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Spectrophotometric and spectrodensitometric determination of paracetamol and drotaverine HCl in combination

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Abstract

Thin-layer chromatography, first derivative, ratio spectra derivative spectrophotometry and Vierordt's method have been developed for the simultaneous determination of paracetamol and drotaverine HCl. TLC densitometric method depends on the difference in Rf values using ethyl acetate:methanol:ammonia (100:1:5 v/v/v) as a mobile phase. The spots of the two drugs were scanned at 249 and 308 nm over concentration ranges of 60–1200 μg/ml and 20–400 μg/ml with mean percentage recovery 100.11% ± 1.91 and 100.15% ± 1.87, respectively. The first derivative spectrophotometric method deals with the measurements at zero-crossing points 259 and 325 nm with mean percentage recovery 99.25% ± 1.08 and 99.45% ± 1.14, respectively. The ratio spectra first derivative technique was used at 246 and 305 nm with mean percentage recovery 99.75% ± 1.93 and 99.08% ± 1.22, respectively. Beer's law for first derivative and ratio spectra derivative methods was obeyed in the concentration range 0.8–12.8 and 0.4–6.4 μg/ml of paracetamol and drotaverine HCl, respectively. Vierordt's method was applied to over come the overlapping of paracetamol and drotaverine HCl in zero-order spectra in concentration range 2–26 and 2–40 μg/ml respectively. The suggested methods were successfully applied for the analysis of the two drugs in laboratory prepared mixtures and their pharmaceutical formulation. The validity of the methods was assessed by applying the standard addition technique. The obtained results were statistically agreed with those obtained by the reported method.

Keywords: Simultaneous determination; TLC densitometry spectrophotometry; Vierordt's method; Paracetamol; Drotaverine HCl

1. Introduction

Paracetamol, (acetaminophen, *N*-acetyl-*P*-aminophenol) is an extensively employed antipyretic analgesic frequently prescribed solely or with other related drugs. Numerous methods have been reported for the simultaneous determination of paracetamol with other active compounds including spectrophotometry [1–5], HPLC [6–8], TLC [9–11], capillary electrophoresis [12], electrochemical [13], flow-injection [14,15], chemiluminescence [16], near infra red [17] and immunological method [18]. Drotaverine HCl, 1-(3,4-diethoxybenzylidene-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline) is used as an antispasmodic combination of drotaverine and paracetamol causes a synergistic effect to each other. Few methods have been reported for quantitative determination of drotaverine HCl alone or in combination with other drugs including spectrophotometry [19–21], electrochemical [22] and HPLC [23–26]. In the literatures no simultaneous determination for paracetamol and drotaverine was published before. In this paper, TLC, first-derivative (1D), ratio spectra derivative spectrophotometry (1DD) and Vierordt's method were reported simultaneous determination of both drugs in synthetic mixtures and a commercial preparation.

2. Experimental

2.1. Apparatus

- Densitometer—Dual wavelength SHIMADZU-flying CS-9301.
- UV lamp—short wavelength 254 nm.
- Thin-layer chromatographic plates precoated with silica gel 60, 10 cm × 10 cm, 0.25 mm thickness, fluorescent at 254 nm (E. Merck, Germany).
- UV/vis Spectrophotometer (Unicom UV 300) Thermo Spectronic, connected to IBM—PC computer loaded with vision 32 software with laser jet HP printer and the data were

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recorded at the following parameters: band width = 1.5 nm; scan speed = intelliscan (nm/min); data interval = normal (nm); smoothing = high.

2.2. Materials and reagents

All chemicals were analytical grade and all solvents were spectroscopic grade.

1. Paracetamol, working standard, was supplied by NODCAR (Cairo, Egypt) and its purity was found to be 99.77 ± 1.12 according to the British pharmacopoeia (2002).
2. Drotaverine HCl, working standard, was kindly supplied by EVA Pharm. Co., Cairo, Egypt, its purity was found to be 99.64 ± 1.46 according to EVA Pharm. Specifications.
3. Anaspasm tablets, EVA pharm. Co., labeled to contain 325 mg paracetamol and 60 mg drotaverine HCl per tablet.
4. Ethyl acetate:methanol:ammonia (10:0.1:0.5 v/v/v) as a mobile phase.
5. Hydrochloric acid, 0.1 M aqueous solution.

