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Novel chemo-fluorimetric methods for determination of bambuterol hydrochloride in presence of the active metabolite terbutaline

Khalid M. Badr El-Din*

Department of Analytical Chemistry, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

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Abstract

Three novel, simple, rapid and sensitive spectrofluorimetric methods were developed for the simultaneous determination of bambuterol hydrochloride (BAM) in presence of its active metabolite and acidic degradation product; terbutaline (TER). Method I based upon calculating the first derivative values (D^1) of the previously stored conventional emission spectra of both drugs. BAM and TER were measured at 302 and 286 nm respectively. Methods II & III built upon estimating the sum amount of both drugs at the isosbestic points (287.5 nm in the conventional emission method and 229.5, 247.5 nm in synchronous emission method). TER was determined using the previously mentioned D^1 methods while BAM concentrations were considered by subtraction. All the optimization parameters were carefully optimized such as Δ λ and diluting solvents. The calibration curves were rectilinear over the range of 0.4 – 8.0 µg mL^{-1} with quantitation limits ranged from 0.246 – 0.273 µg mL^{-1}. The proposed methods were successfully applied to the assay of synthetic laboratory mixtures, commercially available tablets as well as forced acidic stability testing.

Key words

Bambuterol, Synchronous, Spectrofluorimetry, Isosbestic point, First derivative

1. Introduction

Bambuterol hydrochloride (BAM) (l-[3,5-bis(N,N-dimethylcarbamoyloxy) phenyl]-2-t-butylaminoethanol hydrochloride) is a long-acting β adrenoceptor agonist that is used for the treatment of asthma **(Figure 1)** [1]. It is an ester prodrug of terbutaline (TER). It has an affinity for lung tissue, a relative stability to pre-systemic metabolism, and a relative specificity for hydrolysis by butyryl-cholinesterase enzyme (liberating TER) [2]. TER is considered as BAM metabolite from the lung tissue of guinea pig. Thus, there is an evidence for the production of the active metabolite; TER at its desired site of action. As a result, BAM may produce a more significant bronchodilation effect compared to TER [3]. Given once daily, BAM control symptoms in asthmatic patients with lower side effects than those produced from TER given twice or three times daily [4]. Literature review presented numerous analytical methods for the determination of BAM either alone or in combination with other co-formulated drugs such as spectrophotometric [5-12], spectrofluorimetric [13, 14] chromatographic [15-26], electrochemical [27] and electrophoretic [28-30] methods.

Synchronous fluorimetry has been discussed science 1982 [31]. The measurement depends on synchronized scanning of both excitation and emission monochromators either at constant wavelength difference (Δλ) or constant energy difference [31-33]. This technique has many advantages over the conventional fluorimetry such as: decrease in spectral difficulty, spectral overlap and increase in peak sharpness [32, 33]. Coupling mathematical methods with fluorimetry also improves the spectral features of multi-components mixtures with overlapped bands. [32-35].

The present study aimed to the development of a novel, exclusive, simple, rapid and sensitive spectrofluorimetric methods for the simultaneous determination of BAM in presence of its acidic degradation product and active metabolite TER. These methods are considered promising for application to synthetic mixtures, different commercially available tablets and the accelerated stability study in acidic medium. The conventional emission spectra of both BAM and TER were extremely overlapped due to the great similarity of their chemical structures. Thus, to overcome this problem, the conventional emission data was manipulated via calculating D^1 values (at zero crossing points) or determined at the isosbestic points. On the other hand, for sharper peaks and lower interference, synchronous emission spectra were performed at Δ λ = 45 nm. The data stored and computed for the determination of the total amount of both BAM and TER at the isosbestic points of these spectra. TER was determined via the conventional D^1 method, while BAM was calculated by subtraction.

It worth to note that, coupling mathematics with the conventional fluorimetry for the determination of BAM for the first time approves the novelty of the present work. Furthermore, measuring and manipulation of FI (fluorescence intensity) values at isosbestic points either in conventional or synchronous emission spectra support the modernity and exclusivity of this work.

2. Experimental

**** Correspondence: Khalid M. Badr El-Din***

Tel.: *+201006618080* ***; Fax:*** *+20 862369075*

Email Address: *kh.badr77@gmail.com*

2.1. Apparatus

A Perkin Elmer LS 45 Luminescence spectrometer (UK) operating with FL WINLAB™ software. LC–MS/MS studies were carried out on a 6420 Triple Quad instrument (Agilent Technologies, USA). The mass spectrometric data was acquired using Mass Hunter Work station software.

