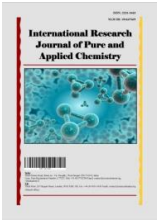

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Stability-indicating UHPLC and TLC-densitometric Methods for the Determination of Tiamulin Fumarate

Shimaa E. Abdel Aziz[1*], Nermin S. Ahmed[2] and Sawsan A. Abdel Razeq[1]

[1]Analytical Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. Author SAAR designed the study and wrote the protocol. Author NSA supervise the analyses of the study. Author SEAA performed the experimental work, statistical analysis and wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Two novel stability-indicating methods were developed for the determination of tiamulin fumarate. in the presence of its degradation products. The first was UHPLC-UV method. Efficient separation was achieved by isocratic elution with a mobile phase of 0.1% aqueous ortho-phosphoric acid (pH 3.5 ± 0.5) and methanol (20:80, v/v) with UV detection at 210 nm. Linearity was obtained in the range of 0.5-10.0 µg mL^{-1} with mean accuracy of 100.40 ± 0.71. The second method was a TLC-densitometric evaluation of a thin-layer chromatogram of the intact drug using a mobile phase of methanol: pentanol: ethyl acetate: 16.5% ammonia (5:4:2:4, by volume). The TLC-plates were scanned densitometrically at 220 nm where R_f values were 0.58, 0.48 and 0.74 for tiamulin F, its acidic and oxidative degradants, respectively. Moreover, the plates were sprayed with 16% sulfuric acid, heated at 105°C for 10 min. where a yellow-coloured band appeared corresponding to the intact drug was scanned densitometrically at 450 nm. While the bands of the two degradants were no longer observed anymore. Tiamulin F was determined in the range of 1.0-10.0 µg/band with mean accuracy of 100.27% ± 1.47 at 220 nm and 99.93% ± 1.38 at 450 nm. The proposed methods

**Corresponding author: E-mail: shimaaebrahim.pharmg@azhar.edu.eg;*

were successfully applied for the determination of drug in marketed oral solution. The obtained results were statistically analyzed and found to be in accordance with those obtained by a reported method.

Keywords: Stability-indicating; UHPLC; TLC-densitometry; tiamulin fumarate.

1. INTRODUCTION

Tiamulin, (*E*)-but-2-enedioic acid; [(1*S*,2*R*,3*S*,4*S*,6*R*,7*R*,8*R*)-4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl-9-oxo-6-tricyclo [5.4.3.0] tetradecanyl] 2-[2(diethylamino) ethyl sulfanyl] acetate fumarate.[1]; Fig. 1. It is a diterpene antimicrobial pleuromutilin antibiotic drug that is used in veterinary medicine particularly for pigs and poultry [2]. It is effective against Mycoplasma and anaerobes. It can be used clinically in treatment of swine dysentery, bacterial pneumonia, porcine intestinal adenomatosis, arthritis and chronic sinusitis [3,4]. Tiamulin F. inhibits bacterial protein synthesis via binding to the peptidyl transferase component of the 50S subunit of ribosomes [2].

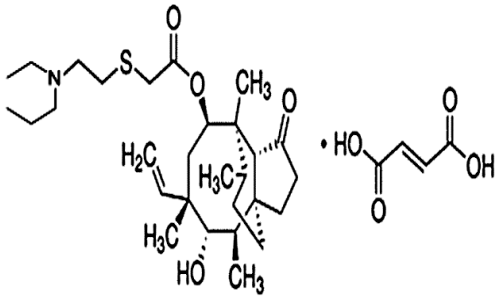


Fig. 1. The chemical structure of Tiamulin F

Several methods were reported for the determination of tiamulin F including TLC-densitometry [5], Ion selective electrode [6,7], HPLC [8-12] and LC/MS [13-18]. It is worthy to mention that comprehensive literature revealed the lack of any stability studies of tiamulin F.

High-performance liquid chromatography (HPLC) is a common liquid chromatography (LC) technique used for qualitative and quantitative analysis of several drugs especially if present in a mixture or in presence of their degradation products, impurities, and other pharmaceutical additives. The UPLC system provide several advantages over conventional High-performance liquid chromatography (HPLC) technique; such as increase selectively and sensitivity with high resolution performance as it based on the use of a novel column material of very small particle size less than 2 µm , short analysis time, cost reduction, decrease solvent consumption and improve separation efficiency , however the major drawbacks of UPLC technique is the higher back pressures which decreases the life of the columns but it can overcome through Increasing the column temperature which reduces the back pressure. Moreover, the particles of less than 2 µm are mostly non-regenerable and, therefore, have a narrow use [19].

The aim of the present work is to develop selective and sensitive UHPLC and TLC-densitometric methods for determination of tiamulin F in the presence of its degradation products. In addition, studying the degradation kinetics of tiamulin F under different stress condition.

2. MATERIALS AND METHODS

2.1 Instruments

- UHPLC (Agilent 1100 series, Waldbronn, Germany) with binary Pump and UV detector, equipped with Phenomenex Kinetex C_{18} (100 × 4.6 mm, 2.6 µm).
- Densitometer model 3; equipped with WinCats software, Camag TLC scanner 3, Camag lincomat 5 autosampler (Switzerland).
- TLC plates pre-coated with silica gel 60 F_{254}, 10 x 20 cm (Merck, Germany).
- Chromatographic tank 10 x 20 cm.
- UV lamp with short wavelength (Wised clean, China).
- IR spectrophotometer (Vector 8201 PC, Germany).
- Mass spectrophotometer (Hewlette Packard, Agilent Technologies, Wilmington, DE).
- Digital pH meter with double junction glass electrode (Hanna, Romania).

2.2 Materials and Reagents

- Pure tiamulin, B. N. 9875624, was kindly supplied by Arab Veterinary Industrial Co.