2.3. Stock solutions

- Paracetamol (1.2 mg/ml) and drotaverine HCl (0.4 mg/ml) were prepared in methanol for TLC method.
- Paracetamol (0.016 mg/ml) and drotaverine HCl (0.008 mg/ml) were prepared in methanol for 1D and 1DD methods.
- Paracetamol (0.04 mg/ml) and drotaverine HCl (0.02 mg/ml) were prepared by dissolving in 2 ml methanol and the volumes were completed with 0.1 M HCl for Vierordt's method.

All solutions must be freshly prepared.

2.4. Laboratory-prepared mixtures

For TLC densitometric method. Mixed standard solutions of paracetamol (60–1200 μg/ml) and drotaverine HCl (20–400 μg/ml) were prepared in methanol with different ratios.

For derivative and ratio derivative methods. Mixed standard solutions of paracetamol (0.8–12.8 μg/ml) and drotaverine HCl (0.4–6.4 μg/ml) were prepared in methanol with different ratios.*For Vierordt's method*. Mixed standard solutions of paracetamol (2–26 μg/ml) and drotaverine HCl (2–20 μg/ml) were prepared in 0.1 M HCl with different ratios.

2.5. Sample preparation

Ten tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 50 mg of paracetamol and 10 mg drotaverine HCl was extracted with methanol by shaking for 10 min. The solution was filtered into 50 ml volumetric flask and the volume was completed with methanol for TLC method, 1D and 1DD method or with 0.1 M HCl for Vierordt's method.

2.6. Procedures

2.6.1. Calibration for TLC densitometric method

Aliquots of standard solution (1.2 mg/ml) equivalent to (0.6–6 mg) paracetamol and aliquots of standard solution (0.4 mg/ml) equivalent to (0.2–2 mg) drotaverine HCl were transferred into two series of 5 ml volumetric flasks and the volume was completed with methanol. Ten microliters of each solution was applied to TLC plate and the plate developed to 8 cm in a chromatographic jar previously saturated for 30 min with the mobile phase ethyl acetate:methanol:ammonia (10:0.1:0.5 v/v/v). The plates were air-dried and the spots were visualized under UV lamp at 254 nm. The chromatograms were scanned with densitometer at 249 nm for paracetamol and at 308 nm for drotaverine HCl under photo mode (reflection); scan mode (zig-zag) and swing width 10 mm. The calibration curves representing the relation ship between the recorded area under the peak and the corresponding concentrations were plotted and the regression equations were recorded.

2.6.2. Calibration for first-derivative and ratio spectra derivative methods

Aliquots of standard solution (0.016 mg/ml) equivalent to (8–128 mg) paracetamol and aliquots of standard solution (8 mg/ml) equivalent to (4–64 mg) drotaverine HCl were transferred into two series of 10 ml volumetric flasks and the volume was completed with methanol. The first-derivative (1D) curves were recorded for each solution using methanol as a blank. The (1D) values were measured at 259 nm for paracetamol and at 352 nm for drotaverine HCl. The ratio spectra derivative (1DD), the zero-order spectra for paracetamol solutions were divided by the spectrum of drotaverine HCl (0.4 μg/ml) and the spectra of drotaverine HCl solutions were divided by the spectrum of paracetamol (0.8 μg/ml). The first-derivative (1D) of each ratio spectra obtained was recorded and the (1DD) values were measured at 246 nm for paracetamol and at 305 nm for drotaverine HCl. The calibration curves were constructed and the regression equations were recorded.

2.6.3. Vierordt's method

Absorbance for standard solutions of paracetamol (2–26 μg/ml) were measured at 243 nm and absorbance for standard solutions of drotaverine HCl (2–20 μg/ml) were measured at 243 and 354 nm using 0.1 M HCl as a blank. *C* values (molar absorptivity) were calculated for each components at these wavelengths. Then the absorbances of the mixed sample solutions were read and the concentration of paracetamol was calculated using Vierordt's equation.

$$C = \frac{A_1\beta_2 - A_2\beta_1}{\alpha_1\beta_2 - \alpha_2\beta_1} = \frac{A_1(\beta_2/\beta_1) - A_2}{\partial_1(\beta_2/\beta_1) - \alpha_2}$$

$$\frac{\beta_2}{\beta_1} = 0.511$$

Then

$$C = \frac{0.511A_1 - A_2}{0.511\alpha_1 - \alpha_2}$$

where C is the molar concentration of paracetamol, α_1 the molar absorptivity of paracetamol at 243 nm = 10,236, α_2 the molar absorptivity of paracetamol at 354 nm = 0, β_1 the molar absorptivity of drotaverine HCl at 243 nm = 18,285, β_2 the molar absorptivity of drotaverine HCl at 354 nm = 9341.25, A_1 the absorbance of paracetamol at 243 nm and A_2 is the absorbance of drotaverine HCl at 354 nm.

