

RESEARCH ARTICLE

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Simultaneous determination of sulpiride and mebeverine by HPLC method using fluorescence detection: application to real human plasma

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Abstract

A new simple, rapid and sensitive reversed-phase liquid chromatographic method was developed and validated for the simultaneous determination of sulpiride (SUL) and mebeverine Hydrochloride (MEB) in the presence of their impurities and degradation products. The separation of these compounds was achieved within 6 min on a 250 mm, 4.6 mm i.d., 5 μm particle size Waters®-C18 column using isocratic mobile phase containing a mixture of acetonitrile and 0.01 M dihydrogenphosphate buffer (45:55) at pH = 4.0. The analysis was performed at a flow rate of 1.0 mL/min with fluorescence-detection at excitation 300 nm and emission at 365 nm. The concentration-response relationship was linear over a concentration range of 10- 100 ng/mL for both MEB and SUL with a limit of detection 0.73 ng/mL and 0.85 ng/mL for MEB and SUL respectively. The proposed method was successfully applied for the analysis of both MEB and SUL in bulk with average recoveries of $100.22 \pm 0.757\%$ and $99.96 \pm 0.625\%$ respectively, and in commercial tablets with average recoveries of $100.04 \pm 0.93\%$ and $100.03 \pm 0.376\%$ for MEB and SUL respectively. The proposed method was successfully applied to the determination of MEB metabolite (veratic acid) in real plasma simultaneously with SUL. The mean% recoveries ($n = 3$) for both MEB metabolite (veratic acid) and SUL were 100.36 ± 2.92 and 99.06 ± 2.11 for spiked human plasma respectively. For real human plasma, the mean% recoveries ($n = 3$) were and respectively.

Keywords: Sulpiride, mebeverine, mebeverine metabolite (veratic acid), HPLC fluorimetric detection, pharmaceutical preparations and human plasma

Introduction

Mebeverine hydrochloride (MEB) 3,4-Dimethoxybenzoic acid 4- [ethyl[2-(4-methoxyphenyl)-1-methylethyl] amino]-butylester (Figure 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 into mebeverine alcohol and veratic acid [1].

British Pharmacopoeia described a non aqueous titrimetric method for determination of MEB in pure form [2].

Sulpiride (SUL) 5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidiny)methyl]-2-methoxy-benzamide (Figure 1) is the

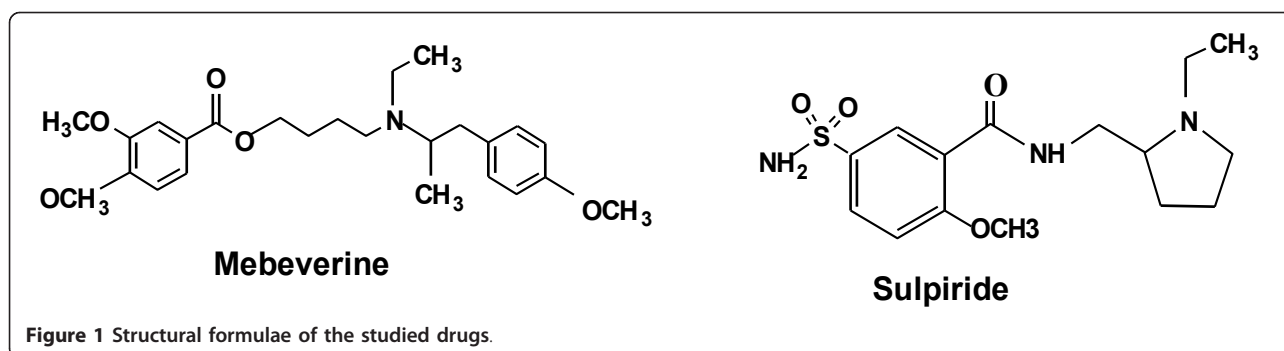
most widely prescribed anti-psychotic drug. It is a selective dopamine D₂ antagonist with antidepressant activities it exerts a mood elevating effect and antiemetic action [1].

Regarding SUL, British Pharmacopoeia described a non aqueous titrimetric method for determination of SUL in pure form [2].

Combination of MEB and SUL is used for treatment gastrointestinal and colic spasms which are a consequence of psychosomatic manifestation of nervous tension, mental stress or anxiety. MEB and SUL were determined in their binary mixture *via* derivative spectroscopy [3,4], first derivative synchronous fluorescence spectroscopy [5], TLC- densitometry [4], HPLC [4-6] and chemometric techniques [7].

The aim of the present study is to establish and develop a novel, sensitive and selective HPLC method for simultaneous determination of MEB and SUL either

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per se or in pharmaceutical preparations and in real human plasma as SUL and MEB metabolite (veratric acid). However, to the best of our knowledge, no analytical methods have been reported for the simultaneous determination of all compounds in the presence of their impurities and degradation products, which might interfere with the analysis of the active ingredients

Experimental

Chromatographic conditions

- HPLC experiments were performed with a Merck Hitachi L- 7100 chromatograph equipped with a Rheodyne injector valve with a 20 μ L loop. The detection of analytes was monitored at emission 365 nm after excitation at 300 nm by L-7485 fluorescence-detector (Darmstadt, Germany). Chromatograms were recorded on a Merck Hitachi D-7500 integrator. Mobile phases were degassed using Merck L 7612 solvent degasser. The separation of these compounds was achieved on Waters[®]-C18 column (250 mm, 4.6 mm i.d., 5 m particle size) using isocratic mobile phase containing a mixture of acetonitrile and 0.01 M dihydrogenphosphate buffer 45:55 (v/v). The analysis was performed at a flow rate of 1 mLmin⁻¹. The pH of the buffer was adjusted with orthophosphoric acid. Prior to any analysis, the mobile phase was filtered using 0.45 μ m filters. The system was equilibrated with the mobile phase before injection.