(AVICO) (Egypt) with purity of 98.25 % as referred by the supplier and it was tested by TLC-densitometry.
- Martimulin oral solution (250 mL), B.N. 14914/1, labeled to contain 125Mg of tiamulin F per ml (Martiros for pharmaceutical industries, Egypt) was kindly supplied by the supplier.
- Acetonitrile, HPLC grade, 2.5 L (Fisher, England).
- Methanol, 2.5 L (Fisher, England).
- Ethanol, 2.5 L (Sigma Aldrich, Germany).
- Pentanol, 2.5 L (Alfa Chemicals, Egypt).
- Chloroform and Acetone, (BDH Chemicals ltd, England).
- Ethyl acetate, N-butanol, Toluene, Iso propanol, Tri-ethyl amine, Dichloromethane, Tetrahydrofurane, and Glacial acetic acid, 1 L (Adwic. Cairo, Egypt).
- Ammonia solution 33%, 1 L (Adwic. Cairo, Egypt); 16.5% aqueous solution.
- ortho-phosphoric acid, 1 L (Adwic. Cairo, Egypt); 0.1% aqueous solution.
- Citric acid, 500 gm (Adwic. Cairo, Egypt); 10% aqueous solution.
- HCl, 2.5 L (Sigma Aldrich, Germany); 0.1 M and 5 M aqueous solution.
- NaOH pellets, 500 gm (Qualikems, India); 0.1 M and 5 M aqueous solution.
- KOH pellets, 500 gm (Nakamaruko Kawasaki, Japan); 5 M methanolic solution [20].
- Sulfuric acid, 2.5 L (Sigma Aldrich, Germany); 16% aqueous solution.
- Hydrogen peroxide solution 33%, 1 L (Piochem, Egypt).

2.3 Standard Solutions

Standard solution of tiamulin F (10 Mg mL^{-1}) was prepared by dissolving 1000 Mg of the pure drug in 100 mL methanol to be used in the TLC-densitometry. This methanolic solution was further diluted two tenth to obtain a standard solution labeled to contain 0.1 Mg mL^{-1} for UHPLC application.

2.4 Preparation of Degraded Solutions

2.4.1 Acidic degradation product

An accurately weighed 250 Mg of pure tiamulin were refluxed with 25 mL 5 M aqueous HCl for 6 h. Then, the solution was cooled to room temperature neutralize and evaporated under vacuum. The dried residues were extracted twice using 10 mL methanol, filtered into 25-mL volumetric flask, and completed to volume with methanol. The prepared acid-hydrolyzed solution derived from ten Mg mL^{-1} tiamulin F. This solution was further diluted to provide a methanolic solution labeled to contain 100 µg mL^{-1} acid degradants.

2.4.2 Oxidative degradation product

An accurately weighed 250 Mg of the pure drug substance were dissolved in 25 ml H_2O_2 (10 %, v/v). Then the solution was gently heated in a boiling water bath to expel the excess H_2O_2. The dried residue was dissolved in 25 mL methanol to obtain a solution derived from ten Mg mL^{-1} tiamulin F. Further dilution with methanol was done to obtain a solution labeled to contain 100 µg mL^{-1} oxidative degradant.

2.5 Procedures

2.5.1 Linearity

2.5.1.1 UHPLC

Aliquots of standard drug methanolic solution (100 µg mL^{-1}) equivalent to (0.005-0.1 Mg) tiamulin F were separately transferred into a series of 10-mL volumetric flasks and diluted to volume with the mobile phase. Triplicate 10 µL injections from each solution were chromatographed on Phenomenex Kinetex C_{18} (100 × 4.6 mm, 2.6 µm) applying isocratic elution using a mobile phase composed of 0.1% aqueous ortho-phosphoric acid (pH 3.5 ± 0.5) and methanol in the ratio of (20:80, v/v) at ambient temperature. Flow rate was 1.0 mL min^{-1} and UV detection at 210 nm. Calibration curve was constructed by plotting the peak areas of tiamulin F versus the drug concentrations and the corresponding regression equation was computed.

2.5.1.2 TLC-densitometry

Accurately measured aliquots equivalent to 1-10 Mg of tiamulin F standard solution (10 Mg mL^{-1}) in methanol were added to 10-mL volumetric flasks to be diluted to the mark with methanol. Triplicate ten µL of each solution were applied to pre-coated (10 x 20 cm) TLC aluminum sheet silica gel 60 F_{254}. The plates were developed in a mobile phase of methanol: pentanol: ethyl acetate: 16.5% ammonia (5:4:2:4, by volume). Then, the plates were allowed to dry in air before densitometric scanning at 220 nm.

Also, the plates were sprayed with 16% aqueous sulfuric acid solution, heated in a vacuum oven at 105°C for 10 min followed by TLC-densitometric scanning at 450 nm. The recorded area under the peaks were plotted against the drug concentration to construct two calibration curves from which the regression equations were calculated.

2.5.2 Assay of laboratory prepared mixtures of the pure tiamulin F and its degradation products

2.5.2.1 UHPLC method

Into a series of 10-mL volumetric flasks, transfer different aliquots of standard drug solution (100 µg mL^{-1}) equivalent to 0.09-0.01 Mg tiamulin F. Then, various aliquots of each (0.1 Mg mL^{-1}) acidic and oxidative degradants equivalent to 0.005-0.045 Mg were added. Flasks were completed to the mark with the mobile phase. Then, 10 µL of each solution was chromatographed on UHPLC column as detailed under "2.5.1. Linearity".

2.5.2.2 TLC-densitometric method

Aliquots equivalent to 9-1 Mg tiamulin F from its standard methanolic solution (10 Mg mL^{-1}) were mixed with different aliquots of each (10 Mg mL^{-1}) acidic and oxidative degradants equivalent to 0.5-4.5 Mg. Each flask was completed to the mark with methanol to be analyzed by TLC-densitometric method as described under "2.5.1. Linearity".

2.5.3 Application to dosage forms

A volume of Martimulin oral solution equivalent to 250 Mg tiamulin F was pipetted into 25-mL volumetric flask. Volume was completed to the mark with methanol to obtain a solution claimed to contain 10 Mg mL^{-1} tiamulin F. Further two tenth dilution was required to obtain a solution labeled to contain 100 µg ml^{-1} tiamulin F. The two prepared solutions (10 Mg mL^{-1}) and (100 µg mL^{-1}) were analyzed by the UHPLC and TLC-densitometric as detailed under "2.5.1. Linearity", respectively. The concentration of the drug was calculated from the corresponding regression equation.