Drotaverine HCl concentration was calculated by reading directly the absorption of the solutions of the mixture in 0.1 M HCl at 354 nm using the recorded regression equations.

3. Results and discussion

3.1. TLC densitometric method

TLC method was used for the simultaneous determination of paracetamol and drotaverine HCl depending on the difference in Rf values. Complete separation was obtained using ethyl acetate:methanol:ammonia (10:0.1:0.5 v/v/v) as a mobile phase. The Rf values of paracetamol and drotaverine HCl were 0.49 ± 0.37 and 0.72 ± 0.01, respectively. The spots were scanned at 249 nm for paracetamol and 308 nm for drotaverine HCl (Fig. 1a and b).

The proposed TLC method is very simple, rapid and use minimal volume of solvents compared with other separation techniques further more, an extremely large numbers of samples can be analyze at the same time without compromising accuracy, the proposed method is suitable for quality control laboratories where economy and time is essential.

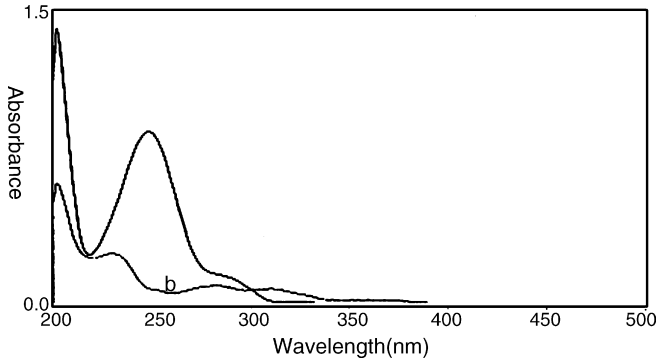

Fig. 2. The absorption spectra of (a) paracetamol (8 μg/ml) and (b) drotaverine Hel (4 μg/ml).

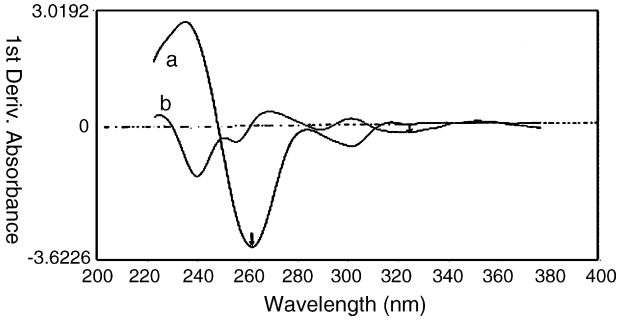

Fig. 3. The first derivative spectra of (a) paracetamol (12.8 μg/ml) and (b) drotaverine Hel (6.4 μg/ml).

3.2. Derivative and ratio spectra derivative method

The zero-order spectra for the two drugs showed marked overlapping (Fig. 2) the (D1) was applied where zero-crossing point at 259 nm for measuring paracetamol (Fig. 3a) and 325 nm for measuring drotaverine HCl (Fig. 3b).

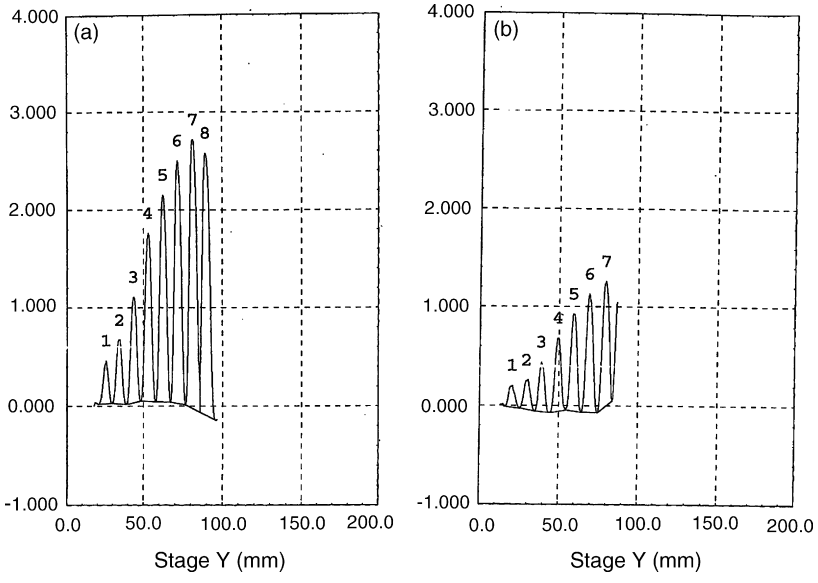

Fig. 1. TLC scanning profile of (a) paracetamol (0.6–12 μg/spot) and (b) drotaverine Hel (0.2–4 μg/spot).