2.2. Materials and reagents

BAM authentic powder (98.79 %) was kindly gifted by multi-apex pharma Company, Badr city - Cairo, TER authentic powder (99.01 %) was kindly provided by SEDICO Company, 6th October - Giza. Methanol, methanol (MS grade), acetonitrile, ethanol, sodium hydroxide and hydrochloric acid were all purchased from Sigma–Aldrich, Germany. Double distilled water. Lactose monohydrate, maize starch, magnesium stearate and cellulose were all purchased from El Nasr chemical Co, Egypt.

2.3. Pharmaceutical formulations

The following dosage forms were analysed; Lelafree® tablets (multi-apex pharma Company, Badr city – Cairo) labelled to contain 10 mg of BAM, Aironyl ® tablets (SEDICO Company, 6th October – Giza) labelled to contain 2.5 mg of TER.

2.4. Standard drug solutions

Ten milligrams of both BAM and TER were accurately weighted, dissolved and completed to 10 ml with methanol to prepare 1.0 mg mL^{-1} stock solutions. Working standard solutions of each drug was prepared by further dilution of the stock solution with same solvent **(Table 1)**.

2.5. General analytical procedures

Aliquots of standard solutions of BAM and TER covering the working concentration ranges **(Table 1)** were transferred into a sequence of volumetric flasks (10 mL). The solutions were diluted with methanol and mixed well. The conventional emission spectra for both drugs were recorded from the range of 250 to 400 nm. The synchronous emission spectra for both drugs were recorded in the range of 200 to 320 nm ($\Delta\lambda = 45$ nm).

2.6. Validation of analytical methods

The studied methods were fully validated agreeing with ICH guidelines [36] with respect to range, linearity, DL (detection limit), QL (quantitation limit), accuracy, precision and specificity.

2.7. Procedures for laboratory prepared mixtures

To prepare mixtures within the concentration range of each compound **(Table 2)**, the exact volumes of BAM and TER stock solutions were transferred into 10 mL volumetric flasks and completed with methanol. The recommended procedures under section 2.5 were then performed.

2.8. Procedure for tablets

Ten tablets containing the named drugs were properly weighed, triturated and mixed well. An amount equivalent to 10 mg of either BAM or TER was dissolved in methanol, sonicated for 5 min and finalized to 10 mL with methanol and carefully filtered. The obtained solutions (1.0 mg mL^{-1}) were further diluted with same solvent to attain the required concentrations **(Table 3)**. Aliquots containing fixed amount of 2.0 µg of the other authentic drug was exactly added and the general analytical procedures were followed.

2.9. Procedures for stress stability study in acidic medium

Forced degradation of BAM in acidic medium was conducted by placing 2.0 mL of the stock solution (1.0 mg mL^{-1}) into a test tube. One mL 1.0 N HCl was added and the resultant solution was heated for 30, 60, 120 and 180 minutes at 70 ± 3 °C in a water bath. The solution was cooled and neutralized using 1.0 N NaOH. Further dilution was accomplished to obtain the working solutions (2.0 µg mL^{-1}) and the general procedures were then followed. The degraded samples were structurally confirmed using LC–MS/MS via direct injection MS scan in positive ESI mode using methanol MS grade as solvent.

3. Results and discussion

3.1. Spectral characterization

Owing to the great structure similarity, the emission spectra of both BAM and TER seriously overlapped **(Figure 2)**. This hindered the use of direct measurements for the simultaneous determination of these drugs in binary mixtures. For this reason, it was required to use other techniques. Accordingly, the first derivative (D^1) for conventional spectra and isosbestic point methods either for conventional or synchronous spectra were successfully used.

3.1.1. Conventional D^1 method

The first derivative of the stored conventional emission spectra of both drugs were computed with $\Delta\lambda = 4$ nm. The amplitude of the first derivative peak of BAM and TER were measured at 302 and 286 nm respectively **(Figure 3).**

3.1.2. Conventional and synchronous isosbestic point methods

At the isosbestic points, the same concentration of two drugs X & Y exhibited the same fluorescence intensities and so did different ratios of X & Y with total concentration equal to X or Y alone. Thus, by measuring the FI value at the chosen isosbestic point, the total concentration of both X and Y could be calculated while the concentration of one of the components in the mixture could be determined using any complementary method.