3. RESULTS AND DISCUSSION

3.1 Degradation of Tiamulin F

Tiamulin F contains a tertiary amine, phenolic OH, sulfur and ester groups that that are expected to be hydrolyzed. Stressed hydrolytic degradation was studied under different stressed conditions; 5 M HCl, 10% H_2O_2, thermal (90 ^{0}C) and UV light at 254 nm for different time intervals. Unfortunately, It is practically insoluble in alkaline medium neither aqueous nor alcoholic solution. Consequently, the stressed alkaline hydrolytic degradation of tiamulin F could not be studied. The drug was found to be photo and thermally stable but susceptible for complete degradation upon acid stress conditions and incomplete oxidative degradation.

The methanolic extract of the bands of tiamulin F and its three degradants were separated on a preparative TLC using the above-mentioned mobile phase. The bands related to the intact tiamulin F and each degradant were scratched, extracted with methanol, and evaporated to dryness. The residues were tested by both IR (using KBr disc) and MS.

The IR spectrum of the intact drug showed characteristic absorption bands for O-H of COOH at 2962 cm^{-1} and C=O of Ester at 1728 cm^{-1}. Both bands had disappeared from the spectra of the acidic degradant (I) and (II) indicate ester cleavage; Fig. 2. While the IR spectrum of the oxidative degradant showed only the bands that are common in the spectra of the intact and the acidic degradants such as C-N and C-OH bands at 1277 cm^{-1} and 3500 cm^{-1}, respectively; Fig. 2. Although oxidative degradation of tiamulin F may involve oxidation of sulfur and nitrogen, the S=O formation was more suggested than N=O formation. This may be attributed to higher stability of the sulfoxide derivative in the neutral medium where N-oxide could not be stabilized. Sulfoxide derivative may be stabilized through conjugation with fumarate through the lone pair of its tertiary amine moiety. Unfortunately, the S=O at 1141 cm-^{1} band could not be distinguished in the IR fingerprint zone. It is noteworthy to mention that maleic acid is not susceptible for oxidation with hydrogen peroxide except in the presence of a catalyst [21].

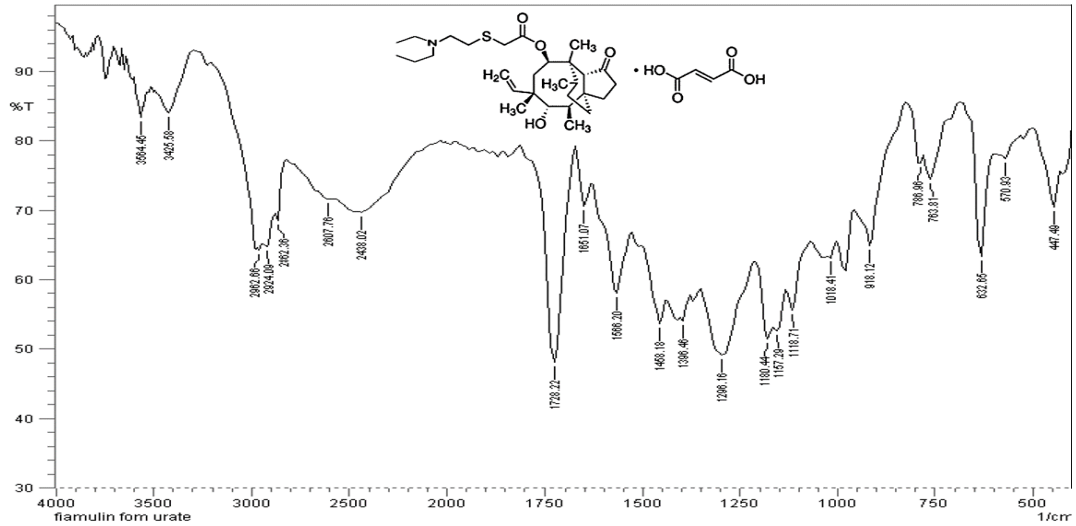
Further confirmation was carried out by MS, where mass spectrum of intact tiamulin F was characterized by the molecular ion m/z at 609.81. While the spectra of the two acid degradants (I) and (II) showed m/z at 148.09 of organosulfur moiety and 461.75 for carboxylic acid derivative, respectively; Fig. 3. It is noteworthy to mention that the acidic degradant (II) exhibits tautomeric ester formation with fumaric acid. The oxidative degradant mass spectrum showed m/z at 625.79

suggesting sulfoxide derivative; Fig. 3. The suggested degradation pathways were presented in Scheme 1.

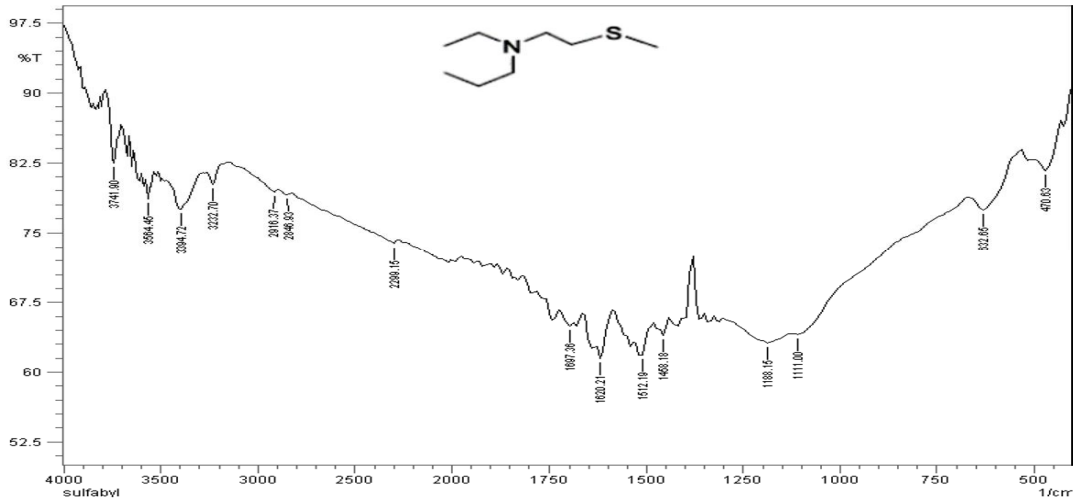
3.2 UHPLC Method

Chromatographic separation of tiamulin and its degradation products was performed using a Phenomenex Kinetex C_{18} (100 × 4.6 mm, 2.6 µm) column. Different mobile phases were tried as 0.1% ortho-phosphoric acid in water-acetonitrile, acetonitrile-methanol, and acetonitrile-phosphate buffer in different ratios. Satisfactory separation was achieved using a mobile phase composed of 0.1% aqueous ortho-phosphoric acid (pH 3.5 ± 0.5) and methanol in the ratio of (20:80, v/v). Different flow rates (0.3-1.5 mL min^{-1}) and wave lengths were tried, where the most sensitive detector response was obtained at 210 nm with and flow rate 1.0 mL min^{-1}.

