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ORIGINAL PAPER

First Derivative Synchronous Fluorescence Spectroscopy for the Simultaneous Determination of Sulpiride and Mebeverine Hydrochloride in Their Combined Tablets and Application to Real Human Plasma

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Abstract A rapid, simple and highly sensitive first derivative synchronous fluorometric method has been developed for the simultaneous analysis of binary mixture of sulpiride (SUL) and mebeverine hydrochloride (MEB). The method is based upon measurement of the synchronous fluorescence intensity of these drugs at $\Delta\lambda = 100$ nm in water. The different experimental parameters affecting the fluorescence of the two drugs were carefully studied and optimized. The fluorescence-concentration plots were rectilinear over the range of 0.05–1 μg/mL and 0.2–3.2 μg/mL for SUL and MEB respectively with lower detection limits (LOD) of 0.006 and 0.01 μg/mL and quantification limits (LOQ) of 0.0.02 and 0.05 μg/mL for SUL and MEB, respectively. The proposed method was successfully applied for the determination of the two compounds in synthetic mixtures and in commercial tablets. The high sensitivity attained by the proposed method allowed the determination of both of SUL and MEB metabolite (veratic acid) in real human plasma samples applying second derivative synchronous fluorometric technique. The mean% recoveries ($n=3$) for both MEB metabolite (veratic acid) and SUL were 99.82± 2.53 and 98.84±6.20 for spiked human plasma respectively, while for real human plasma, the mean% recoveries ($n=3$) were 91.49±4.25 and 91.36±8.46 respectively.

Keywords Sulpiride · Mebeverine · Synchronous fluorimetry · Pharmaceutical preparations and Mebeverine metabolite (veratic acid) · Human plasma

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Introduction

Mebeverine hydrochloride (MEB) 3,4-Dimethoxybenzoic acid 4- [ethyl[2-(4-methoxyphenyl)-1-methylethyl]amino]-butylester (Fig. 1) is a potent direct antispasmodic acting mainly on the smooth muscles of the gastrointestinal tract and particularly effective against the colonic spasm [1].

Mebeverine undergoes rapid and extensive presystemic (first-pass) hydrolysis in the gut and/or the liver [1].

British Pharmacopoeia described a non aqueous titrimetric method for determination of MEB in pure form [2]. Several spectrophotometric methods [3–9], electrochemical methods [10–12] and chromatographic methods [13–21] were reported for its determination either *per se* or in pharmaceutical preparations and biological fluids.

Sulpiride (SUL) 5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-benzamide (Fig. 1) is the most widely prescribed anti-psychotic drug. It is a selective dopamine D_2 antagonist with antidepressant activities it exerts a mood elevating effect and antiemetic action [1].

Regarding SUL, British Pharmacopoeia described a non aqeous titrimetric method for determination of SUL in pure form [2]. Several spectrophotometric methods [22–24], electrochemical methods [25, 26], chromatographic methods [27–34] were reported for its determination either *per se* or in pharmaceutical preparations and biological fluids.

Combination of MEB and SUL is used for treatment gastrointestinal and colic spasms which are a consequence of psychosomatic manifestation of nervous tention, mental stress or anxiety. MEB and SUL were determined in their binary mixture *via* derivative spectroscopy [35–37], TLC- densitometry [37], HPLC [37] and chemometric techniques [38].

The aim of the present study is to establish and develop a novel, sensitive and selective derivative

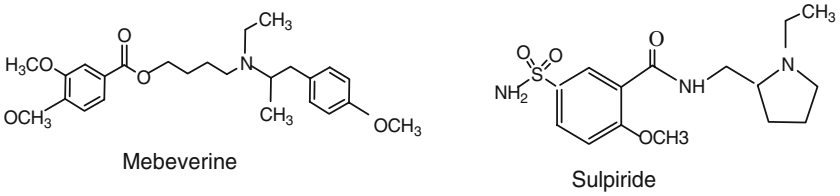


Fig. 1 Structural formulae of the studied drugs

synchronous fluorescence spectroscopic (DSFS) method for simultaneous determination of MEB and SUL either per se or in pharmaceutical preparations and in real human plasma as SUL and MEB metabolite (veratic acid).

The normal synchronous fluorescence spectra of MEB and SUL are greatly overlapped. Such problem led us to utilize a simple first derivative synchronous fluorescence spectroscopy (FDSFS) to solve this problem by measuring peak intensities at 238 and 264 nm for MEB and SUL respectively. The developed method was applied for the simultaneous determination of MEB and SUL in their co-formulated pharmaceutical preparation. On the other hand, MEB is rapidly metabolized to veratic acid, The normal synchronous fluorescence spectra of MEB metabolite and SUL are greatly overlapped. Such problem led us to utilize a simple second derivative synchronous fluorescence spectroscopy (SDSFS) to solve such problem by measuring peak intensities at 253 and 264 nm for MEB metabolite and SUL respectively.

Synchronous fluorescence spectroscopy (SFS) has several advantages over conventional fluorescence spectroscopy, including simple spectra, high selectivity and low interference [39]. Because of its sharp, narrow spectrum, SFS serves as a very simple, effective method of obtaining data for quantitative determination in a single measurement [40].

The combination of SFS and derivatives is more advantageous than the conventional emission spectrum in terms of sensitivity, because the amplitude of the derivative signal is inversely proportional to the band width of the original spectrum [41, 42].