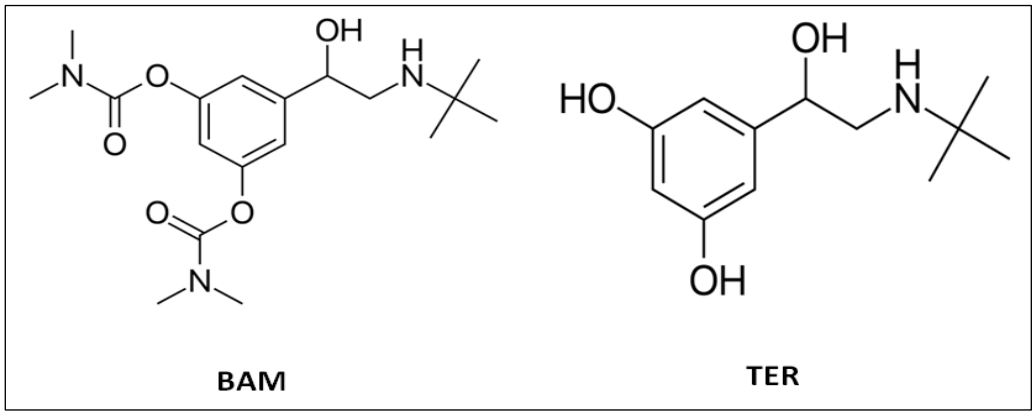

Figure 1: Chemical structure of the studied drugs

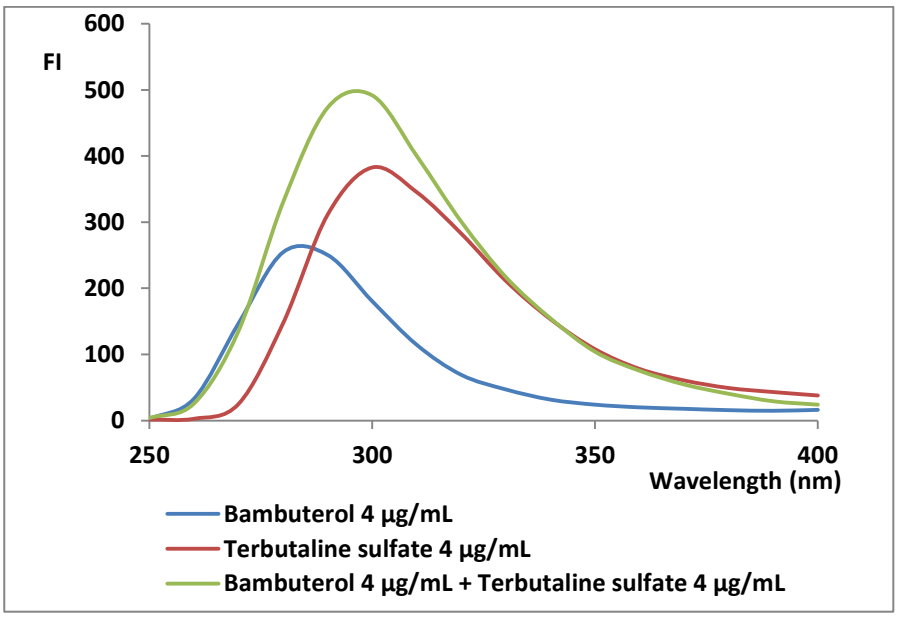

Figure 2: Emission spectra of BAM, TER and their combination

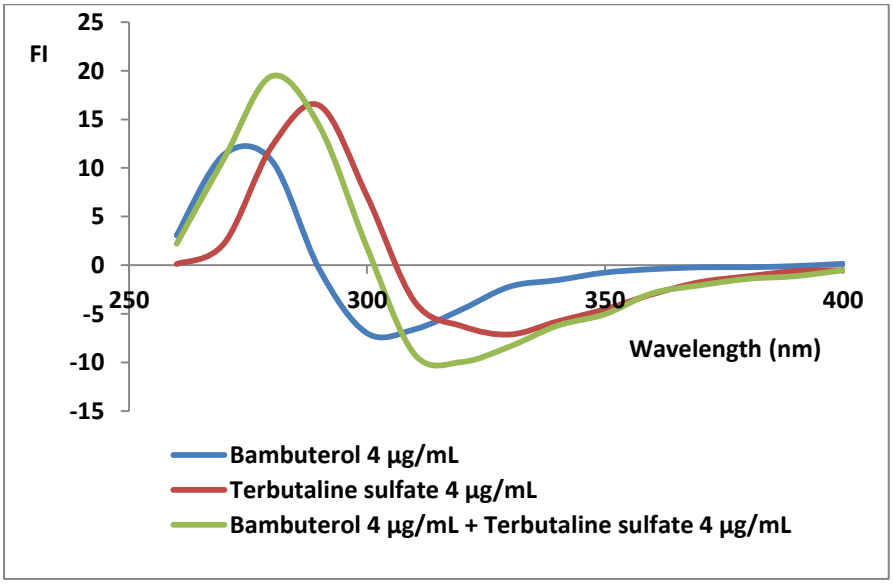

Figure 3: Conventional D^1 emission spectra of BAM, TER and their combination

Table 1: Validation parameters of BAM and TER using the proposed methods

Parameter	Conventional D^1 method		Conventional isosbestic point method	Synchronous isosbestic point method	
	BAM	**TER**	**BAM**	**BAM**	**BAM**
Wavelength (nm)	302	286	287.5	229.5	247.5
Linear range (µg mL^{-1})	0.4 – 8.0	0.4 – 8.0	0.4 – 8.0	0.4 – 8.0	0.4 – 8.0
slope	-1.765	3.726	60.24	12.74	3.55
intercept	-0.12	0.197	6.07	8.41	4.02
SD of intercept	0.044	0.094	1.6	0.35	0.09
r^2	0.9997	0.9998	0.9998	0.9998	0.9999
DL (µg mL^{-1})	0.082	0.083	0.088	0.091	0.083
QL (µg mL^{-1})	0.246	0.249	0.262	0.273	0.251
Accuracy (% recovery* ± RSD)	98.35 ± 0.78	99.90 ± 1.12	101.78 ± 0.99	98.89 ± 1.15	101.49 ± 1.12
Intra-assay precision (% recovery* ± RSD)	99.10 ± 1.49	101.65 ± 0.23	98.27 ± 0.74	98.10 ± 1.01	98.33 ± 0.89
Inter-assay precision (% recovery* ± RSD)	100.63 ± 1.26	101.34 ± 1.41	97.99 ± 1.78	99.11 ± 0.97	102.12 ± 0.74

* Mean of three triplet determinations at three concentration levels

Table 2: Determination of BAM and TER in synthetic laboratory prepared mixtures using the proposed spectrofluorimetric methods

Taken conc. (µg mL^{-1})		% Recovery				
		Conventional D^1 method		Conventional isosbestic point method	Synchronous isosbestic point method	
BAM	**TER**	**BAM (302 nm)**	**TER (286 nm)**	**BAM (287.5 nm)**	**BAM (229.5 nm)**	**BAM (247.5 nm)**
2.0	4.0	98.32	102.56	97.22	102.90	100.62
0.8	2.0	97.44	101.25	100.38	100.87	101.30
4.0	2.0	99.08	99.43	99.89	97.50	98.43
6.0	2.0	100.22	100.55	102.79	99.25	97.41
2.0	6.0	98.77	98.00	100.45	98.88	102.08
