CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.8232674

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

DEVELOPMENT & VALIDATION OF A RP-HPLC METHOD FOR THE ESTIMATION OF ACOTIAMIDE IN COMBINATION WITH AMITRIPTYLINE IN BULK AND PHARMACEUTICAL FORMULATION

Miss. Shraddha Shekhar Patil*, Mrs. Bhuvaneshwari Yogesh Rane*
[1]Student, [2]Assistant Professor
Department of Pharmaceutical Chemistry
KYDSCTC'S College of Pharmacy, Sakegaon Maharashtra, India

Abstract:
Acotiamide and Amitriptyline prokinetic agent with gastrointestinal (GI) motility-enhancing activity. "Development and validation of RP-HPLC method for estimation of Acotiamide and Amitriptyline in Bulk Drug And it`s Dosage form" Attempts were made to develop RP-HPLC method for simultaneous estimation of Acotiamide and Amitriptyline from Tablet. For the RP - Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector Reverse Phase (Agilent) C18 column (4.6mm x 250mm;5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.
Methanol: water (0.05%OPA), (90:10) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 228 nm and flow rate was 0.7ml/min. In the developed method, the retention time of Acotiamide and Amitriptyline was found to be being 4.470 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.
So the proposed methods can be used for the routine quality control analysis Acotiamide and Amitriptyline in bulk drug as well as in formulations.
Keyword: *Acotiamide, Amitriptyline, HPLC.*

Corresponding author:
***Bhuvaneshwari Yogesh* Rane,**
Assistant Professor
Department of Pharmaceutical Chemistry
K.Y.D.S.C.T.S College of Pharmacy, Sakegaon, Bhusawal, Dist- Jalgaon, Maharashtra, India

QR code

Please cite this article in Shraddha Shekhar Patil et al, Development & Validation Of A RP-HPLC Method For The Estimation Of Acotiamide In Combination With Amitriptyline In Bulk And Pharmaceutical Formulation., Indo Am. J. P. Sci, 2023; 10 (07).

1. INTRODUCTION:

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the object in question. During this period significant analytical contributions to chemistry include the development of systematic elemental analysis by Justus von Liebig and systematized organic analysis based on the specific reactions of functional groups.

The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and cesium (Cs) in 1860. Most of the major developments in analytical chemistry take place after 1900. During this period instrumental analysis becomes progressively dominant in the field. In particular many of the basic spectroscopic and spectrometric techniques were discovered in the early 20th century and refined in the late 20th century.

The separation sciences follow a similar time line of development and also become increasingly transformed into high performance instruments. In the 1970s many of these techniques began to be used together to achieve a complete characterization of samples.

Starting in approximately the 1970s into the present day analytical chemistry has progressively become more inclusive of biological questions (bioanalytical chemistry), whereas it had previously been largely focused on inorganic or small organic molecules. Lasers have been increasingly used in chemistry as probes and even to start and influence a wide variety of reactions. The late 20th century also saw an expansion of the application of analytical chemistry from somewhat academic chemical questions to forensic, environmental, industrial and medical questions, such as in histology. Modern analytical chemistry is dominated by instrumental analysis. Many analytical chemists focus on a single type of instrument. Academics tend to either focus on new applications and discoveries or on new methods of analysis. The discovery of a chemical present in blood that increases the risk of cancer would be a discovery that an analytical chemist might be involved in. An effort to develop a new method might involve the use of a tunable laser to increase the specificity and sensitivity of a spectrometric method. Many methods, once developed, are kept purposely static so that data can be compared over long periods of time. This is particularly true in industrial quality assurance (QA), forensic and environmental applications. Analytical chemistry plays an increasingly important role in the pharmaceutical industry where, aside from QA, it is used in discovery of new drug candidates and in clinical applications where understanding the interactions between the drug and the patient are critical.

2. DRUG PROFILE

2.1 Acotiamide

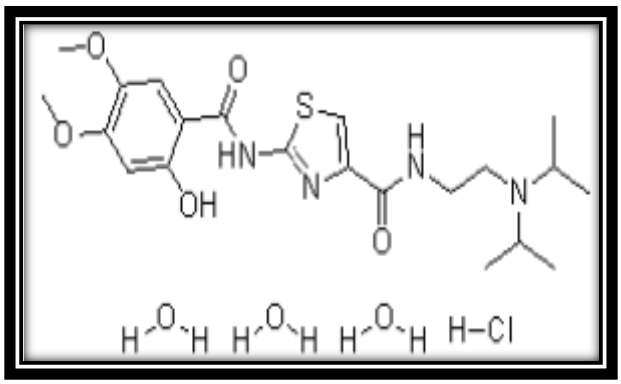

Fig. No. 1: Chemical Strcture of Acotiamide.

2.1.1 Pharmacology

Acotiamide Hydrochloride is the hydrochloride salt form of acotiamide, a prokinetic agent with gastrointestinal (GI) motility-enhancing activity. Although the exact mechanism by which acotiamide exerts its effect has yet to be fully elucidated, this agent appears to inhibit acetylcholinesterase (AchE), an enzyme responsible for the breakdown of acetylcholine (Ach). Increased Ach concentrations lead to an improvement of gastric emptying and GI motility and eventually to a reduction of dyspepsia symptoms.

2.2 Amitriptyline

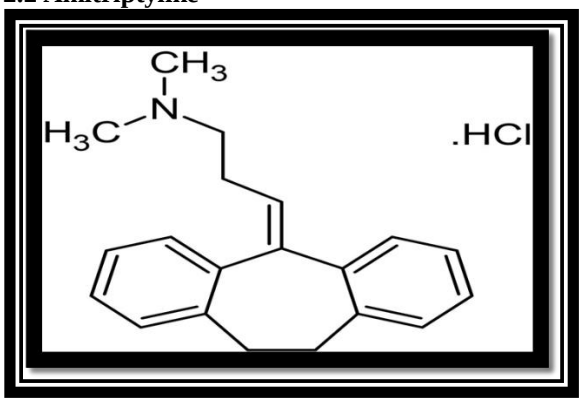

Fig. No 2: Chemical Structure of Amitriptyline

2.2.1 Pharmacokinetics

Amitriptyline is readily absorbed from the gastrointestinal tract (90–95%).[4] Absorption is gradual with the peak concentration in blood plasma reached after about 4 hours.[3] Extensive metabolism on the first pass through the liver leads to average

bioavailability of about 50% (45%[3]-53%[4]). Amitriptyline is metabolized mostly by CYP2C19 into nortriptyline and by CYP2D6 leading to a variety of hydroxylated metabolites, with the principal one among them being (E)-10-hydroxynortriptyline[7] (see metabolism scheme),[4] and to a lesser degree, by CYP3A4.