Reagents and chemicals

-Working reference standard of SUL and MEB were kindly supplied by RAMEDA (The Tenth of Ramadan) Co. for Pharmaceutical Industries & Diagnostic Reagents, 6th of October City, Egypt.

-Colona[®] tablets (Batch No. 09425, labelled to contain 25 mg SUL and 100 mg MEB per tablet) are from RAMEDA (The Tenth of Ramadan) Co. for Pharmaceutical Industries & Diagnostic Reagents, 6th of October City, Egypt.

- Acetonitrile- HPLC grade (Riedel-de Haen, Germany), Phosphoric acid 85% (Riedel-de Haen, Germany).

Analytical grade potassium dihydrogenphosphate and orthophosphoric acid were obtained from Prolabo (Paris, France). HPLC grade methanol (MeOH) was obtained from Prolabo (Paris, France).

- SUL impurities, 2-methoxy-5-sulfamoyl benzoic acid methyl ester (sules) (CAS No. 33045-52-2) and 2-amino-methyl-1-ethylpyrrolidine (sulam) (CAS No. 26116-12-1) were imported from Wuhan Yuancheng Technology Development Co. Ltd., China.

Preparation of standard solutions

Preparation of stock standard solutions Stock standard solutions of MEB, SUL and SUL impurities (200 μ g/mL each) were prepared by accurately weighed 10.0 mg of each of MEB and SUL and transferred to 50 ml measuring flasks. The compounds were separately dissolved in 50 ml of methanol. The stock solutions were further diluted with the mobile phase to obtain the working concentration ranges. The standard solutions were kept in the refrigerator and were found to be stable for 10 days.

Preparation and separation of the hydrolytic degradation products of MEB 0.5 gm MEB powder was transferred into 100 ml stoppered flask. 100 mL 1N NaOH dissolved in methanol was added and the solution was heated 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 left to evaporate under reduced pressure to residue, 30 ml distilled water was added and the oily part was extracted with 60 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) [6]. The primarily separated aqueous layer was acidified with 5N HCl just till acidic to litmus, where a heavy white precipitate of veratric acid was produced, which was then filtered and washed with 30 ml distilled water added in divided portions, The white needles of veratric acid were dried in the oven at 90°C for 1 hr [6]. IR and Mass

spectra were obtained to confirm the identity of the separated degradation products of MEB (veratic acid) as shown in Figure 2.

Stock standard solution of veratic acid (200 $\mu\text{g/mL}$ each) was prepared by accurately weighed 10.0 mg of veratic acid and transferred to 50 ml measuring flask. The compound was dissolved in 50 mL of methanol.

The stock solution was further diluted with the mobile phase to obtain the working standard solution.

Construction of the calibration curves

Aliquots of MEB and SUL covering the working concentration range 10-100 ng/mL for each of MEB and SUL were quantitatively transferred into a series of 10 mL volumetric flasks, after being diluted to 10 mL with the

