IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
February 2020 Vol.:14, Issue:4

Method Development and Validation of Levetiracetam by RP-HPLC

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Bejjanki Anusha *, K R Manohar[1]

**, [1] Department of Pharmaceutical Analysis, Sahasra Institute of Pharmaceutical Sciences, Warangal, Telangana-506007*

Submission: 21 January 2020
Accepted: 29 January 2020
Published: 29 February 2020

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Levetiracetam, Acetonitrile, Chromatography, Retention Time, Prontosil

ABSTRACT

A simple, reproducible and efficient Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the estimation of Levetiracetam in its tablet dosage form. Separation was done by using mobile phase consists of mixture of potassium dihydrogen orthophosphate anhydrous and Sodium 1- heptane sulphonic acid sodium salt anhydrous (pH adjusted to 2.8±0.05 with Orthophosphoric acid): Acetonitrile (90:10, v/v). Chromatography separations were carried out on Prontosil C18 column (150X4.6mn; 5µm) at a flow rate of 1.2 ml/min and UV detection at 215nm and the retention time for Levetiracetam is 3.9minutes. The linear dynamic response was found to be in the concentration of 45µg-270µg/ml. The slope, intercept and correlation coefficient was found to be 0.9999 respectively. The percentage recovery of Levetiracetam was found to be 99.99-100.50%. Proposed method wass found to be simple, accurate, precise and rapid and could be used for routine analysis. This condition is applied only for tablet dosage form. The statistical parameters and recovery studies were carried out and reported.

INTRODUCTION

High Pressure Liquid Chromatography (HPLC) sometimes called High Performance Liquid Chromatography is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The techniques of HPLC are so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation. In reverse phase technique, a non-polar stationary phase is used and the mobile phase is polar in nature. Hence, polar components are eluted first and non-polar compounds are retained for a longer time. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster, columns used in the mode of chromatogram are ODS (Octadecyl silane) or C_{18}, C_8, C_4, etc.

Method development and optimization in liquid chromatography is still an attractive field for theoreticians and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution. Alternate analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intended to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength and quality, for the quantification of the drug substances and drug products. Method validation has received

considerable attention in the literature and from industrial committees and regulatory agencies. Validation is a process of 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. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the method and determine the limits of allowed variability for the conditions needed to run the method.

MATERIALS AND METHODS

LIST OF INSTRUMENTS USED

Table No. 1: List of Instruments Used

Sr. No.	Instruments/Equipment's/Apparatus
1.	A SHIMADZU HPLC with Class-VP version 6.12 SP1 software, UV-Visible Detector (SPD-10A), PUMP (LC-10AT) and (LC-10ATvp).
2.	UV-Visible double beam Spectrophotometer (ELICO).
3.	UV-Visible double beam Spectrophotometer(THERMO)
4.	Electronic Balance (AFCOSET)
5.	Ultra Sonicator (ENERTECH)
6.	LiChroCART-Lichrospher® 100 (C_{18}) RP Column (250 mm x 4mm x 5 µm.)
7.	P^H Analyzer (ELICO)
8.	Triple Quartz Distillation Unit (BOROSIL)
9.	HPLC Injecting Syringe (25 µl) SY25NN (Nylon)

LIST OF CHEMICALS, REAGENTS AND STANDARDS:

Table No. 2: List of Chemicals, Reagents and Standards

Sr. No.	Chemicals / Reagents / Standards	Grade	Batch No	Specification
1	Potassium dihydrogen orthophosphate Anhydrous	AR	DL8S48123	99.5%
2	Methanol	HPLC	R191L04	99.7%
3	Acetonitrile	HPLC	R054B03	99.9%
4	Triple distilled water	NA	NA	NA
5	1-Heptane Sulphonic Acid Sodium Salt Anhydrous	HPLC	NA	NA
6	Ortho-Phosphoric Acid	HPLC	RO53C10	NA