3. MATERIALS AND METHODS:

3.1 Selection and Procurement of Drug

3.1.1 Name of Drug

i. Acotiamide
ii. Amitriptyline

Table No. 2: List of Reagents and Chemicals used

Sr. No.	Name of chemicals
1.	Acetonitrile (HPLC grade)
2.	Methanol (HPLC grade)
3.	0.05% OPA (HPLC grade)
4.	water (HPLC grade)

4. EXPERIMENTAL WORK

4.1 HPLC:

4.1.1 Selection of Analytical Technique

- HPLC was selected as analytical technique for estimation of Acotiamide and Amitriptyline.

a) Selection of stationary phase:

- The column used in this method C18Agilent The configuration of the column is 4.6 x 250 mm, particle size 5 □m. C18 column gives high non polar retentively, symmetric peak shape, highly reproducible and stable ideal for HPLC method.

b) Solubility Studies:

- This study was carried out to find an ideal solvent in which drugs are completely soluble. Various solvents were tried for checking solubility of Acotiamide and Amitriptyline. From solubility studies it was concluded that of Acotiamide and Amitriptyline is freely soluble in Methanol andpoorly soluble in water PH adjusted 0.05%Orthophosphoric Acid, Buffer pH 3.

c) Chromatographic conditions:

- The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

4.2 UV-VIS Spectrophotometer:

UV-VIS Spectrophotometer was selected as analytical technique for estimation of Acotiamide and Amitriptyline. UV absorbance range of 200-400nm..

4.3 Study on the selection of UV spectrum use in uv-vis spectrometer of Acotiamide and Amitriptyline:

Accurately weigh and transfer 10mg Acotiamide and Amitriptyline working standard into 10 ml volumetric flask as about dilute Methanol prepared in completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and from the resulting solution 0.2 ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with Methanol.

4.4 Study on the chromatographic conditions of Acotiamide and Amitriptyline:

Accurately weigh and transfer 5 mg Acotiamide and Amitriptyline working standard into 20 ml volumetric flaskas about dilute Methanol preparedin completely and make volume up to the mark with the same solvent to get 500µg/ml standard (stock solution) and 15 min sonicate to dissolve it and from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol:(0.05%OPA) Water solvent. The resulting 10µg/ml of solution was subjected to chromatographic analyses using mobile phases of different strengths with chromatographic conditions

4.5 Method Development of HPLC:

4.5.1 Preparation of Stock Standard Solution:

- **Standard Solution Stock I : (Acotiamide and Amitriptyline)**

Accurately weight and transfer 5mg Acotiamide and Amitriptyline,working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 500µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase

4.6 Validation of method for analysis of Acotiamide and Amitriptyline:

- The developed method was validated as per ICH guidelines.

4.6.1 Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyst in samples within a given range,

- **Determination:**

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyst concentrations across the claimed range. Area is plotted graphically as a function of analyst concentration. Percentage curve fittings are calculated**.**

- **Preparation of standard stock solution for linearity:**

Average weight of Tablet sample (equivalent to 5 mg of Acotiamide and Amitriptyline) was weighed and transferred to 10 mL volumetric flask &diluents were added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1-0.5 ml of this solution diluted upto 10 ml volumetric flask with diluents was added to make up the volume.

4.6.2 Accuracy (recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay,

- **Preparation of standard stock solution:**

5 mg of Acotiamide and Amitriptyline working standards were weighed and transferred to 10 mL volumetric flask &diluents was added to make up the volume 0.1 ml of this solution diluted upto 10 ml with diluents.

4.6.3 Repeatability:

Precision of the system was determined with the sample. Two replicates of sample solution containing 15 µg/ml of Acotiamide and Amitriptyline were injected and peak areas were measured and %RSD was calculated it was repeated for two times

4.6.4 Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

4.6.4.1 Intra-day precision:

Sample solutions containing5 mg of Acotiamide and Amitriptyline three different concentration (10µg/ml, 15µg/ml, 20µg/ml) of Acotiamide and Amitriptyline were analyzed three times on the same day and %R.S.D was calculated.

4.6.4.2 Inter-day precision:

Sample solutions containing5 mg of Acotiamide and Amitriptyline were analyzed three times on different concentration (10µg/ml,15µg/ml,20µg/ml) Acotiamide and Amitriptyline different days and % R.S.D was calculated. It is usually expressed as standard deviation or relative standard deviation.

4.6.5. Robustness:

The mobile phase composition was changed in (±1 ml/ min-1) proportion and the flow rate was (Fig No:40,41) of Methanol in the mobile phase composition (±1 ml/ min-1) and the change in detection wavelength (±1 ml/ min-1) and the effect of the results were examined.(Fig No: 42,43) and (Fig No:44,45) it was performed using 20µg/ml solution of Acotiamide and Amitriptyline in triplicate

4.7 Analysis of marketed formulation

To determine the content of Acotiamide and Amitriptyline in marketed Injection, Injection were to 100 mg of Acotiamide and Amitriptyline was weighed. The drug was extracted from the inj. with 100 mL Methanol. To ensure complete extraction it was sonicated for 15 min. 0.2 mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted.

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Acotiamide and Amitriptyline in the sample was calculated. The amount of Acotiamide and Amitriptyline per Tablet was obtained from the regression equation of the calibration curve as described in analysis of Tablet formulation are shown in section 8

- Analysis of marketed formulation were also % Label Claim was found to be 99% Satisfactory are concluded

5. RESULT AND DISCUSSIONS:

5.1. Preliminary studies on Acotiamide and Amitriptyline

5.1.1. Melting point

The procured reference standard of Acotiamide and Amitriptyline was found to melt in the range of 216-2170C respectively.