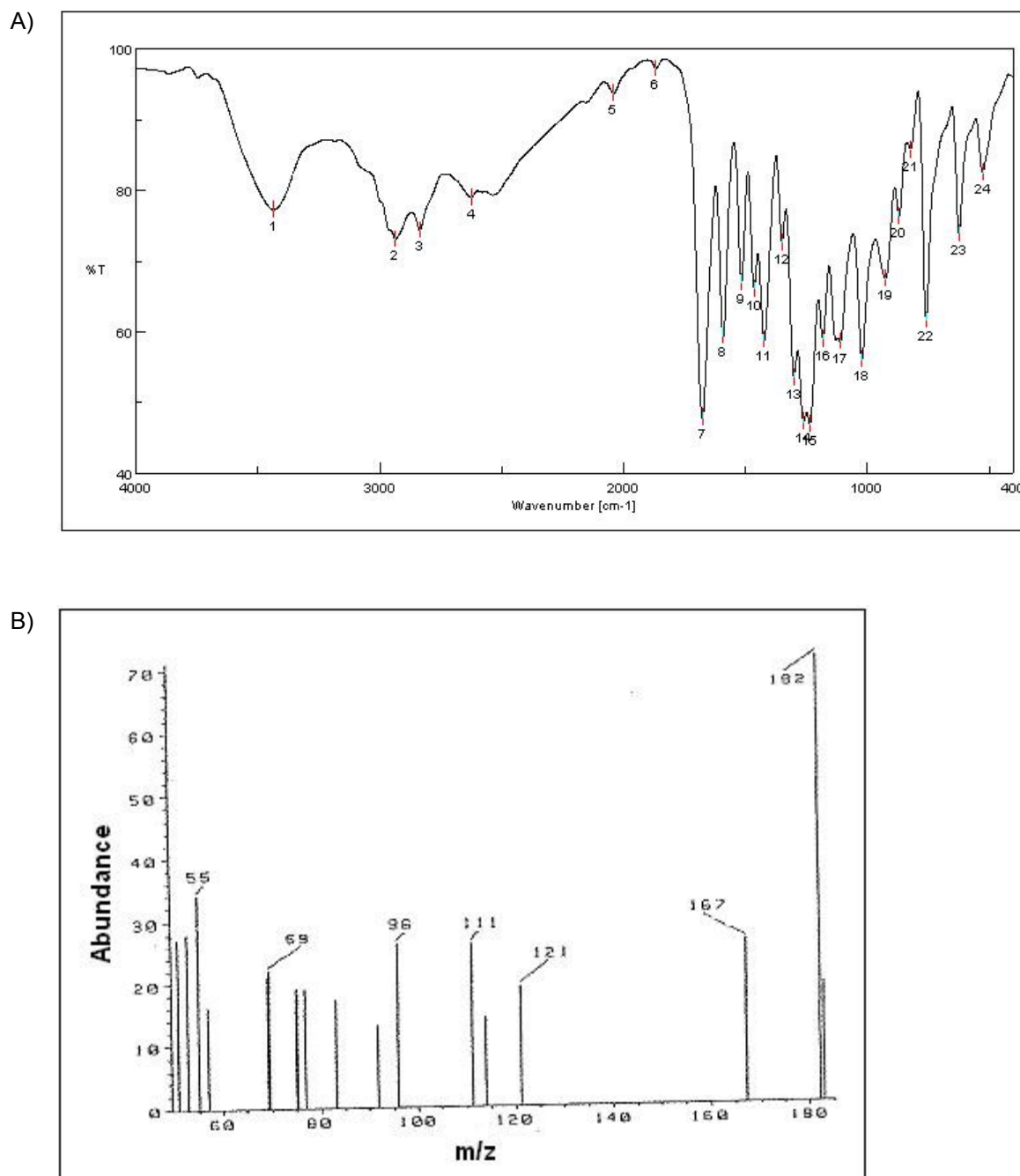


Figure 2 a: IR spectrum of veratic acid. **b:** Mass spectrum of veratic acid (Mol. Wt. = 182).

mobile phase (pH = 4). 20 μ L aliquots were injected (in triplicate) and calibration curve was constructed by plotting the peak area ratio between a drug and the other drug as internal standard against the final concentration of drugs in ng/mL, alternatively, the corresponding regression equations were derived.

Procedure for the synthetic mixture

Aliquot volumes of MEB and SUL standard solutions in the ratio of 4:1 were transferred into a series of 10 mL volumetric flasks. The flasks completed to the volume with the mobile phase. The procedure described under section 2.4 was performed.

Procedure for commercial tablets

10 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 \times 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.

Procedure for spiked human plasma

Suitable aliquots of mixture of MEB metabolite (veratic acid) and SUL stock solutions containing 10.0-50.0 ng/mL and 10.0-100.0 ng/mL respectively were transferred into centrifugation tubes. 1.0 ml of human plasma was added to each tube. Then, alkalization was achieved by addition of 0.1 mL of NaOH (1.0 M) and the tubes were shaken for 1 min. The samples were mixed well using a vortex mixer and then extracted with 3 \times 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 [8]. 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 hours 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

Chromatography

The proposed method permits the separation of MEB and SUL from degradation product and impurity. Figure 3 shows a chromatogram indicating good resolution of MEB (t_R = 5.0 min.) and SUL (t_R = 2.4) in pure form and in tablet extract, whereas Figure 4 show good resolution of MEB, MEB metabolite (veratic acid), SUL and SUL impurity (sules).

In an attempt to optimize the separation of MEB and SUL and their impurities and degradation products in bulk and plasma, the effects of some chromatographic parameters such as mobile phase composition and pH of the buffer solution were investigated.

Effect of the mobile phase composition

Type of Organic Modifier

Acetonitrile was replaced by methanol but it did not give good resolved peaks. Acetonitrile was the organic modifier of choice giving symmetrical narrow peaks.

Ratio of Organic Modifier

The effect of changing the ratio of organic modifier on the selectivity and

retention times of the test solutes was investigated using mobile phases containing concentrations of 40-75% for acetonitrile. Table 1 shows that 45%

