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

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Abstract:
A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for Acotiamide HCl Hydrate In its Pharmaceutical Dosage Form has been developed.
A reverse phase high performance liquid chromatographic method was developed for the Acotiamide HCl Hydrate in its Pharmaceutical Dosage Form has been developed. The separation was achieved by Hypersil BDS C18 (25 cm × 0.46 cm) 5µ, column and Water
(pH 4.5): Methanol: TEA (45:55:0.1) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 222 nm. Retention time of Acotiamide HCl Hydrate was found to be 4.420 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Acotiamide HCl Hydrate 5-15 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Acotiamide HCl Hydrate in its Combined Dosage Form.
The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in Thermal degradation. The proposed method was successfully applied for the estimation of its dosage form.
Keywords: *Acotiamide HCl Hydrate, Stability indicating RP-HPLC Method, Validation.*

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INTRODUCTION:

Acotiamide is *N*- [2- [bis (1-methylethyl) amino] ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl) amino] thiazole-4-carboxamide monohydrochloride trihydrate. It is used in the treatment of Functional Dyspepsia.

Acotiamide Hydrochloride hydrate is a drug which belongs to class of Acetylcholinesterase Inhibitor. Acotiamide Hydrochloride is the hydrochloride salt form of acotiamide, a prokinetic agent with gastrointestinal (GI) motility-enhancing activity. The mechanism by which acotiamide exerts its effect is, by inhibiting 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.

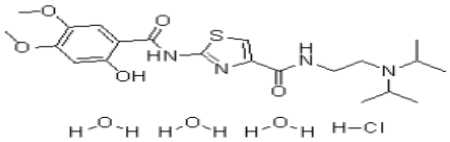

Fig.1: Chemical Strcture of Acotiamide.

From the literature survey it was observed that Two LC-MS methods are reported for the analysis of Acotiamide HCl Hydrate in the Clinical Trial Phase, but no Stability indicating HPLC method reported for this drug in Pharmaceutical dosage form. Therefore, it was thought worthwhile to develop stability indicating-HPLC Method for the Estimation of Acotiamide HCl Hydratein its Pharmaceutical Dosage form.

MATERIAL AND METHOD:

Acotiamide was received from MSN Laboratories, Hyderabad. And other chemicals such as Methanol, Water, HPLC Grade, Acetonitrile, Tri ethyl amine, AR Grade was purchased from Merck specialties pvt, Ltd., Mumbai and used.

Apparatus and Equipment's used in experiment:

Apparatus / Equipment's:

Table1: List of equipment

Components	Volume	Type
Volumetric flasks	10 ml, 25 ml, 50 ml,100 ml	Borosilicate glass type I
Pipettes	1 ml, 2 ml, 5 ml, 10 ml	Borosilicate glass type I
Measuring cylinder	100 ml	Borosilicate glass type I
Beaker	100 ml, 250 ml, 500 ml	Borosilicate glass type I
Whatman Filter	-	Filter Paper No.42

Instrumentation for HPLC

Table 2: Instrumentation of HPLC

Component	Brand / Model / Software	Manufacturer/ Supplier
HPLC	Shimadzu LC-20 AT	Shimadzu
HPLC Column	C18 (25cm x 0.46 cm, 5µm) Hypersil BDS	Waters
Detector	UV	Shimszdu
Ultrasonic Water Bath	Fast Clean	Ultrasonic cleaner
pH meter	-	Electro quip's Digital pH meter

Instrumentation for UV spectrophotometer

Table 3: Instrumentation of UV spectrophotometer

Component	Brand / Model / Software	Manufacturer/ Supplier
UV Visible spectrophotometer	Shimadzu 1800 double beam UV visible spectrophotometer, UV probe 2.33	Shimadzu Corporation, Kyoto, Japan
Cuvette	Quartz cuvette	Shimadzu Corporation, Kyoto, Japan
Analytical Balance	AUX-200	Analab

Experimental Work:

Table 4: RP-HPLC optimized chromatographic conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Water (pH 4.5): Methanol: TEA (45:55:0.1)
Column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	6 min
Injection volume	20 µL
Detection wavelength	222 nm

Preparation of standard solution of Acotiamide HCl Hydrate (10 ppm).

Acotiamide HCl Hydrate Standard stock solution: (100 µg/mL)

A 10 mg of Acotiamide HCl Hydrate was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase.

Preparation of Working Standard solution of Acotiamide HCl Hydrate (10 µg/mL)

Take 1 mL from the Acotiamide HCl Hydrate stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used trials.