Recently, derivative synchronous fluorometry (DSF) has been utilized for determination of several mixtures in their co-formulated dosage forms and biological fluids. Mixtures of cinnarizine and domperidone [43], metoclopramide and pyridoxine [44], aspirin with salicylic acid [45], diflunisal and salicylic acid [46], carvedilol and ampicillin [47], sulpirid and its alkaline degradation [48] have been determined through this approach.

To the best of our knowledge, neither conventional nor synchronous spectrofluorometry has been reported for the analysis of MEB and SUL in their co-formulated tablets. As well as, for the analysis of MEB metabolite (veratic acid) and SUL in spiked and real plasma.

Experimental

Material

Mebeverine and Sulpiride pure samples were purchased from Sigma (St. Louis, Mo, USA) and used as received. Colona® tablets labeled to contain 100 mg of MEB and 25 mg of SUL in a ratio of 4:1 (Batch # 09425) was obtained from commercial source in the local market.

Reagents

All reagents and solvents were of Analytical Reagent Grade, Methanol (Merck, Darmstadt, Germany). Acetate buffer 0.2 M (pH3.6–5.6) was prepared by mixing appropriate volume of 0.2 M acetic acid with 0.2 M sodium acetate. Borate buffers (pH 5.5–13) were prepared by mixing appropriate volumes of 0.02 M boric acid with 0.2 M sodium hydroxide, Dichloromethane (Aldrich, St. Louis, MO, USA). Phosphate buffer (pH 7.4) was prepared by mixing appropriate volume of 0.2 M KH_2PO_4 with 0.1 M sodium hydroxide [43] and sodium hydroxide ((BDH, UK), 0.1 M aqueous solution.

Apparatus

Fluorescence spectra and measurements were recorded using a Perkin -Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp, grating excitation and emission monochromators for all measurements and a Perkin-Elmer recorder. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. Derivative spectra can be evaluated using Fluorescence Data Manger (FLDM) software.

For best resolution and smoothing, number of points of 99 were used for deriving the first derivative spectra. The fluorescence intensities of the first derivative spectra were estimated at 238 and 264 nm for MEB and SUL, respectively.

A pH Meter (Model pHS-3C, Shanghai Leici instruments Factory, China) was used for pH adjustment.

Standard solutions

Stock solutions of MEB and SUL were prepared by dissolving 10.0 mg of the studied compounds in 100 mL

of methanol and were further diluted with the same solvent as appropriate. The standard solutions were stable for 10 days when kept in the refrigerator.

Recommended procedures

Calibration curve

Aliquots of MEB and SUL standard solutions covering the working concentration range cited in Table 1 were transferred into a series of 10 mL volumetric flasks. Two mL of borate buffer (pH 6.5) was added and the solutions were diluted to the volume with water and mixed well. Synchronous fluorescence spectra of the solutions were recorded by scanning both monochromators at a constant wavelength difference $\Delta\lambda = 100$ nm and scan rate of 600 nm min^{-1} using 10 nm excitation and emission windows. The first derivative fluorescence spectra of MEB and SUL were derived from the normal synchronous spectra using FLDM software. The peak amplitude of the first derivative spectra was estimated at 238 nm and 264 nm for MEB and SUL, respectively. A blank experiment was performed simultaneously. The peak amplitude of the first derivative technique was plotted *versus* the final concentration of the drug (μg/mL) to get the calibration graph. Alternatively, the corresponding regression equations were derived.

Procedure for the synthetic mixture

Aliquot volumes of MEB and SUL standard solutions in the pharmaceutical ratio of 4:1 were transferred into a series of 10 mL volumetric flasks. Two mL of borate buffer (pH 6.5) was added and diluted to the volume with distilled water, and mixed well. The recommended procedure described under calibration curve was then performed. The peak amplitude of the first derivative technique was plotted *vs* the final concentration of the drug (μg/mL) to generate the calibration graph. Alternatively, the corresponding regression equations were derived.

Table 1 Performance data of the proposed method

Parameter	Mebeverine	Sulpiride
Concentration range (μg/ml)	0.2–3.2	0.05–1.0
Limit of detection (LOD) (μg/ml)	0.01	0.006
Limit of quantification (LOQ) (μg/ml)	0.05	0.02
Correlation coefficient (r)	0.9999	0.9999
Slope	39.58	140.9
Intercept	16.5	−0.20
standard deviation of the residuals ($S_{y/x}$)	0.352	0.421
standard deviation of the intercept (S_a)	0.227	0.262
standard deviation of the slope(S_b)	0.129	0.472
%RSD	0.318	0.646
% Error	0.112	0.228

$S_{y/x}$, Standard deviation of the residuals; S_a, Standard deviation of the intercept; S_b Standard deviation of the slope

Preparation and separation of the hydrolytic degradation products of MEB

0.5 gm MEB powder was transferred into 100 ml stoppered flask. 100 mL of 1 M NaOH prepared in methanol was added and the solution was heated under reflux at 45°C for 12 hrs till complete degradation of MEB (investigated by the disappearance of MEB spot upon applying qualitative TLC). The solution was allowed to evaporate under reduced pressure to residue, 30 mL distilled water was added and the oily part was extracted with (20×3 ml) ether in divided portions. The ether extract was washed with 20 ml distilled water in two fractions and then the ethereal layer was let to evaporate at room temperature where a colorless oily residue appeared of 4-(ethyl [2-(4-methoxyphenyl)-1-methylethyl] amino) butan-1-ol (mebOH) [49]. The primarily separated aqueous layer was acidified with 5 M HCl just till acidic to litmus, where a heavy white precipitate of veratic acid was produced, which was then filtered and washed with 30 mL distilled water added in divided portions. The white needles of veratic acid were dried in the oven at 90°C for 1 hr. IR and Mass spectra were obtained to confirm the identity of the separated degradation products of MEB.