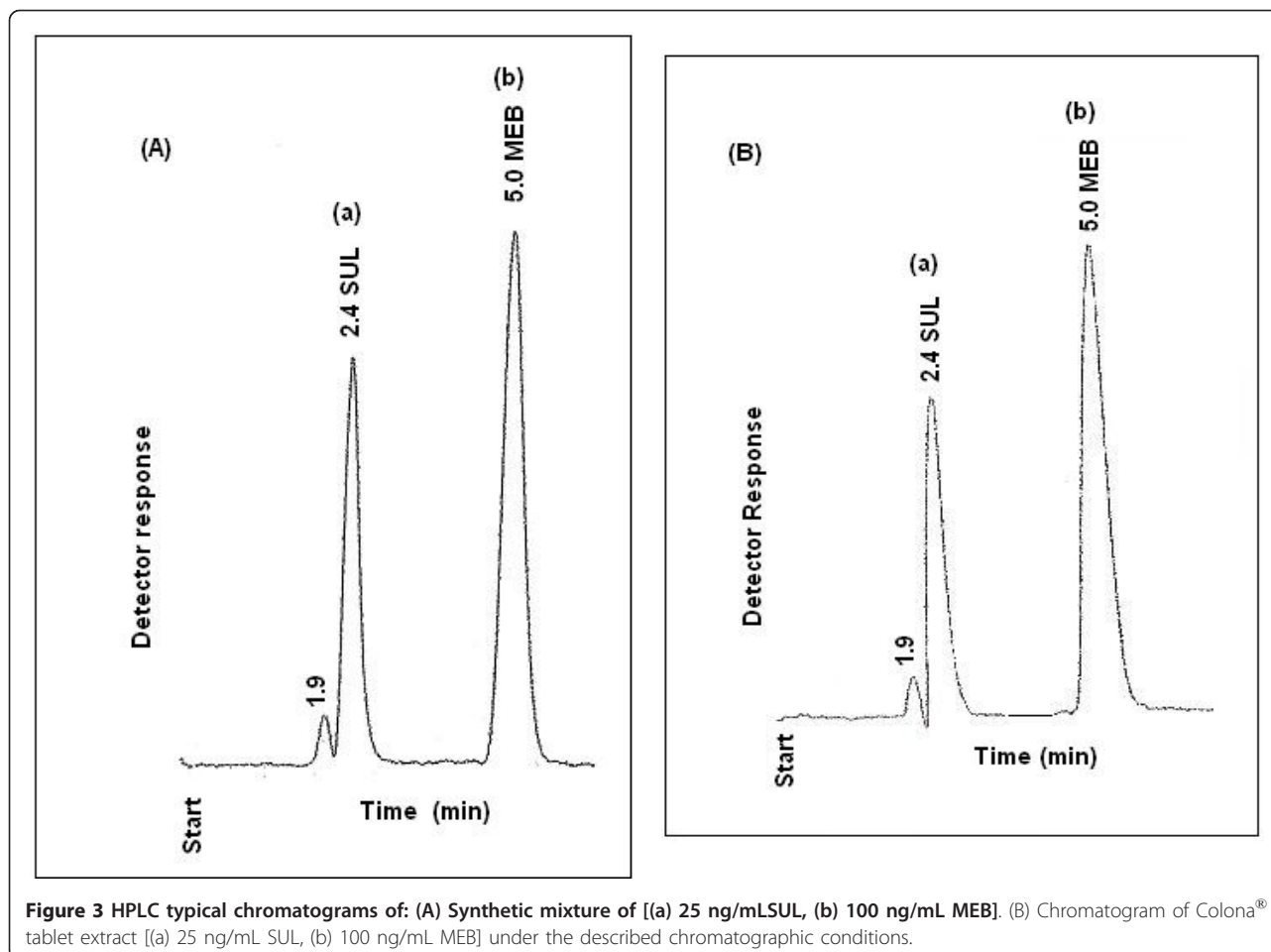
acetonitrile was the best one giving well resolved peaks and highest number of theoretical plates. Ratios less than 45% resulted in peaks with very long unacceptable retention times, whereas ratios higher than 75% resulted in precipitation in the mobile phase.

Effect of pH

The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 3.0-5. Table 1 illustrated that a pH of 4.0 was the most appropriate one giving well-resolved peaks and highest number of theoretical plates. pHs higher than 5.0 produced precipitation in the mobile phase.

Ionic Strength of Buffer

The effect of changing the ionic strength of phosphate buffer on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentration of 0.0075- 0.04 M of phosphate buffer. Table 1 shows that 0.01 M phosphate buffer was found to be the most suitable giving best resolution and highest number of theoretical plates.



Effect of Flow Rate

The effect of flow rate on the formation and separation of peaks of the studied compounds was studied and a flow rate of 1 mL/min was optimal for good separation in a reasonable time (Table 1).

Validation of the Method

Concentration Ranges and Calibration Graphs

Under the above described experimental conditions, a linear relationship was established by plotting peak area ratio for MEB to 0.1 µg/mL SUL as internal standard against MEB concentrations and on the other hand, plot peak area ratio for SUL to 0.1 µg/mL MEB as internal standard against SUL concentrations. The concentration range was found to be 10- 100 ng/mL for each drug. Linear regression analysis of the data gave the following equations:

$$P = 0.018 + 0.04C \quad (r = 0.9999) \text{ for MEB.}$$

$$P = 0.024 + 0.001C \quad (r = 0.9999) \text{ for SUL.}$$

where: C is the concentration of MEB or SUL in ng/mL and P is the peak area ratio. The high value of the correlation coefficient (r-values > 0.999) with small

intercept indicate the good linearity of the calibration graphs. Statistical analysis of the data gave small values of the standard deviation of the residuals, and the % relative error, [9] as shown in table 2.

