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

ISSN 2349-8870
Volume: 5
Issue: 9
355-361
Year: 2018

DEVELOPMENT & VALIDATION OF A STABILITY- INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC (HPTLC) METHOD FOR THE ESTIMATION OF ACOTIAMIDE HYDROCHLORIDE HYDRATE

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Article Received on 28/06/2018 **Article Revised on 18/07/2018** **Article Accepted on 07/08/2018**

ABSTRACT
The objective of the study was to develop & validate a simple, specific, rapid, precise and accurate HPTLC method for the estimation of Acotiamide Hydrochloride. The HPTLC separation was carried out on Merck TLC aluminium sheets Precoated with Silica gel $60F_{254}$ using mobile phase as Chloroform : methanol(6:4v/v) and it was validated as per ICH Q2 (R1) guidelines. Forced degradation study was carried out under different stress conditions. The analysis of the spots was performed at 332nm. A linear data over the range of 200-1000ng/band with a good correlation coefficient of 0.996 unfolds linear relationship between area and concentration in calibration curve. Stress degradation study of Acotiamide Hydrochloride was done according to ICH guidelines Q1A (R2) and the proposed method can be used for the routine analysis of Acotiamide Hydrochloride in bulk and pharmaceutical dosage form.

KEYWORDS: Acotiamide Hydrochloride Hydrate, HPTLC, ICH guidelines, forced degradation studies.

INTRODUCTION
Acotiamide Hydrochloride Hydrate is an anti emetic agent, chemically N-[2-[di(propan-2-yl)amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxmide; trihydrate; hydrochloride. Acotiamide Hydrochloride Hydrate is the hydrochloride salt form of acotiamide, which is a prokinetic agent having gastrointestinal (GI) motility-enhancing activity.[1] Although, the exact mechanism by which acotiamide exerts its effect has yet to be fully elucidated, this agent appears to inhibit acetylcholinesterase (AchE), which is 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. Acetylcholine release via acting as an antagonist on the M1 and M2 muscarinic receptors in the enteric nervous system and inhibiting acetylcholinesterase activity. Acotiamide has been approved in Japan in March 2013 and launched in Japan in June 2013, making it the world's first approved treatment for Functional Dyspepsia in patients.[2]

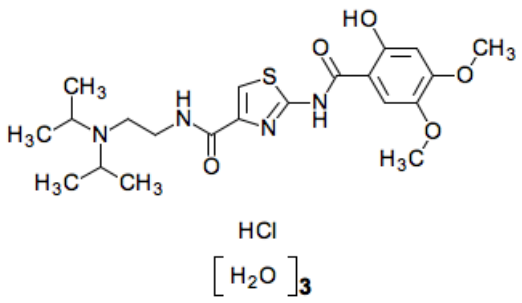

Fig. 1: Structure of Acotiamide Hydrochloride Hydrate.

Acotiamide Hydrochloride Hydrate is not official in IP/BP/USP. Literature survey reveals that few analytical methods have been reported viz., the determination of Acotiamide Hydrochloride Hydrate in Rat plasma by

[LC-MS-MS][3], UHPLC-Q-TOF-MS[4], development and validation of stability indicating RP-HPLC method for Acotiamide Hydrochloride Hydrate in pharmaceutical dosage form[5] but no stability indicating HPTLC Method of Acotiamide Hydrochloride Hydrate has yet been reported.

MATERIALS AND METHODS

Instrumentation

Chromatographic method of Acotiamide Hydrochloride Hydrate was performed on HPTLC (Make- CAMAG, Switzerland) system comprising of Linomat-5 applicator connected to a nitrogen cylinder, a twin trough chamber (20 ×10cm), TLC scanner III(Camag Muttenz, Switzerland), and Win CATS software (version 1.4.2) were used for chromatographic study. Electronic analytical balance (Shimadzu Model AY – 120), was used for all the weighing purpose.

