ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 535–548

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

# Application of first-derivative UV-spectrophotometry, TLC-densitometry and liquid chromatography for the simultaneous determination of mebeverine hydrochloride and sulpiride

Abdel Fattah M. El Walily [a,*], Alaa El Gindy [b], Mohamed F. Bedair [c]

[a] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Alexandria University, P.O. Box Elmesalah 21 521, Khartoum Square Azarita, Alexandria, Egypt*
[b] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Suez Canal University, Ismalia, Egypt*
[c] *Alexandria Company for Pharmaceutical and Chemical Industries, Alexandria, Egypt*

Received 16 September 1998; received in revised form 17 October 1998; accepted 21 June 1999

**Abstract**

Three methods are described for the simultaneous determination of mebeverine hydrochloride (MB) and sulpiride (SU) in combined pharmaceutical tablets. The first method depends on first-derivative ultraviolet spectrophotometry, with zero-crossing measurement method. The first derivative amplitudes at 214.2 and 221.6 nm were selected for the assay of MB and SU, respectively. Calibration graphs follow Beer's law in the range of 10–30 and 2–8 μg $ml^{-1}$, and the linearity was satisfactory ($r = 0.9999$), for MB and SU, respectively. The second method was based on the application of the thin layer chromatographic separation of both drugs followed by the densitometric measurements of their spot areas. After separation on silica gel GF254 plates, using ethanol: diethyl ether: triethylamine (70:30:1 v/v) as the mobile phase, the chromatographic zones corresponding to the spots of MB and SU were scanned at 262 and 240 nm, respectively. The calibration function was established in the ranges of 4–12 μg for MB and 2–8 μg for SU. The third method was an internal standard procedure based on high performance liquid chromatographic separation of the two drugs on a reversed-phase, Bondapak CN column. The detection was done at 243 nm using buclizine hydrochloride as internal standard. All chromatographic methods showed good linearity, precision and reproducibility. No spectral or chromatographic interference from the tablet excipients were found. The proposed methods were successfully applied to the assay of commercial tablets and content uniformity test. The procedures were rapid, simple and suitable for quality control application. 

*Keywords:* First-derivative spectroscopy; Liquid chromatography; Mebeverine hydrochloride; Pharmaceutical tablets; Sulpiride; TLC-densitometry; Zero-crossing measurements

* Corresponding author. Tel.: +203-4833273; fax: +203-4480730.
*E-mail address:* pharmacy.alex.uni.mac@cns-egypt.com (A.F.M. El Walily)

0731-7085/99/$ - see front matter 
PII: S0731-7085(99)00176-4

## 1. Introduction

Mebeverine hydrochloride (MB), 3,4-Dimethoxybenzoic acid 4-[ethyl-[2-(4-methoxyphenyl)-1-methylethyl] amino]butyl ester, is a potent direct antispasmodic acting mainly on the smooth muscles of the gastrointestinal tract and particularly effective against the colonic spasm [1]. Sulpiride (SU), 5-(aminosalfonyl)-*N*-[(1-ethyl -2-pyrrolidinyl)methyl]-2-methoxybenzamide, is an antagonst of the dopamine $D_2$ receptors. It exerts a mood elevating effect and antiemetic action [2]. The manufacturer of compound tablets containing both drugs claimed that the formula is carefully designed to achieve effective relief of gastointestinal and colic spasms specially in presence of psychosomatic manifestation of nervous tension, mental stress or anxiety [3].

MB and SU are official in the BP 1993 [4], while, SU is only official in the European Pharmacopoeia 1997 [5]. The pharmacopoeias described a non-aqueous titration for the determination of both drugs in bulk and in pharmaceutical tablets. Several analytical methods have been reported for assaying (MB) in its dosage forms and these include: HPLC [6–8]; thin-layer chromatography (TLC) [9,10]; derivative [11] and visible spectrophotometric methods [12–14]. HPLC [15,16], TLC [17–19], fluorimetric [20], spectrophotometric [21] and radio-immunoassay [22] methods have been described for SU determination in pharmaceutical tablets. The analytical profile of SU including its stability and some of the analytical methods for the drug determination has been published [23]. No method has been reported for their simultaneous determination in two component mixtures.

The application of derivative techniques to spectroscopy is very useful when signal overlap or interferences exist and it offers a powerful tool for both qualitative and quantitative analysis of mixtures in pharmaceutical analysis [24–27] and biomedical analysis [28,29]. The aim of this work was to demonstrate the capability of the first-derivative ($^1$D) method to resolve and overcome the problem of overlapping spectral bands and allows the simultaneous determination of MB and SU without the need for prior separation. At the same time, the chromatographic procedures (TLC and HPLC) were developed and functional as reference methods. The study compares the three methods for the simultaneous assay of MB and SU in commercial tablets.

## 2. Experimental

### 2.1. Instrumentation

A double beam, self recording Shimadzu (Duisburg, Germany) UV-VIS spectrophotometer Model UV-1601 PC with 1 cm matched quartz cells was used. The spectral band width was 2 nm and the wave length scanning speed was 200 nm $min^{-1}$; $\Delta\lambda = 4$ nm and scaling factor was 6. The response time was 0.02 s in the spectrum mode. The notation for the amplitude measurements in the derivative mode was made according to Fasanmade and Fell [30].

Chromatoplates (20 × 20 cm, aluminium plates precoated with 0.25 mm silica gel GF254) were purchased from E. Merck (Darmstadt, Germany). The samples were applied to the TLC plates using 10 μl Hamilton microsyringe. A Shimadzu dual wavelength flying spot scanner Model CS-9000 was used. The experimental conditions of the measurements were: Wavelength = 262 and 240 nm for MB and SU, respectively; photomode = reflection; scan mode = zigzag; beam size = 12. The integrator conditions were: minimum width = 1 nm; minimum area = 500; swing width = 14; drift line = 10 and peak find filter = 5.

The HPLC (Waters Associates-Milford, MA-USA) instrument consisted of a Model 600 pump, automated gradient controller Model 680, Rheodyne injector and Model 486 tunable absorbance detector or Model 996 photodiode array detector. Peaks data handling was performed with a Waters Millenium 2010 Chromatography Manager Sotware (version 2.15.01).

### 2.2. Materials

Pharmaceutical grade MB (Solvay Duphur B.V., Weesp-Holland) and (SU) (Laboratories Delagrange, Chilly, France) were kindly supplied

by Pharco Pharmaceutical (Alexandria, Egypt) and Memphis Pharm. (Cairo, Egypt) and were certified to contain 99.50 and 99.35%, respectively. They were used without further purification. Buclizine hydrochloride (Select Chemie AG, Zurich, Switzerland) was used as an internal standard (1 mg $ml^{-1}$ solution in methanol) for the HPLC procedure. It was an in-house standard and its purity was certified to be 99.00%. The common tablet excipients were obtained from local sources. Acetonitrile (BDH, Poole, UK) was of HPLC grade; water was doubly distilled from all glass apparatus. Analytical reagent grade ethanol, diethyl ether and triethyl amine were used throughout these experiments. Tablets of Colona, (Batch no. 104), manufactured by RAMEDA (6 October City, Egypt), containing 100 mg MB and 25 mg SU per tablet, were used.