4.0	4.0	100.98	97.45	99.44	98.32	100.34
2.0	0.4	97.21	99.34	101.89	98.45	102.70
0.8	4.0	99.21	100.54	99.59	97.21	101.99
	Mean	98.90	99.89	100.21	99.17	100.61
	SD	1.28	1.69	1.67	1.88	1.85
	SEM	0.45	0.59	0.59	0.67	0.65

Table 3: Application of the proposed methods on tablet formulations

Amount (µg mL^{-1})			% recovery				
			Conventional D^1 method		Conventional isosbestic point method	Synchronous isosbestic point method	
	Added TER	**Analysed BAM**	**BAM (302 nm)**	**TER (286 nm)**	**BAM (287.5 nm)**	**BAM (229.5 nm)**	**BAM (247.5 nm)**
Lelafree® tablets	**0.8**	**2.0**	98.32	98.56	100.89	97.00	100.56
	2.0	**2.0**	97.87	100.01	97.45	99.79	101.27
	6.0	**2.0**	99.01	99.34	97.99	100.21	99.66
		Mean	98.40	99.30	98.78	99.00	100.50
		SD	0.58	0.73	1.87	1.76	0.80
		SEM	0.33	0.42	1.06	1.01	0.47
Aironyl® tablets	**BAM**	**TER**					
	0.8	**2.0**	98.45	100.22	98.23	101.41	97.82
	2.0	**2.0**	101.77	97.55	100.89	99.75	97.77
	6.0	**2.0**	99.01	98.89	97.81	101.32	98.10
		Mean	99.74	98.89	98.97	100.83	97.89
		SD	1.78	1.35	1.69	0.92	0.18
		SEM	1.02	0.77	0.96	0.54	0.11

In the present study, total concentrations of BAM and TER were calculated at the isosbestic points (287.5 nm in conventional emission and 229.5, 247.5 nm in synchronous emission) **(Figure 4, 5)**. TER could be determined using the previously discussed conventional D^1 methods while BAM concentrations were calculated by subtraction.

3.2. Optimization of method parameters

3.2.1. Effect of diluting solvent

Different solvents such as distilled water, methanol, acetonitrile, ethanol, 0.1 M HCl and 0.1 M NaOH, were investigated. it was found that the use of buffer solution with different pH values had no significant effect on the FI. Methanol was found to be most proper solvent with lowermost background noise and highest FI at the carefully chosen wavelengths **(Figure 6)**.