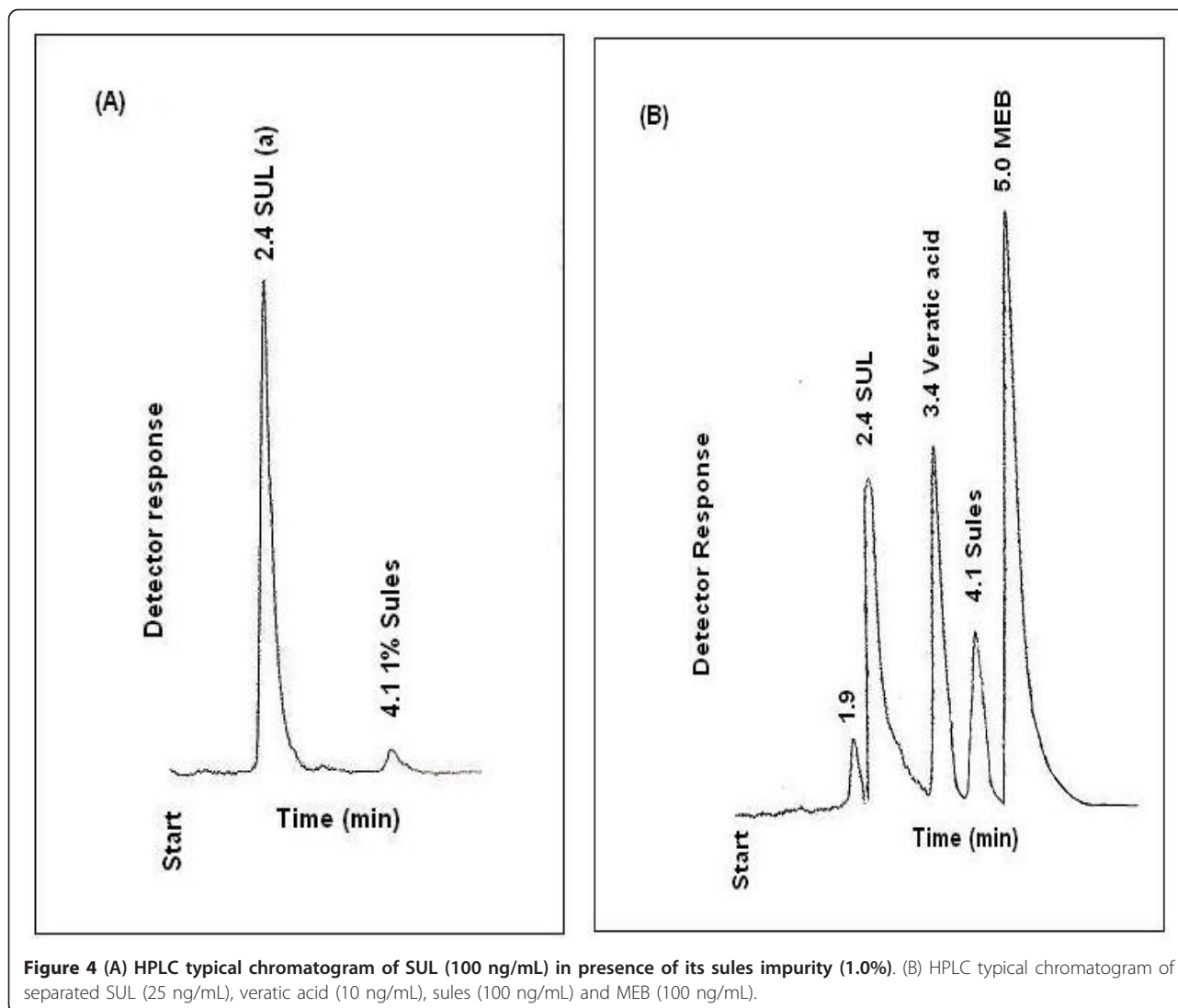
Limit of Quantitation and Limit of Detection

The limits of quantification (LOQ) was calculated according to ICH Q2B recommendations [10]. The limits of detection (LOD) was also calculated according to ICH Q2B recommendations [10]. The results of LOD and LOQ of MEB and SUL respectively are abridged in Table 2.

LOQ and LOD were calculated according to the following equations [10]: LOQ = 10 σ/S

$$\text{LOD} = 3.3 \sigma/S$$

Where, σ is the standard deviation of the intercept of regression line and S is the slope of regression line of the calibration curve. Statistical analysis [9] of the results, obtained by the proposed and the reference method [4] 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 3).



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 [4].

Accuracy and Precision

Repeatability The repeatability was evaluated by applying the proposed method for the determination of three 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.

Application of the method

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 excipients, such as lactose, gelatin, magnesium stearate and starch. These excipients did not interfere with the proposed method (Table 5). The average percent recoveries of different concentrations were based on the average of three replicate determinations. The results shown in Table 4 are in good agreement with those obtained with the reference method [4]. Figure 3 shows a chromatogram indicating good resolved peaks of MEB and SUL.

Degradation product of MEB (veratic acid) and impurity of SUL (sules) of is easily detectable and can be

Table 1 Effect of experimental parameters on the number of theoretical plates of the studied compounds

Parameter	No. of theoretical plates (N)		Resolution (Rs)		
	MEB	SUL	MEB	SUL	
Ratio of organic modifier A/B	40/60	1199	2521	3.20	0.345
	45/55	3407	4691	6.00	3.95
	50/50	2867	1824	5.75	1.77
	55/45	1658	911	3.97	1.20
	60/40	1255	1348	3.97	1.46
	70/30	1946	834	4.90	6.19
	75/25	1487	825	4.20	0.99
pH	3.0	2687	1778	5.04	1.55
	3.5	1138	1510	3.50	1.52
	4.0	3462	4659	6.16	3.70
	4.5	1716	2366	5.10	3.10
	5.0	1345	1630	5.33	3.10
Ionic strength of phosphate buffer [M]	0.0075	2935	2315	5.08	2.24
	0.01	3451	4632	6.16	3.72
	0.02	1614	1251	5.60	2.62
	0.03	1982	1150	6.20	2.50
	0.04	1394	950	5.30	2.40
Flow rate mL/min	0.50	1297	1783	3.6	1.5
	0.75	2356	3252	5.20	2.50
	1.00	3442	4621	6.20	3.90
	1.20	1864	2534	4.75	3.10
	1.50	1534	1963	5.80	3.40

determined quantitatively as shown Figure 4. Therefore, the proposed method can be used for the quality control of the tablets.