### 2.3. Chromatographic conditions

The mobile phase was prepared by mixing acetonitrile and water in the ratio of 75:25. To each liter of mobile phase, 0.1 ml of triethylamine was added and the apparent pH of the solution was adjusted to read 7 using dilute phosphoric acid. All the determinations were performed at ambient temperature (20°C) using cyano reverse phase column (Waters bondapak CN, 10 μm particle size, 150 × 3.9 mm I.D.) with a flow rate of 2 ml $min^{-1}$. The column effluent was monitored at 243 nm, which represents the wavelength at which both drugs have almost the same absorbance. The injection volume was 20 μl.

The TLC plates were developed in ethanol-diethyl ether-triethylamine 70:30:1 v/v/v) solvent. For detection and quantification by scanning densitometry, 10 μl test solution and 10μl of the corresponding standard solution were applied as separate compact spots 10 mm apart and 15 mm from the bottom of a TLC plate. The plate was developed up to the top (over a distance of 16 cm) in the usual ascending way. The chromatographic tank was saturated with mobile phase in the usual mode. After elution the plate was air dried and observed at 254 nm. The starting and the end point for densitometry were marked and the scanning performed as previously described.

### 2.4. Standard solutions and calibration graphs for spectrophotometric measurements

Stock solutions were prepared by dissolving MB and SU in methanol to obtain concentration of 1.0 and 0.1 mg $ml^{-1}$, from both, respectively. The standard solutions were prepared by dilution of stock solutions with 0.1 M hydrochloric acid to reach concentration ranges of 10–30 (in 4 μg increment, $n = 7$) and 2–8 (in 1 μg increment $n = 7$) μg $ml^{-1}$ for MB and SU, respectively. Working standard solution of MB and SU mixtures in 0.1 M hydrochloric acid (containing 2, 5 and 8 μg $ml^{-1}$ of SU and increasing concentrations of MB ranging from 10 to 30 and 10, 20 and 30 μg $ml^{-1}$ of MB with increasing concentration of SU ranging from 2 to 8μg $ml^{-1}$ ($n = 7$)) were prepared from stock solutions of MB and SU in methanol.

#### 2.4.1. UV measurements

The first-order derivative spectra ($^1D$) of the 0.1 M hydrochloric acid working standard containing the varying amount of each drug and those containing mixture of both drugs were scanned in the range of 350–200 nm against 0.1M hydrochloric acid as blank. The values of the $^1D$ amplitudes at 214.2 nm (zero-crossing of SU) were measured for the determination of MB in presence of SU. The $^1D$ spectra of SU and their mixtures with MB were also recorded between 350 and 200 nm and the $^1D$ amplitudes values, at 221.6 nm (zero-crossing for MB) were used for the determination of SU in presence of MB.

### 2.5. Standard solutions and calibration graphs for the chromatographic procedures

#### 2.5.1. For TLC-densitometry

Standard solutions were prepared by dissolving about 100 mg of MB and SU, respectively, into a 100 ml methanol. Different volumes of both standard solutions were applied to the TLC plates in concentration ranges of 4–12 μg ($n = 8$)and 2–8 μg ($n = 7$), respectively. Triplicate applications of the different volumes were made for each solution. The plates were developed in the previously described mobile phase (Section 2.3). The peak

areas were plotted against the corresponding concentration to obtain the calibration graph.

*2.5.2. For HPLC*

Standard solution of MB and SU containing concentration ranges of 10–80 and 2.5–20 μg $ml^{-1}$, respectively, and a fixed concentration of 20 μg $ml^{-1}$ of buclizine hydrochloride (internal standard) were prepared in the mobile phase. Triplicate 20 μl injections were made for each solution. The peak area ratios of drug to the internal standard, were plotted against the corresponding concentrations to obtain the calibration graphs.

*2.6. Sample preparation*

Twenty tablets containing MB and SU as active ingredients were weighed and finely powdered. Portions of the powder equivalent to about 100 mg of MB were weighed accurately, transferred to 100 ml volumetric flasks using methanol. The flasks were completed to volume with methanol.

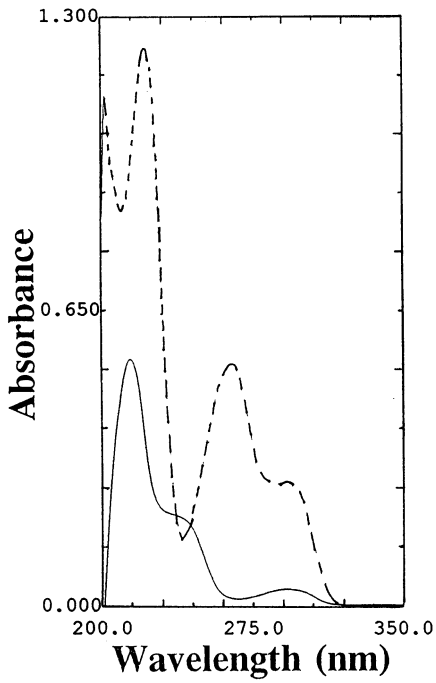


Fig. 1. Absorption (zero-order) UV spectra of 20 μg $ml^{-1}$ MB (----), 5 μg $ml^{-1}$ SU (_____) in 0.1 M hydrochloric acid.

For the derivative and TLC procedures, the suspensions were filtered through a methanolic wetted filter paper and then further diluted, either with 0.1 M hydrochloric acid or methanol, to suit the calibration graphs for the derivative measurements or the TLC procedure, respectively. The HPLC samples were filtered through 0.45 μm membrane filter after the dilution, to suit the calibration graphs, and addition of the internal standards. For the content uniformity test, the same procedure was followed (using one tablet as a sample), except that the mixture was sonicated for 10 min.

## 3. Results and discussion

*3.1. Derivative UV-Spectrophotometry*

The absorption spectra of MB (20 μg $ml^{-1}$) and SU (5 μg $ml^{-1}$) in methanol, were reproduced in Fig. 1. The spectra clearly display considerable overlap; hence, the traditional Vierodet's method and its modification for assaying binary mixtures seems to be impossible. The first order derivative spectra ($^1D$) present spectral features which can be used for the simultaneous determination of MB and SU (Fig. 2). For quantitative work, the amplitude of the derivative peak can be measured in various way. In the present investigation the amplitudes (denoted $h_1$ and $h_2$ in Fig. 2) have been measured with respect to a derivative of zero, which is the true derivative amplitude. Using the amplitudes of the peaks with respect to a derivative of zero of the corresponding first-derivative spectrum ($h_1$ in Fig. 2), MB was quantified at 214.2 nm (zero-crossing, where $dA/d\lambda$ value of SU was zero); similarly, SU was quantified using the amplitude of the derivative spectrum ($h_2$ in Fig. 2) at 221.6 nm MB zero-crossing, where $dA/d\lambda$ value was zero). The measurement of the absolute value of the total derivative spectrum taken at the above mentioned wavelengths afforded the best linear response of the analyte to concentration.