Under the above-optimized conditions, satisfactory chromatographic resolution in a short analysis time was adopted where sharp resolved peaks were obtained at R_t 1.755 ± 0.044 min for the tiamulin F and at 0.957 ± 0.004 min, 2.651 ± 0.006 min for its acid degradant (I) and (II), respectively; Fig. 5. Unfortunately, the peak of the oxidative degradant had overlapped with that of acid degradant (II). The peaks of the degradation products were absent in the chromatograms of the standard drug; indicating that the identified peaks are due to degradation.



(A)



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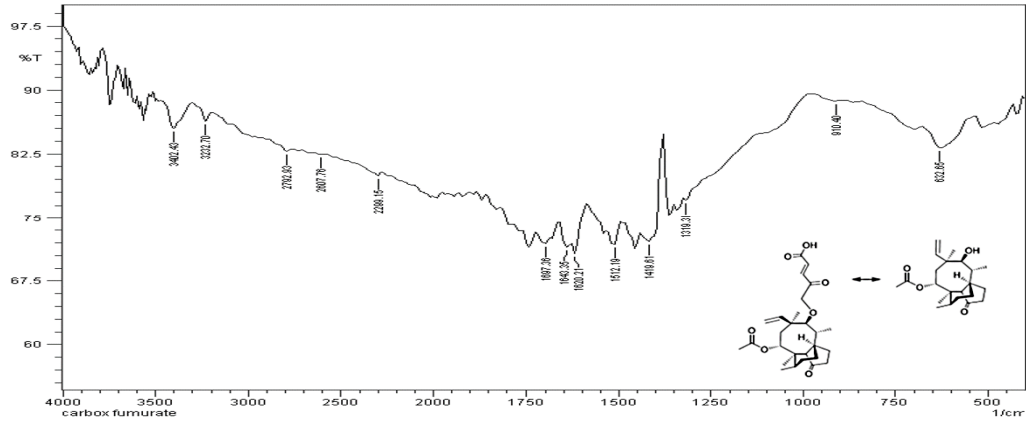
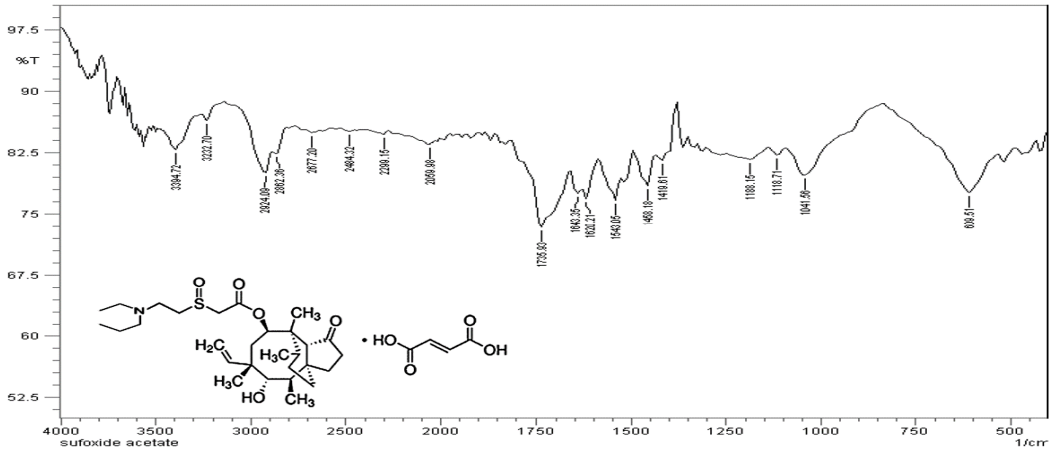
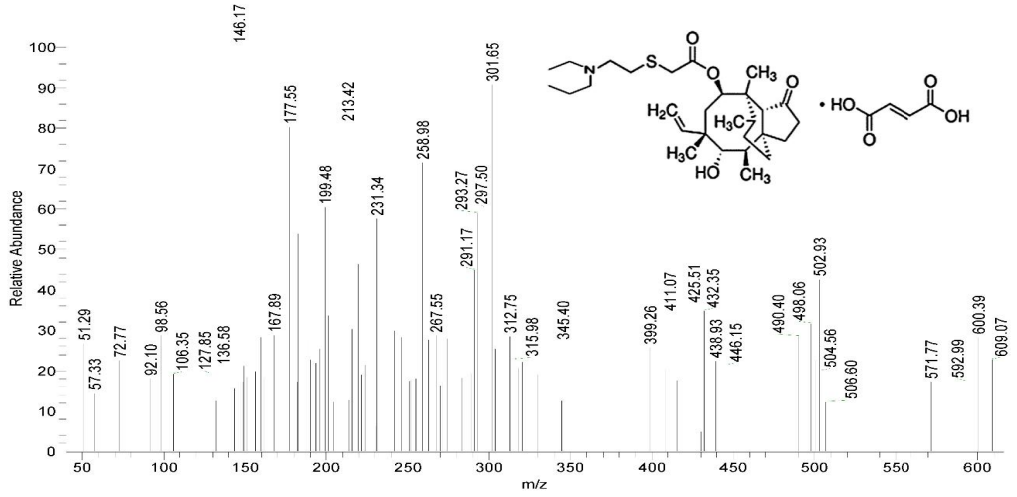


(C)



(D)