Optimized chromatographic conditions:

Table No. 3: Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase (column)	Prontosil C18, 150×4.6mm, 5µm particle size
Mobile Phase	pH 2.8 Buffer: Acetonitrile (90:10 v/v)
Flow rate (ml/min)	1.2 mL/min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	215nm

Preparation of pH 2.8 Buffer solution:

Weigh and dissolve 1.36g of potassium dihydrogen phosphate (KH_2PO_4) and 0.61g of sodium-1-heptane sulphonate in 1000ml of milli-Q-water and adjust the pH to 2.8±0.05 with dilute orthophosphoric acid solution.

Preparation of mobile phase:

1. Mix pH 2.8 Buffer solution and acetonitrile in the ratio 90:10(v/v).
2. Filter through 0.45µm membrane filter and degas for about 10min.

Preparation of standard stock solution:

Weigh accurately and transfer Levetiracetam working standard equivalent to about 90mg of Levetiracetam into a 50ml volumetric flask, add about 35ml of diluent, sonicate to dissolve the material completely, dilute to volume with diluent and mix.

Preparation of standard solution:

1. Pipette 5ml of the above solution into a 50ml volumetric flask, dilute to volume with diluent and mix.

2. Filter through 0.45µm HNN/HVF filter.

Test preparation:

1. Weigh and crush not less than 20tablets using mortar and pestle.

2. Weigh and transfer the tablet powder equivalent to about 180mg of Levetiracetam into a 100ml volumetric flask, add about 75ml of diluent, sonicate for 30minutes with intermediate shaking and dilute to volume with diluent.

3. Centrifuge a portion of above solution at 2500rpm for about 10minutes by using centrifuge tubes with caps.

4. From the above supernatant solution pipette 5ml in to 50ml volumetric flask, dilute to volume with diluent and mix.

5. Filter through 0.45µm HNN/HVF filter.

System suitability:

1. Inject about 10 µL portion of standard solution into the chromatographic system and measure the response of major peak.

2. The USP tailing factor for Levetiracetam peak should be NMT2.0.

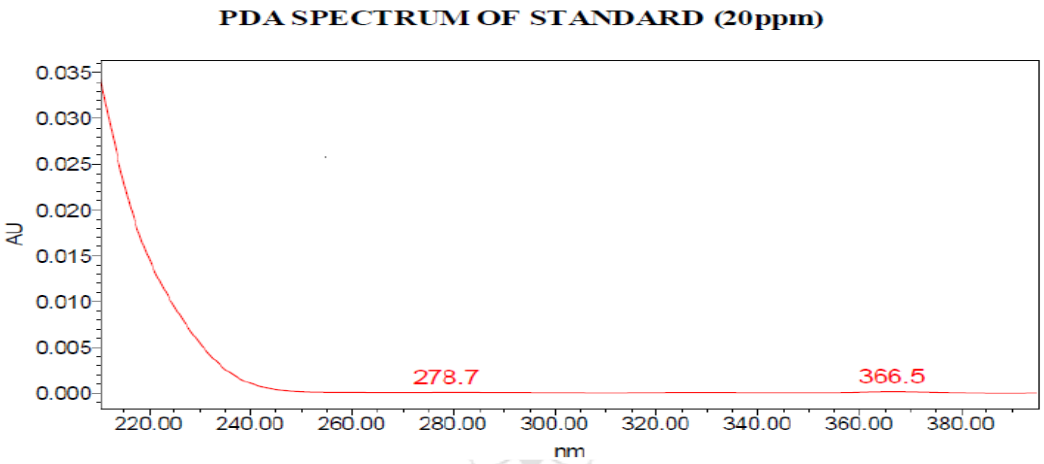
3. The RSD for the area of Levetiracetam peak obtained from the 5replicates injections of standard preparation should be NMT 2.0%.

