	7233
	Robustness(Temp-2)			
	Tailing	NMT 2	1.411	1.364
	Resolution	NMT 2	Nil	3.601
	Theoretical plates	NLT 2500	7515	7300

CONCLUSION

From the results obtained, it was observed that the developed method was proven to be specific, precise, linear, accurate, rugged and robust and is suitable for its intended purpose. So the above work performed gives documented evidence that the analytical method for the Isoniazid and Rifampicin by RP-HPLC in tablet dosage forms will consistently analyze these drugs quantitatively and could be used for routineanalysis.

REFERENCES

1. D. Kealey and P. J. Haines. Analytical Chemistry. 1st edition. New Delhi: Viva Brook Pvt Ltd. 2002;P.1-3.
2. Skoog, West holler crouch. Fundamentals of Analytical Chemistry. 8th edition. Australia: Thomason Brooks/Cole. 2004; P.2-4.
3. Willard, Merit, Dean, Settle. Instrumental Method of Analysis. 7th edition. New Delhi: CS publishers and distributors. 1986; P. 1, 592,622-628,256-264.
4. ICH-Q2A. Validation of Analytical Procedure / Methodology. Geneva, Switzerland: ICH Harmonised Triplicate Guidelines; 1996. P.1-8.
5. Code ICH-Q2B. Validationof Analytical Procedure/Methodology. Geneva, Switzerland: ICH Harmonized Triplicate Guidelines; 1996. P.1-8.
6. Sharma BK. Organic Spectroscopy. 6th edition. New Delhi: GoelPublishing House. 2002; P.75.
7. GurdeepChatwal, Sham Anand. Instrumental Methods of Chemical Analysis. 5th edition. New Delhi: Himalaya Publishing House. 2002;P.25-67.
8. Sharma B.K. Instrumental Method of Chemical Analysis. 18th edition Meerut: Goel Publishing House. 1999; P. 1-23,175-203.
9. Jeffery G. H, Denny R. C, Barnes J. D, Thomas M. Vogel's text book of Quantitative Chemical Analysis. 6th edition. Pearson Education. P. 2-7,216-227.
10. Hdicostas E, Wendawiak B.W. Koch M. Quality Assurance in Analytical Chemistry Training and Teaching. 1st edition. New Delhi: Springer Pvt Ltd. InternationalSpringer Group. 2004; P.204-219.
11. Martindale. The Complete Drug Reference. 3rd edition. London: pharmaceutical press. 1999; P.128-523, 1027.
12. In Indian Pharmacopeia. 6th edition. Published by The Indian Pharmacopeia Commission Indian Pharmacopeia Laboratory Govt. of India. 2010; Vol - II:1515.