Note: During the preparation of MEB hydrolytic degradation products, heating was carried out at 45°C; unlike what stated in the literature (reflux for three hours) [49] to avoid the formation of methyl veratrate.

Stock solutions of MEB degradation product (veratic acid) were prepared by dissolving 10.0 mg of the studied compounds in 100 mL of methanol and were further diluted with the same solvent as appropriate. The standard solutions were stable for 10 days when kept in the refrigerator.

Applications

Procedure for commercial tablets

Ten film coated tablets were bleached with methanol swab and pulverized well. A weighed quantity of the powder equivalent to 100 mg MEB and 25 mg of SUL (in their pharmaceutical ratio of 4:1) was transferred into a small conical flask and extracted with 3×30 mL of methanol. The

extract was filtered into a 100 mL volumetric flask. The conical flask was washed with few milliliters of methanol. The washings were passed into the same volumetric flask and completed to the volume with the same solvent. Aliquots covering the working concentration range were transferred into 10 mL volumetric flasks. The recommended procedure under "Calibration Curve" was performed. The nominal content of the tablets were determined either from a previously plotted calibration graph or using the corresponding regression equation.

Content uniformity testing

The same procedure applied for the analysis of MEB and SUL in tablets was followed using one tablet as a sample. Ten tablets were analysed and the uniformity of their contents was tested by applying the official USP [50] guidelines.

Procedure for spiked human plasma

Suitable aliquots of mixture of MEB metabolite (veratic acid) and SUL stock solutions containing 0.5–2.0 μg/mL and 0.05–0.10 μg/mL respectively were transferred into a series of centrifugation tubes. 1.0 ml of human plasma was added to each tube. Then, 0.1 mL of NaOH (1.0 M) was added and the tubes were shaken for 1 min. The samples were mixed well using a vortex mixer and then extracted with 3×3 mL of ethyl acetate / dichloromethane (5:1 v/v) by centrifugation for 5 min at 4000 rpm. The organic layer was transferred into an evaporating dish and evaporated till dryness. The residue was reconstituted in 3 mL methanol. The procedure described under calibration curve was followed. The nominal content of the drug was determined from the previously plotted calibration graph or using the corresponding regression equation.

Procedure for real human plasma

Three healthy volunteers (women around 35 years old) were administered Colona® tablets after 8 h fasting. Further, 5 mL blood samples were drawn at different time intervals at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 hr into tubes containing 0.5 ml of 2% EDTA solution to prevent blood coagulation. The blood was processed to plasma by centrifugation at 4000 rpm and 15 min. the supernatant plasma samples transferred into test tubes and the procedure mentioned above was followed for the monitoring of drug concentration in plasma.

Results and discussion

Both of MEB and SUL exhibit native fluorescence with λ maximum of 370 nm and 354 nm, after excitation at 235 nm for MEB and SUL respectively (Fig. 2). Both the excitation and emission spectra of MEB and SUL overlapped (Fig. 2). This fact hindered the use of this method for the simultaneous determination of MEB and SUL. This problem is aggravated if it is desired to determine these compounds in their co-formulated preparations and biological fluids.

It was necessary to record first, the normal synchronous spectra for MEB and SUL in order to derive the first derivative synchronous spectra. Figure 3 shows the SF spectra of different concentrations of MEB at 258 nm in presence of constant concentration SUL (1.0 μg/mL), whereas Fig. 4 illustrates the SF spectra of different concentrations of SUL at 240 nm in presence of constant concentration of MEB (3.2 μg/mL).

Therefore the first derivative synchronous fluorescence spectroscopy (FDSFS) technique was chosen for simultaneous determination of both of MEB and SUL in their