5.1.2. Solubility

The drug was found to be

- Freely soluble in methanol, acetonitrile, Methanol
- Insoluble in water

5.1.3. UV Spectroscopy

UV absorption of 20 µg/mL solution of Acotiamide and Amitriptyline in methanol was generated and absorbance was taken in the range of 200-400 nm.λmax

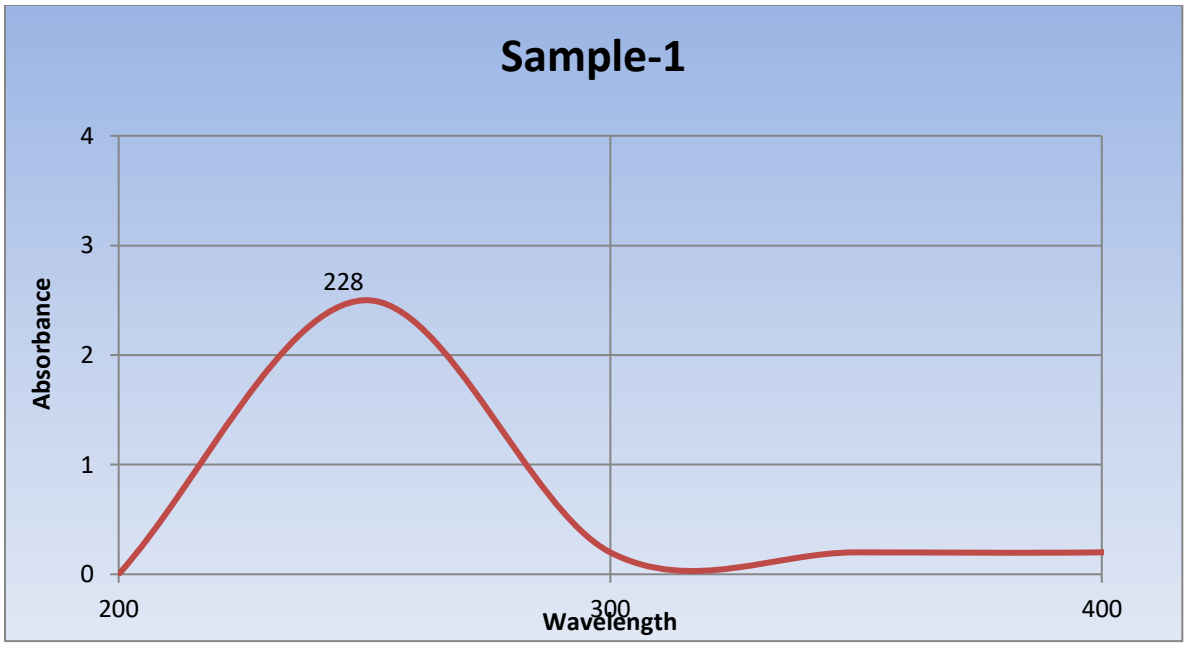

Fig.No. 3 UV spectrum of Acotiamide

Standard solutions were scanned in the range of 200-400nm ,against 10 ml methanol and volume make with methanol solvent system as reference Acotiamide methanol was found to be 228 nm, selected wavelength is 228 nm

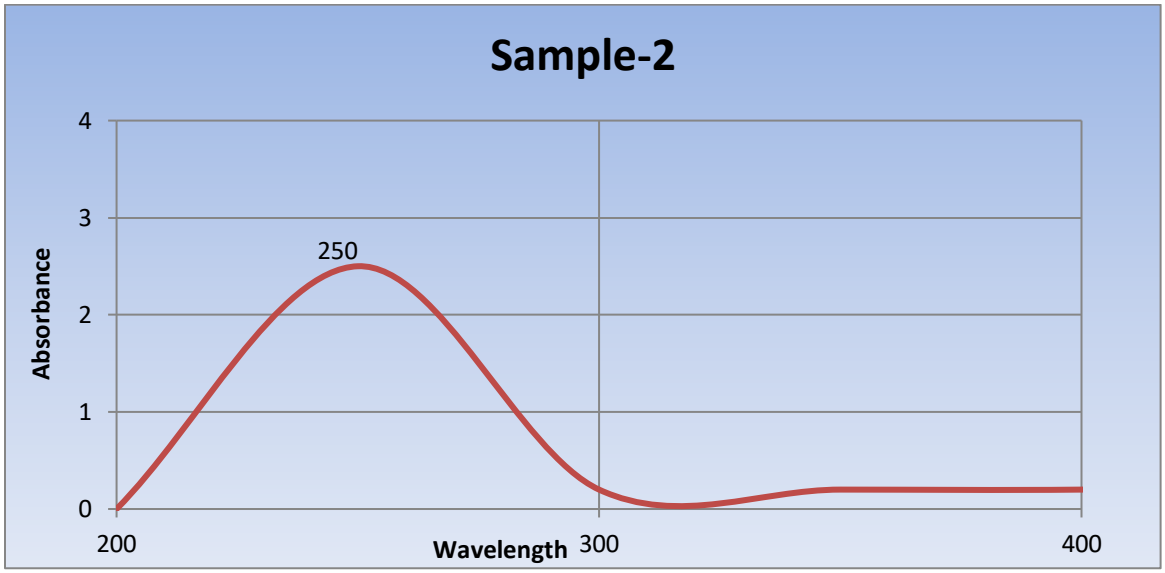

Fig. No 4. UV spectrum of Amitriptyline

Standard solutions were scanned in the range of 200-400nm, against 10 ml methanol and volume make with methanol solvent system as reference Amitriptyline methanol was found to be 250 nm,selected wavelength is 250 nm(Figure No:9)

5.1.4. Studies on the chromatographic behavior of Acotiamide and Amitriptyline

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity & accuracy. The optimized parameters for selected method are as below.

