A Comparative Study on Various Spectrometries with Thin Layer Chromatography for Simultaneous Analysis of Drotaverine and Nifuroxazide in Capsules

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Three spectrophotometric methods including Vierordt's method, derivative, ratio spectra derivative, and thin layer chromatography (TLC)-UV densitometric method were developed for simultaneous determination of drotaverine HCl (DRT) and nifuroxazide (NIF) in presence of its impurity, 4-hydroxybenzohydrazide (4-HBH). In Vierordt's method, ($E^{1\%}_{1\,cm}$) values were calculated at 227 and 368 nm in the zero-order spectra of DRT and NIF. By derivative spectrophotometry, the zero-crossing method, drotaverine HCl was determined using the second derivative at 245 nm and the third derivative at 238 nm, while nifuroxazide was determined using the first derivative at 399 nm and the second derivative at 411 nm. The ratio spectra derivative spectrophotometry is based on the measure of the amplitude at 459 nm for DRT and at 416 nm for NIF in the first derivative of the ratio spectra. Calibration graphs of the three spectrophotometric methods were plotted in the range 1—10 μg/ml of DRT and 2—20 μg/ml of NIF. TLC-UV densitometric method was achieved on silica gel plates using ethyl acetate : methanol : ammonia 33% (10 : 1 : 0.1 v/v/v) as the mobile phase. The *Rf* values were 0.74, 0.50, 0.30±0.01 for DRT, NIF and 4-HBH, respectively. On the fluorescent plates, the spots were located by fluorescence quenching and the densitometrical area were measured at 308 and 287 nm with linear range 0.2—4 μg/spot and 0.6—12 μg/spot for DRT and NIF, respectively. The proposed methods have been successfully applied to the commercial pharmaceutical formulation without any interference of excipients. Mean recoveries, relative standard deviations and the results of the proposed methods were compared with those obtained by applying the alternate methods.

Key words drotaverine; nifuroxazide; Vierordt's method; derivative spectrophotometry; ratio spectra derivative; TLC-UV densitometry

The mixture of drotaverine (DRT) and nifuroxazide (NIF) is well established in pharmaceutical formulation as an antispasmodic-antibacterial drugs. Drotovarine, analogue of papaverine, 1-(3,4-diethoxybenzylidene)-6,7 diethoxy-1,2,3,4-tetrahydroisoquinoline HCl is an effective spasmolytic drug. It is used as an antispasmodic in the management of biliary tract, urinary tract and gastrointestinal spasm by a direct action on smooth muscle. Nifuroxazide, nitrofuran derivative, 2-(5-nitrofurytidene)-4-hydroxybenzohydrazide has the antimicrobial activity of the 5-nitro-2-substituted furans. It is an antiseptic and antimicrobial drug that is poorly absorbed from the gastrointestinal tract and used in the treatment of colitis and diarrhoea.[1,2] There are only a few reports in the literature describing the determination of DRT by spectrophotometry,[3—5] electrochemical[6] and HPLC methods[7—10] in pharmaceutical formulation and biological fluids. NIF was determined by spectrophotometry,[11] electrochemical[11—13] and HPLC[14] and have been demonstrated in formulations or biological fluids.

In the literature, simultaneous determination of DRT and NIF has not yet been developed. Moreover, the absorption spectra of DRT, NIF and 4-hydroxybenzohydrazide (4-HBH) show a marked overlapping. In analytical chemistry, Vierordt's method is based on the solving of a pair of simultaneous equations with two analytes using the absorbance values at two λ_{max} to calculate the concentration.[15—17] Derivative spectrophotometry is an analytical technique of great utility to overcome the interference of some drugs at the zero-crossing points.[18] Ratio spectra derivative has been developed by Salinas,[19] to resolve mixtures of chromophores with overlapped spectra by measuring the amplitude in the first derivative of ratio spectra obtained by using the selected divisor.[20] Thin layer chromatography-UV densitometric method has a favorable advantage, its ability to separate the contents of mixtures, thus eliminating the possibility of interference between active ingredients, excipients or impurities.[21,22]

In the present investigation, we developed an accurate, reproducible and sensitive Vierordt's method; derivative spectrophotometry (first 1D, second 2D, third 3D); ratio spectra derivative (1DD) and TLC-UV densitometric technique for the simultaneous determination of both drugs in laboratory prepared mixtures and the commercial pharmaceutical formulation.

Experimental

Apparatus UNICAM UV 300, thermo Spectronic, with vision 32 software was connected to IBM PC computer used for all the absorbance measurements and treatment of data and hp laser jet 1100 series printer. The absorbencies of all samples were recorded against a solvent blank in 1 cm quarts cuvettes and stored in the computer by scanning at band width=1.5 nm; scan speed=intelliscan; data interval=normal (1 nm); smoothing=high.

Shimadzu, dual wavelength flying spot scanning densitometer CS-9301 PC.

