EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
621-627
Year: 2018

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ACOTIAMIDE HYDROCHLORIDE HYDRATE IN PHARMACEUTICAL DOSAGE FORM

Vinodbhai D. Ladumor*[1], Geeta Chaudhari[1], Priyanka Shah[1] and Suleman S. Khoja[2]

[1]Department of Quality Assurance, Shivam Pharmaceutical Studies and Research Centre, Anand, Gujarat, India.
[2]Resource Person in pharmaceutical Quality assurance and Audit Compliance, Vapi-396191.

***Corresponding Author: Vinodbhai. D. Ladumor**
Department of Quality Assurance, Shivam Pharmaceutical Studies and Research Centre, Anand, Gujarat, India.

Article Received on 08/03/2018 **Article Revised on 29/03/2018** **Article Accepted on 19/04/2018**

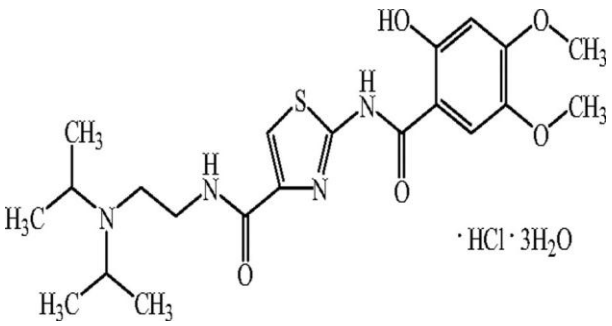
ABSTRACT
A reversed-phase high performance liquid chromatographic method has been developed and validated for estimation of Acotiamide Hydrochloride Hydrate in Pharmaceutical Dosage Form. RP-HPLC method, Column used was 150 x 4.6mm C18, Hypersil BDS with mobile phase containing: Ammonium Acetate (pH 4.5, adjust with 0.1% Tri-ethyl Amine (TEA): Acetonitrile (40:60v/v). The flow rate (1.5 ml/min) and wavelength (315 nm). The retention time was found to Acotiamide Hydrochloride Hydrate was found to be 6.697 ± 0.01 min. Correlation co-efficient for Acotiamide Hydrochloride Hydrate was found to be 0.999. Assay result of marketed formulation was found to be in 99.6 % for Acotiamide Hydrochloride Hydrate. The proposed method was validated with respect to linearity, accuracy, precision and robustness. Percentage recovery for Acotiamide Hydrochloride Hydrate was found to be 99.6 – 100.5%. Analysis proves that the developed method was successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEYWORDS: Acotiamide Hydrochloride Hydrate, HPLC, AMV, ICH,Chromatography.

INTRODUCTION
The IUPAC name of the Acotiamide Hydrochloride Hydrate is N-{2-[Bis (1 methyl ethyl) amino] ethyl}-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]thiazole-4-carboxamide mono hydrochloride trihydrate. With molecular formula and molecular weight.

$C_{21}H_{30}N_4O_5S$. HCl $3H_2O$ and 541.06 g/mol respectively.

The molecular structure of Acotiamide Hydrochloride Hydrate is given in Fig.1.



- Acotiamide Hydrochloride Hydrate is used as Functional dyspepsia (FD).

However no HPLC method has been reported till date for the estimation of Acotiamide Hydrochloride Hydrate using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Acotiamide Hydrochloride Hydrate in Pharmaceutical dosage form using RP-HPLC. The proposed method are optimized and validated as per ICH guidelines.

MATERIALS AND METHODS
Materials
a) Instruments
- Analytical Weighing Balance
- Sonicator
- Micro Balance
- FT-IR spectrophotometer
- HPLC system
- Vacuum Filtration Assembly
- pH Meter
- UV Spectrophotometer
- Vacuum Oven

b) Glassware's
- Beaker
- Conical flask

- Measuring cylinder
- Pipette
- Volumetric flask

c) Chemicals

- Standard Acotiamide Hydrochloride Hydrate Gifted by Amneal Pharmaceutical India Pvt Limited, Ahmedabad. Gujarat,
- The commercial fixed dose **Actapro 100 mg** Tablets 10's manufactured by Lupin Pharmaceutical Ltd. And Marketed By of Sun Pharma Laboratories Ltd was procured from local market. All solvents (HPLC grade) were obtained from S.D. fine chemical.

d) Method

- RP-HPLC Chromatographic method.