Biological applications

The high sensitivity of the proposed method allowed the determination of MEB metabolite and SUL in biological fluids by HPLC method. The method was further applied to the in-vivo determination of both drugs in real human plasma. Sulpiride is absorbed from gastrointestinal 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 (mean 0.18 mg/L) is attained in about 4-7 hours [8]. This value lies within the working concentration range of the proposed method.

After oral administration of mebeverine HCl (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) [11]. Veratric acid (MEB metabolite) is also highly fluorescent compound and the second metabolite is mebeverine alcohol which is non-fluorescent at the studied pH 4 [12]. However, after oral administration of mebeverine HCl (2 mg), only traces of mebeverine were found in plasma with simultaneous appearance of veratric acid. Veratric acid was achieved with considerable concentrations (mean peak plasma concentration of 0.90 µg/mL at (15 min-4 h). The mean percentage recoveries, % Error and % RSD are abridged in Table 6. The results show that mebeverine undergoes rapid and extensive first-pass metabolism involving hydrolysis of the ester function, as shown in Figure 5 and that very trace circulating concentrations of the parent drug are found in humans [12].

The typical chromatograms for the separation of the MEB, veratric acid and SUL in real plasma are illustrated in Figure 6. The plasma samples obtained from the volunteers were investigated using the previously

Table 2 Performance data of the proposed method

Parameter	Mebeverine	Sulpiride
Concentration range (ng/mL)	10-100	10-100
Limit of detection (LOD) (ng/mL)	0.85	0.73
Limit of quantification (LOQ) (ng/mL)	2.57	2.21
Correlation coefficient (r)	0.9999	0.9999
Slope	0.04	0.001
Intercept	0.018	0.024
standard deviation of the residuals ($S_{y/x}$)	0.017	0.0005
standard deviation of the intercept (S_a)	0.012	0.0003
standard deviation of the slope (S_b)	2.23×10^{-5}	7.0×10^{-7}
%RSD	0.756	0.626
% Error	0.267	0.221

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

Table 3 Application of the proposed method to the determination of the studied drugs in the pure form

Parameter	Concentration taken (ng/mL)	Concentration found (ng/mL)	% Found	Reference method [4]
1- MEB	10	10.06	100.56	100.46
	20	20.29	101.44	99.53
	30	30.12	100.40	100.15
	40	39.74	99.36	
	50	49.78	99.55	
	60	60.01	100.02	
	80	79.46	99.33	
	100	100.54	100.54	
X'			100.22	100.04
± S.D.			0.757	0.473
t-test		0.0379 (1.833)		
F-value		2.55 (4.74)		
2- SUL	10	10.08	100.78	100.9
	20	19.80	99.00	99.0
	30	30.10	100.34	100.3
	40	40.05	100.13	
	50	50.00	100.00	
	60	59.71	99.52	
	80	80.43	100.53	
	100	99.82	99.825	
X'			99.96	100.0
± S.D.			0.390	0.92
t-test		0.08 (1.833)		
F-value		2.41 (4.74)		

Values between parenthesis are the tabulated t and F values, respectively at p = 0.05.

Table 4 Application of the proposed method for determination of the studied drugs in their synthetic mixtures and in co-formulated tablets

Sample	Concentration taken (ng/mL)		Concentration found (ng/mL)		Recovery %	
	MEB	SUL	MEB	SUL	MEB	SUL
1-MEB and SUL mixture	40	10	40.23	9.96	100.58	99.59
	60	15	59.38	15.12	98.97	100.82
	80	20	80.53	19.89	100.66	99.47
	100	25	99.86	25.03	99.86	100.11
X'					100.01	99.99
± S.D.					0.787	0.61
%RSD					0.786	0.61
%Error					0.278	0.300
2-Colona[®] tablets^a						
(MEB 100 mg +SUL 25 mg/tab.)	40	10	40.18	10.06	100.45	100.65
Batch # 09425.	60	15	60.26	14.97	100.44	99.77
	80	20	78.92	19.86	98.65	99.30
	100	25	100.63	25.11	100.63	100.42
X'					100.04	100.03
± S.D.					0.930	0.614
%RSD					0.933	0.614
%Error					0.467	0.307

-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 5 Validation of the proposed method for determination of MEB and SUL raw materials using the proposed method

Concentration added (ng/mL)	% recovery	% RSD	% Error
MEB			
Intra-day			
40	100.85 ± 0.636	0.630	0.364
60	100.37 ± 0.490	0.488	0.281
80	100.2 ± 0.854	0.852	0.491
Inter-day			
40	99.75 ± 0.921	0.925	0.534
60	99.08 ± 0.589	0.594	0.343
80	100.53 ± 0.650	0.646	0.373
SUL			
Intra-day			
10	100.23 ± 0.737	0.735	0.424
15	101.03 ± 1.023	1.012	0.584
20	100.06 ± 0.573	0.572	0.330
Inter-day			
10	98.96 ± 0.824	0.832	0.480
15	99.99 ± 0.961	0.961	0.554
20	100.78 ± 0.762	0.756	0.436

N.B. Each result is the average of three separate determinations.

obtained calibration graph or regression equations and the results obtained are shown in Figure 7 showing maximum plasma levels are reached after 3.5 hours for

SUL and veratic acid. Hence, the proposed method allows for the therapeutic drug monitoring for MEB and SUL mixture level in plasma.