List of parameters: photomode=reflection; scan mode=zigzag; swing width=10.

TLC precoated plates 10×10 cm, 0.25 mm thickness with silica gel G60 F254 (E. Merck).

Materials Drotaverine HCl (99.64±1.46), its purity was determined by non aqueous titration according to the compendial method[23] and nifurox-

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azide (99.70±1.18) according to the British and European Pharmacopoeia[24,25] and were supplied by Eva Pharm, Egypth.

4-Hydroxybenzohydrazide, the impurity of nifuroxazide was purchased from Fluke Chemie Gm-bH Sigma-Aldrich.

Drotazide capsules (batch No. 410714, Eva Pharm, Egypt) was labeled to contain 40 mg drotaverine HCl and 200 mg nifuroxazide.

Standard Solutions All solutions were freshly prepared. For spectrophotometry, solutions of DRT (0.02 mg/ml) and NIF (0.04 mg/ml) were separately prepared in 95% ethanol after sonication for 15 min. Serial dilutions were prepared in two series of 10-ml volumetric flasks, containing 1—10 μg/ml of DRT and 2—20 μg/ml of NIF in 95% ethanol and were used for the preparation of calibration curves of spectrophotometric methods. For TLC, solution of DRT (0.4 mg/ml) and NIF (1.2 mg/ml) were separately prepared in 50-ml volumetric flasks using 2 ml dimethylsulphoxide (DMSO) for solubility and volume made up with methanol. Serial dilutions were prepared in two series of 5-ml volumetric flasks containing 0.02—0.4 mg/ml of DRT and 0.06—1.2 mg/ml of NIF in methanol and were used for the preparation of calibration curves of TLC method.

Laboratory prepared mixtures were prepared in different ratios of DRT to NIF mainly (1 : 5) as that of capsule using the same solvent as in the method, containing 1—10 μg/ml of DRT and 2—20 μg/ml of NIF for spectophotometry and containing 0.02—0.4 mg/ml of DRT and 0.06—1.2 mg/ml of NIF for TLC.

4-Hydroxybenzohydrazide solutions were prepared in 95% ethanol or methanol with different concentration in the laboratory prepared mixtures.

Application of the Methods. Modified Vierordt's Method Absorbances of the standard solutions of DRT (1—10 μg/ml) and the standard solutions of NIF (2—20 μg/ml) were measured at 227 and 368 nm in the zero-order spectra using 95% ethanol as a blank. Absorbance values of the 1% solution in 1 cm cell at $\lambda_1$and λ_2 were calculated and used in the two equations:

$$A_1=\alpha_1C_1+\beta_1C_2, \quad A_2=\alpha_2C_1+\beta_2C_2$$

where C_1 and C_2 are the concentrations of DRT and NIF in g/100 ml, respectively. A_1 and A_2 denote the absorbances of the mixture solution. α and β represent ($E^{1\%}_{1\,cm}$) values at each wavelength. The subscripts 1 and 2 refer to λ_1 (227 nm) and λ_2 (368 nm), respectively. The concentration of the two drugs were evaluated using the modified Vierordt's equations in terms of absorbance ratio:

$$C_1=(A_1/\alpha_1)\times(b-m/b-a), \quad C_2=(A_2/\beta_2)\times b(m-a)/m(b-a)$$

Where $m=A_2/A_1$, $a=\alpha_2/\alpha_1$, $b=\beta_2/\beta$.

Derivative Spectrophotometry First Derivative Spectrophotometry: The first derivative spectra of the standard solutions (2—20 μg/ml) of NIF were recorded and the calibration graphs were plotted by measuring the amplitude at 341 and 399 nm.

Second Derivative Spectrophotometry: The second derivative of the standard solutions (1—10 μg/ml) of DRT and standard solutions (2—20 μg/ml) NIF were recorded and the calibration graphs were plotted by measuring the amplitude at 234, 245 nm for DRT and at 368, 390, 411 nm for NIF, respectively.

Third Derivative Spectrohotometry: The third derivative spectra of the standard solutions (1—10 μg/ml) of DRT were recorded and the calibration graphs were plotted by measuring the amplitude at 238 and 250 nm.

Ratio Spectra First Derivative Spectrophotometry To determine DRT, the zero-order absorption spectra of samples (1—10 μg/ml) were recorded and divided by the spectrum of the standard solution of NIF (4 μg/ml) in 95% ethanol. From the obtained ratio spectra, the first derivative spectra were recorded and the calibration graphs for DRT were plotted by measuring the amplitude at 452, 459 and 466 nm. To determine NIF, the stored zero-order spectra of the samples (2—20 μg/ml) were also divided by a spectrum of the standard solution of DRT (10 μg/ml) in the same solvent. The first derivative of the ratio spectra of NIF were recorded by an analogous procedure and the calibration graphs for NIF were plotted by measuring the amplitude at 416 and 423 nm.

