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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF ACOTIAMIDE HYDROCHLORIDE TRIHYDRATE IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT BY DIFFERENT RATIO SPECTRA MANIPULATING METHODS

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ABSTRACT

Back ground: Acotiamide hydrochloride trihydrate is a novel gastroprokinetic drug which has been recently approved for the treatment of patients with functional dyspepsia. **Objective:** Analysis of acotiamide hydrochloride trihydrate in the presence of its oxidative degradation product by simple spectrophotometric methods. **Methods:** The absorption spectra of acotiamide hydrochloride trihydrate (1—15 µg/mL) were divided by spectrum of the degradation product (11 µg/mL) to get the ratio spectra. **Results:** Ratio difference, first derivative of the ratio spectra, and mean centering methods have been developed for manipulating the ratio spectra of acotiamide hydrochloride trihydrate to remove any interference from its degradation product. **Conclusion:** The proposed methods have been successfully applied and validated to the analysis of the drug in its pure or new pharmaceutical dosage form in the presence of its oxidative degradation product. **Highlights:** This study presents the first reported spectrophotometric methods for determination of acotiamide hydrochloride trihydrate in the presence of its oxidative degradation product.

Keywords: Acotiamide hydrochloride trihydrate;ratio difference; first derivative of the ratio spectra; mean centering.

INTRODUCTION

Acotiamide hydrochloride trihydrate (ACT), Figure 1; is recently approved in Japan as the world's first treatment for functional dyspepsia diagnosed by Rome III criteria. It acts as prokinetic agent through enhancement of acetylcholine release from enteric neurons through muscarinic receptor antagonism and acetylcholinesterase inhibition, thereby enhancing gastric emptying and gastric accommodation [1,2].

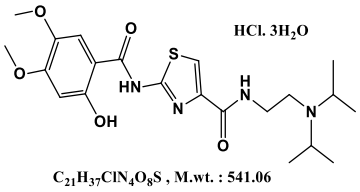

Fig. 1: Structural formula of ACT

Through the last decade, several spectrophotometric methods have been developed to resolve and quantify the drug of interest in presence of its degradation product [3–6]. Among these methods, ratio spectra manipulation seemed to be the most popular methods. To remove the interference from the degradation products in the mixture with their drugs, the spectrum of the mixture could be divided with the spectrum of the degradation product [7–10]. As a result, the spectrum of the degradation product converted into a straight line with constant value [11]. Further manipulation through derivatization or mean centering of the obtained ratio spectra are required to cancel the signals from the degradation product.

To date, no reported spectroscopic methods have been published for the analysis of ACT in the presence of its degradation product. The literature review revealed only three chromatographic procedures for the drug assay [12–14].

The goal of this work is to develop the first spectrophotometric methods for the determination of ACT in the presence of its oxidative degradation product. Three ratio spectra manipulating methods were applied namely; ratio difference (RD) [15–17], first derivative of the ratio spectra (^{1}DD) [18,19], and mean centering (MC) [20–22].

EXPERIMENTAL

Materials and chemicals

Pure ACT (99.0%)— were kindly supplied as gift samples from Al-Andalous for Pharmaceutical Industries (6th of October city, Egypt).

Pharmaceutical formulation—Acofide® tablets; each tablet is claimed to contain 100 mg ACT (Zeria&Astellas, Japan).

Solvent—Methanol (HPLC grade; Sigma-Aldrich, Darmstadt, Germany).

Hydrogen peroxide (30% w/v) — Analytical grade (El-Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt).

Apparatus and software

Spectrophotometer— UV-Vis 1800 (Shimadzu Corp., Tokyo, Japan).

MATLAB—Version 8.2.0.701 (R2013b) with PLS-1 toolbox version 2.1.

Standard solutions

Stock standard solution of ACT(100 µg/mL)—Stock standard solution of ACT was prepared by dissolving 10 mg of the drug powder in 50 mL of methanol and complete to 100 mL with methanol.