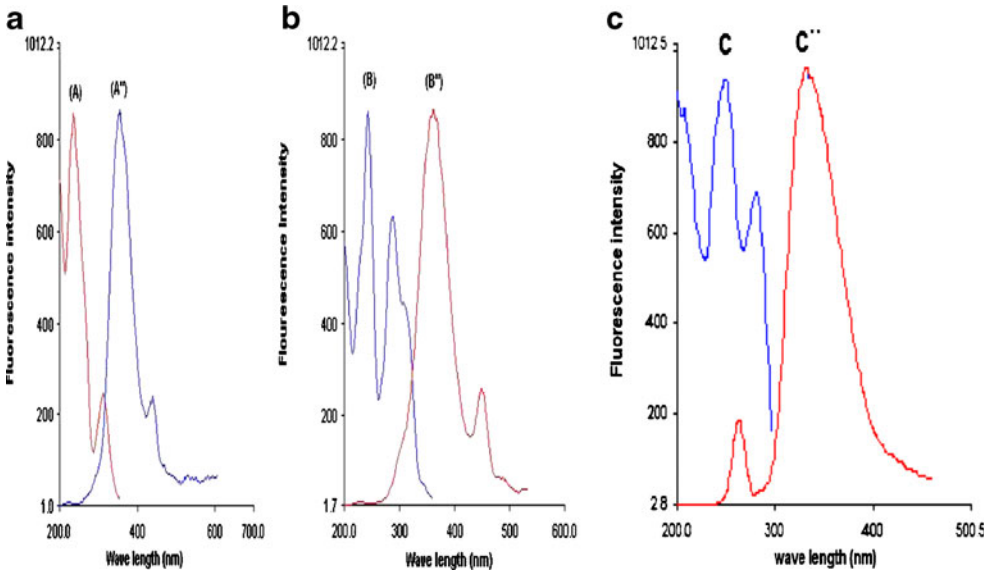


Fig. 2 Native fluorescence spectra at pH 6.5.**a**, (A,A″) are excitation and emission spectra of SUL (1 μg/ml); **b**, (B,B″) are excitation and emission spectra of MEB (4 μg/ml) and (C,C″) are excitation and emission spectra of veratic acid (1 μg/ml)

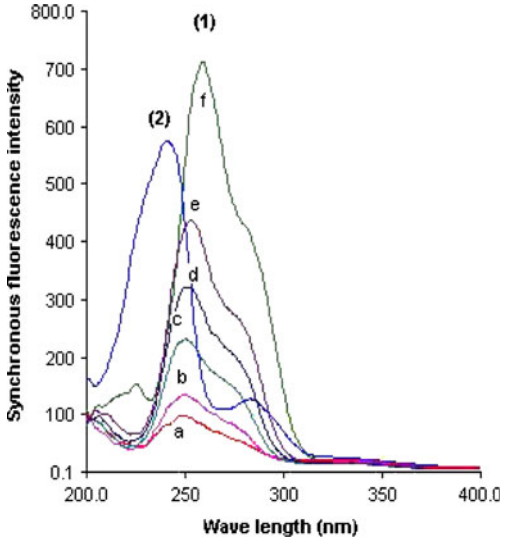


Fig. 3 Synchronous fluorescence spectra of MEB at 258 nm and SUL. (1) **a**-**f** spectra of MEB (0.2–3.2 μg/ml); (2), spectrum of SUL (1 μg/ml) respectively

tablets. Spectra of MEB and SUL were well separated using FDSFS with a zero-crossing technique of measurement (Figs. 5 and 6). Under the experimental conditions the two peaks appeared at 238 and 264 nm for MEB and SUL respectively. Figure 7, illustrates FDSFS for simultaneous determination of synthetic mixture of SUL & MEB.

Optimization of reaction condition

Different experimental parameters affecting the performance of the proposed method were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors included; Δλ selection, pH, type of the diluting solvent, stability time and ionic strength.

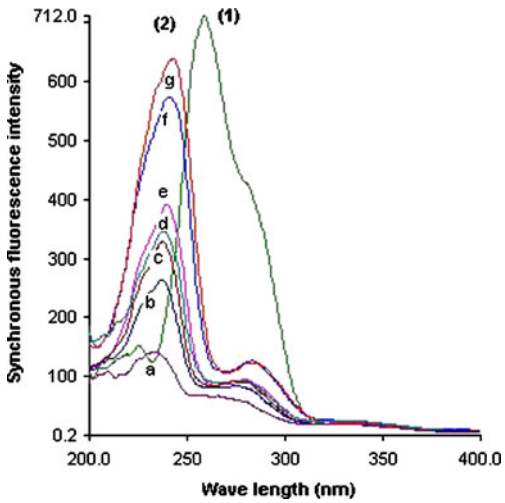


Fig. 4 Synchronous fluorescence spectra of SUL at 240 nm and MEB. (1) spectrum of MEB (3.2 μg/ml); (2), **a**–**g** spectrum of SUL (0.05–1 μg/ml) respectively

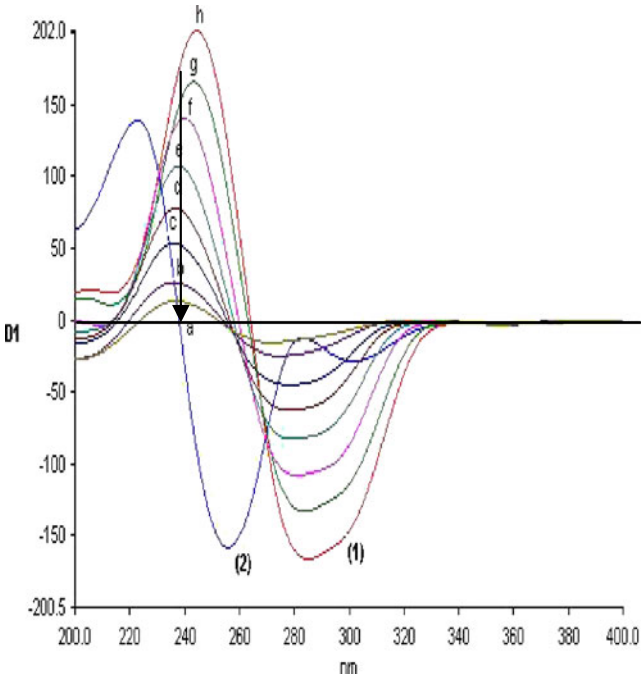


Fig. 5 First derivative synchronous fluorescence spectra of MEB at 238 nm and SUL, (1), **a**–**h** Spectra of MEB (0.2–3.2 μg/ml) ; (2), Spectrum of SUL (1 μg/ml) respectively

Selection of optimum Δλ

The optimum Δλ value is important for performing the synchronous fluorescence scanning technique with regards to its resolution, sensitivity and features. It can directly influence