3.2.2. Effect Δ λ

In synchronous Spectrofluorimetry, both excitation and emission monochromators are scanned concurrently. The selected Δ λ (difference between emission and excitation wavelengths) strongly affect the intensity and position of the peak maxima. In the present work, different Δ λ values ranging from 15 – 80 nm were investigated. The optimum difference was examined by means of interpretation of emission spectra of both drugs over the range of 200 – 320 nm. The Δ λ of 45 nm was selected as the best difference for both drugs.

3.3. Validation of Analytical methods

3.3.1. Linearity, range

Calibration curves for the studied drugs were constructed by plotting the D^1 or FI values of either conventional or synchronous emission against the concentrations. Linear ranges, regression parameters were all itemized in **(Table 1)**.

3.3.2. DL and QL

In agreement with ICH Q2B recommendations [36], the detection limit (DL) was defined as the lowest concentration of the analyte that can be readily detected. While the quantification limit (QL) was defined as the lowest concentration that can be quantitatively measured or the concentration below which the calibration curve is non-linear. The results of DL and QL of BAM and TER by the proposed methods were shown in **(Table 1)**. QL and DL were calculated according to ICH Q2B guidelines:

$$QL = 10\ \sigma / S$$

$$DL = 3.3\ \sigma / S$$

Where: S is the slope and σ is the standard deviation of the intercept of regression line of the calibration curve.

3.3.3. Accuracy

The accuracy was tested on the raw materials by testing triplicate samples of BAM and TER solution. The percentage recoveries were listed in **(Table 1)**. On the other hand, accuracy was checked on the dosage forms by applying standard addition technique. Standard solutions of the studied drugs at different concentrations were added to the tablet samples of the other one. The obtained mean of percentage recoveries were in the range of 98.40- 100.83 with SD values below 2. This is an indication of the high accuracy of the proposed methods **(Table 3)**.

3.3.4. Precision

Intra-assay precision was determined by analyzing variable concentrations of BAM and TER (2.0, 4.0 and 6.0 µg mL^{-1}) in triplicate in one assay batch. The inter-assay precision was measured by examining the same concentrations in triplicate on 3 following days. The percentage recoveries ranged from 97.99 to 102.12 and the low RSD values pointed to the excellent precision of the offered methods **(Table 1)**.

3.3.5. Specificity

The specificity of the methods was studied by observing any interference encountered by the commonly co-formulated excipients. These excipients were mixed with previously prepared synthetic mixtures of BAM (2.0 µg mL^{-1}) and TER (2.0 µg mL^{-1}). The recovery values displayed in **(Table 4)** revealed that these compounds did not interfere with the results of the proposed methods.

3.4. Application

3.4.1. Application for commercial tablets

The proposed methods were successfully applied to BAM and TER assay in their tablet dosage forms. The average percentage recoveries of different concentrations were based on the average of three replicate determinations. The percentage recoveries were all below 102 % **(Table 3)**. These results revealed the perfect exactness and applicability of the offered methods in quality control laboratories.

3.4.2. Application to laboratory prepared mixtures

The proposed methods were successfully applied to the instantaneous determination of BAM with TER in synthetic mixtures having both drugs in different proportions. The concentrations of both drugs in the synthetic mixture were calculated according to the linear regression equations of the calibration graphs. The results displayed in **(Table 2)** indicated high accuracy and selectivity of these methods.

3.4.3. Application to forced stability study in acidic medium

It was reported that, TER is the main acidic degradation product of BAM [37]. The results of the forced acidic degradation were summarized in **(Table 5)** in the form of the percentage of intact drug (BAM) and degradation product (TER) at different times. This degradation followed the suggested pathway illustrated in