Materials

Analytically pure sample of Acotiamide Hydrochloride Hydrate was obtained from Alkem Lab Mumbai, India and spike blend of Acotiamide Hydrochloride Hydrate was prepared from the bulk drug sample (API) with label claim of 100 mg. Analytical reagent grade of Acetonitrile, Methanol and Chloroform were purchased from Loba Chemie, Mumbai, India.

Chromatographic conditions

Stationary phase: Merck HPTLC aluminium plates (10×10 cm, 0.2mm thick), pre coated with silica gel 60 $F_{254.}$

Mobile phase: Chloroform: Methanol (6:4 v/v)

Saturation time: 15 minutes.

Detection wavelength: 332 nm.

Preparation of Solutions

Preparation of Standard Stock Solution

Accurately weighed 10 mg of Acotiamide Hydrochloride Hydrate was dissolved in 10 ml of Acetonitrile to get 1000µg/ ml from which 1ml was diluted to get the final concentration of 100 µg /ml and applied on TLC plate.

Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on the working standard solution of Acotiamide Hydrochloride Hydrate (100µg /ml). Initially, trials were carried out using various solvents in various proportions on normal TLC plates of dimension (5×10 cm), to obtain the desired Rf and shape for drug peak. After several trials, Chloroform: Methanol in the ratio of 6:4 v/v was chosen as the mobile phase, which gave resolved peak with acceptable parameters. Apart from this, other chromatographic conditions like chamber saturation time, run length, sample application volume were optimized.

Selection of Analytical wavelength

From the standard stock solution further dilution was done using Acetonitrile to get the final concentration of 10 µg/ml and scanned over the range of 200-400nm. The spectrum was obtained. It was observed that the drug showed considerable absorbance at 332nm, so it was selected as detection wavelength.

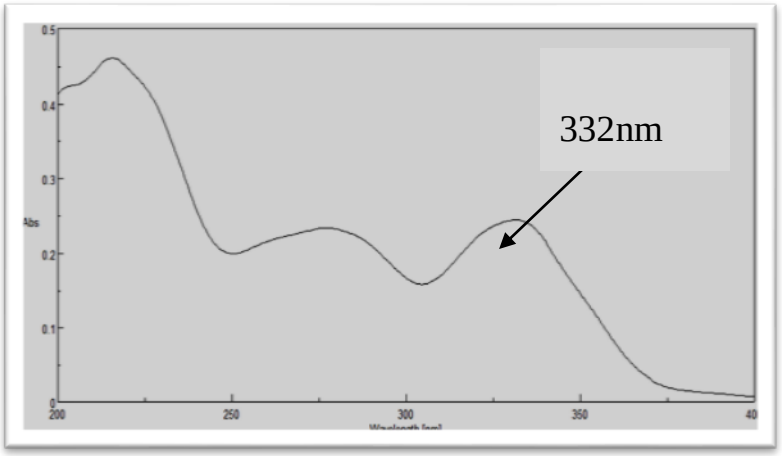

Fig. 2: UV spectrum of Acotiamide Hydrochloride Hydrate 10µg/ml.