In Fig. 3 (A–C), typical sets of the first-derivative spectra of laboratory mixtures of 10, 20 and 30 μg $ml^{-1}$ of MB and increasing concentrations

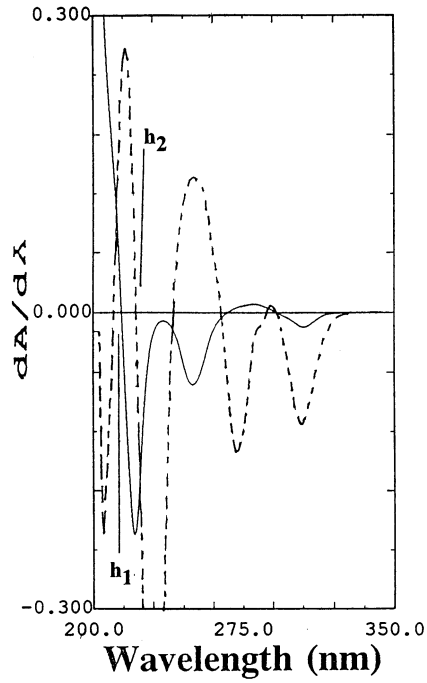


Fig. 2. First-derivative spectra of 20 μg ml$^{-1}$ (MB) (---), 5 μg ml$^{-1}$ (SU) (_____) in 0.1 M hydrochloric acid.

of SU (ranging from 2 to 8 μg ml$^{-1}$, $n = 7$) were shown. The first-derivative spectra of mixtures of 2, 5 and 8 μg ml$^{-1}$ of SU plus an increasing concentration of MB (from 10 to 30 μg ml$^{-1}$, $n = 7$) were shown in Fig. 4 (A–C). The hights at 214.2 nm (zero-crossing, nil contribution from SU) and 221.6 nm (zero-crossing of MB) were proportional to MB and SU concentrations, respectively. Preliminary experiments showed that the height $h_1$ and $h_2$ was proportional to MB and SU concentrations and was hardly affected by the presence of the second component for any ratio of MB: SU over the full range of concentration investigated. In order to test the mutual independence of the analytical signals of each components, i.e. to show that $h_1$ and $h_2$ were independent of SU and MB concentrations, respectively, the following experiments were performed. For each mixture, four calibration graphs were constructed from the derivative signals for the standard of each component in absence and in presence of three different concentrations of the other component. Four calibration graphs were constructed from the $^1$D signals at 214.2 nm for standard samples containing between 10 and 30 μg ml$^{-1}$ of MB, in absence of SU ($P_0$) and in presence of 2.0 ($P_1$), 5.0 ($P_2$) and in presence of 8.0 μg ml$^{-1}$ ($P_3$) of SU. Similarly, four calibration graphs were prepared from $^1$D-derivative signals by measuring at 221.6 nm for standard samples containing between 2 and 8 μg ml$^{-1}$ ($n = 7$) of SU, in absence of MB ($Q_0$) and in presence of 10 ($Q_1$), 20 ($Q_2$) and 30 μg ml$^{-1}$ ($Q_3$) of MB. The experiments showed that the amplitudes height of $^1$D signals at 214.2 nm ($h_1$) and 221.6 nm ($h_2$) were proportional to MB and SU concentrations, respectively. Table 1 summarizes the statistical analysis of the experimental data: the regression equations calculated from the calibration graphs, along with the standard deviations of the slopes and the intercepts on the ordinates. From Table 1, it can be seen that the slopes of the calibration graphs of each component were virtually independent of the second component concentration. Therefore, it can be deduced that the amplitudes of the derivative signals, measured at the selected wavelengths were functionally of the component under determination, in accordance with the theoretical predictions. The linearity of the calibration graphs, the adherence of the system to Beer's law and the negligible scatter of the experimental points were validated by the values of the correlation coefficients of the regression equations and the values of the intercepts on the ordinates, which were close to zero (Table 1). At the same time, if the intercept on the ordinate for the regression lines calculated by the least-squares method is negligible, it is necessary to perform the fitting of the data again according to a function whose intercept on the ordinate is zero and therefore the value of the slope ($b_0$) may be calculated. The results of this study for all the calibration graphs were reported in Table 2. It can be seen that the calculated t-values do not exceed theoretical values and hence the intercept on the ordinate is negligible in all instances. Consequently, the new values of the slopes were calculated (Table 2). In order to verify if the intercepts, a, of the lines of regression were not significantly different from the theoretically expected value ($a = 0$). It was recommended that either construct a joint confi-

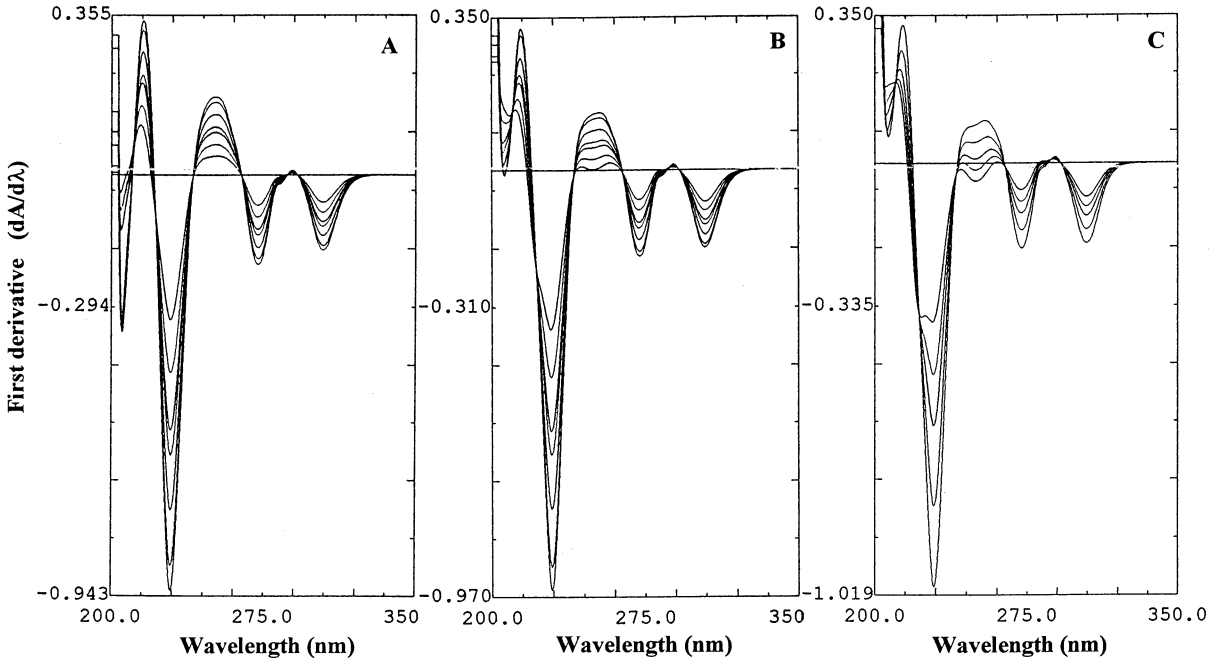


Fig. 3. First-order derivative spectra of mixture containing (A) 2; (B) 5; (C) 8 μg ml$^{-1}$ SU plus increasing amount of MB ranging from 10 to 30 μg ml$^{-1}$ in 0.1 M hydrochloric acid.