5.1.4.1 Chromatographic Trial of Acotiamide and Amitriptyline

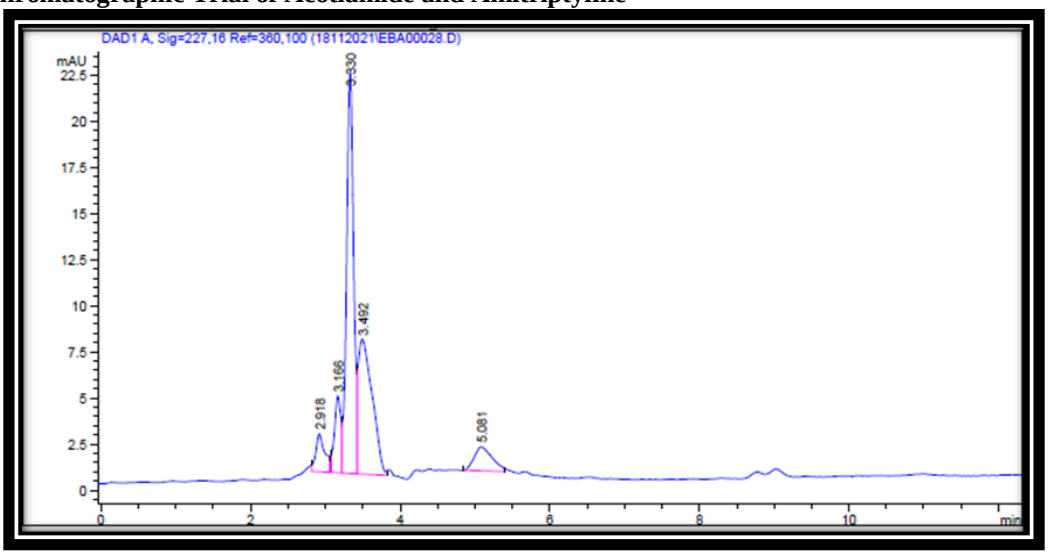

Fig No 5: Chromatogram of Trial I

Table No. 3: Result of Chromatogram of Trial I

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	2.918	17.1340	3174	0.61	0.0000
2	3.330	141.5713	6294	0.97	2.13

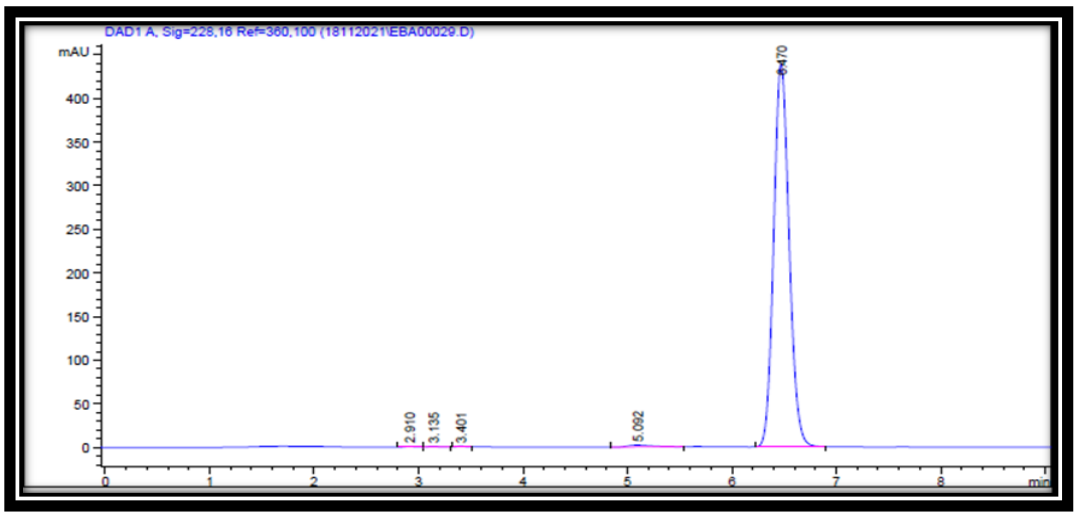

Fig No 6: Chromatogram of Trial II

Table No. 4: Result of Chromatogram of Trial II

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	**6.470**	**4515.3090**	**9448**	**0.86**	**3.73**

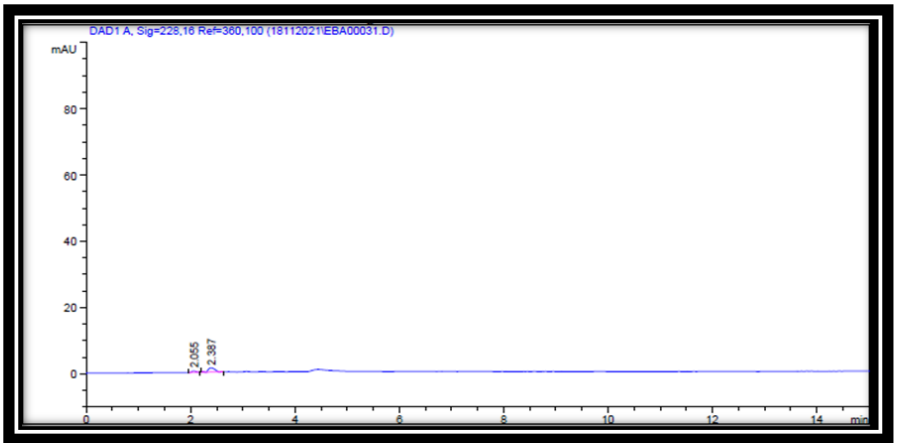

Fig No 7: Chromatogram of Trial III

Table No .5: Result for Chromatogram of Trial III

No.	**RT[min]**	**Area[mV*s]**	**TP**	**TF**	**Resolution**
1	2.055	2.31064	3173	0.61	0.0000
2	2.387	11.89523	1467	0.69	0.33