Standard solution of ACT degradation product—Stock standard solution of ACT degradation product was prepared by dissolved 10 mg of pure ACT powder in 20 mL methanol and transferred to a 100-mL round bottomed flask to which 5 mL of 30% H_2O_2 was added. The solution was heated under reflux at 80 °C for 4 h and evaporated to dryness under vacuum. The obtained residue was extracted with methanol, filtered into a 100-mL volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradation product derived from 100 µg/mL of ACT.

PROCEDURES

Construction of calibration graphs

Aliquots of ACT standard solution equivalent to (10-150) µg were transferred into a series of 10-mL volumetric flasks and diluted to

volume with methanol. The absorption spectra of these solutions were recorded using methanol as a blank. After that, divided by spectrum of the degradation product (11µg/mL) to get the ratio spectra.

For RD**—**The difference between amplitudes of the ratio spectra at 341 and 231 nm were plotted versus the corresponding concentrations of ACT in µg/mL to get the calibration graph and the regression equation was derived.

For ^{1}DD— The first derivative corresponding to each ratio spectrum was recorded, using Δλ = 2 nm and scaling factor 10. Calibration graph of the method was constructed by plotting the amplitude values measured at 276 nm versus the corresponding concentrations in µg/mL and the regression equation was derived.

For MC—The obtained ratio spectra were exported to MATLAB and mean centered. Calibration graph of the method was constructed by plotting the mean centered values of the ratio spectra at 346 nm versus the corresponding concentrations in µg/mL and the regression equation was derived.

Assay of synthetic mixtures

Synthetic mixtures containing the drug and different percentages of its degradation product were prepared. After that, the mixtures were analyzed using the previously described procedure for each method.

Assay of tablet dosage form

Ten Acofide® tablets (100 mg per tablet**)** were weighed and finely powdered. Appropriate weight of the powder equivalent to 10 mg was accurately weighed, transferred to 100- mL conical flask and the volume was made up to 50 mL with methanol. The solution was shaken vigorously for 20 min then sonicated for 30 min and filtered into100-mL volumetric flask. The volume was completed to 100 mL with methanol to produce a stock solution labeled to contain 100 µg/mL of ACT. Further dilutions were done with methanol in order to prepare different concentrations of ACT within the linearity rang. The concentration of ACT was calculated using the previously described procedure for each method.

RESULTS AND DISCUSSION

Accelerated degradation of ACT was achieved upon heating under reflux at 80°C with 30% hydrogen peroxide for 4 h. Complete degradation was confirmed by TLC using mobile phase consisting of heptane–ethyl acetate–methanol–formic acid (2 + 2.5 + 2 + 0.2, by volume) with observation of one spot not related to ACT. The solution was evaporated to dryness under vacuum. The obtained residue was extracted with methanol, filtered, and left to evaporate at room temperature. The structure of the isolated degradation product was interpreted by IR and mass spectrometry.

IR spectrum of the degradation product exhibited disappearance of aromatic-OH band already presented in IR spectrum of ACT at 3499cm^{-1}. Also, appearance of large band at 1741 cm^{-1} that indicated formation of aromatic C=O, as shown in Figure2, 3. Moreover, mass interpretation reveals that the molecular weight of ACT and its degradation product were 450.55 and 466.51, respectively, which indicated formation of N-oxide, as shown in Figure4,5. The predicted structural formula of ACT degradation product is shown in Figure 6.

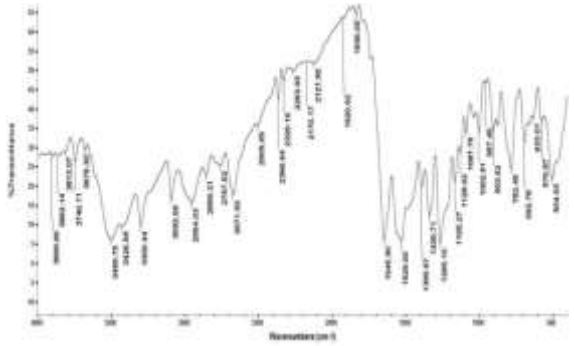

Fig. 2: IR spectrum of ACT on KBr disc.

