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DEVELOPMENT AND VALIDATIONOF OF STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF ACOTIAMIDE HYROCHLORIDE

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Keywords:

Acotiamide hydrochloride, Stability indicating, HPLC, Validation, ICH guideline

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ABSTRACT: A simple and rapid stability indicating HPLC method for acotiamide hydrochloride was developed and validated. The stationary phase used was thermo gold cyno (250 × 4.6 mm) 5 µ with a mobile phase consisting of mixture of acetonitrile and 10 mM KH_2PO_4 in water in the ratio of 80:20 v/v at a flow rate of 0.8ml/min. Detection was carried at 284 nm. The retention time observed was 8.1 ± 0.2 min. The column was maintained at ambient temperature and 20 µl of solutions were injected. The eluted compound was detected by using PDA detector. The linear regression analysis data for calibration plot show good relationship with coefficient of regression value, r^2=0.99 in the concentration range 10-50 µg/ml. The stress degradation studies were performed as per ICH guidelines. Acotiamide hydrochloride degraded with acid/base hydrolysis, thermal, oxidation and photolytic stress. The method was validated as per ICH Q2A (R1) guideline with respect to linearity, accuracy, precision, specificity and robustness. This method can be used for monitoring the stability of acotiamide hydrochloride.

INTRODUCTION: Acotiamide hydrochloride is a new prokinetic drug that is used to treat functional dyspepsia (FD). Extensive literature review reveals that Acotiamide is a drug approved in Japan for the treatment of postprandial fullness, upper abdominal bloating, and early satiation due to functional dyspepsia [1]. It acts as an acetyl cholinesterase inhibitor. Functional dyspepsia (FD) is a highly prevalent condition characterized by symptoms suggested to be of gastro duodenal origin, in the absence of an organic cause that is likely to explain the symptoms. It is generally assumed that FD is heterogeneous condition, which consists of different entities and may require specific management or treatment approaches.

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However, it has proven difficult to identify reliably subgroups of clinically meaningful importance. Functional dyspepsia is treated by two major categories of drugs; acid inhibitors such as H_2-receptor antagonists and proton pump inhibitors (PPIs), and prokinetic drugs which accelerate disturbed GI motility with modifying altered visceral sensitivity [2]. Eradication of *H. pylori* and psychotropic agents such as anxiolytics and antidepressants were added as other options for FD treatment.

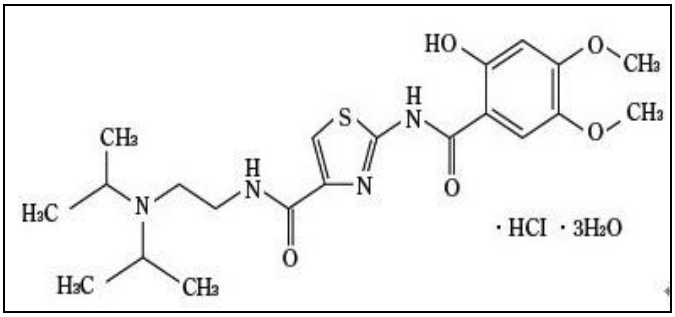

FIG. 1: CHEMICAL STRUCTURE OF ACOTIAMIDE HYDROCHLORIDE

Many studies relating use of acotiamide hydrochloride for the treatment of functional dyspepsia have been reported [3-12]. A method for quantification of acotiamide in Plasma by using UHPLC-MS, LC–MS/MS and NMR along with pharmacokinetic application has been reported [13-15].

MATERIALS AND METHOD:

Instruments: Quantitative HPLC was performed using isocratic High Performance Liquid Chromatography (Jasco HPLC system) with a LC-PU 2080 Plus pump, manual injector with loop volume of 20 µl (Rheodyne), programmable MD 2010 PDA detector and Thermo gold cyno C_8 (250 × 4.6 mm i.d, 5 µm particle size). The HPLC system was equipped with Borwin-PDA software (version1.5). An electronic balance (Shimadzu AY-120), UV-Visible spectrophotometer (make JASCO, model V-550) were used in this study.

Chemicals and Reagents: Analytically working standard of acotiamide was kindly supplied by Alkem laboratories Mumbai. Acetonitrile (HPLC grade) was purchased from Merck specialties Pvt. Ltd., (Mumbai, India). KH_2PO_4 was purchase from Loba chemical. Elga Lab (PURELAB UHQ-II) water purification system.

Detection Wavelength: From the standard stock solution further dilutions were done using acetonitrile and scanned over the range of 200 - 400 nm.

Selection of Mobile Phase: The standard solution of acotiamide hydrochloride (20 µg/ml) was injected into the HPLC system and run in different solvent systems. Different mobile phases like methanol and water, methanol and acetate buffer, acetonitrile and phosphate buffer, methanol and phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the acotiamide hydrochloride.