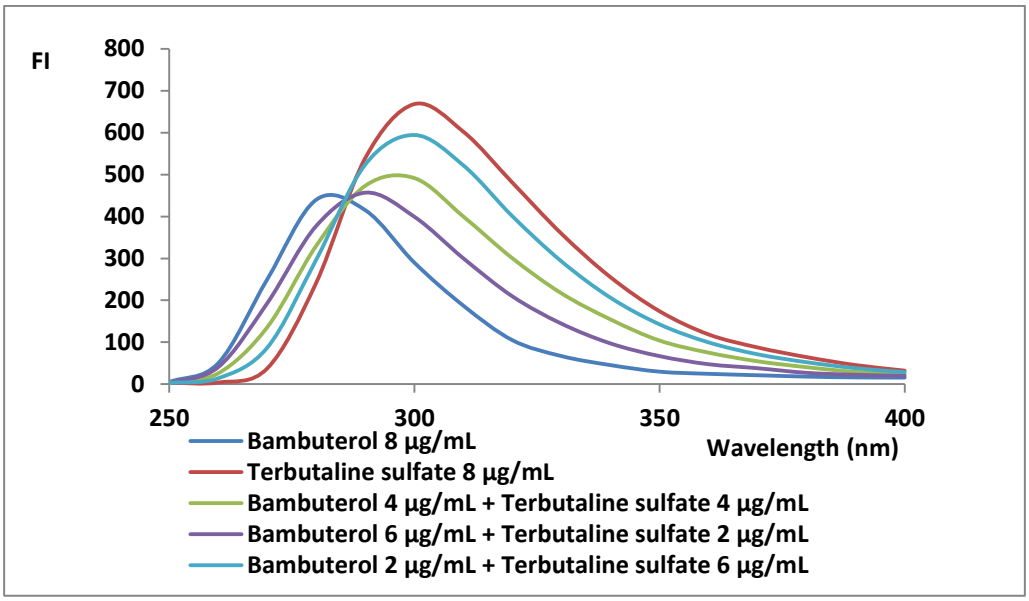

Figure 4: Conventional emission spectra of BAM, TER and their combination showing isosbestic point at 287.5 nm.

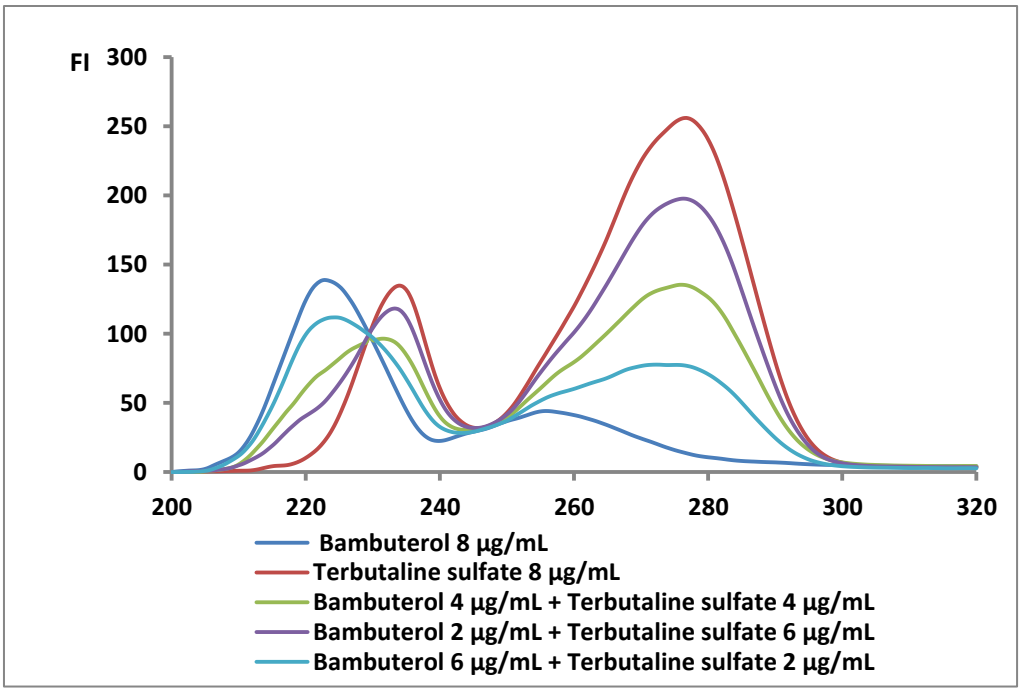

Figure 5: Synchronous emission spectra of BAM, TER and their combination showing isosbestic point at 229.5 & 247.5 nm.

Table 4: Results of the interference of co-formulated excipients

Excipient added	% recovery* ± RSD				
	Conventional D[1] method		Conventional isosbestic point method	Synchronous isosbestic point method	
	BAM (302 nm)	TER (286 nm)	BAM (287.5 nm)	BAM (229.5 nm)	BAM (247.5 nm)
Lactose monohydrate (20 mg)	98.03 ± 0.99	100.22 ± 1.67	99.71 ± 0.88	98.19 ± 1.27	97.78 ± 0.82
Maize starch (20 mg)	97.12 ± 1.11	97.21 ± 0.22	97.12 ± 1.25	101.28 ± 1.11	100.21 ± 1.21
Mg Stearate (20 mg)	97.68 ± 0.34	97.98 ± 1.16	101.88 ± 0.17	101.34 ± 0.78	98.00 ± 0.24
Cellulose (20 mg)	102.76 ± 0.71	98.19 ± 0.44	102.29 ± 0.79	101.03 ± 1.09	99.10 ± 1.36