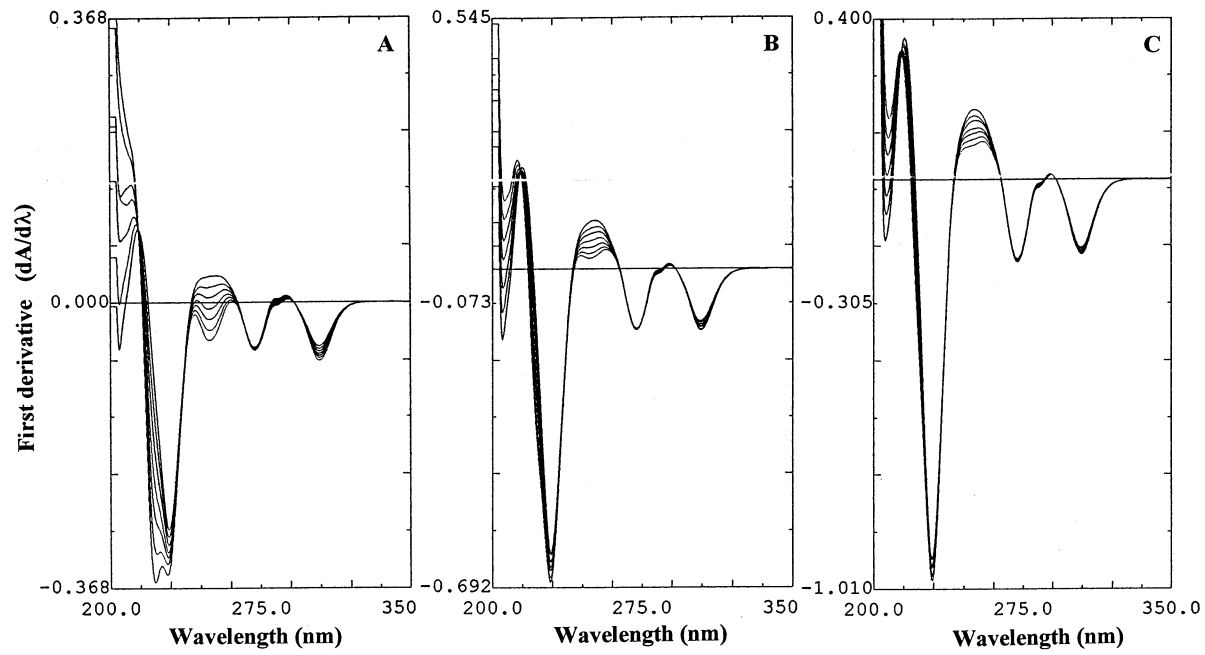


Fig. 4. First-derivative spectra of mixtures containing (A) 10; (B) 20; (C) 30 μg ml$^{-1}$ MB plus increasing amount of SU ranging from 2 to 8 μg ml$^{-1}$ in 0.1 M hydrochloric acid.

Table 1
Statistical analysis of the calibration graphs in the determination of the two binary mixtures of mebeverine hydrochloride (10–30 μg $ml^{-1}$)-sulpiride (2–8 μg $ml^{-1}$) first derivative spectroscopy for $n = 7$ standard specimens

| Drug determined | Other drug present | | Calibration graph | Intercept ($\times 10^{-4}$) S.D. ($\times 10^{-4}$) | Slope ($\times 10^{-3}$) | Corr. Coefficient ($r$) |
|---|---|---|---|---|---|---|
| | Drug | Concentration (μg $ml^{-1}$) | | | | |
| MB | | | $P_0$ | −15.89 (10.47) | 11.25 (0.48) | 0.9999 |
| | SU | 2.00 | $P_1$ | −16.33 (9.77) | 11.21 (0.45) | 0.9999 |
| | SU | 5.00 | $P_2$ | −21.22 (20.44) | 11.22 (0.94) | 0.9998 |
| | SU | 8.00 | $P_3$ | −21.00 (20.61) | 11.24 (0.89) | 0.9999 |
| SU | – | – | $Q_0$ | 3.92 (5.89) | 43.96 (1.09) | 0.9999 |
| | MB | 10.00 | $Q_1$ | 3.93 (15.17) | 43.75 (2.28) | 0.9999 |
| | MB | 20.00 | $Q_2$ | 17.07 (10.57) | 43.54 (1.96) | 0.9999 |
| | MB | 30.00 | $Q_3$ | 4.64 (15.50) | 43.75 (2.89) | 0.9999 |

Table 2
Statistical parameters corresponding to the application of students $t$-test and evaluation of the residual error variance among the calibration graphs of the two binary mixture, mebeverine hydrochloride-sulpiride by applying the analysis of variance (95% level of confidence; 6 degree of freedom)

| Statistical parameters | Calibration graphs of mebeverine hydrochloride | | | | Calibration graphs of sulpiride | | | |
|---|---|---|---|---|---|---|---|---|
| | $P_0$ | $P_1$ | $P_2$ | $P_3$ | $Q_0$ | $Q_1$ | $Q_2$ | $Q_3$ |
| $t_{experimental}$ | 1.52 | 1.67 | 1.04 | 1.02 | 0.67 | 0.25 | 1.52 | 0.30 |
| $t_{theoretical}$[a] | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 | 2.45 |
| $b_0$ ($\times 10^4$) | 11.18 | 11.14 | 11.13 | 11.15 | 43.90 | 43.82 | 43.81 | 43.67 |
| $F_{experimental}$ | $0.248 \times 10^{-3}$ | | | | $0.207 \times 10^{-3}$ | | | |
| $F_{theoretical}$[b] | 3.01 | | | | 3.01 | | | |

[a] Theoretical value of $t$ at the 95% level of confidence.
[b] Theoretical value of $F$ at the 95% level of confidence.