Fig. 2. IR spectrum of (A) Intact tiamulin F, (B) Acid degradant I, (C) Acid degradant II, (D) oxidative degradant



(A)

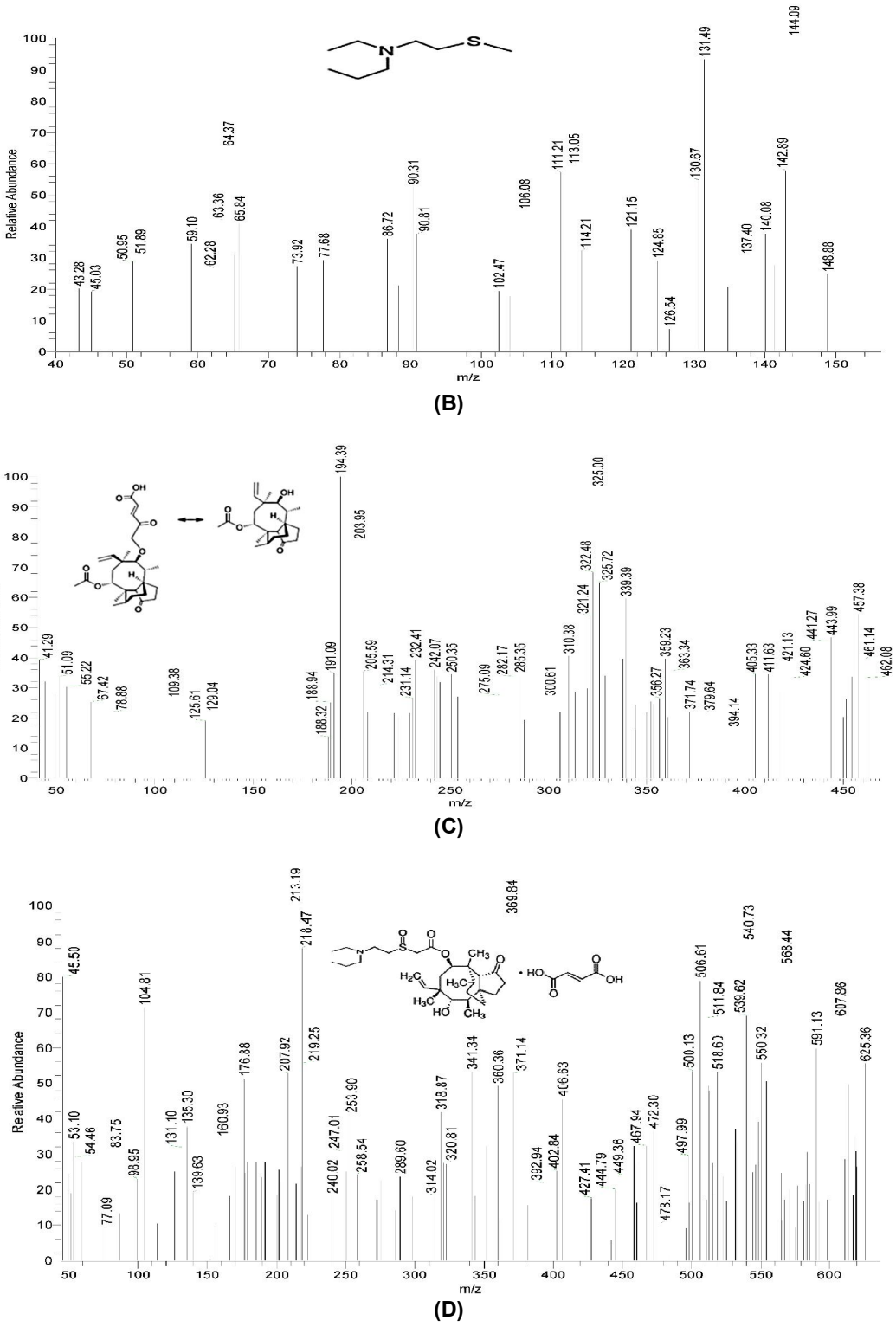


Fig. 3. Mass spectrum of (A) Intact tiamulin F, (B) Acid degradant I, (C) Acid degradant II and (D) oxidative degradant