TLC-Densitometric Method Aliquots of 10 μl of each standard solution (0.02—0.4 mg/ml) of DRT and (0.06—1.2 mg/ml) of NIF were applied to TLC plates using 20 μl Hamilton micro syringe. The plates were developed by ascending migration in a chromatographic tank previously saturated for 20 min with ethyl acetate : methanol : ammonia 33% (10 : 1 : 0.1 v/v/v) as the mobile phase. The plates were dried at room temperature and then the spots were visualized under UV lamp at 254 nm and scanned by the spectrodensitometer at 308 nm for DRT and 287 nm for NIF, respectively. The calibration graphs were plotted representing the relationship between the recorded area under the peak and the corresponding concentration.

Assay of Laboratory Prepared Mixtures in Presence of 4-HBH The laboratory prepared mixtures were analyzed by the spectrophotometry and TLC as under application of the methods in presence of 4-HBH up to 1% in Vierordt's method and up to 10% in derivative, ratio spectra derivative and TLC method. The concentration of DRT and NIF in the mixtures was calculated using the corresponding regression equation.

Assay of Capsules The contents of 10 capsules were accurately weighed and thoroughly ground. For spectrohotometric methods, an amount of the powdered capsules equivalent to 10 mg of DRT and 50 mg of NIF was transferred into 100-ml volumetric flask and treated with ethanol 95% in ultrasonic bath for 15 min and made to volume with the same solvent. For TLC method, an amount of the powdered capsules equivalent to 10 mg of DRT and 50 mg of NIF was transferred into 50-ml volumetric flask and treated with 2 ml DMSO and made to volume with methanol. The solutions were filtered and suitable aliquots of the filtrates were used to quantify the two drugs as described under application of the methods.

Results and Discussion

The original zero-order spectra of DRT, NIF and 4-HBH in 95% ethanol showed a marked overlapping Fig. 1. Different solvents such as methanol, DMSO, dimethylformamide, ethanol, dilute acids and alkalis were tested for complete solubility of NIF and 95% ethanol was found to be suitable for

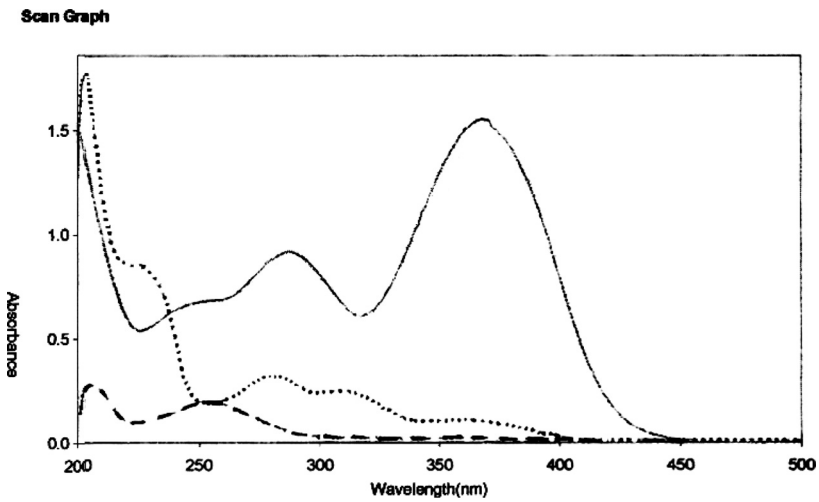

Fig. 1. The Absorption Spectra of Drotaverine (10 μg/ml) ·····, Nifuroxazide (20 μg/ml) —— and 4-Hydroxybenzohydrazide (2 μg/ml) –·–·–

the proposed spectrophotometric methods while the measurements are less affected by the concentration of other component.

Modified Vierordt's Method By the modified version of Vierordt's method, the determination of the two drugs is possible for direct absorbance measurements in their zero-order spectra (Fig. 1) by using the parameters shown in Table 1. The absorbance values of the mixtures were measured at 227 and 368 nm and the equations used have been explained. This method was valid in the concentration range 1—10 μg/ml for DRT and 2—20 μg/ml for NIF. The mean recoveries and the relative standard deviations were obtained as 99.51±0.82% for DRT and 99.83±0.81% for NIF, as shown in laboratory prepared mixtures Table 2.

Vierordt's method could be applied for the determination of DRT and NIF in presence of 4-HBH up to 1% w/w without interference, where the limit specification in the British pharmacopoeia[24] is not more than 0.05%.

Derivative Spectrophotometry First Derivative Spectrophotometry: The first derivative spectra are adequate for determining NIF at 341 and 399 nm with zero-crossing of DRT and 4-HBH, but it did not permit the measurement of DRT (Fig. 2a).

Second Derivative Spectrophotometry: The second derivative spectra permit the measurement of DRT at 234 and 245 nm and NIF at 368, 390, and 411 nm in presence of 4-HBH (Fig. 2b).