dence region for the possible values of both the intercept and the slope [31] or apply the Student's $t$-test at 95% confidence level and six degree of freedom [32]. The confidence region is limited by an ellipse having the point of best fit as center and whose boundary is depending on the magnitude of the experimental error and on the level of significance with which we wish to state that real points lie in the interior of the ellipse. Fig. 5 represent the 95% joint confidence regions for the slopes and intercepts of the regression equations stated in Table 1. The question as to whether the calculated intercepts are not significantly different from zero, was answered by determining whether the calculated ellipses (Fig. 5) contained points for which the intercept (abscissa) was zero. By considering for each ellipse a vertical line through abscissa equal zero, it is clear that points in all lines fall inside the ellipses. Because the values for the correlation coefficients were not sufficient to evaluate the linearity of the calibration graphs. So, the linearity was evaluated by calculation of the relative S.D. of the slope ($S_{brel}$%) [33] (Table 4). Also, the small degree of scatter of the experimental data points around the line of regressions was confirmed by the small values of the variances. For more confirmation, the Student's $t$-test was performed to determine whether the experimental

intercept ($a$) of the above mentioned regression lines were significantly different from the theoretical zero value [32]. The values calculated for $t$ were 1.25 for MB and 0.67 for SU (these values do not exceed the 95% criterion of $t_p = 3.182$ for five samples), so the intercepts are not significantly different from zero. Thus the hypothesis that ($a$) equal zero and the validity of the method were confirmed.

### 3.2. Chromatographic methods

The TLC-densitometric and HPLC methods were developed to provide a specific procedure suitable for the rapid quality control analysis of binary mixtures containing MB and SU, and as reference methods for the derivative procedure.

#### 3.2.1. TLC-densitometric method

Instrumental planar chromatography with precise application of the samples and computer controlled evaluation and quantification of the developed chromatograms, has been considered as reliable for purity control and quantitative drug testing [34]. Experimental conditions, such as mobile phase composition, scan mode and speed and wavelength of detection, were optimized to provide accurate, precise and reproducible results for both MB and SU. The chosen scan mode was the zigzag mode and the wavelengths of scanning were chosen to be 262 and 240 nm for MB and SU, respectively. The greatest differences between the $R_f$ values of the investigated drugs (0.45 and 0.29 for MB and SU, respectively) were obtained by the system containing ethanol-diethyl ether in ratio of 7:3, respectively. Addition of triethylamine in 1% concentration to the above system was essential to prevent tailing and to move the drug spots upward. Migration distance ($\pm$ S.D., $n$ = number of measurements) were $7.20 \pm 0.36$ ($n = 10$) and $4.64 \pm 0.45$ ($n = 10$) cm for MB and SU, respectively. Following three analyses of each of seven different concentrations, the calibration graphs were determined through least squares regression (Fig. 6A and B). The least-squares regression equations obtained for MB and SU determination in the stated concentration ranges (Table 4), respectively, with the corresponding values of correlation coefficients, are: MB: $Y = -0.80 \times 10^2$ ($\pm 4.70 \times 10^2$) $+ 2.47 \times 10^4$ ($\pm 0.54 \times 10^2$) C; $r = 0.9998$; SU: $Y = -3.68 \times 10^2$ ($\pm 3.69 \times 10^2$) $+ 1.76 \times 10^4$ ($\pm 0.68 \times 10^2$) C; $r = 0.9999$, where the concentration of MB and SU are expressed in μg.

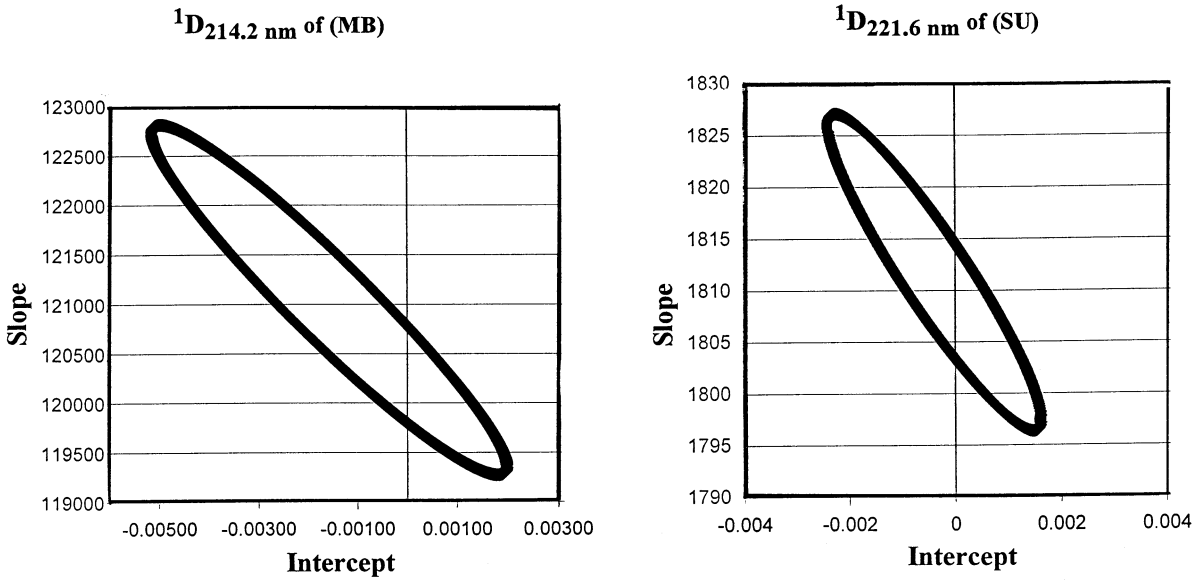


Fig. 5. Joint confidence regions at the $P = 0.05$ level of significant for slopes and intercepts of MB and SU by first-derivative method.

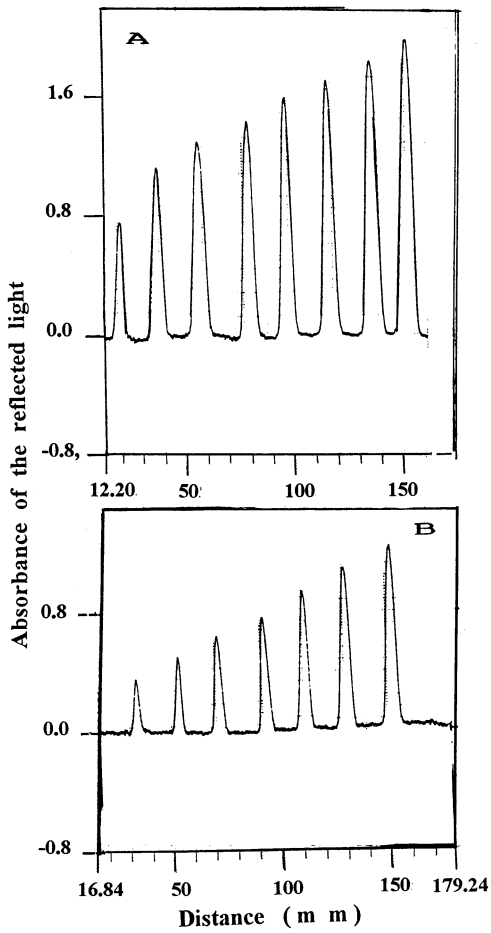


Fig. 6. Scanning profile of TLC chromatogram of different concentrations of (A) mebeverine hydrochloride measured at 262 nm; (B) sulpiride measured at 240 nm.