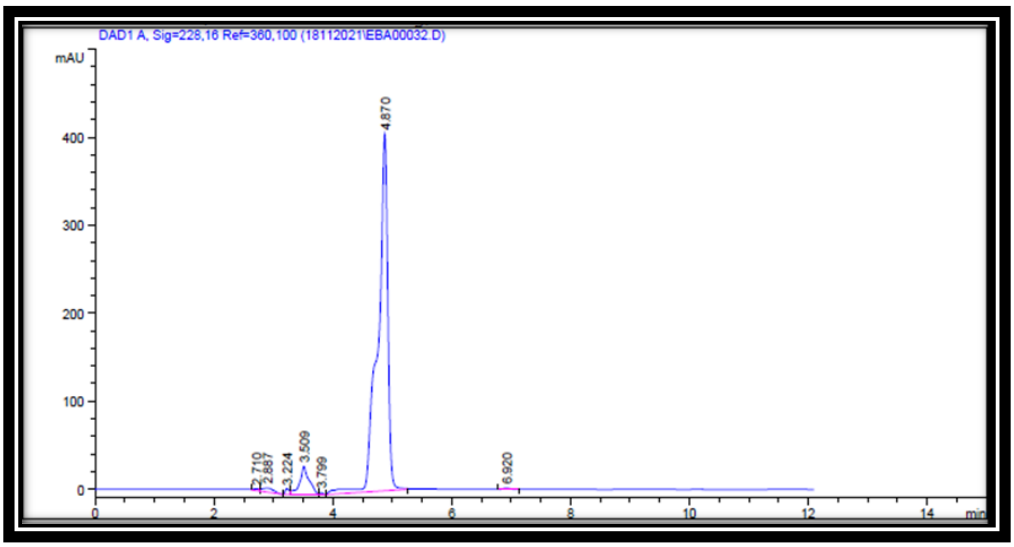

Fig No 8: Chromatogram of Trial IV

Table No .6: Result for Chromatogram of Trial IV

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.509	349.6449	3230	0.77	0.0000
2	4.870	4717.3584	8190	2.25	1.3