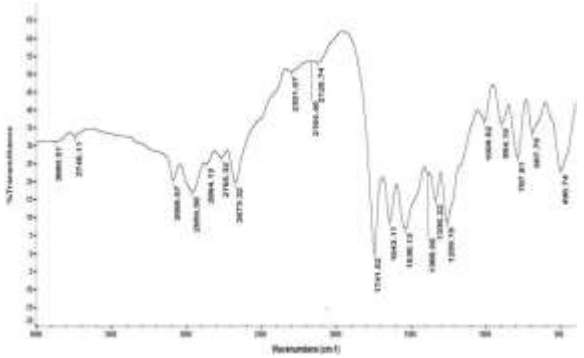

Fig. 3: IR spectrum of ACT degradation product on KBr disc.

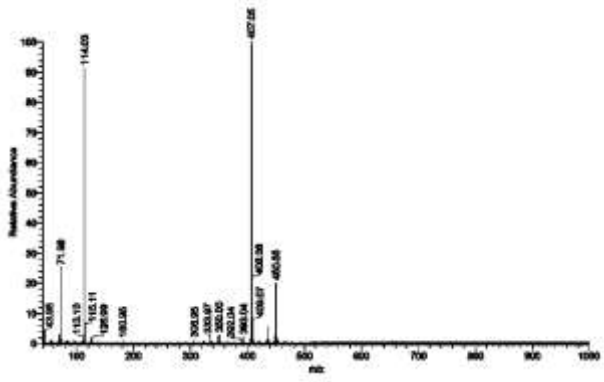

Fig. 4: Mass spectrum of ACT.

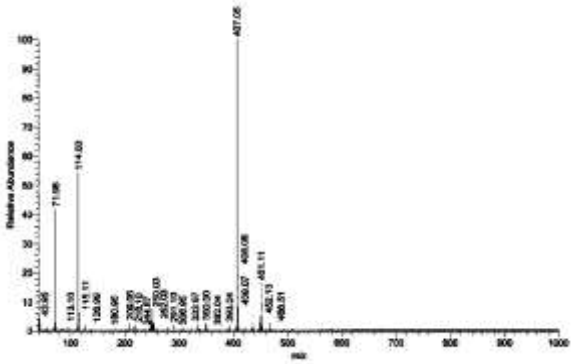

Fig. 5: Mass spectrum of ACT degradation product.

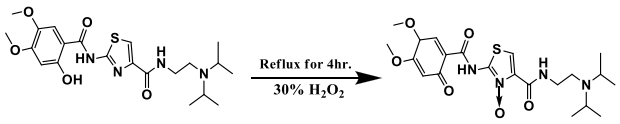

Fig. 6: Proposed degradation pathway of ACT

In spectrophotometry, presence of impurities or degradation products that absorbed UV in the same region of the active drug impeded the direct determination of the drug due to overlapping spectra. After scanning the zero order spectra of ACT and its degradation product (Figure 7), sever overlap has been observed that hindered direct determination of ACT. To remove this interference from the degradation product, the recorded spectra were divided by a spectrum of the degradation product (divisor), as a result, ratio spectra were obtained as shown in Figure 8. In order to choose the best divisor that gave better results regarding selectivity and signal to noise ratio, several concentrations of the degradation product (5, 7, 9, 11, 13 µg/mL) were tried. The divisor of a concentration 11 µg/mL was found to be the best one. Manipulation of the obtained ratio spectra by the proposed methods will remove any interference from the degradation product.

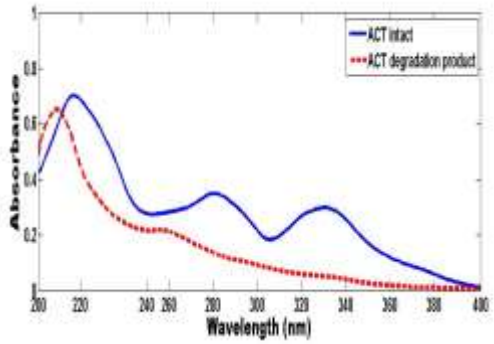

Fig. 7: **Absorption spectra of ACT and its degradation product (11 µg/mL) of each.**

RD—This method based on selection of two wavelengths in the ratio spectra where the difference between them gives a good linearity in correlation with the concentrations. So different wavelength pairs were tried and 341 and 231 nm exhibited the best results. Linear calibration graph was obtained by plotting the difference between amplitudes of the ratio spectra at 341 and 231 nm versus the corresponding concentrations of ACT in the range 1-15µg/mL and the regression equation was derived.