As the TLC procedure depends upon separating MB from SU, direct spectrophotometric measurement of the methanolic extract of MB or SU scratched spots was found to give inconsistent and high results. This has been attributed to the background contribution from the extracted absorbent material. Correction of such background interference has been achieved, usually, using second-derivative ($^2$D) spectrphotometric technique [35]. Unfortunately, due to the high background contribution, not only from the absorbent used but also from, the triethylamine used in the mobile phase, the derivative spectrophotometric technique up to the fourth derivative mode cannot correct for the observed background. Therefore the densitometric measurements were the best way to separate and quantitate the binary mixture of MB and SU using TLC technique.

The proposed TLC method is very simple and rapid and uses a minimal volume of solvents, compared to the other separation techniques.. Further, an extremely large number of samples can be analyzed at the same time without compromising accuracy, the proposed method is thus suitable for quality control laboratories, where economy and time is essential.

### *3.2.2. HPLC method*

Typical chromatogram obtained from the HPLC method was shown in Fig. 7 The HPLC method involves the use of reverse phase cyano column (Bondapak CN), isoc ratic elution of acetonitrile–water (75:25 v/v) with the addition of 0.1 ml of triethylamine $l^{-1}$ of the combined mo-

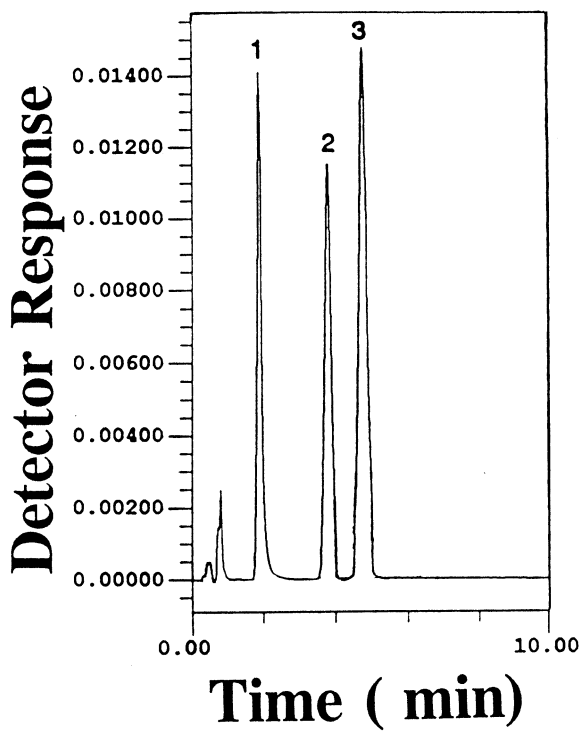


Fig. 7. A typical chromatogram of 20 μl injection of standard mixture of (1) 20 μg $ml^{-1}$ buclizine hydrochloride (IS); (2) 10 μg $ml^{-1}$ SU; (3) 40μg $ml^{-1}$ MB.

Table 3
Statistical data of the regression equation for MB and SU using HPLC procedure

| Parameter | Mebeverine | Sulpiride |
|---|---|---|
| Intercept ($a$) | $-2.67\times10^{-3}$ | $5.67\times10^{-3}$ |
| $tS_a$[b] | $2.30\times10^{-2}$ | $1.18\times10^{-2}$ |
| Slop (b) | 0.049 | 0.122 |
| $tS_b$[a] | $4.56\times10^{-4}$ | $9.38\times10^{-4}$ |
| Correlation coefficient ($r$) | 0.9999 | 0.9999 |
| Variance ($S_0^2$) | $1.46\times10^{-4}$ | $3.85\times10^{-5}$ |

[a] Confidence intervals of the slope ($P=0.05$).
[b] Confidence intervals of the intercepts ($P=0.05$).

bile phase. The mobile phase was chosen after several trials with acetonitrile–water and methanol–water. The apparent pH of the mixed mobile phase was adjusted to be 7 using phosphoric acid. To optimize the assay parameters, the effect of acetonitrile and the apparent pH on the capacity factor ($K'$) values were studied. Due to the basic nature of MB, SU and IS, the addition of the triethylamine (as ion-pair) was essential to prevent tailing for SU peak, broadening to MB peak and to reduce the retention time of both drugs. The above described chromatographic system allows an adequate resolution ($R_s=4.78$ and 3.83) between MB or SU and buclizine hydrochloride (IS) in a reasonable time (Fig. 7) ($R_s$ = resolution). The linearity for MB and SU, over the concentration ranges stated in Table 3, were determined by plotting peak area ratios of the each drug to the internal standard versus concentrations. The analytical data for the calibration graphs are listed in Tables 3 and 4. The relative S.D. (0.28 and 0.46%) of the peak area ratios of MB and SU to the internal standard, derived from replicate ($n=8$) analyses of MB and SU solutions, demonstrate the precision of the chromatographic procedure. The specificity and selectivity of the HPLC system were ascertained by a separate chromatographic analysis of either the excipients mixtures without MB and SU or the alkaline degradation products of both drugs (Fig. 8); no interfering peaks at the retention times of MB or SU and buclizine hydrochloride (internal standard) peaks were observed.

### 3.3. Accuracy and precision of the proposed methods

In order to assess the validity [accuracy ($E_r$%) and precision(RSD%)] of the proposed method for assaying each drug in the presence of the other synthetic mixtures with different proportions of the two drugs were prepared and then assayed using the proposed methods. Five successive determinations of mixtures of MB and SU were carried out. The results obtained for the recovery of both drugs (Table 5) showed that the precision and accuracy were very satisfactory.

Table 4
Concentration ranges, detection limits and relative sensitivity for the proposed methods applied for the determination of mebeverine hydrochloride and sulpiride

| Analytical method | Concentration ranges[a] | Detection limit[a] | Linearity[b] | Relative sensitivity[c] |
|---|---|---|---|---|
| *For mebeverine hydrochloride* | | | | |
| $^1D_{214.2}$ | 10–30 | 0.18 | 0.43 | 0.321 |
| TLC | 4–12 | 0.03 | 0.22 | 0.053 |
| HPLC | 10–80 | 0.56 | 0.38 | 1.000 |
| *For sulpiride* | | | | |
| $^1D_{221.6}$ | 2–8 | 0.03 | 0.25 | 0.250 |
| TLC | 2–7 | 0.05 | 0.39 | 0.417 |
| HPLC | 2.5–20 | 0.12 | 0.31 | 1.000 |

[a] Concentration range and detection limit in μg $ml^{-1}$ for derivative and HPLC procedures and in μg for TLC.
[b] Relative S.D. of the slope.
[c] Calculated relative to the HPLC method.