5.2. Analytical of Method Validation:

1. Linearity:

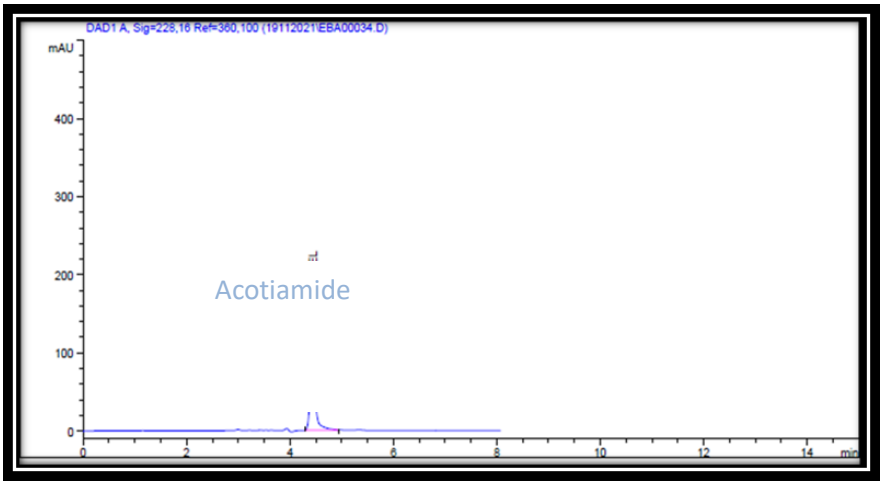

Fig.No.9.Chromatogram of linearity (5 mcg) I

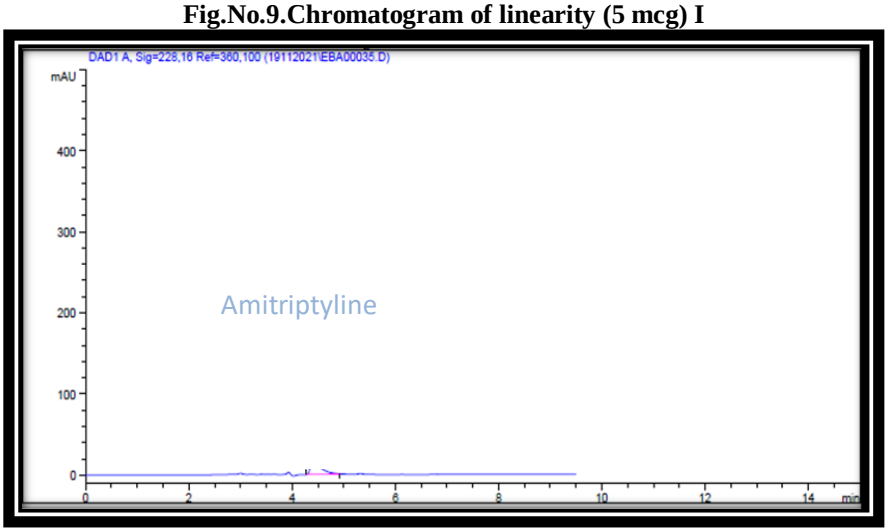

Fig.No.10.Chromatogram of linearity (5 mcg) A

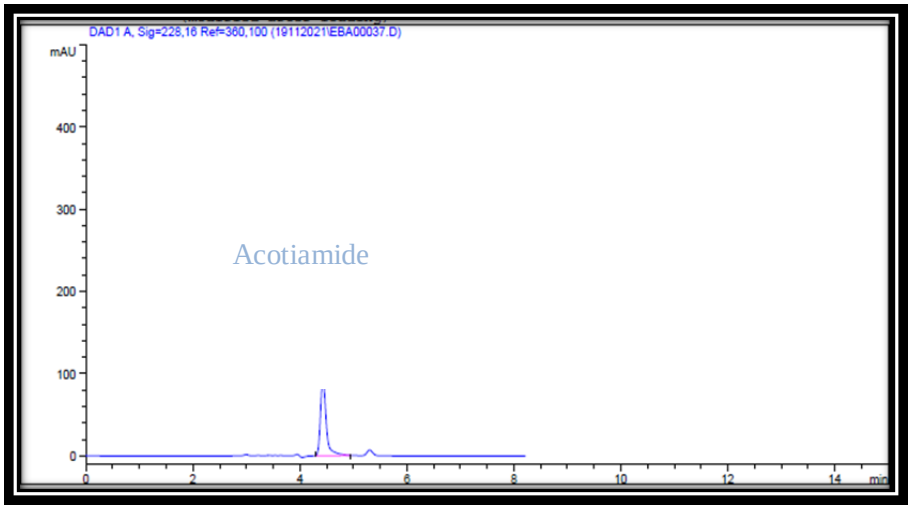

Fig.No.11.Chromatogram of linearity (10 mcg) A

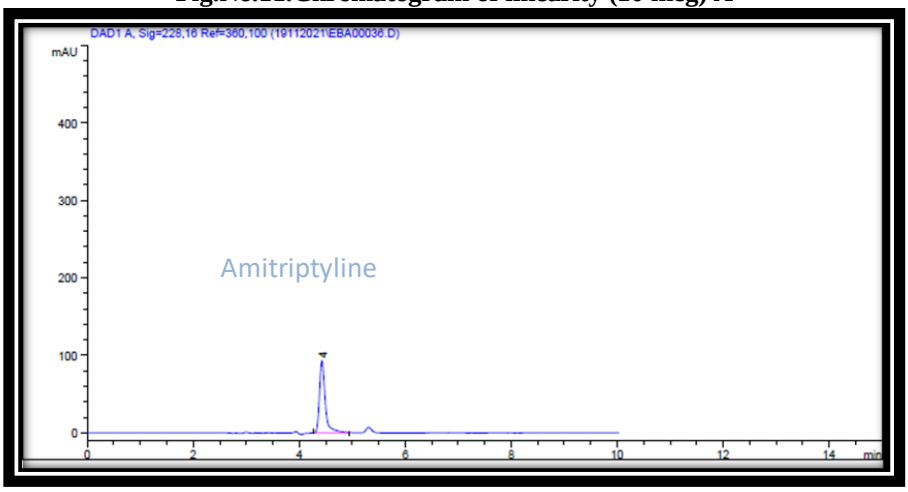

Fig.No.12 Chromatogram of linearity (10 mcg) A

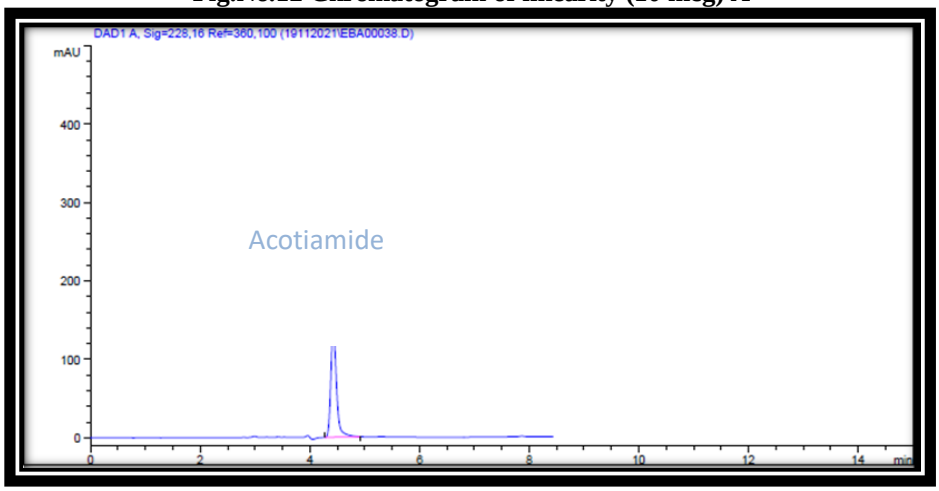

Fig.No.13.Chromatogram of linearity (15 mcg) A

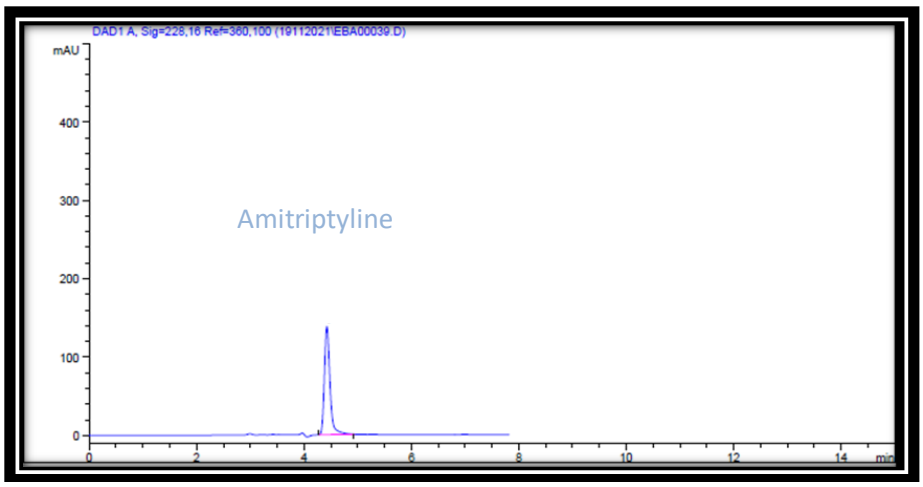

Fig.No.14.Chromatogram of linearity (15 mcg) A

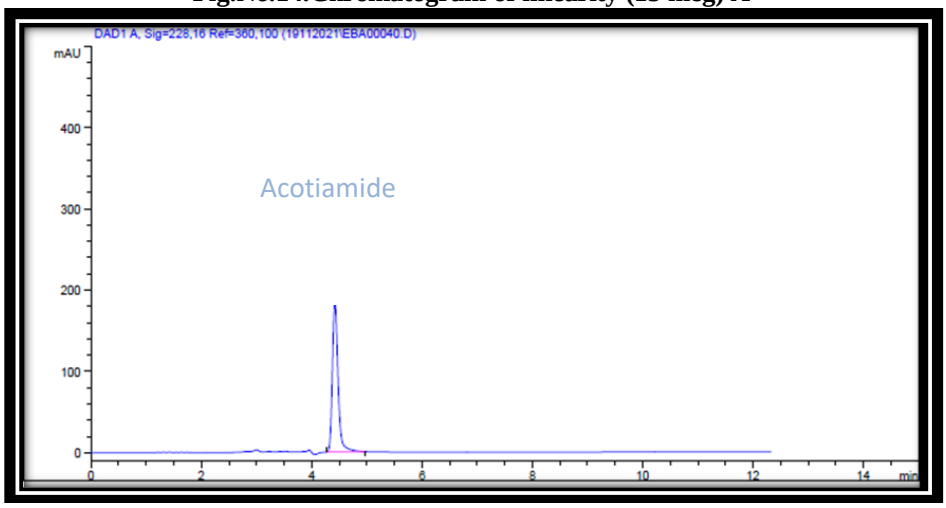

Fig.No.15.Chromatogram of linearity (20 mcg) A

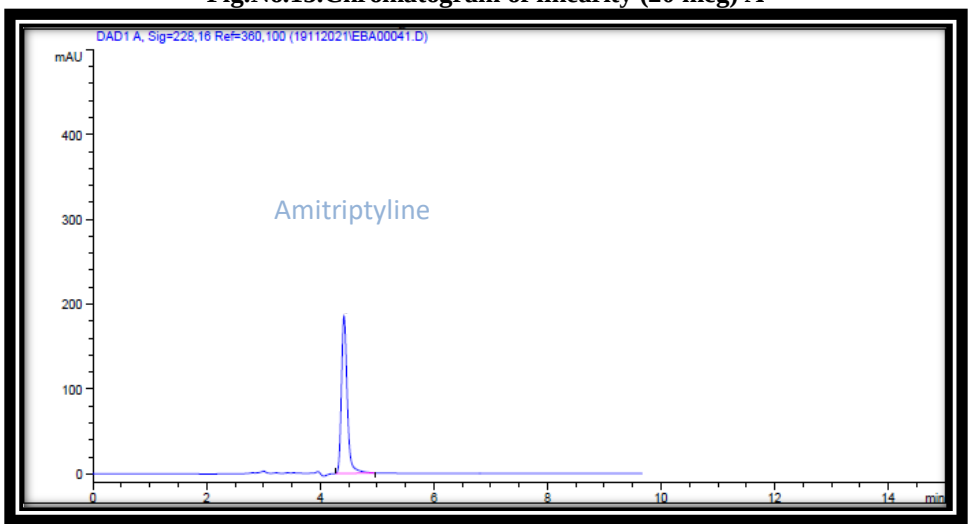

Fig.No.16.Chromatogram of linearity (20 mcg) A

Table No .7: Linearity of Drug

Sr. No.	Concentration µg/ml	Area Acotiamide	Area Amitriptyline
1	5	437.96	435.90
2	10	705.79	702.40
3	15	1005.88	1000.50
4	20	1326.18	1310.80

2. Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Injection solution, a definite concentration of standard drug was added and then its recovery was analyzed (Table No.20). Statistical validation of recovery studies