* Mean of three determinations

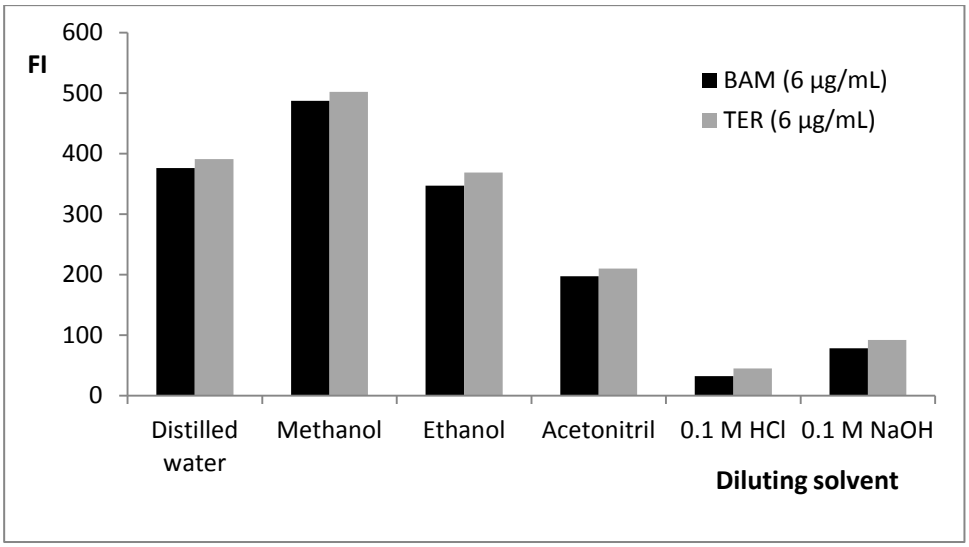

Figure 6: Effect of diluting solvent

Table 5: Results of forced acidic degradation samples

Forced degraded Sample (BAM)		Conventional D[1] method				Conventional isosbestic point method		Synchronous isosbestic point method			
		BAM		TER		BAM		BAM		BAM	
Time (minutes)	C*	C*	%	C*	%	C*	%	C*	%	C*	%
30	**8.0**	6.68	83.5	0.52	6.5	6.67	83.3	6.63	82.9	0.50	6.3
60	**8.0**	5.87	73.4	1.01	12.6	5.84	73.0	5.89	73.6	1.13	14.1
120	**8.0**	5.12	64.0	1.52	19.0	5.15	64.4	5.10	63.8	1.55	19.4
180	**8.0**	4.66	58.3	1.78	22.3	4.62	57.8	4.59	57.4	1.72	21.5