Table 1. Experimental Parameters of Vierordt's Method for Simultaneous Determination of Drotaverine and Nifuroxazide

λ (nm)	Drotaverine		Nifuroxazide	
	α_1	α_2	β_1	β_2
λ_1=227	839		251	
λ_2=368		81		785
Linearity range (μg/ml)	1—10		2—20	

Table 2. Recovery Data for the Synthetic Mixtures Obtained by Using the Proposed Methods in Presence of 4-HBH

	Vierordt's method	Derivative spectrophotometry		Ratio spectra derivative	TLC method
Drotaverine		$2D_{245}$	$3D_{238}$	$1DD_{459}$	
Mean[a)]	99.51	99.87	99.32	99.23	99.49
R.S.D.	0.82	1.01	0.60	0.79	1.24
S.E.	0.27	0.34	0.20	0.29	0.41
Nifuroxazide		$1D_{399}$	$2D_{411}$	$1DD_{416}$	
Mean[a)]	99.83	99.37	99.45	99.32	99.62
R.S.D.	0.81	0.67	0.68	0.72	1.25
S.E.	0.27	0.22	0.23	0.24	0.42

a) Average of the analysis of 9 mixtures in different ratios of DRT : NIF.

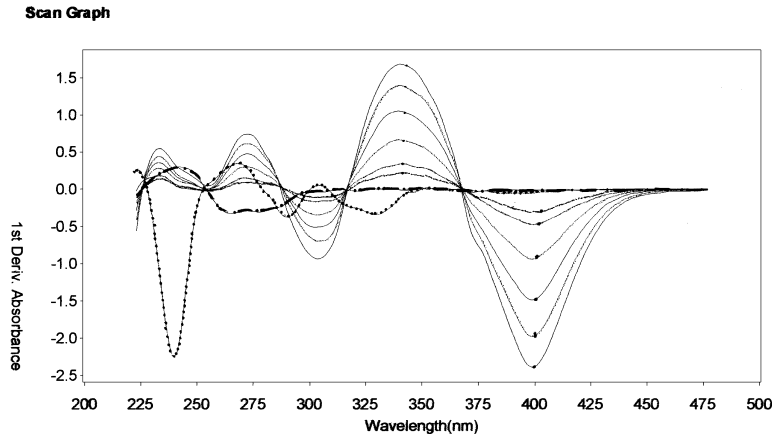

Fig. 2a. The First Derivative Spectra of Drotaverine (8 μg/ml) ·····, Nifuroxazide (2—20 μg/ml) —— and 4-Hydroxybenzohydrazide (2 μg/ml) –·–·–

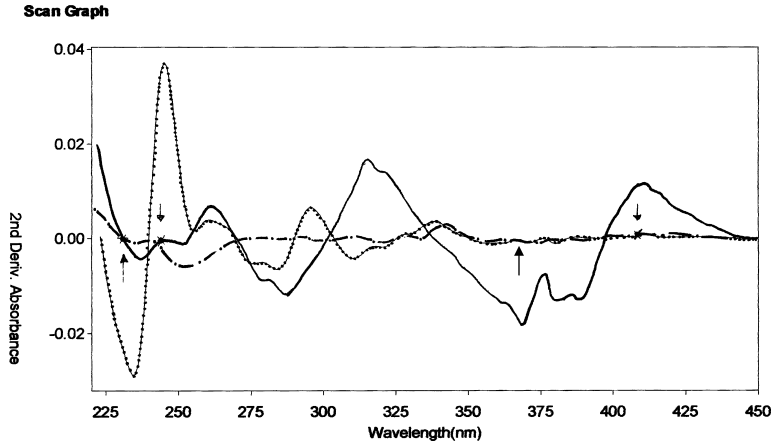

Fig. 2b. The Second Derivative of Drotaverine (10 μg/ml) ·····, Nifuroxazide (2—20 μg/ml) —— and 4-Hydroxybenzohydrazide (2 μg/ml) –·–·–

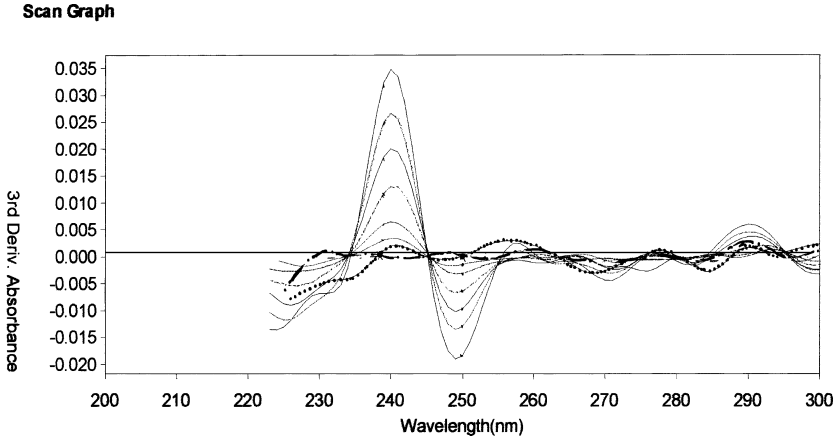

Fig. 2c. The Third Derivative Spectra of Drotaverine (1—10 μg/ml) ——, Nifuroxazide (20 μg/ml) ····· and 4-Hydroxybenzohydrazide (2 μg/ml) –·–·–