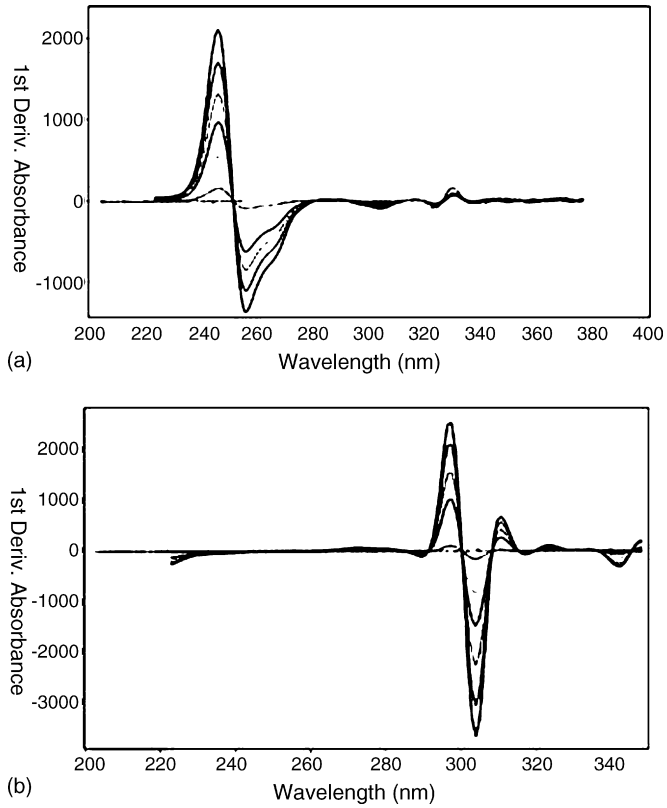

Fig. 4. (a) The first derivative of ratio spectra of paracetamol (0.8–12.8 μg/ml) and (b) the first derivative of ratio spectra of paracetamol (0.4–6.4 μg/ml).

The application of the first derivative of ratio spectra (1DD), paracetamol and drotaverine HCl could be determined at 246 and 305 nm, respectively (Fig. 4a and b).

Careful choice of the divisor and the working wavelength were of great importance so different concentration of paracetamol (0.8, 8, 12.8 μg/ml) and drotaverine HCl (0.4, 4, 6.8 μg/ml) were tried as the divisor, The best one was (0.8 μg/ml) for paracetamol and (0.4 μg/ml) for drotaverine HCl, as it produces minimum noise and gives better results in accordance with selectivity.

3.3. Vierordt's method

As shown in (Fig. 2b); drotaverine HCl in the zero-order spectra can be determined without interference with paracetamol in their mixtures by reading directly, the absorption of the solutions of the mixture in 0.1 M HCl at 354 nm. Application of Vierordt's method for the determination of paracetamol was made in binary mixtures by measuring the absorbance at the selected wavelengths 243 and 354 nm in the zero-order spectra of the mixture in 0.1 M HCl. The analytical parameters, validation results, linearity ranges and regression equations of the proposed methods are shown in (Table 1).

3.4. Quantification, accuracy and precision of the proposed methods

Accuracy was measured by analysis of five concentration within the linearity range of each drug and the recoveries were calculated ± relative standard deviation (Table 1).

