WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 1261-1267. Research Article ISSN 2277– 7105

CHROMOGENIC-VISIBLE-SPECTROPHOTOMETRIC QUANTIFICATION OF ACOTIAMIDE IN BULK DRUG AND ITS FORMULATION

Imam Pasha S.*[1], Murali Balaram Varanasi[1] and Ibrahim Mohammed[2]

[1]Sultan -Ul-Uloom College of Pharmacy, Banjara Hills, Road No.3, Hyderabad-500 034, Telangana, India.

[2]Pratap Narendar Reddy College of Pharmacy, Shamshabad, Hyderabad-509 325, Telangana, India.

Article Received on
30 October 2017,
Revised on 20 Nov. 2017,
Accepted on 10 Dec. 2017
DOI: 10.20959/wjpr201717-10469

***Corresponding Author**
Imam Pasha S.
Sultan -Ul-Uloom College of Pharmacy, Banjara Hills, Road No.3, Hyderabad-500 034, Telangana, India.

ABSTRACT

Simple, precise, economic and less time consuming visible spectroscopic method for quantification of Acotiamide was developed by using MBTH, Ferric chloride. The absorption maximum of the chromogen was found to be 630 nm. The developed method was obeying Beer Lambert's law in the range of 10-100 µg/ml concentration. The method has also been statistically evaluated and the results were within the limits. Molar absorptivity, Sand ell's sensitivity and correlation –co-efficient were found to be 1.4596×10^{12}, 0.107 and 0.997 respectively and the method is free from the interferences of other additives present in the formulation.

KEYWORDS: Acotiamide , MBTH,Visible,Spectroscopic, Analytical method.

1. INTRODUCTION

Acotiamide is (*N*-[2-[bis(1-methylethyl) amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl) amino] thiazole-4-carboxamide. Acotiamide acts as an acetyl cholinesterase inhibitor.[1] It is under global development by Zeria Pharmaceutical Co. Ltd and Astellas Pharma Inc. for the treatment of patients with functional dyspepsia.[3,4] It exerts its activity in the stomach via muscarinic receptor inhibition, resulting in enhanced acetylcholine release and inhibition of acetyl cholinesterase activity.[2] The Fig.1 represents the structure of Acotiamide.

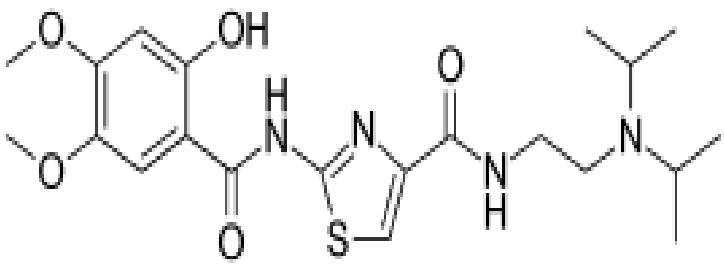

Fig. 1: Structure of Acotiamide.

2. Theoretical analysis

Only few HPLC methods[5,8] & LC/MS/MS methods[6,7] for quantitative determination of Acotiamide were reported in the literature. No visible spectrophotometric method for quantitative determination of Acotiamide in bulk drug samples and its formulations was reported. The aim of the present work is to develop and validate rapid, economical and sensitive visible spectrophotometric method for quantitative determination of Acotiamide in bulk drug samples and its formulations. In the present investigation new visible spectrophotometric method was developed for Acotiamide by using Chromogenic reagent namely MBTH.

3. EXPERIMENTAL

3.1 Instruments

SHIMADZU-1700 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements.

Digisun model DI-707 pH meter was used for all the pH measurements.

3.2 Chemicals and Reagents

All the chemicals used were of analytical grade.

3.3 Preparations

3-methyl-2-benzothiazolinone hydrazine hydrochloride (MBTH): was prepared by dissolving 100 mg of MBTH in 50 ml of distilled water.

Ferric Chloride ($FeCl_3$)(0.3% w/v): was prepared by dissolving 300 mg of ferric chloride hexa-hydrate in 100 ml of distilled water.

Sodium Hydroxide (NaOH) (2% w/v): was prepared by dissolving 2 gms of NaOH in 100 ml of distilled water.

3.4 Procedures

3.4.1 Preparation of standard drug solution

A standard drug solution of Acotiamide was prepared by dissolving 100 mg of drug in 100 ml of methanol in a standard volumetric flask to obtain a stock solution of 1 mg/ml.

3.4.2 Preparation of sample solution

A quantity of the powder from tablets equivalent to 100 mg of drug was dissolved in 100 ml methanol, filtered and analyzed by taking an aliquot and treated as per the procedure for standard.

3.4.3 Analysis of bulk drug Samples

Aliquots of standard drug solution (1.0-6.0 ml) were transferred into series of 10 ml graduated test tubes, 2 ml of ferric chloride and 1 ml of MBTH were added to each test tube, mixed well and volume was made up to 10 ml with methanol. The absorbance of resulting solution was measured at 630 nm against reagent blank prepared simultaneously and a linear graph was obtained. The amount of Acotiamide present in the sample solution was computed from its calibration curve.