Table 3. Calibration Data of the Simultaneous Determination of Drotaverine and Nifuroxazide by the Proposed Methods

Method	nm	Linearity range	Regression equation[a)]	Regression coefficient r^2
Derivative		μg/ml		
2D	245	1—10	$y=0.35\times10^{-2}C_{DRT}-2\times10^{-4}$	0.9999
3D	238	1—10	$y=0.32\times10^{-2}C_{DRT}-2\times10^{-4}$	0.9995
1D	399	2—20	$y=12.06\times10^{-2}C_{NIF}-2\times10^{-4}$	0.9998
2D	411	2—20	$y=0.05\times10^{-2}C_{NIF}-1\times10^{-4}$	0.9996
Ratio spectra derivative		μg/ml		
	459	1—10	$y=2.62\times10^{2}C_{DRT}-0.42\times10^{2}$	0.9989
	416	2—20	$y=2.73\times10^{2}C_{NIF}-1.16\times10^{2}$	0.9991
TLC		μg/spot		
	308	0.2—4	$y=16.95C_{DRT}+4.23$	0.9971
	287	0.6—12	$y=19.94C_{NIF}+14.01$	0.9994

a) $y=bc+a$ where b=the slope, a=the intercept, C_{DRT} and C_{NIF}=the concentration in μg/ml or μg/spot.

Third Derivative Spectrophotometry: The third derivative spectra are adequate for determining DRT at 238 and 250 nm with zero-crossing of NIF and 4-HBH, but it did not permit measurement of NIF (Fig. 2c).

Laboratory prepared mixtures and capsules were analyzed by measuring DRT only at 245 nm in the second derivative and at 238 nm in the third derivative, while NIF was measured only at 399 nm in the first derivative and at 411 nm in the second derivative using the corresponding regression equations in Table 3. The mean recoveries and the relative standard derivations for derivative spectrophotometry of DRT and NIF in laboratory prepared mixtures in presence of 4-HBH up to 10% w/w are shown in Table 2.

Ratio Spectra First Derivative Spectrophotometry Figures 3a and b indicate the ratio spectra and first derivative of the ratio spectra for the determination of DRT using 4 μg/ml of NIF as a divisor. Three calibration graphs were plotted by measuring the amplitude at 452, 459 and 466 nm, and were tested between (1—10 μg/ml) of DRT. Similarly, Figs. 4a and b indicate the ratio spectra and first derivative of the ratio spectra for the determination of NIF using 10 μg/ml of DRT as a divisor. Two calibration graphs were plotted by measuring the amplitude at 416 and 423 nm, and were tested between (2—20 μg/ml) of NIF. For selecting a divisor in appropriate concentration, which is very important factor in practice, some divisor concentrations were tested in the determination. The standard solution of 10 μg/ml of DRT and 4 μg/ml of NIF were found suitable as a divisor. By application of the ratio spectra derivative for the determination of the two drugs in laboratory prepared mixtures and capsules, the calibration graphs and the regression equations were only used which were obtained by measuring at 459 nm for DRT and 416 nm for NIF, respectively, as shown in Table 3. The mean recoveries and the relative standard deviations of the ratio spectra were obtained as 99.23±0.79% for DRT and 99.32±0.72% for NIF in laboratory prepared mixtures, respectively (Table 2). The method was valid in presence of 4-HBH up to 10% w/w of NIF.

TLC-UV Densitometric Method TLC-UV densitometric method was developed for provide a specific procedure suitable for quality control analysis of DRT and NIF in presence of its impurity 4-HBH. Several mobile phases were prepared and tested for efficient separation of the three components. Ethyl acetate : methanol : ammonia 33% (10 : 1 : 0.1 v/v/v) was found to be the best mobile phase and the *Rf* values±S.D. were 0.74±0.01, 0.50±0.01, 0.30±0.01 for DRT, NIF and 4-HBH, respectively. The plates were scanned densitometrically at the selected wavelengths 308 nm for DRT and 287 nm for NIF, and the area under the peak corresponding to concentration (0.2—4 μg/spot) of DRT and (0.6—12 μg/spot) of NIF were recorded Figs. 5a and b. The linearity ranges, regression equations and regression coefficients for quantitative analysis were summarized in Table 3. In order to demonstrate the validity and applicability of the present TLC method, recovery studies were performed by analyzing laboratory prepared mixtures with different composi-