Procedure:

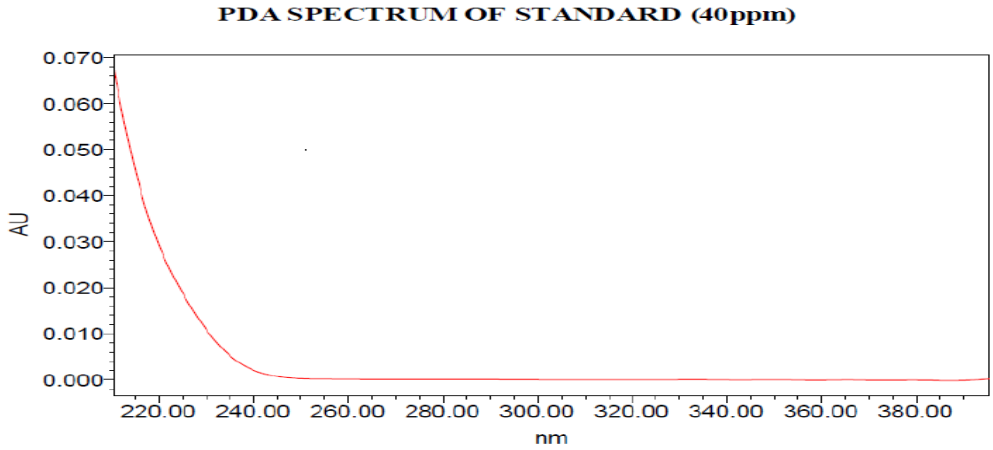
Inject about 10µL portion of diluent and test preparation into the chromatograph, record the chromatogram and measure the response of major peak.

RESULTS

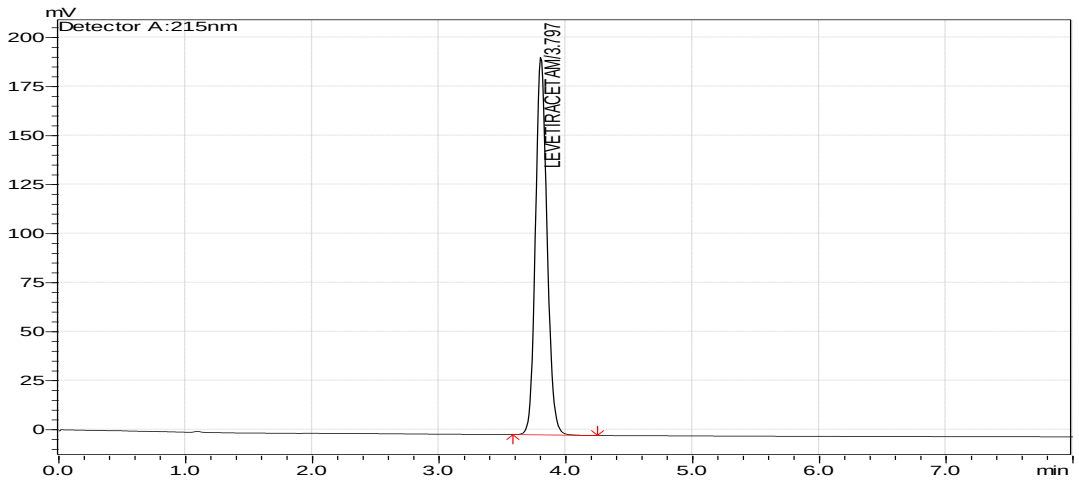
Chromatogram of Standard (20ppm):