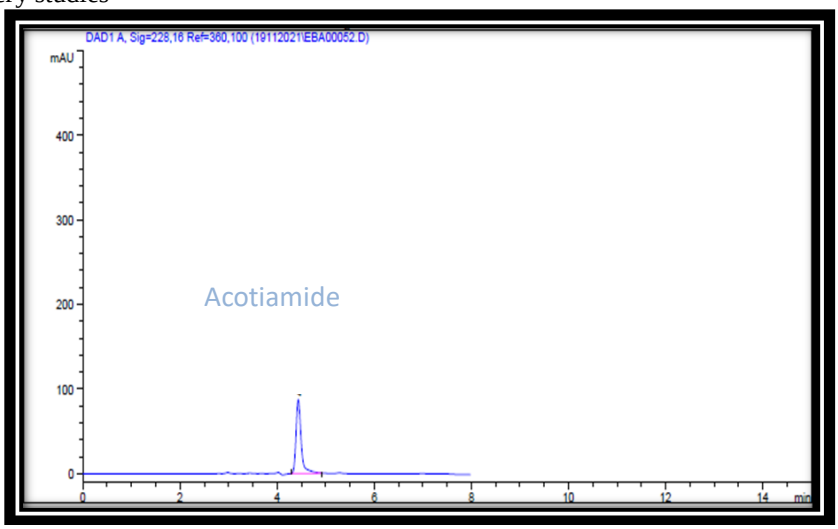

Fig. No.17. Chromatogram of Accuracy

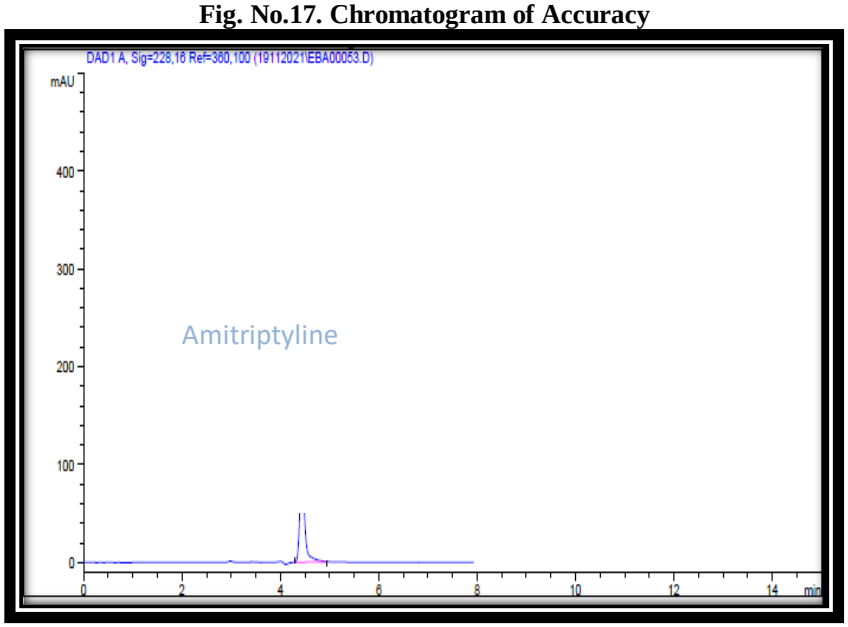

Fig. No 18. Chromatogram of Accuracy

Table No. 8. Result of Recovery data for Acotiamide and Amitriptyline

Drug	Sr No	Level (%)	Amt taken (µg/ml)	Amt. Added (µg/ml)	Area. mean ±S.D	Amt. recovered Mean±S.D	%Recovery Mean±S.D.
Acotiamide	1	80%	5	4	8.9 ± 0.004	3.9 ± 0.004	99.74 ± 0.10
Amitriptyline	2	100%	5	5	9.98 ± 0.026	4.98 ± 0.02	99.53 ± 0.53

3. Precision

Table No. 9: Precision Study

Conc. (µg/mL)	Intra-day %Amount found (µg/mL)		Inter-day %Amount found (µg/mL)	
	Mean ± S.D. (n = 3)	%R.S.D	Mean ± S.D. (n = 3)	%R.S.D
12	99.30 ± 0.61	0.61	99.30± 0.46	0.46
16	99.13 ± 0.52	0.52	98.84 ± 0.60	0.61
20	98.65 ± 0.48	0.49	98.75± 0.36	0.37

4. Repeatability

The six replicates of 16 µg/mL sample solution of Acotiamide and Amitriptyline were analyzed in UV-Spectrophotometer. The concentrations of the drug were calculated from linear regression equation. The %RSD of repeatability study was found to be0.56 i.e. less than 2 which indicate that this passes parameter.