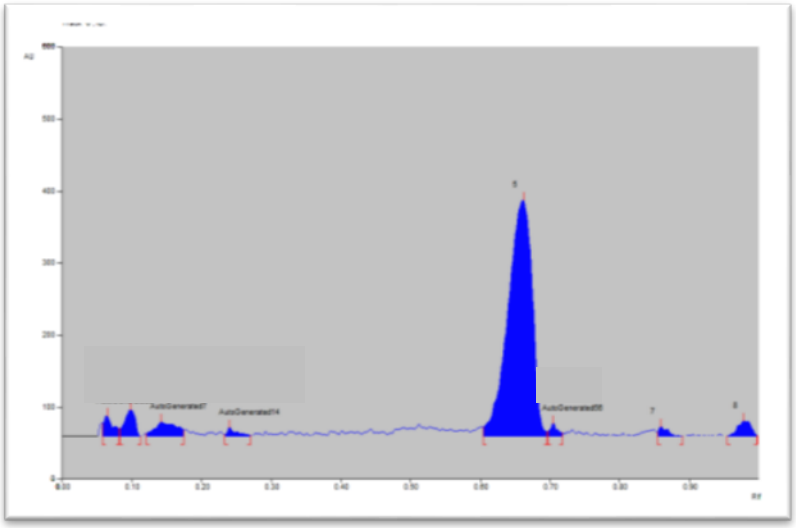

Fig. 3: Densitogram of standard solution of Acotiamide Hydrochloride Hydrate 1000ng/band (RF= 0.64± 0.03).

Stress degradation study of bulk drug

The forced degradation studies were carried out as per ICH guidelines[6] to assess the stability indicating property and selectivity of the developed HPTLC method. The stress degradation studies were carried out under ICH recommended conditions. Stress degradation of Acotiamide Hydrochloride Hydrate was carried out by exposing the bulk sample to hydrolytic, oxidative, photolytic, and thermal stress conditions. The aim was to study the ability of the proposed method to measure the analyte response in presence of its degradation products, if any.

Acidic hydrolysis

Acid induced forced degradation was performed by adding 1ml of 0.1 N Hydrochloric acid (HCl) to volumetric flask containing 1 ml of Acotiamide Hydrochloride Hydrate standard solution (100µg/ml). The volume was made up to10 ml with Acetonitrile and kept for 4 hr in dark place. From the resulting solution, final solution of 6µl spot (600ng/band), 8µl spot (800ng/band) were applied at TLC plate with the help of applicator.

Alkali hydrolysis

Alkali induced forced degradation was performed by adding 1ml of 0.1 N Sodium Hydroxide base (NaOH) to volumetric flask containing 1 ml of Acotiamide Hydrochloride Hydrate standard solution (100µg/ml). The volume was made up to 10 ml with Acetonitrile as mobile phase and kept for 4 hr in dark place. From the resulting solution, final solution of 6µl spot (600ng/band), 8µl spot (800ng/band) were applied at TLC plate with the help of applicator.

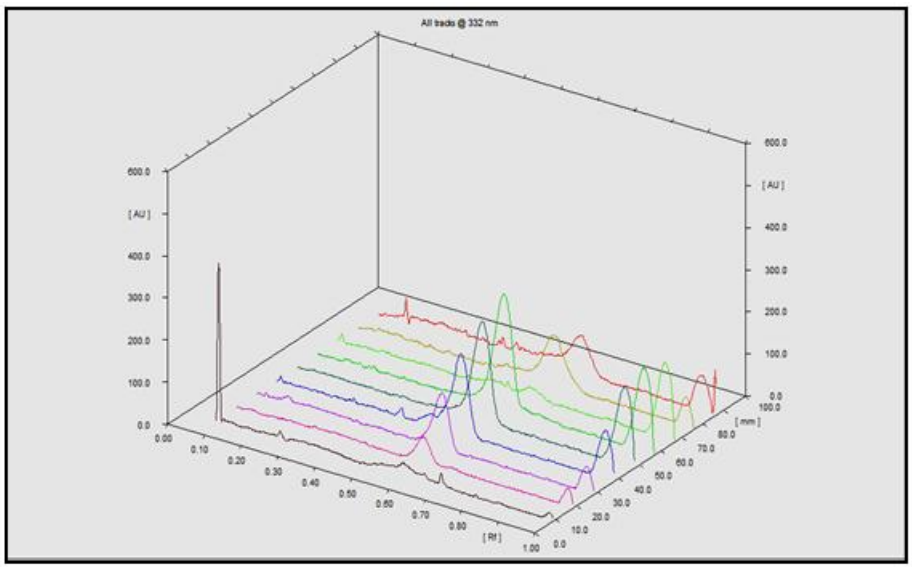

Fig. 4: 3D display of Alkaline hydrolysis and densitogram of Acotiamide Hydrochloride Hydrate.