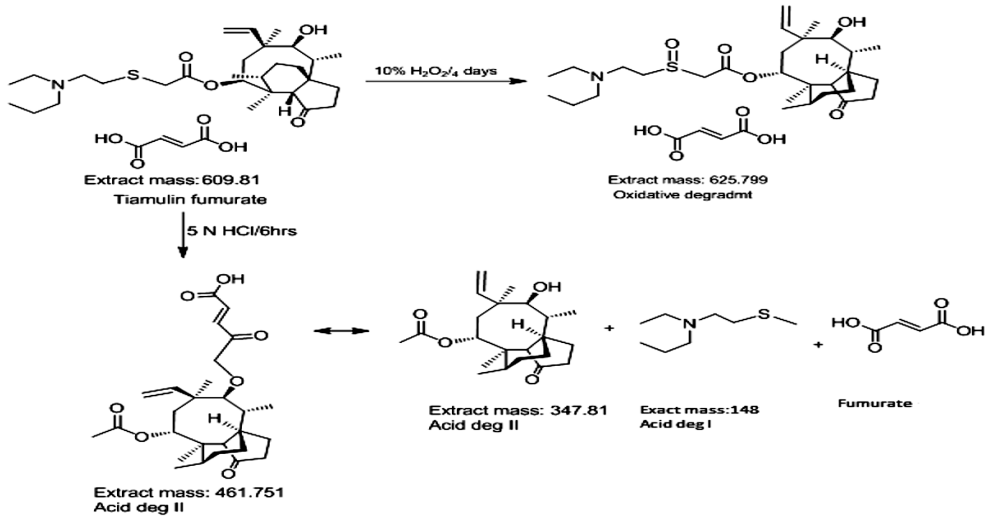
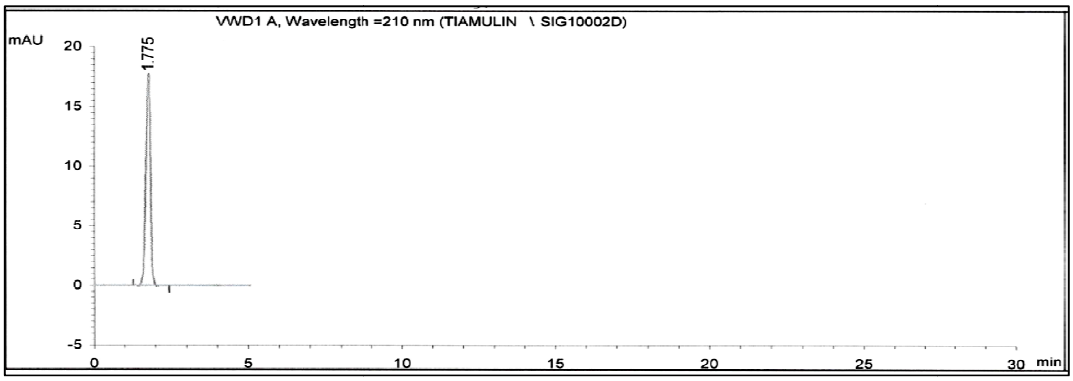
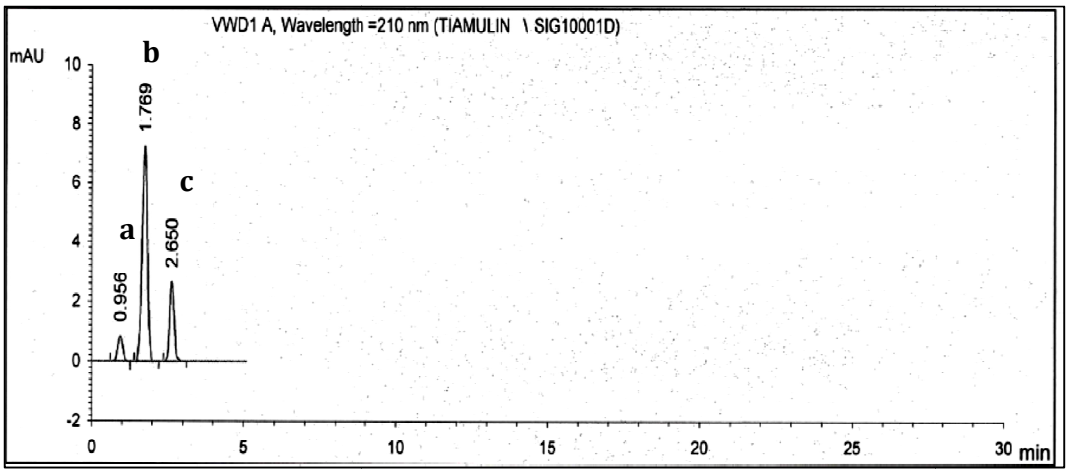


Fig. 4. Proposed degradation pathways of tiamulin fumarate



(A)



(B)

Fig. 5. UHPLC chromatogram at 210 nm for (A) tiamulin F (6 µg mL^{-1}) and (B) mixture of intact tiamulin F and its degradants (3:2 µg mL^{-1})

3.3 TLC-densitometric Method

It was evident that there were no reports about the determination of tiamulin in the presence of its degradation products by TLC-chromatography. Hence, a stability-indicating TLC-densitometric method was proposed. To achieve effective separation of tiamulin from its degradation products, several solvent mixtures having a wide range of elution strengths were tried such as methanol: butanol: chloroform: glacial acetic acid (5:4:2:0.2, by volume), toluene: ethyl acetate: methanol: ammonia 33%: water (1:5:5:1:1, by volume), 10% citric acid: n-hexane: ethanol (8:0.1:0.1, v/v/v) [5], methanol: n-butanol: chloroform: 16.5% ammonia (5:4:2:4, by volume) were attempted. Complete separation of the drug from its degradation products was carried out using a mobile phase of methanol: pentanol: ethyl acetate: 16.5% ammonia) (5: 4: 2: 2, by volume); Fig. 6.

Furthermore, different wavelengths (210, 230 and 254 nm) were tried where the most reliable results were obtained at 220 nm. The plates were densitomerically scanned at 220 nm. The R_f values were 0.58 for tiamulin F and 0.74 for its oxidative degradant. While its acidic degradants (I) and (II) appeared at R_f 0.48, 0.74, respectively; Fig. 7. Also, the plates were sprayed with 16% sulfuric acid and heated at 105°C for 10 min., yellowish-brown bands appeared only with the intact bands but not with the bands of the degradants. Upon densitometric scanning at 450 nm, the R_f values of tiamulin F was markedly shifted to 0.87; Fig. 7.

3.4 Method Validation

The two proposed methods were validated according to ICH guidelines [22].

3.4.1 Linearity

A linear correlation was obtained between the peak area and the corresponding drug concentration in the range of 0.5-10.0 μg mL^{-1} by UHPLC and 1.0-10.0 μg/band by TLC-densitometry. The regression parameters were calculated and presented in Table 1.

3.4.2 Accuracy

The mean accuracy of the proposed methods was tested using triplicate of three concentrations of the cited drug within the linearity range. It was found to be 100.40 ± 0.71 for UHPLC method. While for the TLC-densitometric method, the accuracy was found to be 100.27 ± 1.47 at 220 nm and 99.93 ± 1.38 at 450 nm.

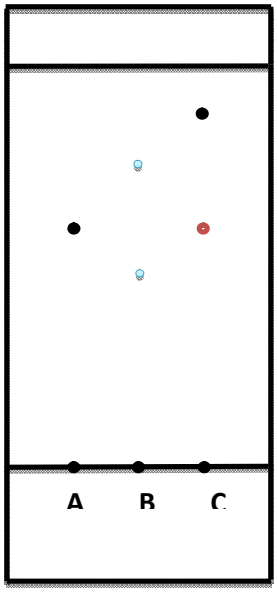


Fig. 6. Thin layer chromatogram of (A) tiamulin F, (B) its acidic degradants and (C) its oxidative degradant using methanol: pentanol: ethyl acetate: 16.5% ammonia (5:4:2:4, by volume) as a mobile phase