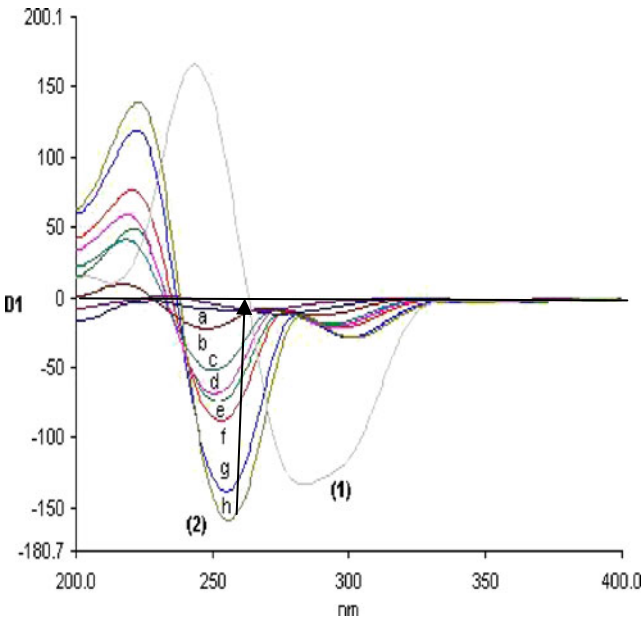


Fig. 6 First derivative synchronous fluorescence spectra of SUL at 264 nm and MEB, (1), Spectrum of MEB (3.2 μg/ml) ; (2), **a**–**h** Spectra of SUL (0.05–1 μg/ml) respectively

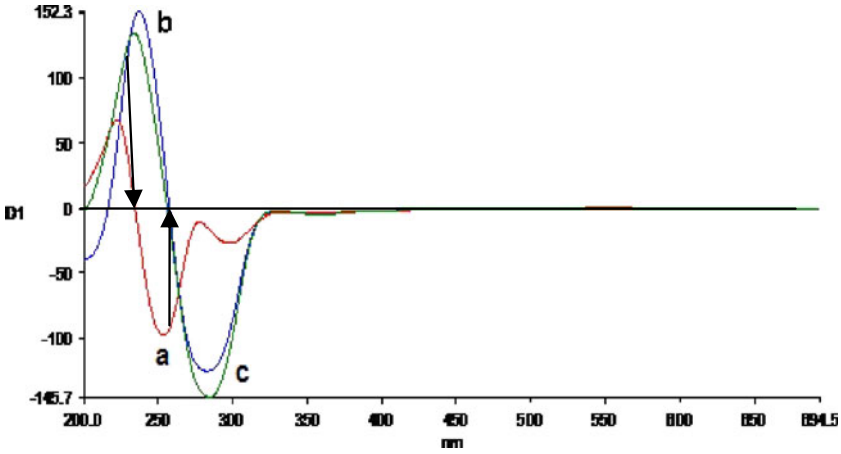


Fig. 7 First derivative synchronous fluorescence spectra of **a**, SUL (0.5 μg/ml) at 264 nm.; **b**, MEB (2.0 μg/ml) at 238 nm.; **c**, synthetic mixture of SUL (0.5 μg/ml) and MEB (2.0 μg/ml)

synchronous spectral shape, band width and signal value. For this reason a wide range of Δλ (20, 40, 60, 80, 100 and 120 nm) was examined. When Δλ was less than 100 nm, the spectra shapes were irregular with weak fluorescence intensities and noisy. On the other hand, when Δλ was more than 100 nm, overlapping of the two peaks with poor separation was achieved. Therefore, Δλ of 100 was chosen as optimal for separation of MEB and SUL mixtures, since it resulted in two distinct peaks with good regular shapes and reduced the spectral interference caused by each compound in the mixture.

Selection of optimum pH

The influence of pH on the synchronous fluorescence intensities of the two drugs was studied using different buffers covering the whole pH range, e.g. acetate buffer over the pH range of 3.6–5.6 and borate buffer over the pH range 5.5–13. The synchronous fluorescence intensity of MEB and SUL is increased gradually upon increasing the pH values up to 6.4 and remained constan until pH6.6, further increase in pH resulted in a gradual decrease in the synchronous fluorescence intensities, after which it extremely decreased at pH 13 (Fig. 8). Therefore, borate buffer of pH 6.5 was used throughout the study.

Effect of volume of buffer

Increasing volume of borate buffer (pH 6.5) resulted in a gradual increase in the synchronous fluorescence intensities of MEB and SUL up to 2 mL after which the fluorescence intensities remained constant till 4 mL. Therefore, 2 mL of borate buffer (pH 6.5) was chosen as the optimum buffer volume throughout the study.

Effect of diluting solvent

Dilution with different solvents including water, methanol, isopropanol, dimethyl sulfoxide (DMSO), tetrahydrofurane (THF) and dimethyl formamide (DMF) was employed. Each of DMSO, DMF and THF decrease the fluorescence intensities of both compounds. This may be attributed to heavy atom effect and inter system crossing process. Of the all tested solvents water gave the highest synchronous fluorescence intensities compared with the other solvents, more over its use adds another advantage of the method. Thus, water was chosen as the diluting solvent through out the study.

Effect of time

The effect of time on the stability of the synchronous fluorescence intensity of the drugs was also studied. It was found that the fluorescence intensity developed instanteously and remained stable for more than 2 h.