Neutral hydrolysis

Neutral hydrolysis was performed by adding 1ml of Acotiamide Hydrochloride Hydrate standard solution(100µg/ml) and was mixed with 1 ml of water in a 10 ml volumetric flask and the volume was made up to the mark with Acetonitrile as mobile phase. Solution was kept for 24 hr in a dark place.

From the resulting solution, final solution of 6µl spot (600ng/band), 8µlspot (800ng/band) were applied at TLC plate with the help of applicator.

Oxidative hydrolysis

Oxidative degradation was performed by adding 1 ml of Hydrogen peroxide (H_2O_2, 30% v/v) to volumetric flask containing 1ml of Acotiamide Hydrochloride Hydrate standard solution (100µg/ml). The volume was made up to 10 ml with mobile phase and kept for 24hr in dark away from light.

From the resulting solution, final solution of 6µlspot (600ng/band), 8µl spot (800ng/band) were applied at TLC plate with the help of applicator.

Thermal degradation

Thermal degradation study was performed by keeping the drug sample of Acotiamide.

Hydrochloride Hydrate in oven at 60ºC and the sample was withdrawn after 4 hrs. It was appropriately weighed and dissolved in mobile phase to get a solution of 100µg/ml of Acotiamide Hydrochloride Hydrate and then applied.

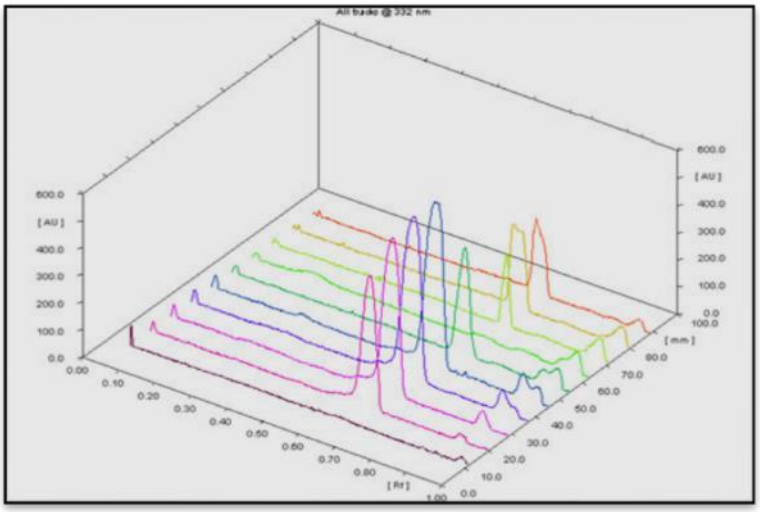

Fig. 5: 3D display of Thermal degradation and densitogram of Acotiamide Hydrochloride Hydrate.

Photo-degradation studies

Photolytic degradation studies were carried out by exposing the sample of drug to UV light up to 200 watt hours/square meter & subsequently to fluorescent light illumination not less than 1.2 million lux hours. Sample was accurately weighed, dissolved in mobile phase to get a solution of 100µg/ml. From the resulting solution, final solution of 6µl spot (600ng/band), 8µl spot (800ng/band) were applied subsequently for both the parameters viz., UV & Fluorescence at TLC plate with the help of applicator.