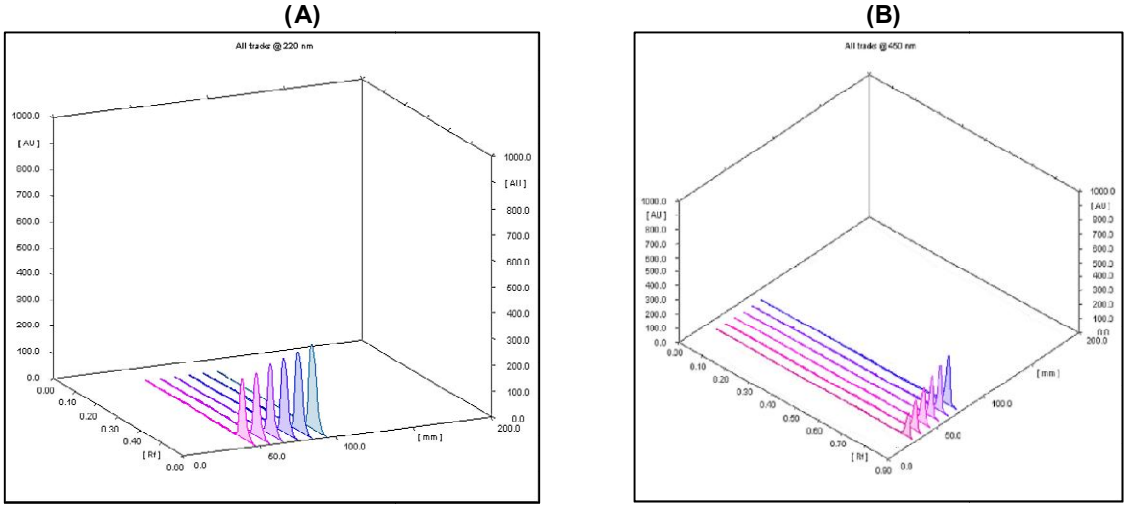


Fig. 7. TLC-densitometric chromatogram of tiamulin F (1.0-10 µg/band) at (A) 220 nm and (B) 450 nm

3.4.3 Precision

It was also evaluated by calculating the intraday RSD%, which ranged between 0.21 and 1.29% and interday RSD% range was 0.23 - 1.47% over a period of three weeks. These results indicated the repeatability and reproducibility of the proposed methods; Table 1.

3.4.4 Selectivity

It was assessed by analyzing laboratory prepared mixtures of tiamulin F with its acidic and oxidative degradants in different ratios. The proposed methods were valid for the determination of the pure drug in the presence of up to 95% and 90% of its degradation products without any interference for UHPLC and TLC-densitometric methods; respectively. The obtained recoveries were 99.67 ± 0.83 for UHPLC, 100.47 ± 1.24 at 220 and 100.27 ± 1.08 at 450 nm for TLC-densitometry; Table 2.

The validity of the proposed methods was further assessed by applying the standard addition technique. The mean recovery of added was 100.55% ± 0.93 for UHPLC and 100.62% ± 1.48, 99.78 % ± 1.15 for TLC-densitometry method at 220 nm and 450 nm, respectively; Table 3. These obtained results were statistically [23] compared with those obtained from a reported TLC-densitometric method [5]; no significant difference was found between the proposed and reported methods at probability of 95%; Table 3.

Table 1. Regression parameters and assay validation results for the determination of tiamulin F by the proposed methods

Parameters	UHPLC method	TLC-densitometric method	
		220 nm	450nm
Linearity range	0.5-10.0 (µg mL^{-1})	1.0-10.0 (µg / band)	
Regression parameters			
Slope ± S.D.	13.27±0.069	1078.55±7.52	810.92±7.45
Intercept ± S.D.	0.43±0.42	741.85±45.69	9473.52±45.24
S.D. of residual	0.56	58.73	58.13
Correlation coefficient	0.9998	0.9998	0.9998
Accuracy (R%± S.D.)	100.40±0.71	100.27±1.47	99.93±1.38
Precision (RSD%, n=9)			
-Intraday	0.57 to 1.29	0.22 to 0.99	0.56 to1.27
-Interday	0.23 to 0.38	0.87 to 1.47	0.31 to 0.74

Table 2. Determination of tiamulin F in mixtures with its acidic degradation products using the proposed methods

UHPLC method				TLC-densitometric method				
Intact (µg mL^{-1})	Degradant added (µg mL^{-1})		R% of Intact	Intact (µg/band)	Degradant added (µg/band)		R% of Intact	
	(µg mL^{-1})	%			(µg/band)	%	220 nm	450 nm
9	1	90	100.47	9	1	10	100.27	100.28
8	2	80	99.47	8	2	20	102.07	100.83
7	3	70	100.22	7	3	30	100.88	101.05
6	4	60	100.81	6	4	40	100.56	98.65
5	5	50	99.79	5	5	50	101.08	101.22
4	6	40	100.04	4	6	60	98.05	101.45
3	7	30	99.00	3	7	70	100.50	99.25
2	8	20	99.08	2	8	80	101.68	98.85
1	9	10	98.15	1	9	90	99.09	100.93
Mean ± S.D.			99.67 ±0.83	Mean ± S.D.			100.47 ±1.24	100.28 ±1.08

Table 3. Statistical analysis of determination of tiamulin F in Martiamulin oral solution by the proposed methods in comparison with the reported method

Parameters	UHPLC method	TLC-densitometric method		Reported method**
		220 nm	450 nm	450 nm
Mean %	101.43	101.81	101.46	101.27
S.D.	0.45	0.43	0.37	0.33
Variance	0.19	0.18	0.14	0.11
N	5	5	5	5
t – test *	0.64	2.19	0.83	-
F – test *	1.00	1.01	1.23	-
Standard addition Mean% ± S.D.	100.55±0.93	100.62±1.48	99.78 ± 1.15	-

**The theoretical t- and F- values at p=0.05 were 2.31 and 6.39, respectively; **The reported method [5] is a TLC-densitometry using 10% citric acid solution – n-hexane - ethanol (80: 1: 1, v/v/v) as mobile phase and detection at 450 nm*