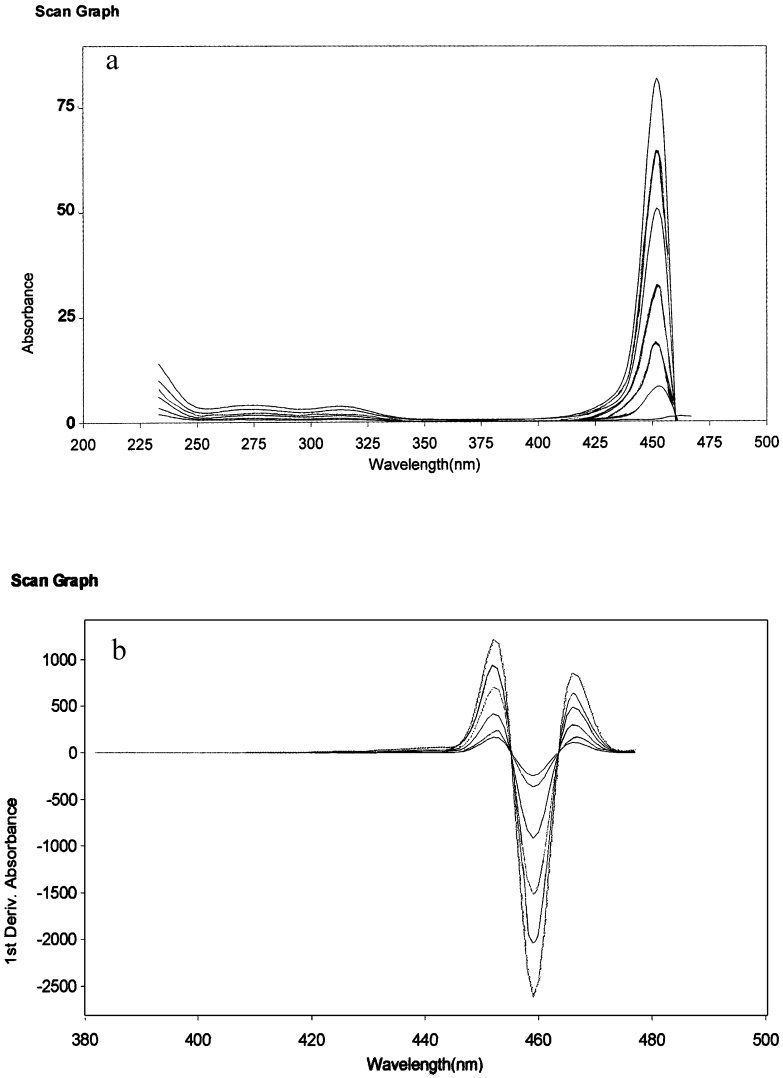

Fig. 3. Ratio Spectra (a) and First Derivative of the Ratio Spectra (b) of Drotaverine (1—10 μg/ml) Using (4 μg/ml) of Nifuroxazide as a Divisor