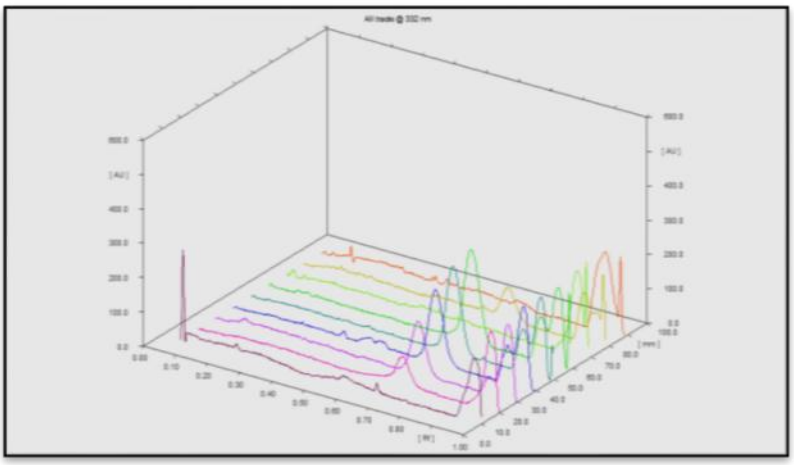

Fig. 6: 3D display of UV degradation and densitogram of Acotiamide Hydrochloride Hydrate.

Result of forced degradation studies

Table 1: Summary of stress degradation of Acotiamide Hydrochloride Hydrate.

S.No.	Stress degradation conditions at 332 nm	Conditions	Percentage recovery%	Peak purity	
				r(s,m)	r(m,e)
1.	Acid hydrolysis	(0.1N, HCl), 4 Hrs	80.54	0.999537	0.999987
2.	Alkaline hydrolysis	(0.1 N HCl, 4 Hrs)	77.20	0.999998	0.999961
3.	Oxidation	(30% H_2O_2), 24 Hrs	73.08	0.999994	0.999982
4.	Neutral hydrolysis	24 Hrs	59.31	0.999981	1.000000
5.	Dry heat	60ºC, 4 Hrs	94.97	0.999983	0.999975
6.	UV light	(200 Watt hours/Square meter)	62.62	0.999997	0.999890
7.	Fluorescence light	(1.2 million lux hours)	94.79	0.999938	0.999920

Method Validation

The method validation was done as per ICH Q2 (R1) guidelines.[7]

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.990, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

Linearity was observed for the range of concentrations 200 -1000ng/band of Acotiamide Hydrochloride Hydrate was applied on TLC plate and calibration curve was obtained against peak area Vs concentration. The equation was found to be y= 8.055x -945.13 for Acotiamide Hydrochloride Hydrate and correlation coefficient (R^2) was found to be 0.996.

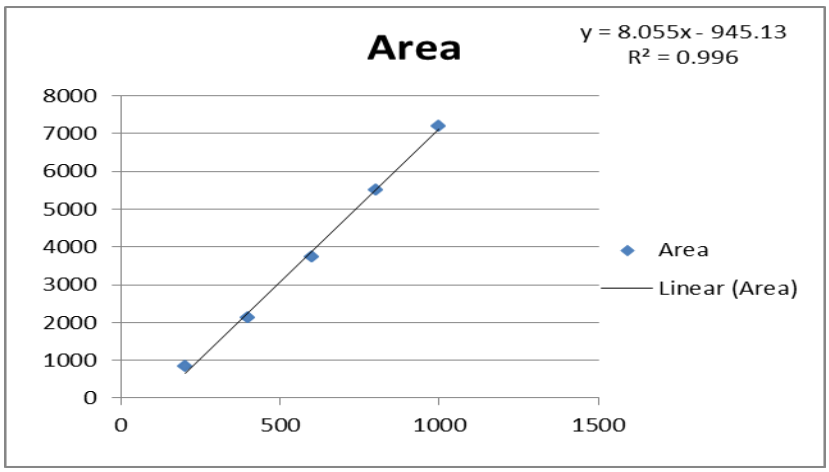

Fig. 7: Calibration curve of Acotiamide Hydrochloride Hydrate.