Optimized Chromatographic Conditions: The mobile phase consisted of acetonitrile and 10 mM KH_2PO_4 in water the ratio of 80:20 v/v. It was then filtered through 0.45 µ membrane filter paper using vacuum filtration assembly and then sonicated using ultrasonic water bath for 15 min. The flow rate of mobile phase was maintained at 0.8 ml/min. The column and the HPLC systems were kept at ambient temperature.

Preparation of Solutions:

Preparation of Standard Stock Solution: Prepared standard stock solution of acotiamide hydrochloride in acetonitrile (100 µg/ml). Further dilution was made in mobile phase to get final solution of acotiamide hydrochloride in the range 10 to 50 µg/ml.

Preparation of Sample Solution: 20 Tablets were weighed and average weight was determined. An accurately weighed tablet powder equivalent to 25mg of acotiamide hydrochloride was transferred into 25ml volumetric flask dispersed in acetonitrile, sonicated for 10 min and volume was made up to the mark. Solution was filtered using whatmann filter paper and diluted to obtain 10 µg/ml solution.

Forced Degradation Studies: In order to determine whether the method is stability indicating, Forced degradation studies were carried under condition of acid, alkaline and neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A (R2) and Q1B guidelines. The analysis was carried out by HPLC with a PDA detector. For each study, two samples were prepared: the drug solution and working standard solution of acotiamide hydrochloride subjected to degradation condition. Stress degradation conditions were optimized with respect to time of exposure and strength of reagent so as to obtain degradation in the range of 10 to 30%. Dry heat and photolytic degradation were carried out in solid state.

Acid Hydrolysis: Acid induced degradation was performed by adding 1 ml of 0.1N Hydrochloric acid (HCl) to volumetric flask containing 1 ml of acotiamide hydrochloride standard solution (100 µg/ml). The volume was made up to 10 ml with mobile phase and kept for 24 h in dark place. Final solution (10 µg/ml) was injected.

Alkaline Hydrolysis: Alkali induced forced degradation was performed by adding 1 ml of 0.1N sodium hydroxide (NaOH) to volumetric flask containing 1 ml of acotiamide hydrochloride standard solution (100 µg/ml). The volume was made up to 10 ml with mobile phase and kept for 24 h in dark place.

Neutral Hydrolysis: Neutral hydrolysis was performed by adding 1 ml of acotiamide hydrochloride standard solution (100 µg/ml) was mixed with 1ml of water in 10 ml of volumetric flask and the volume was madeupto the mark with mobile phase. Solution was kept for 24 h dark place. Final solution (10 µg/ml) was injected.

Oxidative Hydrolysis: Oxidative degradation was performed by adding 1 ml of Hydrogen peroxide (H_2O_2, 6% v/v) to volumetric flask containing 1 ml of acotiamide hydrochloride standard solution (100 µg/ml). The volume was made up to 10 ml with mobile phase & kept for 24 h protected from light.

Degradation under Dry Heat: Dry heat study was performed by keeping acotiamide hydrochloride in oven at 60 ºC. A sample was withdrawn after 4 h, weighed and dissolved in mobile phase to get solution of 10 µg/ml of acotiamide hydrochloride and then injected.

Photo - Degradation Studies: Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours /square meter and subsequently to fluorescent light illumination not less than 1.2 million lux hours.

Sample was weighed, dissolved in acetonitrile to get concentration of 1000 µg/ml. and further dilutions were made with acetonitrile to get final concentration (10 µg/ml).

RESULTS AND DISCUSSION:

Selection of Analytical Wavelength: The standard solution was scanned over range of 200-400 nm to obtain a spectrum. The drug showed considerable absorbance at 284 nm.

Selection of Mobile Phase: After several trials, acetonitrile and 10 mm KH_2PO_4 in water in the ratio of 80:20 v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatogram and System Suitability Parameter of Drug: The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug (10 µg/ml) was injected into the system. The retention time for the drug was found to be 8.1 ± 0.2 min. System suitability parameters of acotiamide hydrochloride hydrate are summarized in **Table 1**.