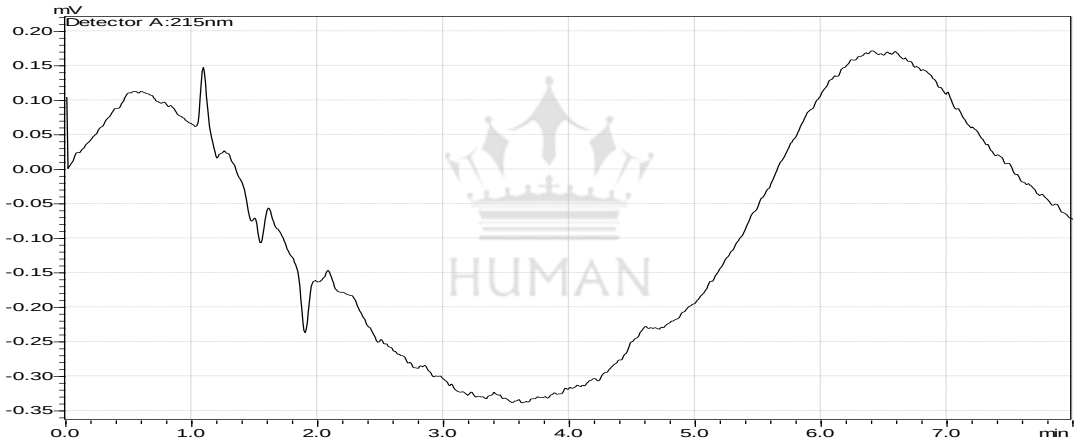
Chromatogram of Standard (40ppm):



SELECTION OF PH OF THE BUFFER (pH 2.8 BUFFER):