3.4.4. Analysis of Formulations

A volume of 1 ml of the sample solution was transferred into 10 ml graduated test tube, 2 ml of ferric chloride and 1 ml of MBTH were added to the test tube, mixed well and volume was made up to 10 ml with methanol. The absorbance of resulting solution was measured at 630 nm against reagent blank prepared simultaneously.

4. RESULTS AND DISCUSSION

4.1 Optimization of parameters for Method

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen.Acotiamide has amino group in its molecular structure making it possible to undergo oxidative coupling of the drug with MBTH in ferric chloride solution in the proposed method. The effect of temperature of the reaction, quantity, concentration and order of addition of various reagents were studied, optimized after several experiments with respect to maximum sensitivity, color stability,

adherence to Beer's law and other optimum conditions are incorporated in the procedure. Optimum conditions established for the method are presented in table 2.

4.2 Optical Characteristics

Linearity

By using the method of least squares, regression analysis was performed to evaluate the slope (m), intercept (b) and correlation coefficient (r^2) was computed from various concentrations and the results are presented in Table 2**.** The graph (Fig 2) showed negligible intercept as described by the regression equation y = mx + b where y is the absorbance and x is the concentration in µg/ml.

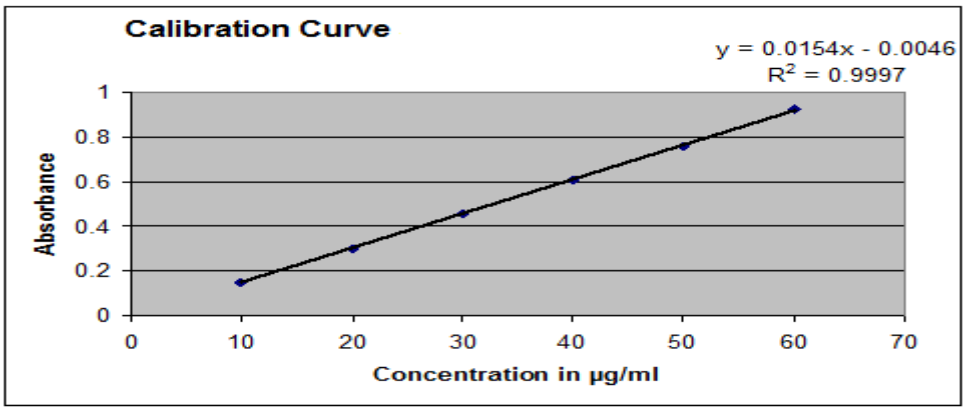

Fig 2: Calibration Curve.

The optical characteristics such as molar absorptivity, Beer's law limits, absorption Maxima and Sandell's sensitivity are presented in Table 2.

Table 2: Optical characteristics.

S. NO	Parameter	Value
01	λ_{max} (nm)	630
02	Beer's law range (µg/ml)	10-100
03	Molar extinction coefficient(L.mole^{-1} cm^{-1})	1.4596×10^{12}
04	Sandell's sensitivity (µg/cm^2/0.001)	0.107
05	Regression equation (y = mx + c) * Slope (m) Intercept (c)	 0.0015 -0.0046
06	Correlation coefficient (r)	0.9997
07	Precision (%Relative Standard Deviation)	0.77
08	Standard Error Of Mean	0.1174

4.3 Accuracy and Recovery

Commercially available tablets of Acotiamide (Table 3) were analyzed by the proposed method and as additional check on the accuracy of the method, recovery experiments were also conducted by spiking known amounts of pure drug in preanalysed formulation and the recovery was calculated in each of the case using the regression line equation developed under the Linearity experiment. Assay results of the proposed method was compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The means of the proposed method are not significantly different from that of reference method ($P > 0.05$). The assay and accuracy results were presented in Table 4. The interference studies indicated the common additives and excipients present in formulations did not interfere with the proposed method.

Table 3: Commercially Available Formulations of Acotiamide.

Generic Name	Proprietary Name	Dosage Form	Content
Acotiamide	Acogut, Lupin	Tablets	100 mg
	Actapro, sun Pharma	Tablets	100 mg
	Acotrust, Dr Reddy	Tablets	100 mg
	Acopep, Zydus Cadilla	Tablets	100 mg

Table 4: Evaluation of Acotiamide in pharmaceutical dosage forms (n=6).

Sample[a]	Labelled Amount (mg)	Amount obtained (mg)[b]		Percentage Recovery[b,c]
		Proposed method	Reference method[5]	Proposed Method
A_1	100	98.94.05±0.47	97.27±0.73	98.02±0.08
A_2	100	99.10±0.12	95.62±0.39	99.32±0.09

a - A_1 and A_2 are the tablets from different batches (Calutide, Cipla, Mumbai).

b – Mean ± SD of 6 determinations.

c – 40 mg of pure drug was added and recovered.

For the sample One-way ANOVA with post-test followed by Dunnett multiple comparison test was performed. The results showed that $P > 0.05$ and the means of the proposed method are not significantly different from that of reference method.