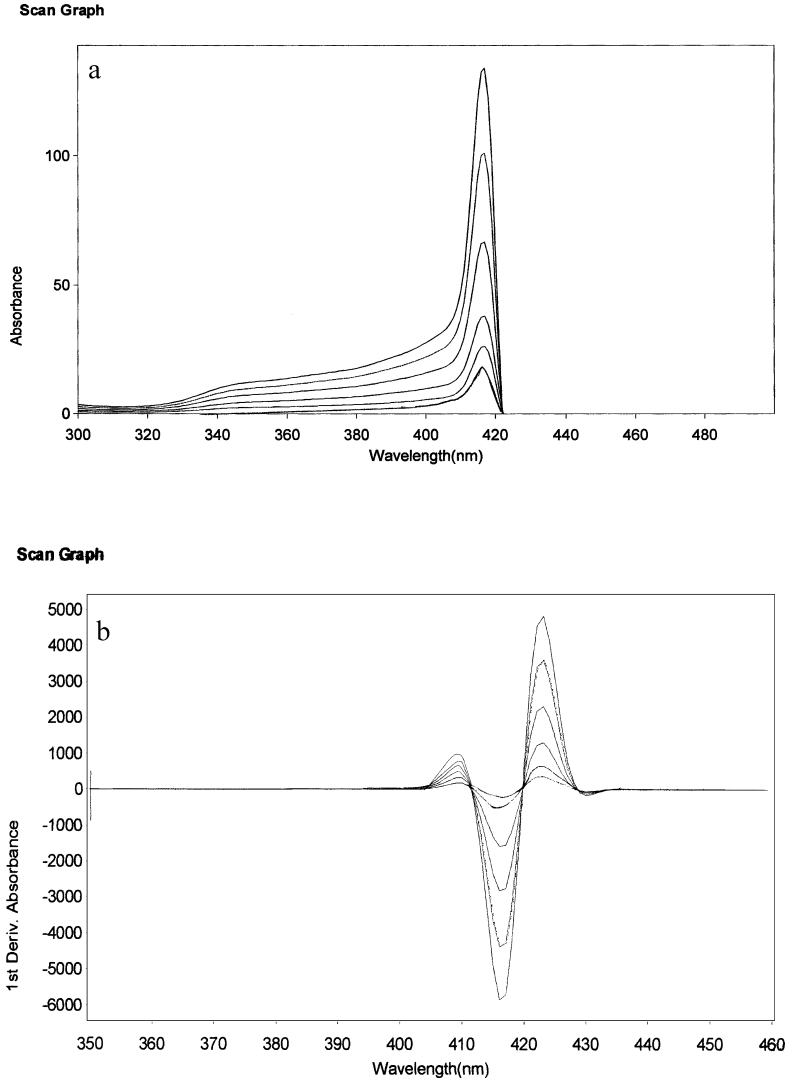

Fig. 4. Ratio Spectra (a) and First Derivative of the Ratio Spectra (b) of Nifuroxazide (2—20 μg/ml) Using (10 μg/ml) Drotaverine as a Divisor

tion ratios in presence of 4-HBH up to 10%. The mean recoveries and the relative standard deviations of DRT and NIF were found as 99.49±1.24% and 99.62±1.25%, respectively, as in Table 2.

Validation of the Methods Accuracy was tested by analyzing nine different mixtures of samples in presence of 4-HBH and calculating the recovery of each drug using the corresponding regression equations Table 2.

Precision of intra- and inter-days assays were estimated by the percent of the relative standard deviations of the proposed methods between 0.60—1.24% and 0.67—1.25% for DRT and NIF, respectively, on the analysis of freshly prepared solution on 3 d.

A good coincidence of the capsules was observed for the assay results by the application of the proposed methods. The ingredients commonly found in commercial pharmaceutical formulation as silicon dioxide and magnesium stearate did not interfere. Statistical data was obtained by using the student's *t*-test and *F*-test and found no significant difference between performance of the proposed and the reported methods[23] as regards to accuracy and precision in Table 4. For further study of the validity of the present methods, the recovery experiments were carried out by analyzing samples of capsules formulation by applying the standard addition technique. The precision of the standard added was confirmed by RSD less than 2% in the range of 0.44—1.17% for the two drugs.

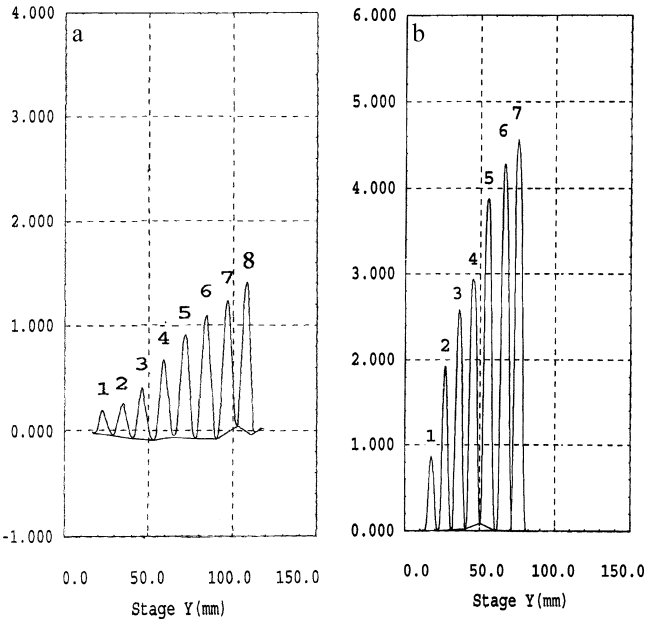

Fig. 5. (a) TLC Scanning Profile of Drotaverine (0.2—4 μg/Spot) at 308 nm and (b) TLC Scanning Profile of Nifuroxazide (0.6—12 μg/Spot) at 287 nm

Conclusion

The main purpose of this study was to develop accurate, precise and economic methods for the simultaneous determination of DRT and NIF. Vierordt's method, derivative, ratio derivative spectrophotmetry, and TLC-densitometric method were applied without using any prior chemical pretreatment in the presence of the strongly overlapping spectra. Accurate results were obtained by utilizing the proposed methods for the quantitation of DRT and NIF and a good agreerment with the results obtained by the reported methods was found. These proposed methods are suitable for the pharmaceutical analysis in quality control laboratories.