Table 6 Application of the proposed method for determination of the studied drugs in spiked and real human plasma

Sample	Concentration taken (ng/mL)		Concentration found (ng/mL)		Recovery %	
	Veratic acid	SUL	Veratic acid	SUL	Veratic acid	SUL
1-Spiked plasma sample						
a- Intra-day precision						
	10	10	10.32	9.62	103.19	96.23
	25	50	24.34	51.04	97.36	102.09
	50	100	50.28	99.50	100.55	99.50
	X'				100.36	99.27
	± S.D.				2.92	2.95
	%RSD				2.91	2.95
	%Error				1.68	1.69
b- Inter- day precision						
	50	100	50.56	95.32	101.12	95.30
	50	100	51.10	98.57	102.20	98.57
	50	100	49.95	101.24	99.90	101.24
	X'				101.07	98.37
	± S.D.				1.15	2.97
	%RSD				1.13	3.02
	%Error				0.65	1.74
2- Real plasma sample						
	100	100	91.87	92.48	91.87	92.48
	100	100	90.12	93.76	90.12	93.76
	100	100	86.25	87.98	86.25	87.98
	X'				89.41	91.40
	± S.D.				2.87	3.03
	%RSD				3.21	3.321
	%Error				1.85	1.91

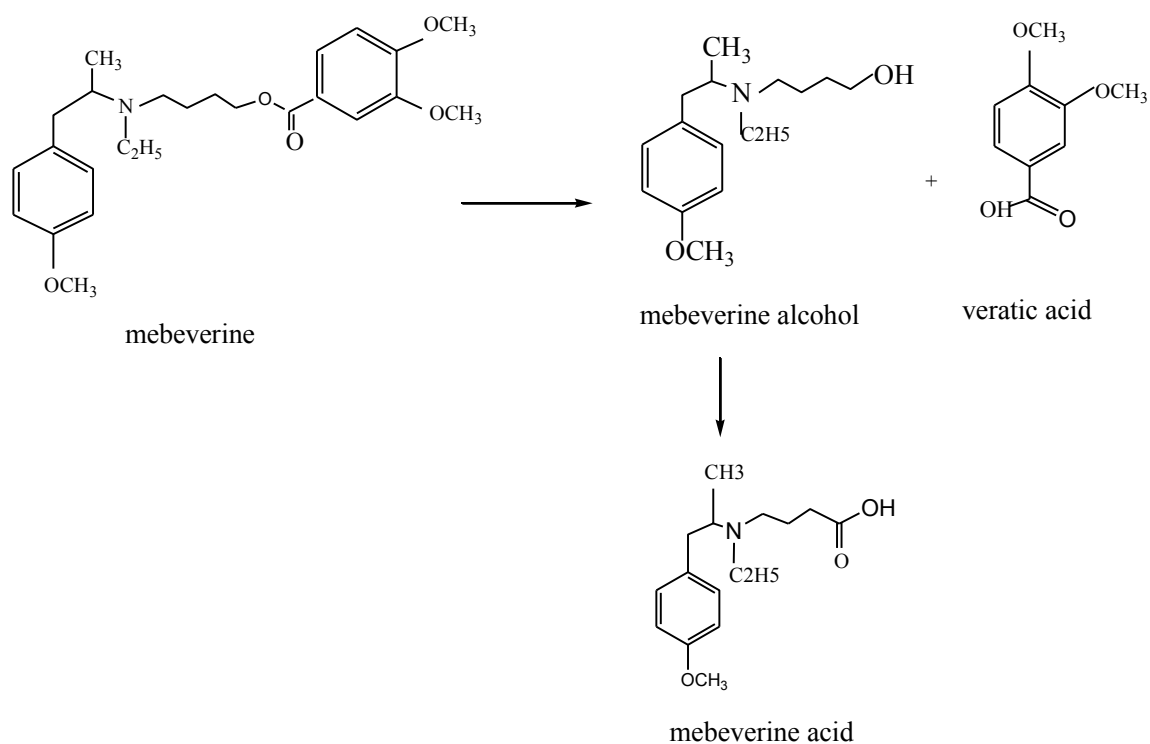


Figure 5 In vivo hydrolysis of mebeverine into mebeverine alcohol and veratic acid and the subsequent oxidation of mebeverine alcohol to mebeverine acid.