Methods

Working Standard preparation

- **Solution Preparation of Acotiamide Hydrochloride Hydrate: (100 µg/ml)**
  About 10 mg of Acotiamide Hydrochloride Hydrate API was weight and dissolve in 100 ml of Water with 15 minutes sonication.
- **Sample Preparation for marketed formulation**
  Transferred 5 tablets in to 250mL volumetric flask and added 170-175 mL of water, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablets were checked visually if they got dispersed and then volume was made up to mark with water and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate was transferred to 100mL of volumetric flask and volume was made up to mark with water. (ACT-100ppm)

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters

Mode of chromatography: Reversed Phase Chromatography.
Mode of Elution: Isocratic.
Flow Rate: 1.5 ml/min.
Oven: Oven Temperature: 25° ± 2°C.
Detector: Type: UV detector.
Wavelength: 315nm.
Column: 150 x 4.6mm C18, Hypersil BDS.
Sample Volume: 20µl.
Run time: 10 min.
Mobile Phase: Ammonium Acetate (pH 4.5, adjust with 0.1% Tri-ethyl Amine (TEA): Acetonitrile (40:60 %v/v).

System Suitability Parameters

Table 1: System Suitability Test Parameters for Acotiamide Hydrochloride Hydrate.

Sr. No.	System suitability parameter	Acotiamide Hydrochloride Hydrate
1	Retention time (min)	6.693
2	Resolution (R)	-
3	Theoretical plate number (N)	14364
4	Tailing factor (T)	1.0

Linearity and Range (n=3)

- The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.
- The range of analytical method is the interval between the upper and lower levels of analytes that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.
- The linearity was determined at five levels over the range of 50-150 µg/ml for Acotiamide Hydrochloride Hydrate. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (y axis) versus Concentration (x-axis) was plotted.

PRECISION

Repeatability

Six replicate of 100ug/ml concentration of Acotiamide Hydrochloride Hydrate were prepared and chromatographic were recorded at the optimized condition. SD and RSD were calculated.

Intraday Precision and Interday Precision

Variations of results within the same day (intra-day), variation of results between days (inter-day) were analyzed. Intra-day precision was determined by analyzing both standard solutions for three times in the same day. Interday precision was determined by analyzing the drugs daily for three days. %RSD was calculated.

Accuracy (% Recovery)

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 5 tablet powder were weighed and analysis was carried out as per assay. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard

deviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.
LOD = 3.3 × σ /s
LOQ = 10 × σ /s

Robustness
The robustness of the method was established by making deliberate minor variations in the following method parameter
a) Flow rate: ±0.4 ml/min
b) Change in the ratio of component in the mobile phase: ± 4%.
c) pH of mobile phase: ±0.4

RESULT
Validation Parameter
Linearity and Range
Linear correlation was obtained between peak area and concentration of Acotiamide Hydrochloride Hydrate in the range of 50-150 µg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r).

Table 2: Linearity data for Acotiamide Hydrochloride Hydrate.

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	50.0	2456353	0.9999
80	80.0	3936338	
100	100.0	4922869	
120	120.0	5907264	
150	150.0	7416583	

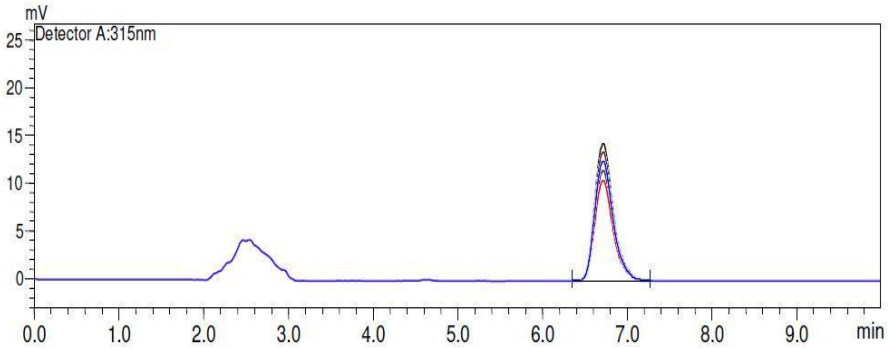


Figure 2: Overlay chromatogram Acotiamide Hydrochloride Hydrate.