Analytical performance

The first derivative synchronous fluorescence spectroscopy (FDSFS)—concentration plots for the two drugs were linear

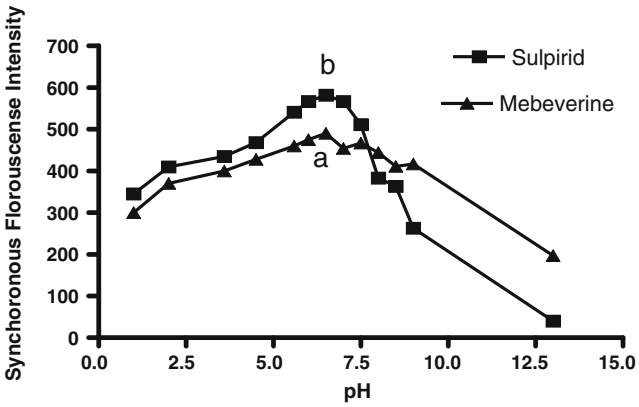


Fig. 8 Effect of pH on the synchronous fluorescence intensity. a, MEB(2 μg/ml), b, SUL (1 μg/ml)

Table 2 Application of the first derivative synchronous fluorimetry to the determination of the studied drugs in the pure form

Parameter	Concentration taken(μg/ml)	Concentration found (μg/ml)	% Found	Reference method [37]
1-MEB	0.2	0.200	100.00	100.46
	0.4	0.400	100.00	99.53
	0.8	0.797	99.60	100.15
	1.2	1.206	100.53	
	1.6	1.603	100.17	
	2.0	1.998	99.89	
	2.4	2.386	99.43	
	3.2	3.209	100.27	
X’			100.07	100.04
± S.D.			0.318	0.473
t-test		0.126 (1.833)		
F-value		1.48 (4.74)		
2-SUL	0.05	0.0499	99.37	100.9
	0.1	0.099	99.23	99.0
	0.2	0.199	99.37	100.3
	0.4	0.398	99.49	
	0.5	0.502	100.36	
	0.6	0.605	100.79	
	0.8	0.802	100.25	
	1.0	0.996	99.58	
X’			99.76	100.0
± S.D.			0.645	0.92
t-test		0.470 (1.833)		
F-value		2.266 (4.74)		

Figures between parenthesis are the tabulated t and F values, respectively at p=0.05 (52)

over the concentration range cited in Table 1. Linear regression analysis of the data gave the following equations:

$$^{1}D = 16.5 + 39.58C \quad (r = 0.9999),$$

for MEB at 238 nm With $S_a = 0.227$ and $S_b = 0.129$

$$^{1}D = -0.2 + 140.9C \quad (r = 0.9999),$$

for SUL at 264 nm With $S_a = 0.262$ and $S_b = 0.472$

Where ^{1}D is the first derivative synchronous fluorescence spectroscopy, C is the concentration of the drug (μg/mL) and r is correlation coefficient. The limits of quantification (LOQ) was calculated according to ICH Q2B recommendations [51]. The limits of detection (LOD) was also calculated according to ICH Q2B recommendations [51]. The results of LOD and LOQ of MEB and SUL respectively are abridged in Table 1.

Table 3 Application of the proposed method for determination of the studied drugs in their synthetic mixtures

Sample	Concentration taken (μg/ml)		Concentration found (μg/ml)		Recovery %	
	MEB	SUL	MEB	SUL	MEB	SUL
MEB and SUL mixture	0.8	0.2	0.796	0.197	99.49	98.54
	1.6	0.4	1.612	0.403	100.75	100.83
	2.0	0.5	1.996	05.05	99.81	100.91
	2.4	0.6	2.396	0.595	99.85	99.16
X’					99.97	99.85
± S.D.					±0.541	±1.19
%RSD					0.541	1.19
%Error					0.270	0.595

Each result is the average of three separate determinations

Table 4 Validation of the proposed method for determination of MEB and SUL raw materials using FDSF mode

Concentration added (μg/ml)	% recovery	% RSD	% Error
MEB			
Intra-day			
0.8	101.00±1.6	1.58	0.91
1.2	101.20±0.624	0.61	0.35
2.0	99.77±0.320	0.32	0.18
Inter-day			
0.8	99.10±0.9	0.90	0.52
1.2	100.06±1.10	1.09	0.63
2.0	99.96±0.35	0.35	0.20
SUL			
Intra-day			
0.2	97.58±1.62	1.66	0.95
0.3	99.98±0.38	0.38	0.21
0.5	100.72±0.461	0.45	0.26
Inter-day			
0.2	97.26±1.55	1.59	0.92
0.3	98.30±1.47	1.49	0.86
0.5	102.0±0.624	0.61	0.35

LOQ and LOD were calculated according to the following equations [51]:

$$LOQ = 10\sigma/S$$
$$LOD = 3.3\sigma/S$$

Where, σ is the standard deviation of the intercept of regression line and S is the slope of regression line of the calibration curve. The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation. The results are abridged in Table 1. Statistical analysis [52] of the results, obtained by the proposed and the reference method [37] using Student's *t*-test and variance ratio F-test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2). The reference method is based on HPLC separation of the two drugs on a reversed phase, Bondapak CN column and UV detection was done at 243 nm using buclizine hydrochloride as internal standard [37].