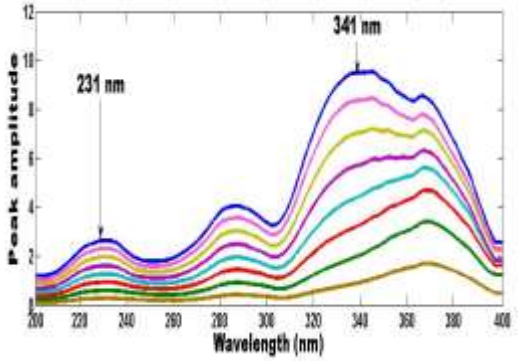

Fig. 8: Ratio spectra of ACT (1-15 µg/mL) using 11 µg/mL of degradation product as a divisor.

¹DD—Each ratio spectrum was transformed into its first derivative using Δλ = 2 nm and scaling factor 10 (Figure 9). Linear calibration graph was obtained by plotting the amplitudes of the first derivative of the ratio spectra at 276 nm versus the corresponding concentrations of ACT in the range 1-15µg/mL and the regression equation was derived.

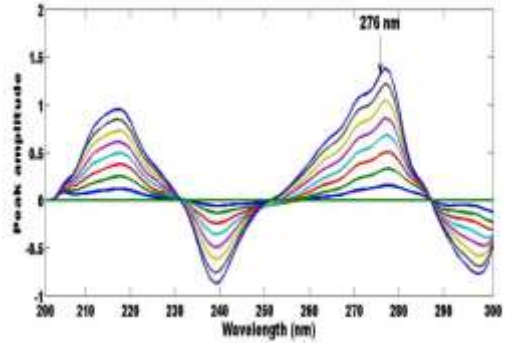

Fig. 9: First derivative of the ratio spectra of ACT(1-15 µg/mL) using 11 µg/mL of degradation product as a divisor.

MC—Ratio spectra were transferred into MATLAB and mean centered (Figure 10**)**. Linear calibration graph was obtained by plotting the mean centered values at 346 nm versus the corresponding concentrations of ACT in the range 1-15µg/mL and the regression equation was derived.

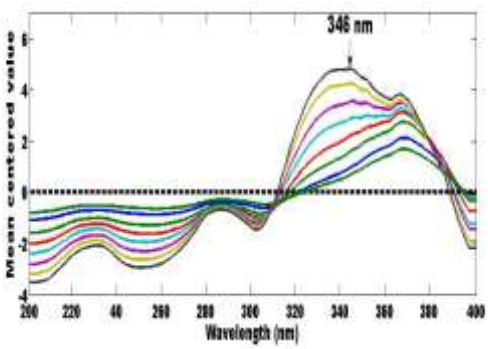

Fig. 10: Mean centering of thee ratio spectra of ACT (1-15 µg/mL) using 11 µg/mL of degradation product as a divisor.

Validation of the described methods was performed in a compliance with International Conference of Harmonization (ICH) guidelines [23]. All methods exhibited good linearity for ACT in a concentration range 1-15 µg/mL. Values of slope, intercept and coefficient of determination (r^2) were presented in Table 1. Residual standard deviation of the regression line (Sa) and slope were used for calculation the Limit of detection (LOD = 3.3 Sa / slope) and Limit of quantitation (LOQ = 10 Sa / slope), The obtained results were presented in Table 1. Accuracy was calculated as a mean percent recovery of three determination for three concentration levels (5, 9, 13 µg/mL) and the results were presented in Table 1. Moreover, standard addition technique was applied to assess the accuracy and there was no interference from excipients (Table 2).Precision was calculated as a relative standard deviation of three determination for three concentration levels (5, 9, 13 µg/mL) within one day for repeatability and on three successive days for intermediate precision and the results were presented in Table 1.The specificity of the methods was proved by the analysis of synthetic mixtures of the drug and its degradation product (Table 3).