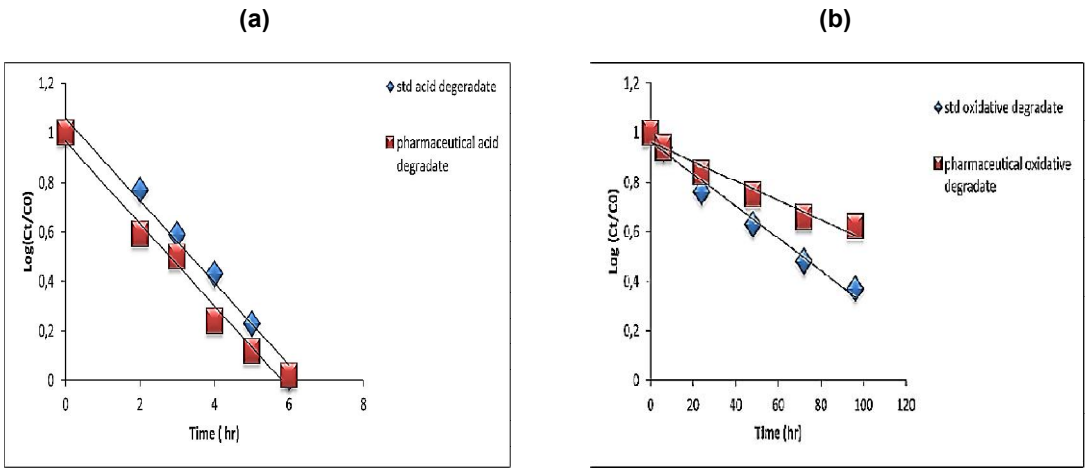


Fig. 8. Kinetics plots of 1 Mg mL^{-1} of (a) acid degradation and (b) oxidative degradants of tiamulin F at 220 nm

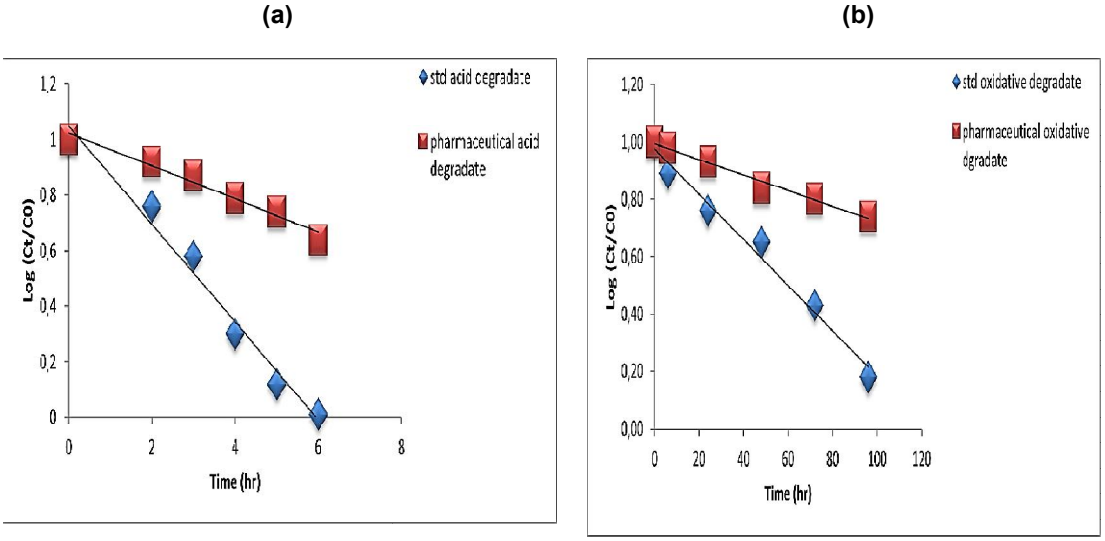


Fig. 9. Kinetics plots of 1 Mg mL^{-1} of (a) acid degradation and (b) oxidative degradants of tiamulin F at 450 nm

Table 4. Summary of the results of the degradation kinetics studies of tiamulin fumarate by the proposed densitometric method

Parameter	Acidic induced degradation		Oxidation induced degradation	
	Pure drug	Dosage forms	Pure drug	Dosage forms
Order of reaction	Pseudo-first order			
		at 220 nm		
Rate constant (k_1)	0.27	0.47	0.02	0.02
t ½	2.56	1.47	29.98	29.98
		at 450 nm		
Rate constant (k_1)	0.28	0.09	0.04	0.01
t ½	2.46	7.71	16.63	79.34

3.4.5 Stability of standard solution

The stability of standard tiamulin F solutions (10 Mg mL^{-1}) in methanol were evaluated by the proposed TLC-densitometric method. This was carried out through storing on laboratory bench and in the refrigerator at 4°C. The solutions were found to be stable for one week at room temperature and for two weeks in refrigerator.

4. KINETICS STUDY

The drug was found to be stable on light and thermal stress condition but susceptible for degradation upon acid and oxidative stress conditions. The stability studies could provide many important information about factors affecting the stability of the dosage forms or the bulk powder of a pure drug. For example, the changes that might occur with time during the production and storage processes. The proposed TLC-densitometric method was applied to study the kinetics of the degradation of tiamulin F upon both reflux with 5 M HCL and oxidation by 10% H_2O_2. The remaining concentration of the intact was determined over 6 hours for acid degradation, and within 4 days for oxidative degradation. The experimental investigation showed that both acid-hydrolyzed and oxidative degradation of tiamulin F following pseudo first order kinetics [24] for the drug in bulk powder and dosage forms; Figs. 8, 9. The rate and half-life of the degradation kinetics of tiamulin F under the studied acidic and oxidation degradation conditions are illustrated in Table 4.

5. CONCLUSION

Two chromatographic methods UHPLC and TLC-densitometry were applied for determination and stability indication of tiamulin F. It was found to

be susceptible only for degradation upon acid and oxidative stress conditions. Identification of oxidative and acidic degradants was accomplished, and degradation pathway was suggested. The degradation kinetics studies were carried out using the proposed TLC densitometric method. Validation of the proposed methods was carried out according to ICH guidelines; the methods were proved to be accurate, precise, and rugged. The high sensitivity and selectivity of the methods were bonuses for routine analysis (batch analysis and stability tests).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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