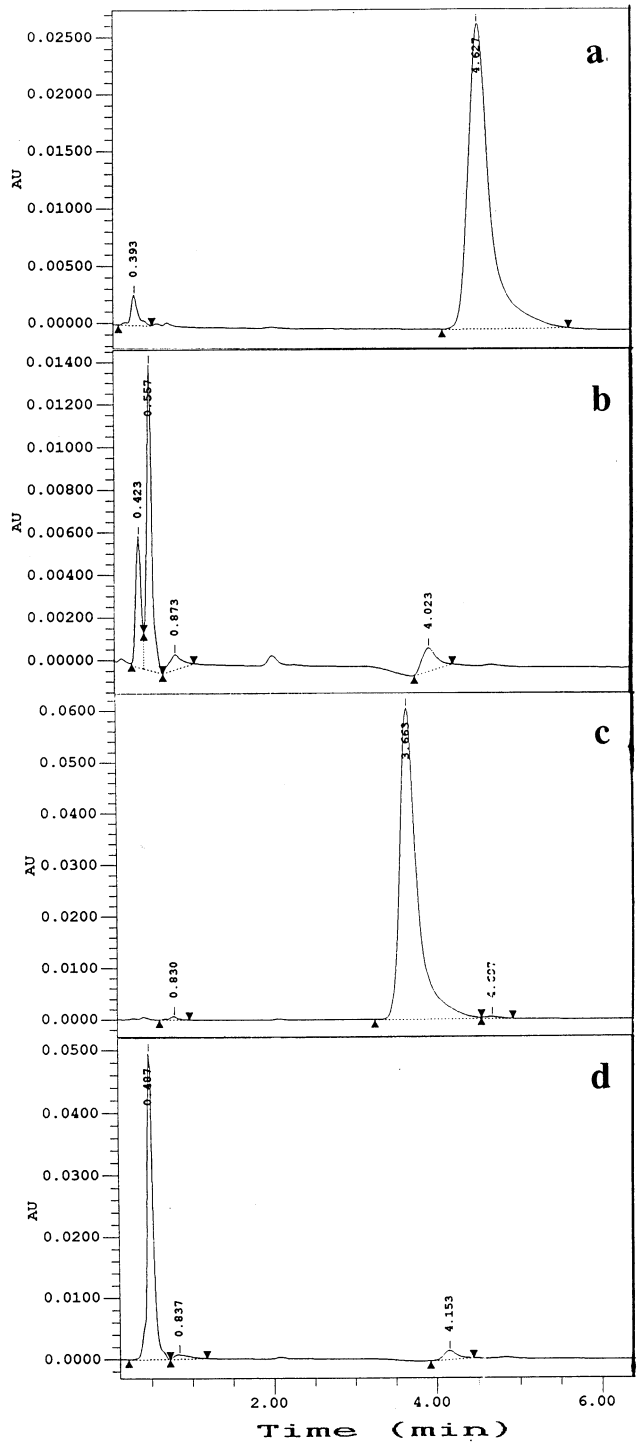


Fig. 8. Typical chromatogram of (A) intact MB (B) alkaline decomposed MB (C) intact SU and (D) alkaline decomposed SU.

Table 5
Precision and accuracy for the determination of mebeverine hydrochloride (MB) and sulpiride (SU) by the proposed methods

| Drug | Added[d] | Found (μg ml$^{-1}$) | | |
|---|---|---|---|---|
| | | Mean ± S.D.[a] | RSD%[b] | $E_r$(%)[c] |
| *I. Derivative method* | | | | |
| MB | 20.000 | 20.040 ± 0.110 | 0.55 | −0.20 |
| SU | 5.000 | 4.975 ± 0.010 | 0.20 | 0.50 |
| *II. TLC method* | | | | |
| MB | 10.000 | 9.990 ± 0.015 | 0.15 | 0.10 |
| SU | 2.500 | 2.495 ± 0.001 | 0.04 | 0.20 |
| *III. HPLC method* | | | | |
| MB | 60.000 | 60.120 ± 0.150 | 0.25 | −0.20 |
| SU | 15.000 | 14.895 ± 0.038 | 0.26 | 0.70 |

[a] Mean and S.D. for three determinations.
[b] Relative S.D.
[c] Percentage relative error.
[d] The concentration of the simulated mixtures was mentioned in μg ml$^{-1}$ for both derivativeand HPLC method and in μg for TLC method.

### 3.4. Comparison of the results obtained by derivative and chromatographic procedures.

For full comparison between the derivative and the chromatographic procedures, five standard mixtures containing MB and SU were prepared and analysed using the three proposed procedures. There is a linear correlation between the results obtained by the derivative spectrophotometry and each of the chromatographic procedures over the concentration ranges analysed. The fitted curves can be expressed by the following equations:

$$Y_{MB} = 0.9792X_{MB} + 0.0805 \ (n = 6;\ r = 0.9994)$$

$$Y_{MB} = 0.9885Z_{MB} + 0.0473 \ (n = 6;\ r = 0.9998)$$

$$Y_{SU} = 0.9994X_{SU} - 0.0117 \ (n = 6;\ r = 0.9994)$$

$$Y_{SU} = 0.9994Z_{SU} + 0.0217 \ (n = 6;\ r = 0.9997)$$

where $Y$ are the derivative assay values and $X$ and $Z$ the TLC and HPLC values, respectively. By the examination of the four equations, the slopes and intercepts were found to close to the unity and zero values, respectively. Therefore, the differences observed between the derivative method and the two chromatographic procedures results only from the variability of measurements.

### 3.5. Application to a commercial formulation of tablet

The methods were applied to the determination of MB and SU in tablets of Colona® (RAMEDA, 6 October City, Egypt) which comprise the binary mixture (100 mg MB and 25 mg SU). Five replicate determinations were made. Satisfactory results (Table 6) were obtained for the recovery of both drugs and were in a good agreement with the label claims. The recovery of the three procedures was tested by adding known amount (standard addition) of MB and SU to the commercial tablets. No significant differences were found between the results obtained by the three procedures for the same batch, at the 95% confidence level (Student's $t$- and $F$-ratio tests). As the dosage form of MB and SU is not pharmacopoeial yet, the values given by the derivative procedure were compared with both the chromatographic procedures (TLC and HPLC). The statistical evaluation indicated that there was no significant difference between the methods used.