Acotiamide HCl Hydrate Sample stock solution: (100 µg/mL)

Take tablet Powder equivalent to 10 mg of Acotiamide HCl Hydrate was weighed and transferred to a 100 mL volumetric flask. Add 60 ml Mobile Phase and shake for 15 min and volume was make up to the mark with mobile phase. The solution was filtered through Whatman filter paper no.42

Preparation of Working Sample solution of Acotiamide HCl Hydrate (10 µg/mL)

Take 1 mL from the Acotiamide HCl Hydrate stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase.

RESULT AND DISCUSSION:

Method Development:

No of mobile Phase and their different proportions were tried and finally was selected as Water (pH 4.5): Methanol: TEA (45:55:0.1) which give good resolution, acceptable system suitability parameter. The result of system suitability parameter given in table no 5 and optimized chromatographic condition given in table no:4

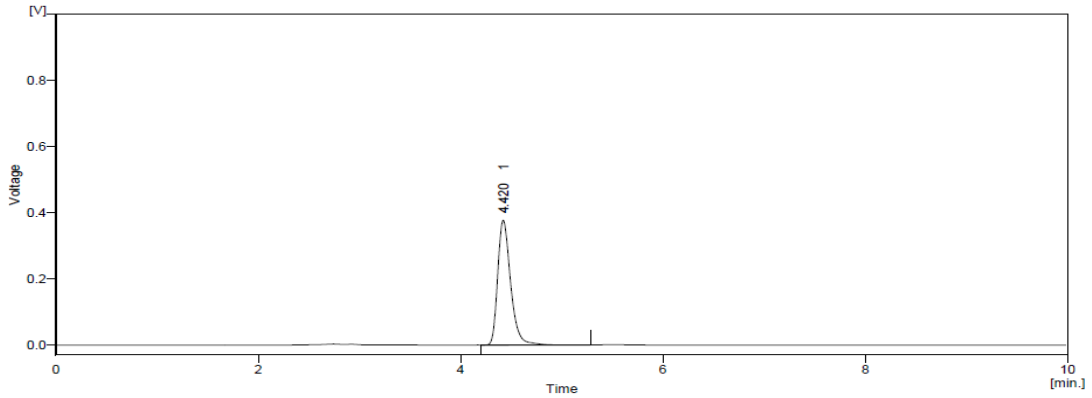

Fig. 2: Chromatogram of Acotiamide HCl Hydrate 10 ppm in water (pH 4.5): Methanol: TEA :(45:55:0.1) (Flow rate:1.0 ml/min)

Table 5: system suitability Parameter.

Parameters	Acotiamide HCl Hydrate
Retention Time	4.420
Theoretical Plates	6088
Symmetry Factor	1.516

Method Validation:

Accuracy: For Acotiamide HCl Hydrate

5 µg/ml drug solutions were taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Acotiamide HCl hydrate was calculated at each level and % recoveries were computed.

Table 6: Recovery data for Acotiamide HCl Hydrate

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S. D
1	80 %	5	4	3.955	98.867	100.089 ± 1.127
2		5	4	4.043	101.086	
3		5	4	4.013	100.315	
4	100 %	5	5	4.957	99.134	99.765 ± 0.648
5		5	5	5.021	100.428	
6		5	5	4.987	99.733	
7	120 %	5	6	6.013	100.223	99.742 ± 0.491
8		5	6	5.955	99.242	
9		5	6	5.986	99.760	

Precision

I. Repeatability

The data for repeatability of peak area measurement for Acotiamide HCl Hydrate (10 µg/ml) based on six measurements of same solution of Acotiamide HCl Hydrate (10 µg/ml). The % RSD for Acotiamide HCl Hydrate was found to be 0.488

Table 7: Repeatability data for Acotiamide HCl Hydrate

Acotiamide HCl Hydrate				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S. D
1.	10	3276.127	3275.72±15.999	0.488
		3281.194		
		3289.861		
		3273.166		
		3288.090		
		3245.882		

II. Intraday precision

Standard solution containing (5,10,15 µg/ml) of Acotiamide HCl Hydrate were analyzed three times on the same day and % R.S.D was calculated

Table 8: Intraday precision data for estimation of Acotiamide HCl Hydrate

	Acotiamide HCl Hydrate		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S. D
1	5	1607.936 ± 23.171	1.144
2	10	3262.256 ± 26.219	0.804
3	15	4896.893± 23.384	0.477

III. Interday precision

Standard solution containing (5,10,15 µg/ml) of Acotiamide HCl Hydrate were analyzed three times on the different day and % R.S.D was calculated

Table 9: Interday precision data for estimation of Acotiamide HCl Hydrate

	Acotiamide HCl Hydrate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S. D**
1	5	1610.194 ± 21.841	1.356
2	10	3266.893± 14.352	0.439
3	15	4890.796± 26.570	0.543

Linearity:

The linearity for Acotiamide HCl Hydrate was assessed by analysis of combined standard solution in range of 5-15 µg/ml.5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Acotiamide HCl Hydrate (100 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml for Acotiamide HCl Hydrate.