TABLE 1: SYSTEM SUITABILITY PARAMETER

Name	RT(Min)	Conc. (µg/ml)	Area	Theoretical Plates	Asymmetry
Acotiamide Hyrochlroide	8.1	10	201856.5	2391	1.47

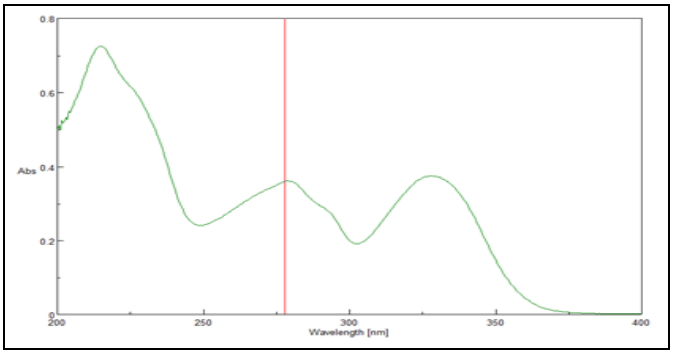

FIG. 2: ABSORBANCE SPECTRUM OF ACOTIAMIDE HYDROCHLORIDE (10µg/ml)

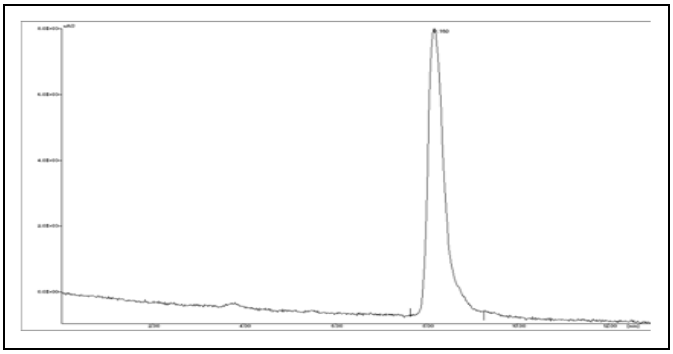

FIG. 3: STANDARD CHROMATOGRAM OF ACOTIAMIDE HYDROCHLORIDE (10µg/ml)

Stress Degradation Studies of Bulk Drug: Stress degradation were performed as per ICH Q1A (R2) guideline [16]. At every injection of stress degraded samplepeak purity studies were done to check presence of co-eluting other substance.

Acid Hydrolysis: Under acid hydrolysis, 84.53% acotiamide hydrochloride was recovered with no peak of degradation.

Alkaline Hydrolysis: After alkaline hydrolysis, 89.60% acotiamide hydrochloride was recovered with no peak of degradation.

Oxidative Hydrolysis: Oxidative hydrolysis studies showed about 88.11% acotiamide hydrochloride was recovered with no peak of degradation.

Neutral Hydrolysis: After neutral hydrolysis, 94.01% acotiamide hydrochloride was recovered with no peaks of degradation.

Degradation under Dry Heat: After the dry heat degradation, 88.69% acotiamide hydrochloride was recovered with no peaks of degradation.

Photo-Degradation Studies: After the photo degradation study under UV light 84.20% and Fluorescence light 89% acotiamide hydrochloride hydrate was recovered with no peak of degradation

Validation of Analytical Method: The method was validated as per ICH Q2 (R1) guidelines [17].

Specificity: The specificity was carried out by determining the peak purity values which were found to be more than 996, indicating that there is non-interference of any impurities or degradation product at retention time of analytical peak. The study was performed by Borwin software.

Linearity: Linearity was tested for the range of concentrations 10 - 50 µg/ml. Each sample in five replicates was analysed and peak areas were recorded. The areas were plotted against the corresponding concentrations of acotiamide hydrochloride to obtain the calibration curve. **Fig. 4** and **5** represents the linearity and calibration curve for acotiamide hydrochloride respectively.

Range: The linearity range of was found to be 10-50 µg/ml.

Assay: Percent assay for acotiamide hyrochlroide was found out to be 98.13%.

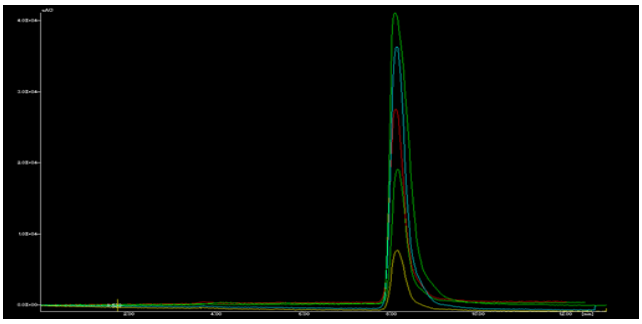

FIG. 4: LINEARITY OF ACOTIAMIDE HYROCHLROIDE (10-50 µg/ml)

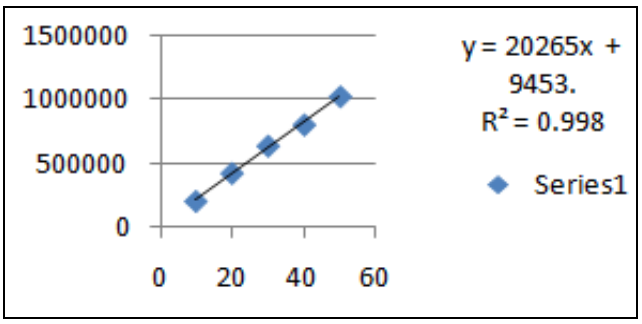

FIG. 5: CALIBRATION CURVE FOR ACOTIAMIDE HYROCHLROIDE

Accuracy: The accuracy study was performed recovery studies were carried out by adding standard drug to marketed sample at three different levels 80%, 100% and 120%. The accuracy study was performed for % recovery of acotiamide hydrochloride.