Analysis of synthetic mixture of MEB and SUL

The proposed method was applied for the simultaneous determination of MEB with SUL in synthetic mixtures containing different concentrations of both drugs in a ratio of 4:1 (Fig. 7). The relative fluorescence intensities of first derivative technique were measured for both drugs. The first derivative signal of MEB was measured at 238 nm which is considered as zero crossing point for SUL and the first derivative signal for SUL was measured at 264 nm which is the zero crossing point for MEB. The concentrations of both drugs in the synthetic mixture were calculated according to their linear regression equation of the calibration graphs. The results indicate high accuracy of the proposed method as shown in Table 3.

Validation of the method

The validity of the method was tested regarding; linearity, specificity, accuracy, repeatability and precision according to ICH Q2B recommendations [51].

Linearity

The regression plots showed a linear dependence of ^{1}D values on drug Concentration(μg/ml) over the range cited in Table 1.

Table 5 Application of the proposed method for determination of the studied drugs in their co-formulated preparations

Preparation	Concentration taken (μg/ml)		Concentration found (μg/ml)		Recovery %	
	MEB	SUL	MEB	SUL	MEB	SUL
Colona® tablets[a] (MEB 100 mg+SUL 25 mg/ tab.) Batch # 09425.	0.5	0.125	0.500	0.126	100.0	100.52
	1.0	0.250	0.991	0.249	99.12	99.66
	1.5	0.375	1.520	0.374	101.33	99.94
	2.0	0.500	1.989	0.500	99.47	100.00
X`					99.97	100.03
± S.D.					±0.946	±0.360
%RSD					0.940	0.350
%Error					0.473	0.179

Each result is the average of three separate determinations. [a] Product of Rameda Co. for Pharmaceutical Industries and Diagnostic Reagents 6th of October City. Egypt

Table 6 Content uniformity testing of MEB and SUL in co-formulated tablets using the proposed method

Parameter	Percentage of the label claim in Colona® tablets	
	MEB	SUL
	98.90	100.70
	100.50	99.30
	101.00	102.10
	102.10	101.90
	99.89	100.90
Data	99.46	100.50
	100.58	102.80
	100.90	102.20
	100.00	100.20
	98.8	100.40
X^-	100.20	101.17
%RSD	1.00	1.12
%Error	0.31	0.35
Acceptance value (AV) (50)	2.42	2.71
Max. allowed AV (L1) (50)	15.00	

Accuracy

The proposed methods were applied for the determination of authentic sample of MEB and SUL over the concentration range cited in Table 2 in order to determine their accuracy. The results obtained were in good agreement with those obtained using reference method [37]. Using the Student's *t*-test and the variance ratio F-test, [52] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2). The validity of the methods was proved by statistical evaluation of the regression lines, using the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and standard deviation of the slope (S_b). The results are abridged in Table 1. The small values of the figures point out to the low scattering of the points around the calibration lines and high precision.

Precision

Repeatability

The repeatability was evaluated by applying the proposed method for the determination of two concentrations of MEB and SUL in pure forms on three successive times, and the results are abridged in Table 4. The low %Error and low % RSD indicates high accuracy and high precision of the proposed method respectively.

Intermediate precision

Intermediate precision was performed through replicate analysis of MEB and SUL in pure form. The results are shown in Table 4, for a period of 3 successive days.

Robustness of the method

The robustness of the proposed method is demonstrated by the constancy of the synchronous fluorescence intensities with the deliberated changes in the experimental parameters such as pH, 6.5±0.1 for MEB and SUL respectively and change volume of borate buffer 2 ml±1. These minor changes that may take place during the experimental operation didn't greatly affect the fluorescence intensity of the mixture.

Pharmaceutical applications

Selectivity

The proposed method was applied for the determination of the studied drugs in their co-formulated tablets. The specificity of the method was investigated by observing any interference encountered from the common tablet excepients, such as lactose, gelatin, magnesium stearate and starch. These excepients did not interfere with the proposed method (Table 5).

Table 7 Application of the proposed method for determination of the studied drugs in spiked and real human plasma

Sample	Concentration taken (μg/ml)		Concentration found (μg/ml)		Recovery %	
	Veratic acid	SUL	Veratic acid	SUL	Veratic acid	SUL
1-Spiked plasma sample	0.5	0.2	0.49	0.19	97.46	92.54
	1.0	0.5	1.03	0.52	102.5	104.95
	2.0	1.0	1.99	0.99	99.5	99.06
X`					99.82	98.84
± S.D.					2.53	6.20
%RSD					2.53	6.27
%Error					1.46	3.5
2-Real plasma sample						
	1.0	0.22	0.870	0.215	87.00	97.9
	1.0	0.22	0.920	0.180	92.00	81.8
	1.0	0.22	0.9547	0.207	95.47	94.4
X^-					91.49	91.36
±S.D.					4.25	8.46
%RSD					4.64	9.26
%Error					2.68	5.34