Table 1
Analytical parameters and validation results of the simultaneous determination of paracetamol and drotaverine HCl by TLC, 1D, 1DD and Vierordt's methods

Paracetamol	TLC method		1D method		1DD method		Vierordt's—uv method	
	Parameters	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine
Wavelength (nm)	249	308	259	325	246	305	243	354
Linearity range (μg/ml)	60–1200	20–400	0.8–12.8	0.4–6.4	0.8–12.8	0.4–6.4	2–26	2–40
Regression equation								
Slope	11.2900	16.9510	0.2730	0.0379	3.8100	8.1694	0.0677	0.0235
Intercept	9.7821	4.2279	0.1175	0.0070	0.2871	0.9507–	–0.0031	0.0035
Regression coefficient	0.9954	0.9971	0.9983	0.9979	0.9997	0.9993	0.9999	0.9998
Accuracy ± R.S.D.[a]	0.75 ± 99.08	98.94 ± 0.73	98.63 ± 0.34	99.22 ± 0.92	99.56 ± 1.74	98.74 ± 0.69	98.56 ± 0.56	99.36 ± 1.14
Precision ± R.S.D.[b]								
Inter day	98.75 ± 0.62	99.00 ± 0.66	98.78 ± 0.49	99.57 ± 0.91	99.11 ± 0.62	98.95 ± 0.53	98.90 ± 0.52	98.90 ± 0.70
Inter day	98.70 ± 1.22	1.1 ± 099.9	1.3 ± 99.80	99.75 ± 1.2	1.48 ± 99.72	1.38 ± 99.64	0.84 ± 99.31	1.65 ± 99.80
LOD	0.26	0.12	0.54	0.32	0.53	0.07	0.89	0.92
LQD	0.86	0.41	1.8	1.05	1.76	0.22	2.95	3.06

[a] Average of $n = 5$.
[b] Average of $n = 9$.

Table 2
Statistical comparison between the proposed methods and the reported method for the determination of paracetamol and drotaverine HCl in the laboratory prepared mixtures

Items	TLC method		1D method		1DD method		Vierordt's method		Reported method	
	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol
Mean[a]	100.11	100.15	99.25	99.45	99.75	99.08	99.24	99.43	99.77	99.64
S.D	1.91	1.87	1.08	1.14	1.39	1.22	0.61	0.67	1.12	1.46
S.E	0.64	0.62	0.36	0.38	0.46	0.41	0.20	0.30	0.46	0.60
Variance	3.65	3.50	1.17	1.30	1.93	1.49	0.37	0.45	1.25	2.13
t (2.16)[b]	0.43	0.59	0.89	0.33	0.03	0.91	1.06	0.51	–	–
F (4.82)[b]	2.92	1.60	1.07	1.64	1.54	1.43	3.38	4.73	–	–

[a] Average of $n = 9$ for the proposed method and $n = 6$ for the reported method.
[b] Theoretical values of t and F at $P = 0.05$.

Table 3
Statistical comparison between the proposed methods and the reported method for the determination of paracetamol and drotaverine HCl in anaspasm tablets

Items	TLC method		1D method		1DD method		Vierordt's method		Reported method	
	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol	Drotaverine	Paracetamol
Mean[a]	102.56	97.25	102.52	96.93	102.21	97.18	102.51	97.92	103.1	96.95
S.D.	0.72	0.67	0.98	0.78	0.83	1.36	1.25	1.28	0.73	1.47
S.E.	0.32	0.30	0.44	0.35	0.37	0.60	0.56	0.57	0.33	0.66
Variance	0.52	0.45	0.96	0.61	0.69	1.85	1.56	1.64	0.53	2.16
t (2.306)[b]	1.17	0.41	1.05	0.91	1.94	1.04	0.91	1.11	–	–
F (5.19)[b]	1.02	4.80	1.81	3.54	1.30	1.17	2.94	1.32	–	–

[a] Average of $n = 5$ for the proposed method and $n = 5$ for the reported method.
[b] Theoretical values of t and F at $P = 0.05$.

Precision of repeatability and reproducibility (intra day and inter day) were measured for three concentrations of each drug on 3 days and the recoveries ± relative standand deviations were calculated (Table 1).

Selectivity of the proposed methods were checked by analyzing nine laboratory prepared mixtures of paracetamol and drotaverine HCl in different ratios with satisfactory mean recoveries and small relative standard deviations in (Table 2) and shows the statistical comparison of analytical results for samples by the proposed methods and B.P. [25] for paracetamol, non-aqueous titration method for drotaverine HCl and A_{max} at 263 and 280 nm for the mixture, respectively [26].

The proposed methods have been applied to commercial tablets and was no evidence of interference from the excipients and the results obtained were represented in (Table 3) .The validity of the proposed methods were assessed by applying the standard addition technique. Calculated t and F values are less than the theoretical ones at 95% confidence limit indicating that there is no significant difference with respect to accuracy and precision (Table 3).

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