4.4 Chemistry of the colored species formed

Acotiamide has an amino group in the molecular structure making it possible to undergo oxidative coupling of the drug with MBTH in ferric chloride solution in method.

Under the reaction conditions, MBTH loses two electrons and one proton on oxidation forming the electrophilic intermediate which has been postulated to be the active coupling species. The intermediate reacts with amine by electrophilic attack on the aromatic ring of the amine and the resulting intermediate is spontaneously oxidized with an oxidant to form the colored species via oxidative coupling mechanism. Proposed scheme of complex formation is represented in Fig 2.

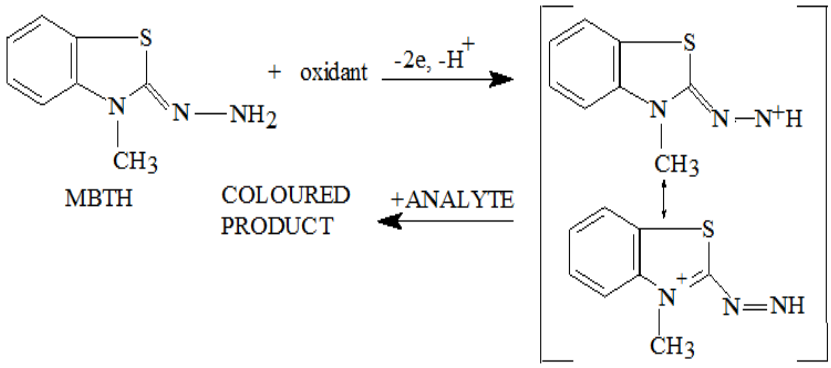

Fig 2: Proposed scheme of Complex formation.

5. CONCLUSION

The proposed visible spectrophotometric method enables quantitative determination of Acotiamide in bulk drug samples and tablets. Efficient visible spectrophotometric detection at the respective absorption maxima enabled determination with no interference from the excipients. The calibration curve was linear over a concentration range of 10-100 µg/ml for the proposed method. The relative standard deviation's (R.S.D.) was less than 1% and average recovery was above 99%. The proposed method is fast, sensitive, precise, accurate, and efficient and can be used for analysis in quality control laboratories.

6. REFERENCES

1. Guoguang Xiao, Xiaoping Xie, Juan Fan, Jianjun Deng, Shan Tan, Yu Zhu, Qin Guo and Chaomin Wan, Efficacy and Safety of Acotiamide for the Treatment of Functional Dyspepsia: Systematic Review and Meta-Analysis, *The Scientific World Journal, 2014; PP 9.*
2. Kazuyoshi Yoshii, Masamichi Hirayama, Toshifumi Nakamura, Ryoko Toda, Junko Hasegawa, Mineo Takei, Yukinori Mera, Yoshihiro Kawabata, Mechanism for distribution of Acotiamide, a novel gastroprokinetic agent for the treatment of functional

dyspepsia, in rat stomach, *Journal of Pharmaceutical sciences*, 2011; 100(11): 4965-4973.

3. H. Kusunoki, K. Haruma, N. Manabe, H. Imamura, T. Kamada, A.Shiotani, J. Hata, H. Sugioka, Y. Saito, H. Kato, J. Tack. Therapeutic efficacy of Acotiamide in patients with functional dyspepsia based on enhanced postprandial gastric accommodation and emptying: randomized controlled study evaluation by real-time ultrasonography, *Wiley Online Library, 2012; 24(6): 540–551.*
4. K. Matsueda, M. Hongo, J. Tack, H. Aoki, Y. Saito, H. Kato, Clinicaltrial: dose-dependent therapeutic efficacy of Acotiamide(Z-338) in patients with functional dyspepsia – 100 mg t.i.d. is an optimal dosage, Wiley Online Library, 2010; 22(6): 618–e173.
5. Vani.R, Sunitha.M, Analytical method development and validation for the determination of Acotiamide hydrochloride using reverse phase HPLC method in bulk and tablet dosage form, World journal of pharmacy and pharmaceutical sciences, 6(10): 768-775.
6. Jing Li, Rui Huang, Zhi Wang, Haijun Qu, Meijuan Sun, Zhenhuan Zhao, Development and Validation of a Sensitive and Specific LC–MS-MS Method for the Determination of Acotiamide in Rat Plasma, Journal of Chromatographic Science, 2016; 54(6): 1004–1009.
7. Patel.P.N, Kalariya.P.D, Swamy.C.V, Gananadhamu.S, Srinivas.R, Quantitation of Acotiamide in rat plasma by UHPLC-Q-TOF-MS: method development, validation and application to pharmacokinetics, Biomed Chromatography., 2016; 30(3): 363-368.
8. Mohit Thummar, Prinesh N. Patel, Gananadhamu Samanthula, Srinivas Ragampeta, Stability-indicating assay method for Acotiamide: Separation, identification and characterization of its hydroxylated and hydrolytic degradation products along with a process-related impurity by ultra-high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry, Wiley Online Library, 2017; 31(21): 1813–1824.