References and Notes

1) Martindale, "The Complete Drug Reference," 34th ed., Part 1, Sean C. Sweetman, the Pharmaceutical Press, London, 2005, p. 237, 1683.
2) Wilson and Gisvold's, Arnold R. Martin, "Textbook of Organic Medicinal an Pharmaceutical Chemistry," 10th ed., Chap. 7, Jaime N. Delgado and William A. Remers, Lippincott-Raven, Philadelphia, 1998, p. 202.
3) Geher J., Szabo E., *J. Pharm. Biomed. Anal.*, **6**, 757—764 (1988).
4) Knaub V. A., Kartashov V. A., *Farmatsiya*, **38**, 46—49 (1989).
5) Daabees M. G., *Anal. Lett.*, **33**, 639—656 (2000).
6) Fulop M., Kaloy K., Toth A., *Magy-Kem. Foly.*, **92**, 468—473 (1986).
7) Mezei J., Kuttel S., Szentmiklosi P., Marton P., Racz I., *J. Pharm. Sci.*, **73**, 1489—1491 (1984).
8) Bolaji O. O., Onyeji C. O., Ogungbamila F. O., Ogunbona F. A., Ogunlana E. O., *J. Chromatogr. Biomed. App.*, **133**, 93—97 (1993).
9) Girgis E. H., *J. Pharm. Sci.*, **82**, 503—505 (1993).
10) Lalla J. K., Shah M. U., Jain M. B., Sharma A. H., *J. Pharm. Biomed. Anal.*, **11**, 385—388 (1993).
11) Szuminska E., Cisak A., *Acta Pol. Pharm.*, **47**, 1—2 (1990).
12) Radi A., El-Laban S., Kenawy I. M. M., *Anal. Sci.*, **14**, 607—608 (1998).
13) Radi A., El-Ries M. A., *Anal. Sci.*, **15**, 385—388 (1999).

Table 4. Statistical Comparison between the Assay Results of the Proposed Methods and the Compendial Method in Drotazide Capsules

	Vierordt's method	Derivative spectrophotometry		First derivative of ratio spectra	TLC method	Compendial[c] method[23]
Drotaverine		$2D_{245}$	$3D_{238}$	$1DD_{459}$		
Mean[a]	97.86	98.00	97.96	98.02	97.80	97.94
S.D.	0.87	0.73	0.85	0.79	0.80	0.55
S.E.	0.33	0.28	0.32	0.30	0.30	0.21
Variance	0.76	0.53	0.72	0.62	0.64	0.30
t-test (2.18)[b]	0.20	0.17	0.05	0.22	0.38	
F-test (4.95)[b]	2.53	1.77	2.40	2.07	2.13	
Nifuroxazide		$1D_{399}$	$2D_{411}$	$1DD_{416}$		
Mean[a]	97.80	98.03	97.87	98.23	97.89	98.00
S.D.	0.72	0.31	0.74	0.41	0.83	0.55
S.E.	0.27	0.12	0.28	0.16	0.31	0.21
Variance	0.52	0.10	0.55	0.17	0.69	0.30
t-test (2.18)[b]	0.58	0.12	0.37	0.87	0.29	
F-test (4.95)[b]	1.73	3.00	1.83	1.76	2.30	

a) Mean of $n=7$. *b*) Theoretical *t*-values and *F*-ratios at $p=0.05$. *c*) Spectrophotometric method by measuring DRT in chloroform at 360 nm and NIF in 0.1 M sodium hydroxide at 450 nm against their standards.

14) Broquaire M., Guinebault P. R., *J. Liq. Chromatogr.*, **4**, 2039—2061 (1981).
15) Gillam and Stern's, "Absorption Spectroscopy in Organic Chemistry," published by Edward Arnold LTD, London, 1970, pp. 212—219.
16) Erk N., Onur F., *Anal. Lett.*, **30**, 1503—1515 (1997).
17) Dinc E., *J. Pharm. Biomed. Anal.*, **21**, 723—730 (1999).
18) Berzas Nevado J. J., Rodriguez Flores J., Villasenor Llerena M. J., *Anal. Lett.*, **27**, 1009—1029 (1994).
19) Salinas F., Berzas Nevado J. J., Espinosa Mansilla A., *Talanta*, **37**, 347—351 (1990).
20) Erk N., *J. Pharm. Biomed. Anal.*, **20**, 155—167 (1999).
21) Xie H.-Z., Dong C., Fen Y.-L., Liu C.-S., *Anal. Lett.*, **30**, 79—90 (1997).
22) Bebawy L. I., El-Kousy N. M., *J. Pharm. Biomed. Anal.*, **20**, 663—670 (1999).
23) The reported method (Eva Pharm Co., Egypt), non-aqueous titration for drotaverine HCl and spectrophotometry for Drotazide capsule, by personal communication.
24) "British Pharmacopoeia," Vol. II, Her Majesty's Stationery Office, London, 2003, p. 1321.
25) "European Pharmacopoeia," 5th ed., Vol. 2, Council of Europe Strabourg, France, 2004, p. 2099.