Range

200-1000ng/band

Assay

Assay was performed on the blend of bulk drug plus excipients from which an equivalent weight of 10mg of Acotiamide Hydrochloride Hydrate was accurately weight and transferred to 10ml volumetric flask. Acetonitrile was added and filtered. Dilutions were made to get the final concentration of 100µg/ml. Assay was done by the extrapolation of peak area from respective calibration curve and it was found to be 89.18%.

Accuracy

To determine the accuracy of the method, recovery studies were carried out by addition of standard drug to pre-analysed sample solution in triplicate 50%, 100% and 150%.

The concentration of sample chosen was in ng/band of standard. The recovery percentage of the drug was calculated by slope and intercept of the linearity plot of drugs. The results obtained for accuracy is shown in the table.

Table 2: Recovery studies of Acotiamide Hydrochloride Hydrate.

Level	Conc (ng/band)	Area (avg)	Conc. Recovered (ng/band)	% Recovery
50	300	1396.507	290.70	96.83
100	400	2127.103	381.40	99.99
150	500	2996.917	489.38	97.87

Precision

The precision to the method was demonstrated by intra-day and inter-day studies. For precision evaluation, a standard solution of fixed concentrations in 3 replicates of 3 standard solutions were analyzed on the same day in order to record any intra-day variations in the results and percentage RSD was calculated. For the inter-day, 3 standard solutions were analyzed on three consecutive days and percentage RSD was calculated.

Table 3: Intra-day & Inter-day precision studies of Acotiamide Hydrochloride Hydrate.

Conc (ng/band)	Condition	Mean area (avg)	SD	%RSD
600	Intra-day	3754.683	1.28	1.10
600	Inter-day	3785.667	48.60	41.30

*Average of 3 determinations.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively.

Where σ is the standard deviation of the lowest response of linearity and S is the slope of the calibration curve of the analyte. The LOD and LOQ were found to be 7.66 ng/ band and 23.23 ng/band.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which saturation time (15mins) ± 5min., Mobile phase composition, Time from spotting to development (immediate), detection wavelength and the effects on the peak area was noted. The %RSD values of all robustness parameters were examined and found to be within the limit of 2%, showed that the proposed method was robust.

Table 4: Summary of validation studies.

Sr. no.	Validation parameters	Acotiamide Hydrochloride Hydrate
1.	Linearity	Y =8.055x-945.13 $R^{2=}$ 0.996
2.	Range	200-1000ng/band
3.	Precision A)Inter-day B)Intra-day	(%RSD) 1.28 1.10
4.	Accuracy 50% 100% 150%	(% Recovery) 96.83 99.99 97.87
5.	LOD	7.66 ng/band
6.	LOQ	23.23ng/band
7.	Specificity	Specific
8.	Robustness	Robust

DISCUSSION

The methods available in literature based on [LC-MS-MS][3], UHPLC-Q-TOF-MS[4], development and validation of stability indicating RP-HPLC method for the determination of Acotiamide Hydrochloride Hydrate.[5] To best of our knowledge there are no stability indicating HPTLC method for Acotiamide Hydrochloride Hydrate in pharmaceutical dosage form.[5] Hence, we have developed stability indicating HPTLC method for the determination of Acotiamide Hydrochloride Hydrate wherein the stress conditions were optimized to achieve 10-30% degradation in acidic, alkali, neutral, oxidation, thermal and photolytic conditions.

CONCLUSION

A simple and rapid method was developed and validated. The method was based on the separation of the drug on plate pre coated with silica gel 60 F_{254} using Chloroform and Methanol as mobile phase in the ratio of 6:4 v/v followed by scanning in absorbance mode at 332nm. The optimised condition gave compact spot for Acotiamide Hydrochloride Hydrate at Rf value of 0.64±0.3 respectively. The method has been successfully validated according to ICH Q2R1 Guidelines.

ACKNOWLEDGEMENT

Authors are thankful to the Principal and the Management of AISSMS College of Pharmacy, Pune for providing the necessary facilities for research work and Alkem Lab Mumbai, India for providing API.

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