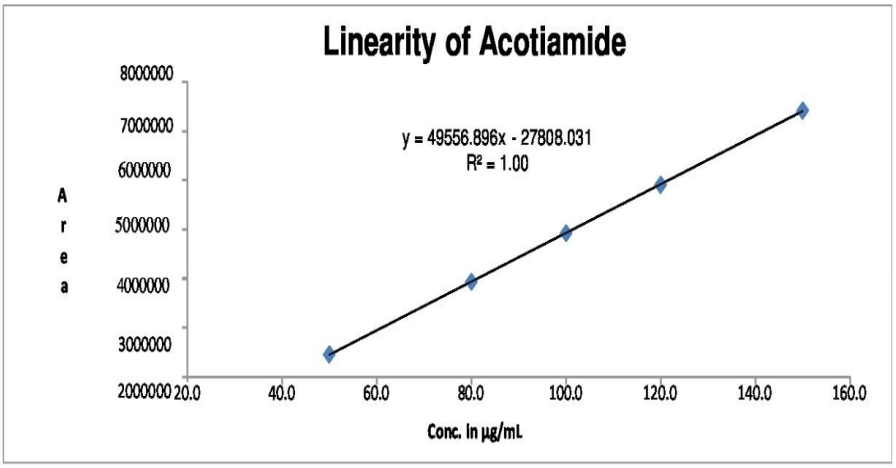


Figure 3: Calibration curve of Acotiamide Hydrochloride Hydrate.

ACCURACY
Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Acotiamide Hydrochloride Hydrate was found to be 99.6 – 100.5 %.

Table 3: Recovery Data of Acotiamide Hydrochloride Hydrate.

Accuracy Level %	Set no.	Amount Added (mg)	Amount Recovery (mg)	% Recovery	Mean	% RSD
80	1	400.00	395.87	99.0	99.6	0.8
	2	405.00	407.07	100.5		
	3	395.00	392.40	99.3		
100	1	505.00	510.43	101.1	100.5	0.8
	2	500.00	503.68	100.7		
	3	497.50	495.27	99.6		
120	1	602.50	600.66	99.7	99.8	0.7
	2	605.00	599.63	99.1		
	3	599.00	601.90	100.5		

PRECISION

Repeatability (Method precision, n=6)

Repeatability

The data for repeatability of Acotiamide Hydrochloride Hydrate is shown in Table 4.The % RSD for Repeatability data was found to be 0.3%

Table 4: Repeatability of Acotiamide Hydrochloride Hydrate.

Sr. no	Area	Mean	SD	% RSD
1	4895214	4896562	14602.4	0.3
2	4889564			
3	4875236			
4	4895552			
5	4905236			
6	4918569			

Intraday precision

The data for intraday precision for Acotiamide Hydrochloride Hydrate is shown in Table-5. The % RSD For intraday precision was found to be 0.233%.

Table 5: Intraday precision for Acotiamide Hydrochloride Hydrate (n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50	2446226±5634.5	0.23
2	100	4893755±10170.2	0.21
3	150	7372720±19477.6	0.26
Mean			0.233

Table 8: Change the flow rate.

Standard repetitions (n=6)	1.3 mL/min	1.7 mL/min
	ACT	ACT
Mean Area ± SD	5373980±26830	4447712±24372
% RSD	0.5	0.5

Table 9: Change the mobile phase composition

Standard repetitions (n=6)	38:62	42:58
	ACT	ACT
Mean Area ± SD	4916958±20834	4918241±43185
% RSD	0.4	0.9

Interday precision

The data for Interday precision for Acotiamide Hydrochloride Hydrate is shown in Table-6. The % RSD For intraday precision was found to be 0.80%.

Table 6: Interday precision for Acotiamide Hydrochloride Hydrate (n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50	2454495±17658.3	0.72
2	100	4913262±37777.5	0.77
3	150	7401025±68654.2	0.93
Mean			0.80

Limit of Detection And Limit of Quantification

The Limit of detection (LOD) and Limit of quantitation (LOQ) of Acotiamide Hydrochloride Hydrate as mention below Table -7.

Table 7: Results of LOD and LOQ.

Drug	Acotiamide Hydrochloride Hydrate
LOD	0.689
LOQ	2.088

ROBUSTNESS

Robustness

The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, Mobile phase pH and flow rate of the mobile phase.

Table 10: Change the mobile phase pH.

Standard repetitions (n=6)	4.3	4.7
	ACT	ACT
Mean Area ±SD	4902679±13249	4901544±16502
% RSD	0.3	0.3

System Suitability tests

Table 11: System Suitability Test Parameters Fot Acotiamide Hydrochloride Hydrate.

Sr.No.	System suitability Parameter	ACT
1	Retention time (min)	6.693
2	Resolution (R)	-
3	Theoretical plate number (N)	4896562
4	Tailing factor (T)	1.0

Assay preparation (Marketed formulation)
Label claim: ACT-100mg.

Sample stock solution
Transferred 5 tablets in to 250mL volumetric flask and added 170-175 mL of water, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablets were checked visually if they got dispersed and then volume was made up to mark with water and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate was transferred to 100mL of volumetric flask and volume was made up to mark with water (ACT-100mcg/ml).