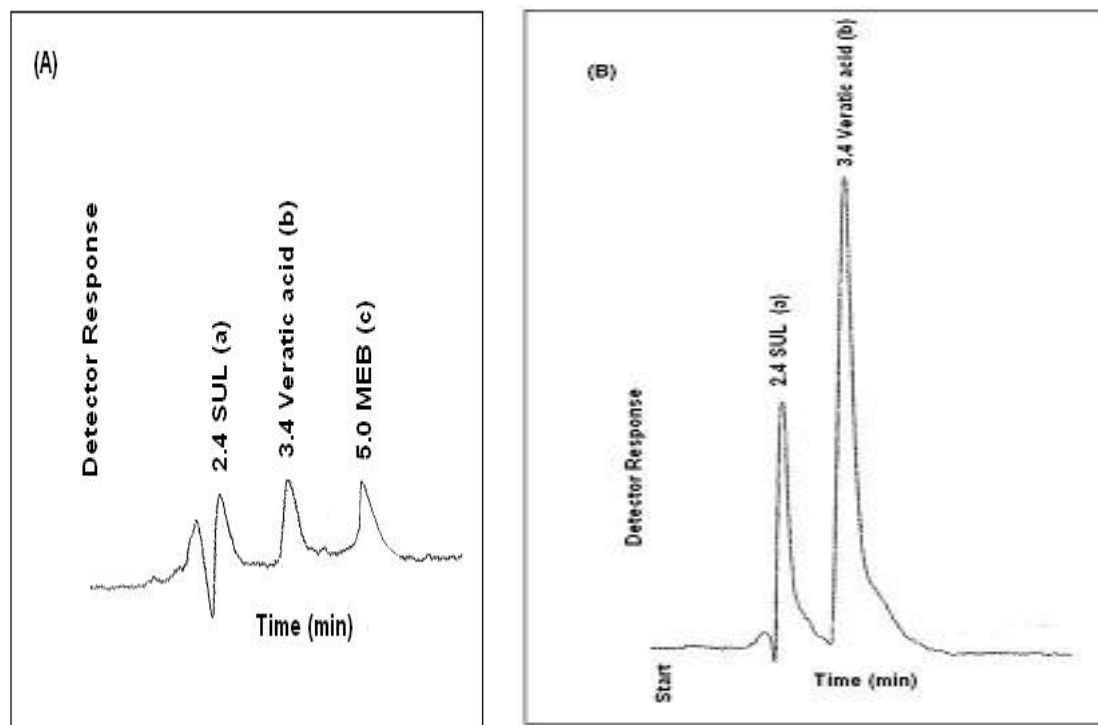


Figure 6 (A) Plasma samples obtained 1.0 hr after oral administration of Colona® tablet under the chromatographic conditions. (B) Plasma samples obtained 3.5 hr after oral administration of Colona® tablet under the chromatographic conditions.

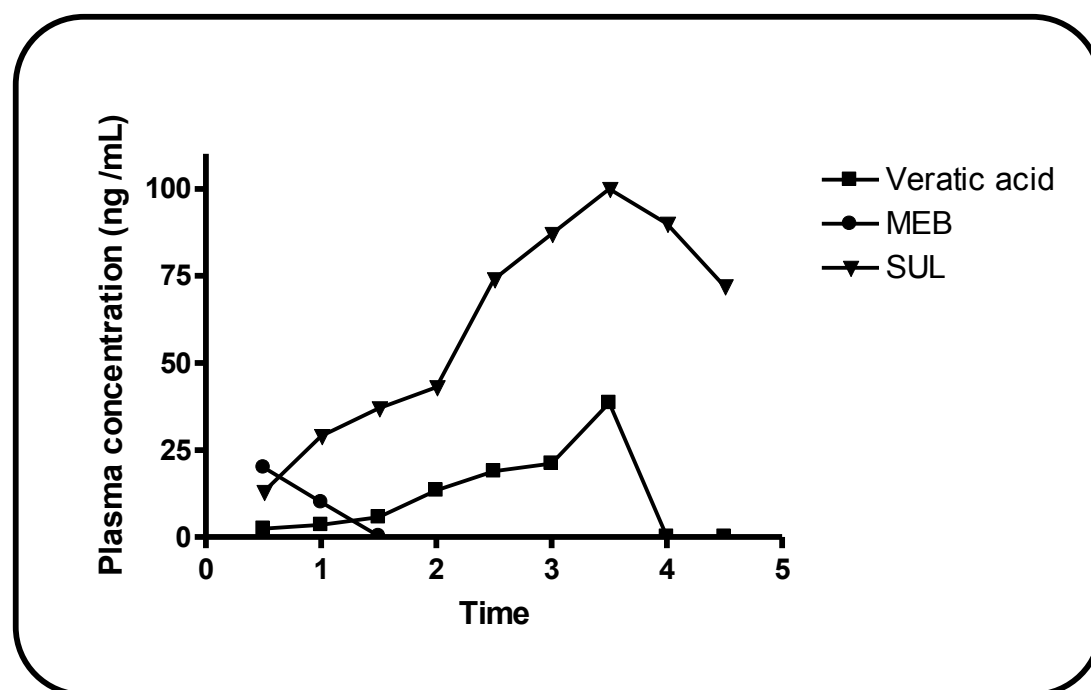


Figure 7 Plasma concentration - time profiles of MEB, veratic acid and SUL after oral administration of Colona[®] tablets [2 x(100 mg MEB + 25 mg SUL)].

Conclusion

A new simple and sensitive method was explored for the simultaneous determination of MEB and SUL in their combined tablets. The HPLC method using fluorescence detection, 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 0.85 and 0.73 ng/mL for MEB and SUL respectively with good accuracy. Moreover, 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.

Authors' contributions

MIW designed the proposed method and analyzed the data statistically. MKS and NME proposed, planned and supervised the whole work. NME coordinated the study and modified the text. SMS carried out the experimental work. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 3 October 2011 Accepted: 14 February 2012

Published: 14 February 2012

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doi:10.1186/1752-153X-6-13

Cite this article as: Walash *et al.*: Simultaneous determination of sulphiride and mebeverine by HPLC method using fluorescence detection: application to real human plasma. *Chemistry Central Journal* 2012 **6**:13.

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