## 4. Conclusions

The derivative ($^1$D), the TLC and HPLC procedures were shown to be reproducible and sensitive

Table 6
Determination of mebeverine hydrochloride (MB)–sulpiride (SU) combination in synthetic mixtures and commercial tablets by the three proposed methods

| | Analytical method Mean found ± S.D.[a] | | |
|---|---|---|---|
| | TLC | Derivative | HPLC |
| *Synthetic mixtures* | | | |
| For (MB) content | 100.13 ± 1.22 | 99.64 ± 0.86 | 100.04 ± 0.54 |
| For (SU) content | 100.32 ± 1.42 | 99.47 ± 0.83 | 100.07 ± 0.64 |
| *Colona tablets (Batch Number 104)* | | | |
| For MB content | 100.20 ± 1.20 | 99.52 ± 0.76 | 99.95 ± 0.61 |
| | $t = 1.29$ | (2.179)[b] | 1.18 |
| | $F = 2.49$ | (6.160)[b] | 1.55 |
| For SU content | 100.10 ± 1.08 | 99.48 ± 0.66 | 100.00 ± 0.69 |
| | $t = 1.32$ | | 1.46 |
| | $F = 2.68$ | | 1.09 |
| *Recovery*[c] | | | |
| For MB | 100.57 ± 1.20 | 99.85 ± 0.50 | 100.13 ± 0.61 |
| For SU | 99.95 ± 1.13 | 100.05 ± 0.74 | 99.94 ± 0.68 |

[a] Mean and S.D. for seven determinations; percentage recovery from the label claim amount.
[b] Theoretical values for $t$ and $F$.
[c] For standard addition of 50% of the nominal content ($n = 7$).

in the analysis of mebeverine hydrochloride and sulpiride in simple binary mixture. The derivative procedure has been validated with respect to simple binary mixtures of mebeverine hydrochloride and sulpiride. In addition to the advantage of the used derivative procedure, both chromatographic techniques (TLC and HPLC) avoid the interference from the existence of any degradation products of the two drugs. At the same time, the analytical results confirm that the derivative spectrophotometry offers accuracy and precision with the added advantages of the low cost, speed and simplicity. Therefore, the proposed derivative procedure is likely to be very suitable for the analysis of MB and SU.

## Acknowledgements

The authors are grateful to Alexandria Pharmaceutical Company (Alexandria, Egypt) for granting permission to use their laboratory facilities for the project. The authors are also thankful to Ms. Amany Moustafa for typing the manuscript.

## References

[1] J.E.F. Reynolds (Ed.), Martindale- The Extra Pharmacopoeia, 31st Edition, Pharmaceutical Press, London, 1996, pp. 735, 1227.
[2] R.F. Spano, M. Trabucchi, G.V. Corsini, G.L. Gessa, in: Sulpiride and Other Benzamide, Experimental and Clinical Pharmacology, Ravan Press, New York, 1979.
[3] Personal communication, RAMEDA (The Tenth of Ramadan Co., 6th of October City, Egypt).
[4] British Pharmacopoeia 1993, Her Majesty's Stationary Office, Pharmaceutical Press, London, pp. 404, 995, 1833.
[5] European Pharmacopoeia 1997, third ed., Maisonneuve, Sainte-Ruffine, France, p. 1566.
[6] J.A. De Schutter, F. De Croo, G. Van der Weken, W. Van den Bossche, P. De Mocrlose, Chromatographia 20 (1985) 185–192.
[7] G. Hoogewijs, D.L. Massart, J. Chromatogr. Biomed. Appl. 50 (1986) 391–398.
[8] O. Al-Deeb, B.M. Al-Hadiya, N.H. Foda, Chromatographia 44 (1997) 427–430.
[9] J.A. De Schutter, G. Van der Weken, W. Van den Bossche, P. De Moerloose, J. Chromatogr. 350 (1985) 135–144.
[10] J.A. De Schutter, G. Van der Weken, W. Van den Bossche, P. De Moerloose, Chromatographia 20 (1985) 739–742.
[11] M.M. Bedair, M.A. Korany, M.A. Abdel Hay, A.A. Gazy, Anal. Lond. 115 (1990) 449–453.

[12] F.M. Abdel Gawad, Egypt J. Anal. Chem. 3 (1994) 129–134.
[13] E.M. Hassan, A.A. Gazy, M.M. Bedair, Drug Dev. Ind. Pharm. 21 (1995) 633–638.
[14] M.N. Reddy, K.V.S. Rao, D.G. Sankar, K. Sridhar, Ind. Drugs 33 (1996) 604–606.
[15] N. Verbiese-Genard, M. Hanocq, M. Van Damme, L. Molle, Int. J. Pharm. 2 (1979) 155–166.
[16] F. Bressolle, J. Bres, J. Chromatogr. Biomed. Appl. 42 (1985) 391–399.
[17] F. Bressolle, J. Bres, S. Brun, E. Rechenq, J. Chromatogr. 174 (1979) 421–433.
[18] W. Han, L. Jiang, L. Gao, G. Zhou, W. Jia, Zhongguo Yaoxue Zazhi 24 (1989) 605–607 through Anal. Abst. 52 (1990) 12E53.
[19] K. Dross, C. Sonntag, R. Mannhold, J. Chromatogr. 639 (1993) 287–324.
[20] M. Buna, J.J. Aaron, P. Prognon, G. Mahuzier, Anal. Lond. 121 (1996) 1551–1555.
[21] Z. Liu, J. Zhang, Y. Fenxi Zazhi 13 (1993) 279, through Anal. Abst. 56 (1994) 4G86.
[22] M.T. Cardoso, P. Pradelles, J. Labelled Compd. Radiopharm. 19 (1982) 1103–1109.
[23] D. Pitre, R. Stradi, G. Nathansohn, in: K. Florey (Ed.), Sulpiride in Analytical Profiles of Drug Substances, vol. 17, 1988, p. 607.
[24] A.M. El Walily, S.F. Belal, E.A. Heaba, A.M. El Kersh, J. Pharm. Biomed Anal. 13 (1995) 851–856.
[25] A.M. El Walily, S.M. Blaih, M.H. Barary, M.A. El Sayed, H.H. Abdine, A.M. El Kersh, J. Pharm. Biomed. Appl. 15 (1997) 1923–1928.
[26] A.M. El Walily, Acta Pham. Hung. 67 (1997) 89–97.
[27] A.M. El Walily, J. Pharm. Biomed. Appl. 16 (1997) 21–30.
[28] A.M. El Walily, M.A. El Sayed, M.A. Korany, S.M. Galal, J. Clin. Pharm. Ther. 17 (1992) 101–105.
[29] A.G. Eshra, E.M. Hassan, A.M. El Walily, J. Clin. Pharm. Ther. 18 (1993) 331–335.
[30] A.A. Fasanmade, A.F. Fell, Analyst 110 (1988) 1117–1124.
[31] V.V. Nalimov, The application of mathematical statistics to chemical analysis, Pergamon Press, Oxford, 1963, p. 189.
[32] B. Morelli, Anal. Chim. Acta 209 (1988) 175–184.
[33] S. Torrado, S. Torrado, R. Gadoringa, J. Pharm. Biomed. Anal. 12 (1994) 383–387.
[34] B. Renger, in: Proceedings of the Sixth International Symposium on Instrumental Planar Chromatography, Institute for chromatography, Bad Duerkheim, 1991, p. 291.
[35] A.F. Fell, Proc. Anal. Div. Chem. Soc. 15 (1978) 260–265.