Working sample preparation
Take 1ml from sample stock solution into a 10ml and make up with mobile phase. (ACT-100 mcg/ml).

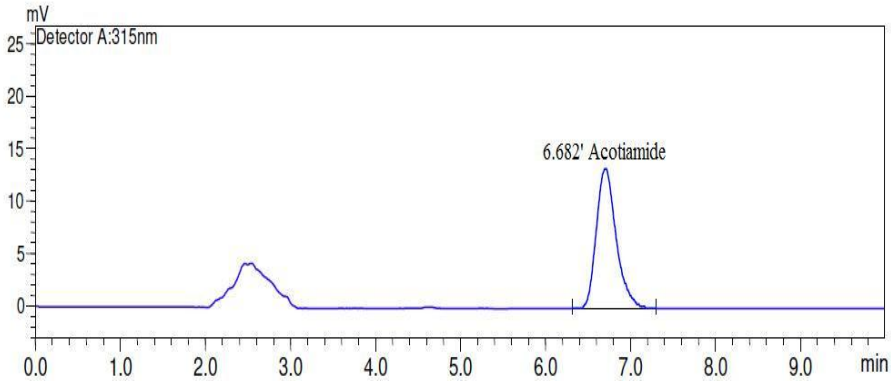


Figure 4: Injection of marketed formulation.

Peak Table
Table 12: Injection of marketed formulation

Sr. No	Peak name	Retention time	Area	Tailing factor	Theoretical Plates	Resolution time
1	Acotiamide	6.682	4876041	1.0	7785	-

Observations
In formulation sample preparation, main peak is found symmetrical and good in shape.

% Assay Results from Formulation
Table 13

Sr. No.	Sample name	% Assay of ACT
1	ActaPro (Acotiamide Hydrochloride Hydrate 100mg) Tablet	99.6%

SUMMARY OF REGRESSION PARAMETERS
Table 14: Summary of Regression Parameters for Acotiamide HCl Hydrate.

Sr. No.	Parameters	Acotiamide HCl Hydrate	REMARK
1	Linearity (µg/ml)	50-150 µg/ml	Linear
2	%Recovery	99.6-100.5	Accurate
3	Precision (%RSD)	0.3%.	Precise (%RSD < 2)
	Repeatability (n=6)		
	Intra-day (n=3)	0.23%	
	Inter-day (n=3)	0.80%	
4	LOD (µg/ml)	0.689	Sensitive
5	LOQ (µg/ml)	2.088	Sensitive
6	Specificity	Specific	Specific (No interference)
7	Robustness	Robust	(No difference in result)

DISCUSSION
A simple, accurate and precise RP-HPLC method for the simultaneous estimation of Acotiamide Hydrochloride Hydrate in Pharmaceutical Dosage form has been developed and validated. Ammonium Acetate (pH 4.5, adjust with 0.1% Tri-ethyl Amine(TEM):Acetonitrile (40:60 % v/v) Separation of drugs was carried out using mobile phase at 10 min. run time and 315nm. The Rt value for Acotiamide Hydrochloride Hydrate were found to be 6.697 ± 0.01 min.

The drug response with respect to peak area was linear over the concentration range 50-150µg/ml Acotiamide Hydrochloride Hydrate. The percentage recovery of Acotiamide Hydrochloride Hydrate was found to be 99.6-100.5%

The %RSD values for intra-day precision study and inter-day study were ≤ 2.0%, confirming that the method was sufficiently precise. The limit of detection and limit of quantitation were found to be 0.689 µg/ml and 2.088µg/ml for Acotiamide Hydrochloride Hydrate.

The %RSD values of Robustness study were ≤ 2.0%, confirming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of the sample. Interference studies reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed method.

So it is concluded that the developed method is specific. The system test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the determination of Acotiamide Hydrochloride Hydrate in pharmaceutical dosage form.

CONCLUSION

A simple, economic, specific and robust RP-HPLC method has been developed and validated for the estimation of Acotiamide Hydrochloride Hydrate in pharmaceutical dosage form. There was no interference from any excipients in the determination of drugs in tablets which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2(R1) guideline so we can conclude that method is Specific, Linear, Accurate and Precise. Hence it can be successfully used for the routine analysis of Acotiamide Hydrochloride Hydrate in pharmaceutical dosage form.

