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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF ACOTIAMIDE HYDROCHLORIDE USING REVERSE PHASE HPLC METHOD IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new method was established for Acotiamide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Acotiamide by using Agilent C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (60:40 v/v) Methanol: Acetonitrile, detection wavelength was 235nm. The instrument used was Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.825 mins. The % purity of Acotiamide was found to be 100.27%. The system suitability parameters for Acotiamide such as theoretical plates and tailing factor were found to be 4023 and 1.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Acotiamide was found in concentration range of 20ppm-100ppm and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 100.56%, %RSD for repeatability was 0.17, % RSD for intermediate precision was 0.1. The precision study was precision, robustness and repeatabilty. LOD value was 0.303µg/mL and LOQ value was 1.011µg/ml. Hence the suggested RP-HPLC method can be used for routine analysis of Acotiamide in API and Pharmaceutical dosage form.

KEYWORDS: Acotiamide C18, Acotiamide, RP-HPLC.

INTRODUCTION

Acotiamide is N-[2-[bis (1-metyletyl) amino] etyl]-2-[(2-hydroxy-4,5-dimetoxybenzoyl) amino]tiazol-4-carboxamide (WHO) (Fig. 1). It is used in the treatment of Functional

dyspepsia It helps to relieve the symptoms like bloating after meal, epigastric pain/discomfort and early satiety. Acotiamide increases the release of acetylcholine, a chemical that can increase the motility of the intestine.This drug is not official in any pharmacopoeia.[1-3]

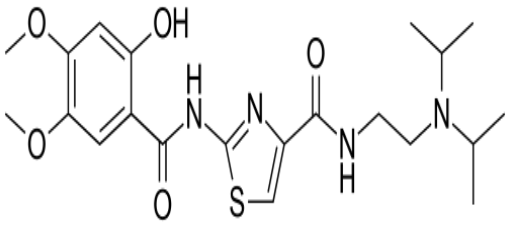

Fig. 1: Chemical Structure of Acotiamide.

According to literature survey few spectrophotometric,[2,7,14,15] HPLC[3,7,9,10,11,12,18,19,20] and HPTLC[13] methods have been reported for the determination of ACT in single and in combination with other drugs. However very few HPLC methods were reported for the estimation of ACT in tablet dosage form. The aim of present work was to develop and validate as per ICH guidelines,[23] a sensitive HPLC method that can be applied for estimation of ACT.

MATERIALS

ACT was received gratis from Dr. Reddys, Hyderabad and was used as received. HPLC grade acetonitrile was purchased from SD Fine Chem Pvt. Ltd. (Mumbai, Maharashtra). Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit. Waters total recovery vials (Waters, Milford, MA, USA) were of glass type 1, class A with 950 µL maximal injectable volumes. All other chemicals were of analytical reagent grade.

Experimental Work

Chromatographic conditions

The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA).

Isocratic elution of the mobile phase Methanol & ACN in the ratio of 60:40 v/v with the flowrate of 1 ml/min. Separation was performed on a Agilent C_{18} (250 x 4.6 mm i.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Waters

Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µm membrane filter and degassed by sonication before use. Mobile phase was used as diluents.

The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 10 min and a column temperature was maintained at 35°C. The volume of injection was 10 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluents were detected ACT at 235nm. The developed method was validated in terms of specificiy, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of ACT as per ICH guidelines.

Preparation of standard solution

10 mg Acotiamide working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solution

10 mg of Acotiamide tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

RESULTS AND DISCUSSION

Method Development

Number of mobile phase and their different proportions were tried and finally was selected as Methanol and acetonitrile in the ratio of 60:40 v/vappropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results of system suitability parameters were shown in table 2. The chromatogram of working standard solution is shown in Fig 3. The summary of Chromatographic conditions were given in table 1.

Table 1: Summary of Chromatographic conditions.