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve for Acotiamide HCl Hydrate was found 0.999.

The regression line equation for Acotiamide HCl Hydrate is

For Acotiamide HCl Hydrate **y = 320.0x + 33.54**

Table 10: Linearity data for Acotiamide HCl Hydrate

Sr. No	Concentration (µg/ml)	Area
1	5	1630.946
2	7.5	2407.569
3	10	3291.461
4	12.5	4009.444
5	15	4830.575

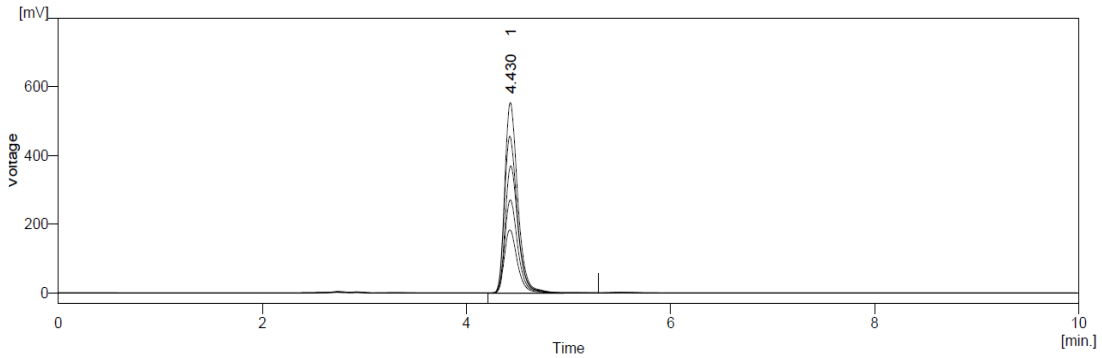

Fig. 3: Overlay chromatogram of different concentrations of Acotiamide HCl Hydrate

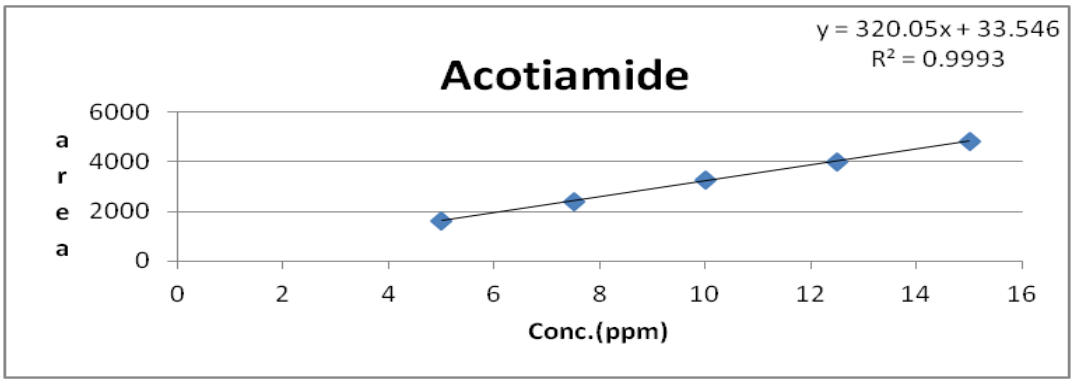

Fig. 4: Calibration Curve of Acotiamide HCl Hydrate (5-15 µg/ml).

Specificity:

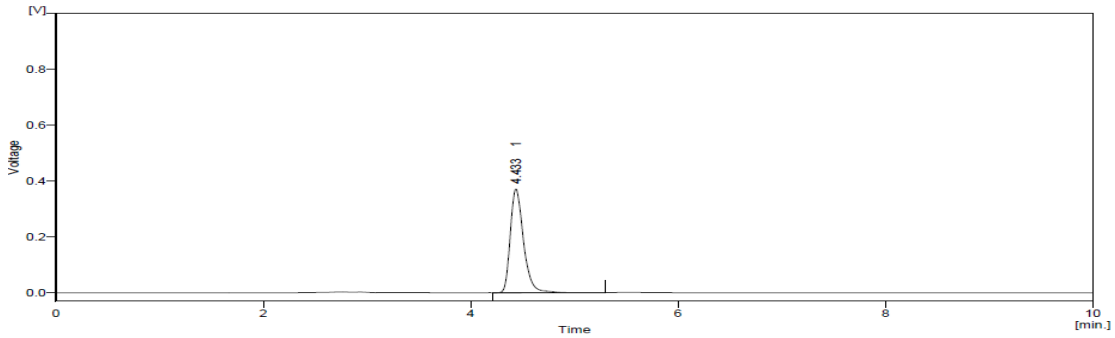

Fig. 5: Chromatogram of Acotiamide HCl Hydrate std

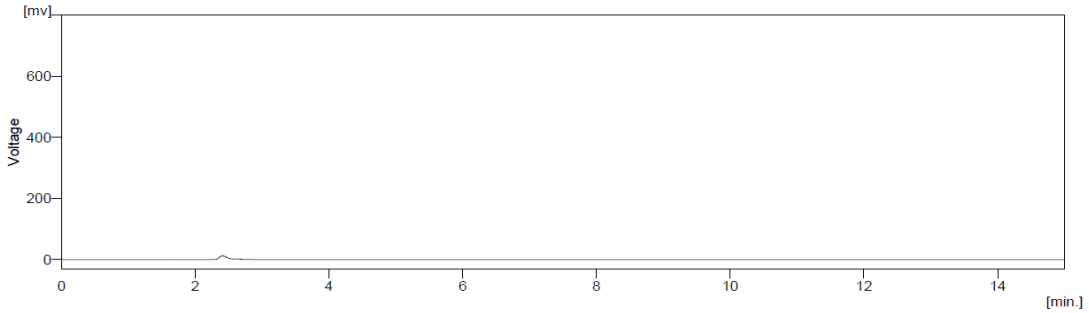

Fig. 6: - Chromatogram of Blank

The Chromatograms of Acotiamide HCl Hydrate standard shows no interference with the Chromatogram of Blank, so the Developed method is Specific.

LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 11: Limit of Detection and Limit of Quantitation data for Acotiamide HCl Hydrate

Acotiamide HCl Hydrate	
Limit of Detection	**Limit of Quantitation**
LOD = 3.3 x (SD / Slope) = 3.3 x (39.261/320.05) = 0.405 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (39.261/320.05) = 1.127 µg/ml

Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 4.7 and 4.3
3. Ratio of Mobile phase was changed (±2) Water: Methanol (43:57) and Water: Methanol

Table 12: Robustness data for Acotiamide HCl Hydrate

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	3393.596	3202.327	3302.814	3130.020	3335.950	3189.504
2	3357.399	3148.928	3380.504	3149.650	3315.879	3194.171
3	3433.420	3228.865	3393.596	3048.741	3390.192	3232.104
% R.S. D	1.120	1.275	1.461	1.721	1.148	0.729

Analysis of marketed formulation by developed method

Sample Stock Solution (Acotiamide HCl Hydrate 100 µg/mL):

Take Tablet powder equivalent to 10 mg of Acotiamide HCl Hydrate was transferred to a 100 ml volumetric flask, add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (Acotiamide HCl Hydrate 10 µg/mL):

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase

Inject above Solution 20 µl for Assay Analysis.

Table 13: Analysis on marketed formulation

Tablet	Acopep Tablet
Label claim	**Acotiamide HCl Hydrate (100mg)**
Assay (% of label claim*) Mean ± S. D.	97.387±2.206

The assay results were comparable to labelled value of drug in dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

FORCED DEGRADATION STUDY

I. Acid degradation

Acid degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. 2 ml of 0.1 N HCl solutions was added and mixed well and put for 5 hrs at RT. than neutralize with 2 ml 0.1N NaOH Then the volume was adjusted with diluent to get 10µg/ml for Acotiamide HCl Hydrate

II. Base degradation

Base degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 3 hrs at RT.

than neutralize with 2 ml 0.1 N HCl Then the volume was adjusted with diluent to get 10µg/ml for Acotiamide HCl Hydrate.

III. Oxidative degradation

Oxidation degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. 2 ml of 3% H2O2 solutions was added and mixed well and put for 6 hrs at RT. Then the volume was adjusted with diluent to get 10µg/ml for Acotiamide HCl Hydrate.

IV. Photo degradation

Photo degradation studies were performed by transferring1 ml of stock solution to 10 ml of volumetric flask. Then the volumetric flask was kept in UV chamber for 12 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Acotiamide HCl Hydrate.

V. Thermal degradation

Photo degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Then the volumetric flask was kept in an oven at 80^0C Temperature for 8 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Acotiamide HCl Hydrate.