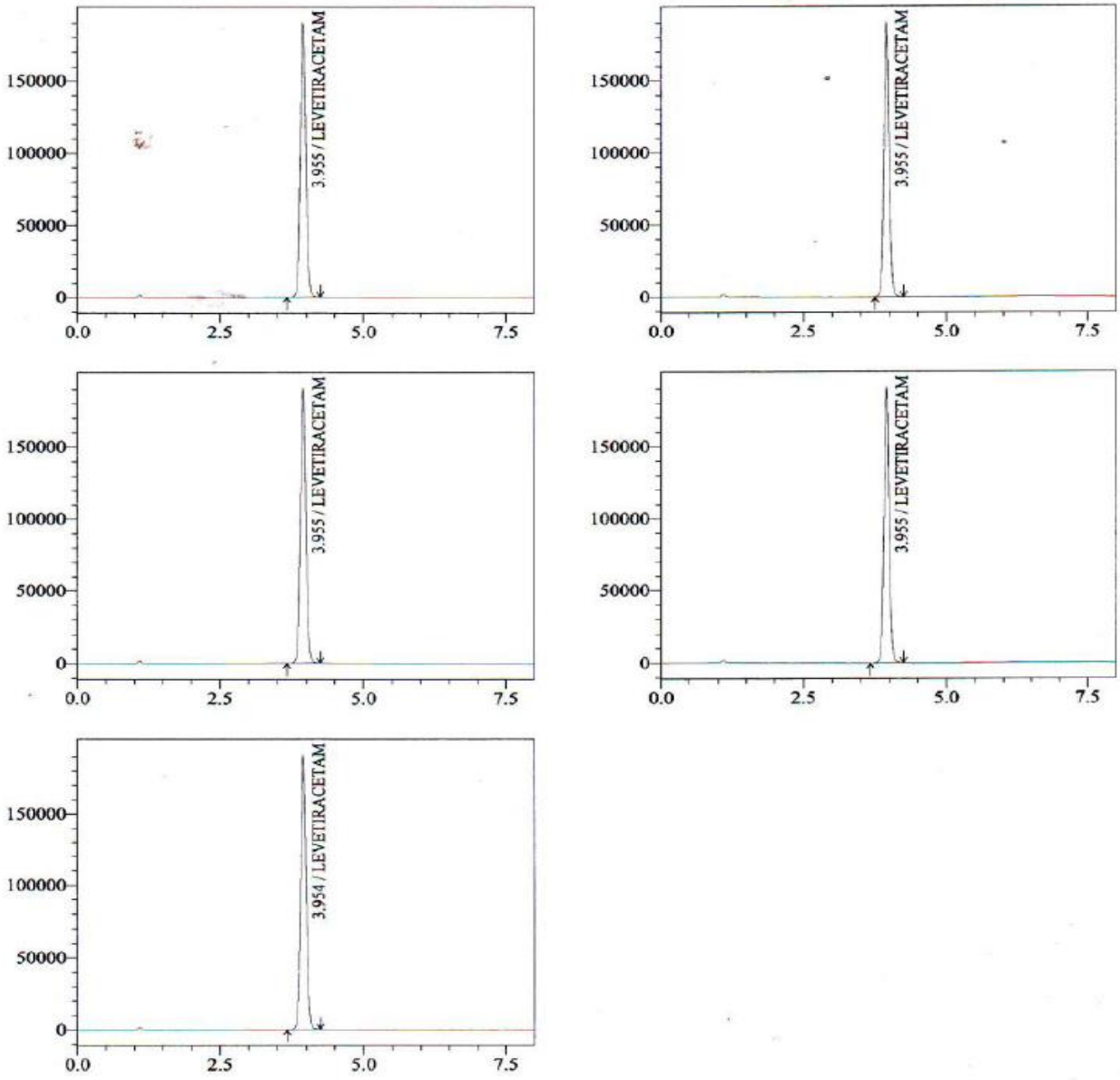


SYSTEM SUITABILITY:



Injection	Retention time	Area	Theoretical plates	Tailing factor
BLANK	0.0	0	0	0.00

SYSTEM SUITABILITY



<< Detector A >>
ID#1 Compound Name: **LEVETIRACETAM**

Data File	ate Acquire	Sample Name	Sample ID	Ret. Time	Area
00704890.lcd	07/26/10	LEVETIRACETAM	STANDARD- 1	3.955	1216054
00704891.lcd	07/26/10	LEVETIRACETAM	STANDARD- 2	3.955	1215432
00704892.lcd	07/26/10	LEVETIRACETAM	STANDARD- 3	3.955	1216616
00704893.lcd	07/26/10	LEVETIRACETAM	STANDARD- 4	3.955	1217255
00704894.lcd	07/26/10	LEVETIRACETAM	STANDARD- 5	3.954	1218497
				3.955	1216771
				0.0	0.1
				0.000	1177

DISCUSSION

The new analytical method for the HPLC method was established for Levetiracetam then optimized and then applied on pharmaceutical dosage forms.

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of pH 2.8 buffer and Acetonitrile in the ratio 90:10 v/v gave a better resolution and sensitivity.

The detection was carried out by using PDA detector at 215nm. Among the several flow rates tested, the flow rate of 1.2ml was found to be the best for Levetiracetam with respect to retention times and theoretical plates.

The retention time is 3.9 for Levetiracetam. The asymmetry factor or the tailing factor was found to be 1.0 for Levetiracetam, which indicates symmetrical nature of the peak.

System suitability parameters such as retention time, tailing factor, capacity factor and number of theoretical plates were calculated. The number of theoretical plates was found to be above 5000 for Levetiracetam, which indicates efficient performance of the column. The retention time of Levetiracetam was found to be within the limits of 3.8-4.0 minutes. These parameters represent the specificity of the method.

From the linearity studies, the specified concentration range was determined. It was observed that Levetiracetam was linear in the range of 25% to 150% for the target concentrations.

The regression equation of Levetiracetam concentration over its peak area ratio was found to be Y=12084X+8581 (R^2 = 0.9999) where Y is the peak area ratio and X is the concentration of Levetiracetam (µg/mL). The linearity range of 45µg-270µg/ml for Levetiracetam was found to obey linearity with the correlation coefficient of 0.9999.

The validation of the proposed method was verified by system precision and method precision. The %RSD for system precision of Levetiracetam was 0.0. The validation of proposed method was verified by recovery studies. The percentage recovery range was found between 100.1-100.5% for Levetiracetam. This is a good index of accuracy, specificity and repeatability of the method.