Table No. 10: Repeatability Study

Conc. (µg/mL)	%Amt. found Mean ± S.D. (n = 6)	% R.S.D
16	98.95 ± 0.55	0.56

5. Sensitivity

For the LOD and LOQ 50, 60,70,80,90 and100 µg/mL concentration solutions were analyzed.

Table No. 11: Sensitivity

LOD (µg/mL)	LOQ (µg/mL)
0.142	0.430

6. Ruggedness

Table No. 12: Ruggedness Data

Analyst	% Amount found (n = 6)	% RSD
I	98.95	0.53
II	98.92	0.53

5.3 System Suitability Test

As shown in table, no. of high theoretical plate, peak symmetry and proper retention time indicates that proposed method was suitable for determination Acotiamide and Amitriptyline.

Table No. 13: System Suitability Test

System Suitability Parameters	Proposed Method
Retention Time (t_R)	4.4
Theoretical Plate (N)	13217
Tailing Factor (T)	1.17

6. SUMMARY AND CONCLUSION:

Acotiamide and Amitriptyline prokinetic agent with gastrointestinal (GI) motility-enhancing activity. "Development and validation of RP-HPLC method for estimation of Acotiamide and Amitriptyline in Bulk Drug And it`s Dosage form" Attempts were made to develop RP-HPLC method for simultaneous estimation of Acotiamide and Amitriptyline from Tablet. For the RP - Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector Reverse Phase (Agilent) C18 column (4.6mm x 250mm;5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

Methanol: water (0.05%OPA), (90:10) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 228 nm and flow rate was 0.7ml/min. In the developed method, the retention time of Acotiamide and Amitriptyline was found to be being 4.470 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.

So the proposed methods can be used for the routine quality control analysis Acotiamide and Amitriptyline in bulk drug as well as in formulations.

7. ACKNOWLEDGEMENT

The authors are thankful to the Principal, KYDSCTC'S College of Pharmacy, Sakegaon Maharashtra, India. necessary facilities for research work.

8. CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

9. REFERENCES:

1. Connors AK. A Text Book of Pharmaceutical Analysis. A Wiley Interscience publication, 3rd edition 2005; 373-400.
2. Ahuja S, Ahuja S: High Pressure Liquid Chromatography. Comprehensive Analytical Chemistry. Elsevier; 2006.
3. Amesham Biosciences: Reversed Phase Chromatography. Principles and Methods ; 6-8.
4. Dorsey JG, Cooper WT: Retention mechanisms of bonded-phase liquid chromatography. Anal. Chem. 66th edition 1994; 857A-867A,.
5. Tanford CW : Physical chemistry of macromolecules. 1961.
6. How do I develop an HPLC method. Available from: www.sge.com
7. Snyder LR, Kirkland JJ, Glajch JL: Practical HPLC Method Development. 2nd ed. 2001.
8. Sethi PD: HPLC Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers & Distributors, first edition 2001.
9. Lindholm J: Development and Validation of HPLC Methods for Analytical and Preparative Purposes. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. 2004; 995
10. McCown SM, Southern D, Morrison, B.E. Solvent properties and their effects on gradient elution high performance liquid chromatography. Experimental findings for water and acetonitrile. J. Chromatogr.1986; 352: 493-509.
11. Scott PWR: Liquid Chromatography for the Analyst. New York: Marcel Dekker Inc. 1994:1-10.
12. Stanley BJ, Foster CR, Guiochon G: On the Reproducibility of Column Performance in Liquid Chromatography and the Role of the Packing Density. J. Chrom. A. 1997; 761: 41-51.
13. Christain GD: Analytical Chemistry. John Wiley & Sons Inc, 6th edition 2001.

14. Nledner W, Karsten M, Stelner F, Swart R: Automating Method Development with an HPLC System Optimized for Scouting of Columns, Eluents and Other Method Parameters. Pittcon presentation; 2008.

15. 20. Kar A: Pharmaceutical Drug Analysis. First edition 2001; 565-592.

16. Beckett AH, Stenlake JB: Practical Pharmaceutical Chemistry. CBS Publishers and Distributors, first edition 2002; 157-171.

17. Skoog DA, West DM, Holler FJ, Crouch SR: Fundamentals of Analytical Chemistry. 8th edition 2004; 973.

18. Sethi P D. High Performance Liquid Chromatography. Quantitative Analysis of Pharmaceutical Formulation. Ist Ed. New Delhi: CBC Publication and Distributors; 2001.p.36.

19. Sethi PD. High Performance Liquid Chromatography. Quantitative Analysis of Pharmaceutical Formulations. Ist Ed. New Delhi CBS Publication and Distributors, 2001. p. 60-61.

20. Sethi PD. High Performance Liquid Chromatography. Quantitative Analysis of Pharmaceutical Formulations. Ist Ed. New Delhi CBS Publication and Distributors, 2001. P.116-120.

21. Skoog D, Holler F, Nieman T. Principles of Instrumental Analysis. 5th Ed. New Delhi: Thomson Books; 2006.p.11-16.

22. Cindy Green, RAC. A Step By Step Approach to Establishing a Method Validation. Journal of Validation Technology August 2007; 13(4):p.317-323.

23. D. S. Jain, G. Subbaiah, M. Sanyal and P. Shrivastav, A high through and selective method for the estimation of valproic acid an antiepileptic drug in human plasma by tandem LC- MS/MS, Talanta 72 (2007) 80–88.

24. Guideline on validation of bioanalytical methods, European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP), London 2009;81-83

25. ICH Harmonized Tripartite guideline, validation of analytical procedures: Text and Methodology Q2 (R1) Current Step 4 version, November (2005),5-9

26. Henry TR, The history of valproate in clinical neuroscience, Psychopharmacology Bulletin, 37(2), 2003, supplement 2, 5–16

27. Shiba M, Hashi S, Nakanishi H, Masuda S, Katsura T, and Yano I, Detection of 22 antiepileptic drugs by ultra-performance liquid chromatography coupled with tandum mass spectrometry applicable to routine therapeutic drug monitoring, Biomedical Chromatography, 26, 2012, 1519–1528.

28. LGC, In-House Method Validation: A Guide for Chemical Laboratories, 2003.