Table 14: Acotiamide HCl Hydrate std for stability

Drugs	Area
Acotiamide HCl Hydrate	3171.579

Table 15: Acotiamide HCl Hydrate % Degradation result

Parameter	Standard		Sample	
	Area	% Degradation	Area	% Degradation
Acid	2526.76	20.33	2527.55	20.31
base	2593.12	18.24	2483.09	21.71
oxidation	2330.06	26.53	2386.14	24.76
Photo	2544.04	19.79	2451.91	22.69
Thermal	2494.87	21.34	2429.53	23.40

CONCLUSIONS:

RP-HPLC method was developed for estimation of Acotiamide HCl hydrate. In RP-HPLC method, good resolution and separation of two drugs was achieved. Water (pH 4.5): Methanol: TEA (45:55:0.1) was used as mobile phase. Retention time of Acotiamide HCl hydrate was found to be 4.420 min with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Acotiamide HCl hydrate in tablets.

Forced degradation study of Acotiamide HCl hydrate was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit.

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REFERENCES:

1. Pramod kerkar, “Introduction to functional dyspepsia disease”, March 2017,
2. https://www.epainassist.com/abdominal-pain/stomach/dyspepsia
3. “Drug profile of Acotiamide HCl Hydrate”, August 2017,
4. https://www.drugbank.ca/drugs/DB2482
5. “Drug profile of Acotiamide HCl Hydrate”, August 2017,
6. http://www.medkoo.com/products/4524
7. “Drug profile of Acotiamide HCl Hydrate”, August 2017,
8. https://pubchem.ncbi.nlm.nih.gov/compound/Acotiamide_HCl#section=Substances-by-Category
9. “Drug profile of Acotiamide HCl Hydrate”, August 2017,
10. http://apisynthesisint.blogspot.in/2016/01/acotiamide-hydrochloride-trihydrate.html
11. Kasture AV., Mahadik KR., Wododkar SG and More HN. A Text Book of Pharmaceutical Analysis; 17th Edn; Nirali Prakashan, Pune, 2002, pp 48-57.
12. Shethi PD. HPLC-Quantitative Analysis of Pharmaceutical Formulations; CBS Publishers & Distributers, New Delhi, 1996, pp 3-46.
13. Chatwal GR. Instrumental Method of Chemical Analysis; Part-1, Himalaya Publishing House,5th Edition,2002, pp 2.624-2.631.

14. Robinson JW, Skelly Frame EM and Frame GM. Undergraduate Instrumental Analysis; 6th Edition, Marcel Dekker, 2005, pp 806.
15. Ahuja S and Scypinski S. Handbook of Modern Pharmaceutical Analysis; Academic Press 3, 2001 pp 356-367.
16. FDA Guidance for Industry, "Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration", Rockville, US Department of Health and Human Services, 2000.
17. ICH, "Validation of Analytical Procedures, Methodology, Q2 (R1)", International Conference on Harmonization, IFPMA, Geneva 1996.
18. Brummer H, "How to Approach A Forced Degradation Study."*Life sci. tech bul.* **2011.**
19. Patel PN, Kalariya PD, Swamy CV, Gananadhamu S, Srinivas R. "Quantitation of acotiamide in rat plasma by UHPLC-Q-TOF-MS; method development, validation and application to pharmacokinetics." *Biomed. Chrom.* **2016,** *30(3),* 363-368.
20. Li J, Huang R, Wang Z, Qu H, Sun M, Zho Z. "Development and Validation of a Sensitive and Specific LC–MS-MS Method for the Determination of Acotiamide in Rat Plasma.; *J. chrom. Sci*. **2016,** *54-56,* 1004-1009.
21. Guang QJ, Biao L, Jie SL, Cho CY, Ju LF. Detection method of related substances in chemical drug acotiamide hydrochloride hydrate. Chinese Patents CN 201510124739¸2015.
22. Jianjin H, Xiaojun L, Bo S, Yan Y. Synthetic method of acotiamide hydrochloride hydrate. Chinese Patents CN 201410024859, 2014.
23. Guang QJ, Biao L, Jie SL, Cho CY, Ju LF. Detection method of related substances in chemical drug acotiamide hydrochloride hydrate. Chinese Patents CN 201510124739¸2015.
24. Gong Q, Zhai Z, Li G, Si. Preparation method of acotiamide hydrochloride. Chinese patents CN 201410281884, 2015.
25. Ying Z, Yanli P, Yang J, Shi G. Acotiamide hydrochloride-controlled release tablet and preparation method thereof. Chinese Patents CN 201610848023, 2014.
26. Jie Y, Wang Z. Acotiamide tablet and preparation method. Chinese patents CN 201510758663, 2015.