Concentration of assay sample chosen was 20 µg/ml. These solutions were injected into HPLC system in triplicate to obtain the chromatogram. The drug concentrations were calculated by using linearity equation of acotiamide hydrochloride. The results obtained are shown **Table 2**.

TABLE 2: RECOVERY STUDIES OF ACOTIAMIDE HYDROCHLORIDE

Recovery Level %	Amount taken (µg/ml)	Amount added (µg/ml)	Total concen-tration (µg/ml)	Mean peak Area	% Recovery	% RSD
80	20	16	34	734029.3	99.31953	0.689999
100	20	20	40	821113.3	100.1307	1.232838
120	20	24	44	913731.7	101.4151	1.646053

Precision: The precision was demonstrated by intra-day and inter-day studies. In the intra-day studies, 3 replicates of 3 standard solutions (10, 20 and 30 µg/ml) were analyzed in a same day and percentage RSD was calculated **Table 3**. For the inter-day variation studies, 9 replicates of 3 standard solutions (10, 20 and 30 µg/ml) were analyzed on 3 consecutive days and percentage RSD was calculated **Table 4**.

TABLE 3: INTRA-DAY PRECISION STUDY OF ACOTIAMIDE HYDROCHLORIDE

Conc. (µg/ml)	Area	Mean Area	SD	% RSD
10	210688	210969.3		
	211320		0.158724	0.15
	210900			
20	401030	408050.7		
	409017		6590.846	1.6
	414105			
30	613910	612123.3		
	610650		0.271804	0.27
	611810			

TABLE 4: INTER-DAY PRECISION OF ACOTIAMIDE HYDROCHLORIDE

Conc. (µg/ml)	Area	Mean Area	SD	% RSD
10	210890	210367.7		
	209067		0.559418	0.564251
	211146			
20	411390	411159.3		
	404693		1.567762	1.58179
	417395			
30	613454	609455.3		
	613365		1.126567	1.14149
	601547			

Limit of Detection (LOD): LOD is calculated from the formula:

$$LOD = 3.3\ \sigma / S$$

Where, σ = standard deviation of Y intercept
S = slope of the calibration curve.
LOD of acotiamide hydrochloride = 0.6 µg/ml

Limit of Quantification (LOQ): The Quantitation limit is expressed as:

$$LOQ = 10\ \sigma / S$$

LOQ of acotiamide hydrochloride was found to be 1.9 µg/ml

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, mobile phase ratio, wavelength were altered and the effects on the peak area were noted in **Table 5**.

TABLE 5: SUMMARY OF ROBUSTNESS PARAMETER

S. no.	Parameter	Area	Mean	% RSD
1		410201	406654.3	
	Plus Flow Rate	408526		1.172142
	(0.85)	401236		
2		404103	408217	
	Minus Flow Rate	410312		0.872828
	(0.75)	410236		
3		416230	410604	
	Plus Mobile Phase	412302		1.617101
	Composition	403280		
	(82-18)			
4		402103	404371.3	
	Minus Mobile	401210		1.16808
	Phase	409801		
	Composition			
	(78-22)			
5		406397	411022.7	
	Plus Wavelength	406798		1.865405
	286	419873		
6		410320	412427	
	Minus	419302		1.479237
	Wavelength 282	407659		

Summary of Validation Study:

TABLE 6: SUMMARY OF VALIDATION PARAMETER

S. no.	Validation parameter	Acotiamide hydrochloride
1	Linearity Equation	y = 20265x + 9453.6
	(r2)	0.998
	Range	10-50 µg/ml.
2	Precision (% RSD)	
	Interday	0.159617
	Intraday	0.564251
3	Accuracy	% Recovery
	80	99.31953
	100	100.1307
	120	101.4151
4	Limit of Detection	0.6 µg/ ml
5	Limit of Quantitation	1.9µg/ ml
6	Specificity	Specific
7	Robustness	Robust

DISCUSSION: There was no interference from blank at the retention time of acotiamide hydrochloride.

CONCLUSION: The developed method is stability indicating, since the drug peak was found to be pure as confirmed by peak purity profiling studies. The developed method is simple, rapid and stability indicating can be used for routine quality control as well as assessing the stability of acotiamide hydrochloride.

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CONFLICT OF INTEREST: Nil

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