* Concentration in µg mL^{-1}

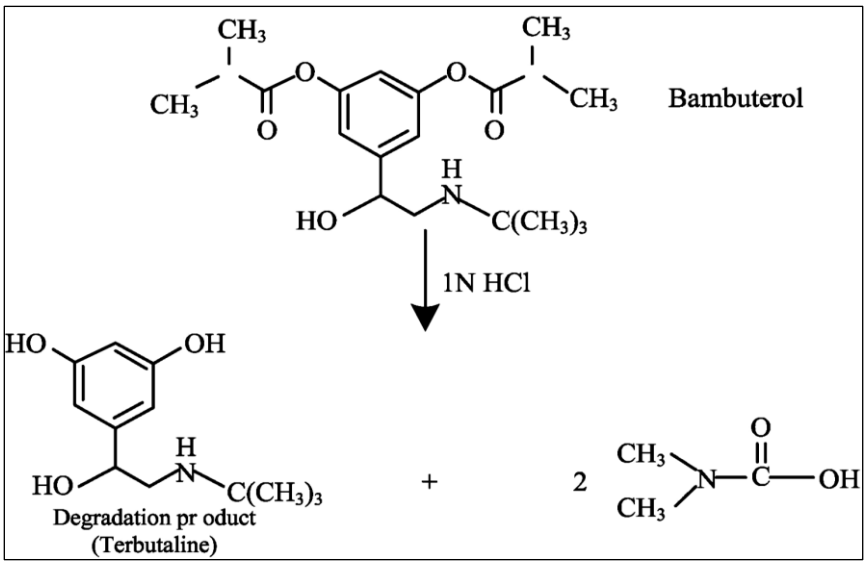

Figure 7: Acidic degradation pathway of BAM

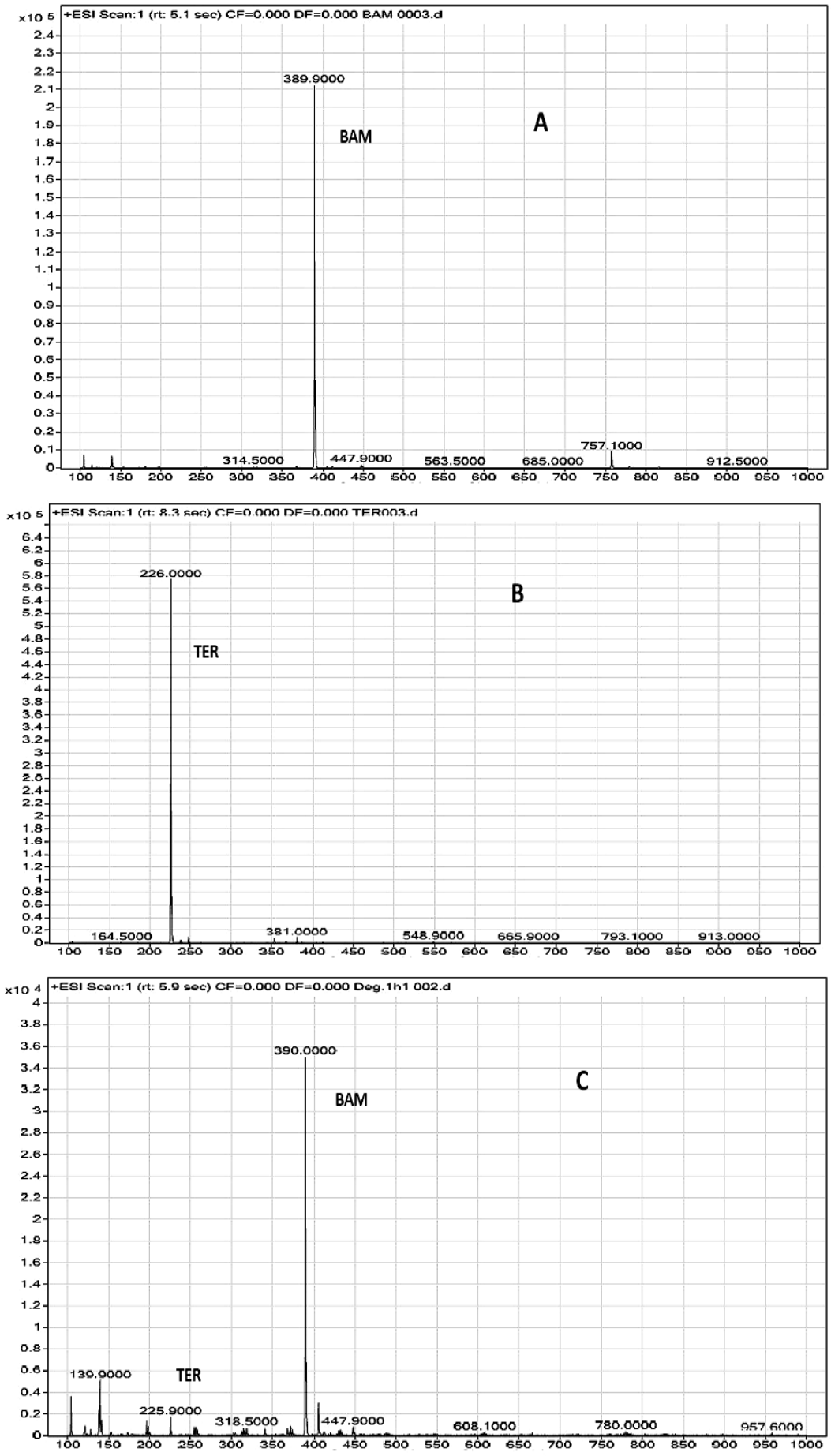

Figure 8: LC-MS spectra of (a) Authentic BAM, (b) Authentic TER and (c) BAM sample after acidic degradation (for 1 hour).

(Figure 7). As a confirmatory step, the degradation pathway was confirmed using LC-MS/MS for the intact and degraded samples **(Figure 8)**.

4. Conclusion

The present work describes three validated spectrofluorimetric methods depending on measuring either the zero crossing D^1 (for conventional) or the FI values (for both conventional and synchronous) emission spectra of BAM and TER after excitation at 225 nm. It is considered the first conventional chemo-spectrofluorimetric method, furthermore the first use for the isosbestic point manipulation for BAM analysis. The proposed methods have many advantages such as:

- Rapid, time saving, cheap and do not require elaborate handling or derivatization compared to the chromatographic techniques.
- Referring to their simplicity and good sensitivity, the offered methods could be used in the determination of cited drugs in QC laboratories as well as in stability testing.

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