S. No.	Parameter	Description/Value
1.	Stationary Phase	Agilent C18 (250X4.6X5)
2	Mobile Phase	Methanol and Acetonitrile in the ratio of 60:40 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	235 nm
5	Detector	Photo diode array
6	Injection	autosampler -Waters, model 717 plus
7	Rt's	2.825min
8	Injection volume	10 µl
9	Column Temperature	35 °C
10	Run time	10 mins
11	Diluent	Mobile Phase

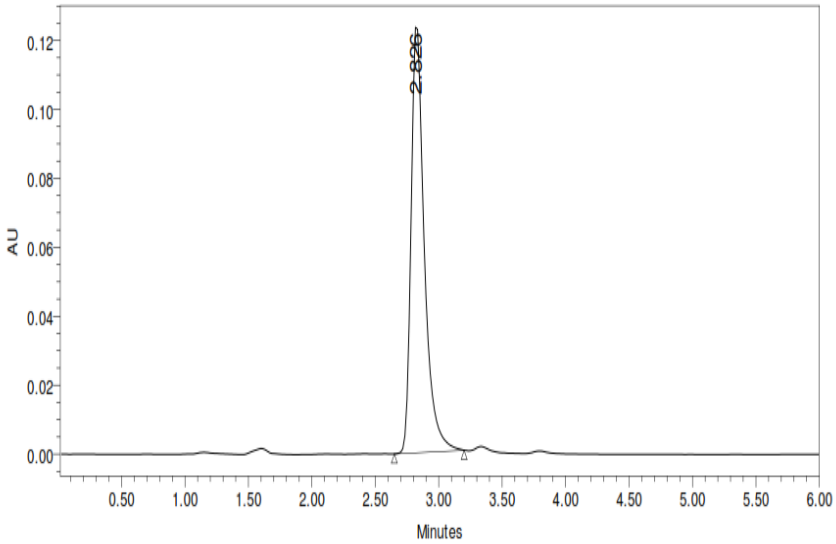

Fig. 3: Typical Chromatogram of Acotiamide.

Table 2: System suitability parameters.

S. No.	Parameter	Result
		Acotiamide
1	Retention Time	2.833
2	Tailing	1.4
3	Theoretical Plates (n)	4023

Method Validation

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100%and 150% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 3.

Table 3: Results of Accuracy.

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1093514.6	5	4.96	99.91%	100.56%
100%	2246802.7	10	9.98	99.18%	
150%	3407885.8	15	15.02	99.60%	

Precision

The intraday and interday precision of the proposed method was determined by analyzing standard solution of ACT at concentration 60ppm, 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Table 4: Results of Precision (%Assay).

Name : Acotiamide

	Name	RT	Area	Height (µV)
1	Acotiamide	2.823	895311	125747
2	Acotiamide	2.827	896783	122578
3	Acotiamide	2.828	895237	124365
4	Acotiamide	2.828	894206	124057
5	Acotiamide	2.825	895085	125410
Mean			895324	
Std. Dev.			927.8	
% RSD			0.10	

Linearity

Calibration graph was constructed by plotting peak area vs concentration of ACT and the regression equation was calculated. The calibration graphs were plotted over 5different linear concentrations in the range of 20-100ppm for ACT. Aliquots (10 µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n =6)]. The linearity graphs were shown in fig 4.

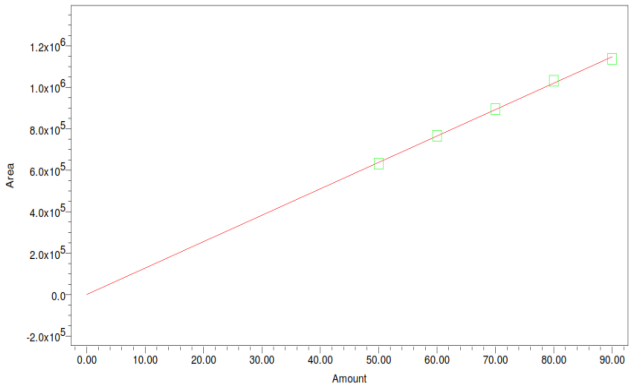

Fig 4: Linearity of Acotiamide r^2 = 0.998.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of ACT were determined by calculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD value for ACT was found to be 0.303µg/mL. LOQ value for ACT was found to be 1.011 µg/mL respectively.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine ACT in tablet dosage form. The results obtained for ACT were comparable with corresponding labeled amount. The results were tabulated in table 4.

CONCLUSIONS

The proposed method has advantage of simplicity and convenience for the separation and quantitation of ACT in the formulation which can be used for the assay of the dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for estimation of Acotiamide in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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