ACKNOWLEDGEMENT

Words of gratefulness are expressed for God; My family; Dr. Harish Kakrani, Mrs. Geeta Chaudhari;, Dr Hatel Solanki, Dr. Upma Trivedi, Mrs Zankhana Sheth, Ms. Priyanka Shah, Ms Devshree Parmar, Ms Seena Patel, Ms. Hinal Patel of Shivam pharmaceutical studies and research centre, Anand, Gujarat, India; All Other Departmental Staff members of Shivam pharmaceutical studies and research centre, I very thankful to S. Kant Healthcare Ltd. Vapi(Gujarat) For providing training and project work support. I am very Thankful to Mr. Suleman S. Khoja for always helping in research article related material and during development phase of research program. I am very much thankful to Mr. krunal parikh, Amneal Pharmaceuticals India Pvt Limited, Ahmedabad for Providing Gift sample of Drug Molecule. Last but not least would like to thank Gujarat Technological University, Ahmedabad for providing a platform to develop student with expert advice in Research Week Program.

REFERENCES

1. Skoog DA, "Introduction to UV Spectroscopy in, Principle of instrumental analysis", Thomson Brooks/Cole publication, 5th, 301: 893-934.
2. ICH Q1A(R2) Stability Testing of new Drug Substances and Products.
3. Guidance for industry, analytical procedures and method validation U.S. Department of Health and human Services FDA, August, 2000. (www.fda.gov/guidance/index.htm).
4. Blessy M, Patel R D, Prajapati P N, Agawam Y K, "Development of forced degradation and stability indicating studies of drugs" *J. of pharmaceutical analysis*, 2014: 4(3): 159-165.
5. Siriki PK, "method validation process and parameters" pharma. information. (pharmainfo.net)
6. Validation of Analytical Procedures: Methodology, ICH Guideline, 1996; Q2(R1): 1-8.
7. Chauhan A., Mittu B. and Chauhan P. "Analytical Method Development and Validation: A Concise Review" Journal of Analytical & Bioanalytical Techniques, 2015.
8. Dr. Kasture AV., Wadodkar SG, Mahadik KR., and More HN. Introduction to Instrumental Techniques; 12th Edn, Nirali Prakashan, Pune, 2002.
9. Skoog DA., Holler JE., and Crouch S. Principles of Instrumental Analysis; 6th Edn; Thomson Brooks Cole. Belmont, CA, 169–173.
10. Kalra K., "Method Development and Validation of Analytical Procedures" Dev Bhoomi Institute of Pharmacy and Research, Dehradun, Uttarakhand, India, 2011.
11. ICH Q2 (R1) Validation of Analytical Procedure: Text & Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.
12. Raghav R, JosepH JC, "chromatographic methods of analysis: high performance liquid chromatography." Encyclopedia of pharmaceutical technology. USA, 2007; 526-537.
13. Dr. Sankar SR. Text book of pharmaceutical analysis; 4th Edn; Rx Publications, Tirunelveli, India, 2010; 1-2.
14. Acotiamide drug profile: http://www.drugbank.ca/drugs/DB12482
15. Chemistry Dashboard | Acotiamide hydrochloride: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=VQEKQYLTAIVCBWT
16. Report on the Deliberation Results February 15, 2013, https://www.google.co.in/url?q=https://www.pmda.go.jp/files/000153467.pdf&sa=U&ved=0ahUKEwjV4pGOx9PWAhWPL1AKHY7CC1oQFggtMAA&usg=AOvVaw28nIgPJlyWa8MFwhAnqYaf
17. Acotiamide hydrochloride hydrate drug profile: http/info.pada.go.jp/

18. Available Brands in Market of this Drug: https://www.medplusmart.com/compositionProducts/Acotiamide-100 MG/30426
19. Mary L.Nowlan,Lesley J.Scott, ”Erratum to:Acotiamide first Global Approval, Springer International Publishig, Switzerland, 2014; 74: 1059.
20. Acotiamide Hydrochloride drug profile: https://pubchem.ncbi.nlm.nih.gov/compound/Acotiamide_HCl#section = NLM-Curated-PubMed-Citations
21. Khoja SS, Khoja S, Chauhan PH, Khoja FS;A review on USFDA warning letter and violation observed in Pharmaceutical Industry; Pharma Tutor, 2016; 4(12): 33-36.
22. Drug Profile: Acotiamide Hydrochloride Hydrate - pharmacodia.com https://www.pharmacodia.com/yaodu/html/v1/chemicals/d2350a54d774001d6078e326b4488878.html
23. Khoja S. S., Chauhan P. H., Patel M. N. Jani H. D. A Review on High Performance Liquid Chromatography Analytical method development. J Pharm Sci Bioscientific Res., 2016; 6(5): 6.