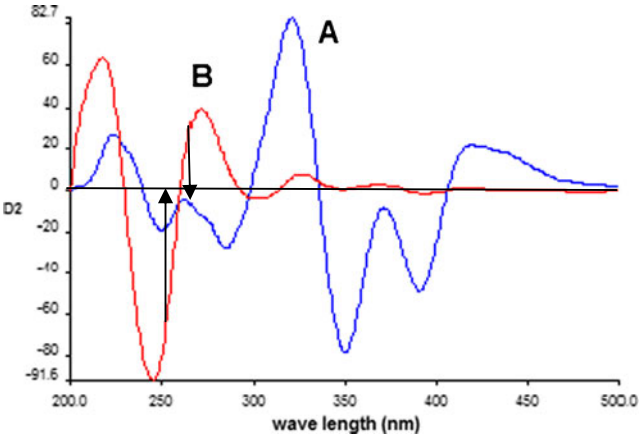


Fig. 9 (a) plasma blank, (b) Second derivative synchronous fluorescence spectrum of 1 μg/ml of (MEB metabolite) veratic acid at 253 nm and 0.022 μg/ml of SUL at 264 nm in real human plasma at 3.5 h

Content uniformity testing

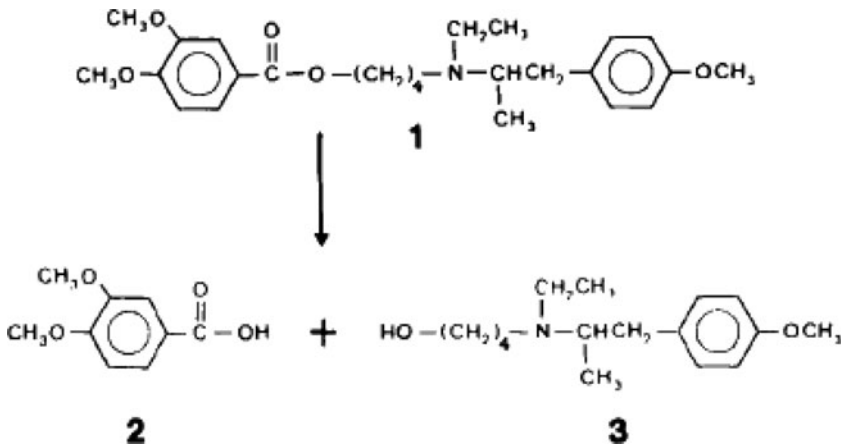
Owing to the high precision of the proposed method and its suitability for analysis of the studied drugs in their dosage forms with sufficient accuracy, the method is ideally suited for content uniformity testing which is time consuming process when using conventional assay techniques. The steps of the test were adopted according to the commercially available tablets and it was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated good drug uniformity as shown in (Table 6).

Biological applications

The high sensitivity of the proposed method allowed the determination of MEB metabolite and SUL in biological fluids by SDSFS method. The method was further applied to the in-vivo determination of both drugs in real human plasma. Sulpiride is absorbed from gastro-intestinal tract. Following oral ingestion of a single oral dose of 50 mg of SUL, a peak plasma concentration of 0.03–0.60 mg L^{-1} (mean 0.18 mg L^{-1}) is attained in about 4–7 h [48]. This value lies within the working concentration range of the proposed method.

After oral administration of mebeverine HCI (270 mg) to fasted human volunteers, measurable concentrations of the drug were not found in plasma. By contrast, the metabolite veratric acid achieved considerable concentrations (mean peak plasma concentration of 13.5 μg/mL at (40–80 min) [53]. Veratic acid (MEB metabolite) is also highly fluorescent compound and the second metabolite is mebeverinic alcohol which is non-fluorescent at the studied pH value (6.5) [54]. However, after oral administration of mebeverine HCI (2 mg), only traces of mebeverine were found in plasma. Veratric acid again achieved considerable concentrations (mean peak plasma concentration of 0.90 μg/mL at (15 min-4 h). The results show that rnebeverine undergoes rapid and extensive first-pass metabolism involving hydrolysis of the ester function, and that negligible circulating concentrations of the parent drug are found in humans [53].

Scheme Chemical structure of mebeverine (1) and its hydrolysis products veratric acid (3,4-dimethoxybenzoic acid;(2) and mebeverinic alcohol (4-[ethyl-(2-[4-methoxyphenyl]—l-methylethyl)amino] butanol;(3)



The second derivative signal of veratic acid and SUL—concentration plot in spiked human plasma was applied over the working concentration. Linear regression analysis of the results gave the following equations:

$^2D = 18.5 + 39.9C (r = 0.9991)$ for veratic acid.

$^2D = 1.35 + 110.5C (r = 0.9985)$ for SUL respectively.

^2D is Second derivative synchronous fluorescence spectroscopy (SDSFS), C is concentration in μg |mL and r is the correlation coefficient. The (SDSFS) is specific for the determination of veratic acid (MEB metabolite) at 254 nm and SUL at 264 nm. The proposed method was applied to human plasma by studying the accuracy as percent relative error and precision as relative standard

deviation. The results are abridged in Table 7. Figure 9 illustrates the second derivative synchronous fluorescence spectroscopy (SDSFS) in real human plasma.

Conclusion

A new simple and sensitive method was explored for the simultaneous determination of MEB and SUL in their combined tablets. The first derivative synchronous spectrofluorimetric method, by virtue of its high sensitivity, could be applied to the analysis of both drugs in their co-formulated dosage forms and biological fluids. It was possible to measure concentrations as low as 0.01 and 0.006 μg/mL for MEB and SUL respectively with good accuracy Moreover, first derivative spectrofluorimetric technique enables the determination of MEB in the presence of SUL by applying the zero-crossing technique in the spectra without prior separation steps in their pure form. Also, the proposed method allowed therapeutic monitoring of MEB metabolite (veratic acid) in real human plasma without interference with SUL. Moreover, the proposed method is simple, time saving and other advantage of the method is the use of low cost reagent.

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