Placebo interference studies were made by injecting placebo alone, then the standard and the placebo along with the standard. The graphs were shown in chromatogram. They did not show any interference of placebo at the RT of the analyte peak.

Robustness studies were made by varying the flow rate by 20% and also by performing filter validation studies on to types of filters.

Study of ruggedness was made by conducting the study on different Shimadzu system and by two analysts.

CONCLUSION

A simple, reproducible and efficient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Levetiracetam in its tablet dosage form. Separation was done by using mobile phase consists of Mixture of Potassium Dihydrogen ortho Phosphate anhydrous and Sodium 1- Heptane Sulphonic acid sodium salt anhydrous (pH adjusted to 2.8±0.05 with Orthophosphoric acid):Acetonitrile (90:10,v/v). Chromatography separations were carried out on Prontosil C18 column (150X4.6mn; 5µm) at a flow rate of 1.2 ml/min and UV detection at 215nm and the retention time for Levetiracetam is 3.9minutes. The linear dynamic response was found to be in the concentration of 45µg-270µg/ml. The slope, intercept and Correlation coefficient was found to be 0.9999 respectively. The percentage recovery of Levetiracetam was found to be 99.99-100.50%. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis. This condition is applied only for tablet dosage form. The statistical parameters and recovery studies were carried out and reported.

ACKNOWLEDGEMENTS

I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

I wish to extend my sincere thanks to my beloved guide Sri. K R Manohar, Associate Professor, Department of Pharmaceutical Analysis, Sahasra Institute of Pharmaceutical Sciences, Warangal for his encouragement which made me to finish this work.

ABBREVIATIONS

HPLC	High performance Liquid Chromatography
%	Percent
PDA	Photodiode Array
ICH	International Conference for Harmonization
GR	General reagent
C18	Octadecyl
UV	Ultraviolet
ml	Milliliter
Min	Minute
MeOH	Methanol
µl	Micro Liter
µ	Micron
µg	Micro gram
ppm	Parts per million
nm	Nanometer
RSD	Relative Standard Deviation
Fig	Figure

REFERENCES

1. Bently and Drivers, "Textbook of Pharmaceutical Chemistry," 8th Edition, 1985, O'Brein, Oxford University Press, Pg. No. 1 – 3.
2. Napoleon A.A. "Pharmaceutical Titrimetric Analysis theory and practical," Kalaimani Publishers and Distributors, Kanchipuram, Pg. No. 1.1 – 1.4
3. Ewing, G.W, "Instrumental Methods of Chemical Analysis", 2nd Edition, 1960, MC Graw Hill Book Company, Page No. 1-3.
4. Gurdeep R Chatwal and Sham K Anand, "Instrumental Methods of Chemical Analysis 5th Edition, Himalaya Publishing House, Pg No. 1.2 – 1.5.
5. U.S.P. Asian Edition, United Pharmacopoeial Convention Inc, Rockville, 2005, Page No. 2386-2389.
6. James W Munson, "Modern Methods of Pharmaceutical Analysis, 2001, Medical Book Distributors, Mumbai. Page No. 17-54.
7. Sethi P.D. HPLC 'High Performance Liquid Chromatography and Quantitative analysis Pharmaceutical Formulations", 1st Edition, CBS Publishers and Distributors; 2001. Page No 3-5
8. Erwing, G. W., "Instrumental Methods of Chemical Analysis,' 2nd Edn.,1960 McGraw Hill Book Company, Pg, 3-5
9. Jenkins GL., Knevel, A.M and Digangi, F. E., "Quantitative Pharmaceutical Chemistry". 7th Edn. McGraw Hill Book Co., New York, Edition 1977, Pg No 297-299.
10. Beckett A. H and Stenlake J. B., "Practical Pharmaceutical Chemistry" 4th Edition. Part II, CBS Publisher and Distributor, New Delhi, 1997, Pg No 277-278.