Table 1: Regression and validation data for determination of ACT by the proposed methods.

Parameter	RD	¹DD	MC
Slope ± SE	0.4462 ± 0.0034	0.0884 ± 0.0004	0.3030 ± 0.0018
Intercept ± SE	0.2058 ± 0.0313	0.0643 ± 0.0036	0.1887 ± 0.0162
Coefficient of determination(r^2)	0.9997	0.9999	0.9998
Accuracy (mean %R) *	100.33	99.68	99.39
Repeatability (%RSD)*	0.832	0.775	0.581
Intermediate precision (%RSD)*	1.047	0.963	0.852
LOD (µg/mL)	0.301	0.174	0.229
LOQ (µg/mL)	0.913	0.527	0.695
Range (µg/mL)	1-15	1-15	1-15

***Average of three determinations for three concentrations repeated three times.**

Table 2: Determination of ACT in Acofide® tablets by the proposed methods and application of standard addition technique:

Method	Acofide® tablets	Standard addition technique			
	% Recovery* ± %RSD	Pharmaceutical(µg/mL)	Pure added (µg/mL)	Pure found (µg/mL)	% Recovery**
RD	100.03 ± 0.738	5	5	5.095	101.91
			7	7.063	100.90
			9	9.120	101.34

	Mean ± %RSD				101.38 ± 0.497
	100.34 ± 0.485	5	5 7 9	5.036 6.982 8.939	100.72 99.74 99.32
^{1}DD	Mean ± %RSD				99.93 ± 0.720
	100.35 ± 0.426	5	5 7 9	5.010 6.897 8.860	100.21 98.53 98.44
MC	Mean ± %RSD				99.06 ± 1.005

***Average of five determinations.**
****Average of three determinations.**

Table 3: Determination of ACT in synthetic mixtures with its degradation product by the proposed methods

ACT (µg/mL)	Degradation product (µg/mL)	Degradation product %	% Recovery of ACT		
			RD	^{1}DD	MC
13	2	13.33	101.20	100.48	100.97
11	4	26.67	100.26	100.55	100.20
9	6	40.00	99.39	100.01	99.78
7	8	53.33	98.42	99.82	99.40
5	10	66.67	99.74	99.48	100.22
3	12	80.00	98.40	99.43	97.83
Mean ± %RSD			99.57 ± 1.089	99.96 ± 0.478	99.73 ± 1.073

The proposed methods were successfully applied for determination of ACT in Acofide® tablets. Statistical analysis was performed between the obtained results and those obtained by the reported HPLC method [14] by applying *t*-test and *F*-test, and no significant differences were noticed, as shown in Table 4.

Table 4: Statistical comparison of the results obtained by applying the proposed method and the reported method [14].

Parameters	RD	^{1}DD	MC	Reported method*
N**	5	5	5	5
$\bar{X}$***	100.03	100.34	100.35	100.55
%RSD	0.738	0.485	0.426	0.941
Variance	0.544	0.237	0.183	0.895
t-test (2.306)****	0.968	0.447	0.426	
F-test (6.388)****	1.645	3.783	4.907	

*HPLC determination on C18 column using mobile phase consists of methanol: 20 mmol/L ammonium acetate aqueous solution pH 6.8(45: 55, v/v).** Number of experiments.
*** The mean of percent recovery of pharmaceutical preparation.
**** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05).

With regard to the previous discussion, RD has superiority over the other methods in being the simplest one regarding manipulation of data (one step method). RD overcomes the limitation of other methods that required extra-processing steps by MATLAB or derivatization steps.

CONCLUSIONS

In this work, three ratio spectra manipulating methods namely; RD, ^{1}DD and MC were presented as the first spectrophotometric methods for determination of ACT in the presence of its oxidative degradation product. The developed method is simple, rapid, accurate and sensitive and does not need any sophisticated instrumentation and separation steps.

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