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UV Spectrophotometric Determination of Paracetamol in Presence of Drotaverine Hydrochloride

Ola Mostafa Abdallah¹, Noha Salem Rashed¹, Ahmed El-Olemy², Asmaa Ibrahim Hosam Eldin^{1*}

¹Analytical Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.

²Analytical Chemistry Department, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt.

*Corresponding author: Asmaa Ibrahim Hosam Eldin, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr city, Cairo, Egypt, Tel.: +202-01018468987

E-mail address: drasma13687@yahoo.com

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ABSTRACT

Objective: The aim of this study is to develop three simple, specific and accurate spectrophotometric methods manipulating ratio spectra for the determination of paracetamol in binary mixture with drotaverine hydrochloride in tablet dosage form. **Methods:** In these methods, the absorption spectra of paracetamol were divided by 8 µg/ml of drotaverine hydrochloride to get the ratio spectra. In the first method (ratio difference), the difference in peak amplitudes of the ratio spectra were measured at 262 and 272 nm. The second method is a ratio subtraction which is based on determination of paracetamol at 248 nm after subtraction of interference exerted by drotaverine hydrochloride. In the third method (mean centering), the mean centered values of the ratio spectra were measured at 262 nm. **Results:** The proposed methods were accurate, precise and selective for determination of paracetamol in presence of drotaverine hydrochloride in pure form and in pharmaceutical dosage forms. Beer's law was obeyed in the concentration range of 1–10 µg/ml in all methods. **Conclusion:** The developed methods were used to determine the studied drug in bulk powder, laboratory prepared mixtures and pharmaceutical dosage form with good accuracy and precision. All methods were validated according to ICH guidelines and the results obtained were statistically compared to those obtained from a reported method and were found to be in good agreement.

Key Words: Drotaverine hydrochloride, Mean centering, Paracetamol, Ratio difference, Ratio subtraction, UV Spectrophotometric.

INTRODUCTION

Paracetamol (PAR) is N-(4-hydroxyphenyl) acetamide (Figure 1a). Its molecular weight is 151.16 and its molecular formula is C₈H₉NO₂. It is very slightly soluble in cold water, considerably more soluble in hot water, soluble in methanol, ethanol, dimethylformamide and ethyl acetate. It melts at 169-170.5 °C. It is an analgesic and antipyretic drug with weak anti-inflammatory activity.

Drotaverine hydrochloride (DRH) is 1-[(3,4-diethoxyphenyl)methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (Figure 1b). Its molecular weight is 433.97 and its molecular formula is C₂₄H₃₁NO₄.HCl. It is pale yellow crystals, soluble

ethanol. It melts at 197-200 °C. It is commonly prescribed as antispasmodic agent.¹

There are various publications which describe various analytical methods for determination of PAR either present in single or in combination with other active ingredients in pharmaceutical formulations and in biological fluids. The reported analytical methods are based on the use of spectrophotometry²⁻⁶, spectrofluorimetry⁷⁻¹⁰ and liquid chromatography.¹¹⁻¹⁹

In this work, three different spectrophotometric methods manipulating ratio spectra were developed namely; ratio difference, ratio subtraction and mean centering of ratio spectra methods, aiming for the determination of PAR in presence of DRH without previous separation.

MATERIALS AND METHODS

Apparatus

Shimadzu, UV-Vis 1601PC spectrophotometer (Tokyo, Japan), connected with UV probe program with two matched 1 cm path-length quartz cell.

Samples

Pure samples. Paracetamol pure sample was kindly supplied by Egyptian Co. for Pharmaceutical and Chemical Industries (10th of Ramadan City), Cairo, Egypt. Its purity was assigned as 99.92% as referred by the supplier.

Drotaverine hydrochloride pure sample was kindly supplied by Alexandria Pharmaceutical Company, Egypt. The purity was assigned as 99.95% as referred by the supplier.

Pharmaceutical preparation. Dropar tablets[®], each tablet claimed to contain 500 mg of paracetamol and 80 mg of drotaverine hydrochloride, manufactured by Fair Deal Corporation (FDC) company, Mumbai, Maharashtra, India), purchased from local market.

Chemicals and reagents

Methanol (AR-Grade; Sigma-Aldrich).

Standard solutions

Stock solutions of both drugs (1mg/ml) were prepared separately by dissolving 100 mg of PAR and DRH in 25 ml of methanol and the volumes of each were completed to 100 ml with the same solvent.

Working standard solutions were prepared by further dilution of their stock solutions with methanol.

Methods

Construction of calibration graphs

Aliquots of standard PAR solution (100 µg/ml) equivalent to (1-10 µg) were introduced into a series of 10-ml volumetric flasks then completed to volume with methanol. The absorption spectra of the prepared solutions were scanned from 200-400 nm using methanol as a blank and saved in the computer. The retrieved spectra of PAR were divided by that of DRH (8 µg/ml) to get the ratio spectra.

Ratio difference method: The difference in the peak amplitudes of the ratio spectra was measured at 262 and 272 nm. The measured difference values in peak amplitudes were plotted versus the final concentrations of PAR to get the calibration graph and the regression equation was derived.

Ratio subtraction method: The constant (amplitude value at 360 nm in the ratio spectra) was subtracted from each ratio spectrum, followed by multiplication of the

obtained spectra by the divisor spectrum. The amplitude values were measured at 248 nm in the final spectra. The measured amplitudes were plotted versus the final concentrations of PAR to get the calibration graph and the regression equation was derived.

Mean centering of ratio spectra method: The ratio spectra (from 200 to 300 nm) were mean centered and the mean centered values were measured at 262 nm. The measured mean centered values were plotted versus the final concentrations of PAR to get the calibration graph and the regression equation was derived.

Assay of laboratory prepared mixtures

Aliquots of standard PAR solution (100 µg/ml) and standard DRH solution (100 µg/ml), in the specified range, were introduced into a series of 10-ml volumetric flasks and diluted to volume with methanol. Procedure for each method was applied and the concentrations of PAR in the prepared mixtures were determined from the corresponding regression equation for each method.

Application to pharmaceutical preparation

The contents of five Dropar tablet[®], each labeled to contain 500 mg of PAR and 80 mg of DRH, were weighed, powdered and mixed well. An accurately weighed quantity of the powder equivalent to one tablet was introduced into a 100-ml volumetric flask and volume was adjusted up to the mark with methanol. The flask was sonicated for 15 minutes with vigorous shaking then filtered. The clear filtrate was claimed to contain 5mg/ml of PAR and 0.8 mg/ml of DRH. One ml of the clear filtrate was transferred to 100-ml volumetric flask and diluted to volume with methanol to obtain a solution labeled to contain 50 µg/ml of PAR and 8 µg/ml of DRH. Procedure for each method was repeated using aliquots covering the working concentration range. PAR concentrations were calculated from the corresponding regression equation for each method.

RESULTS AND DISCUSSION

Spectral characteristics and methods development

Zero-order absorption spectra of PAR and DRH (Figure 2), show severe overlap, which does not permit direct determination of PAR in presence of DRH.

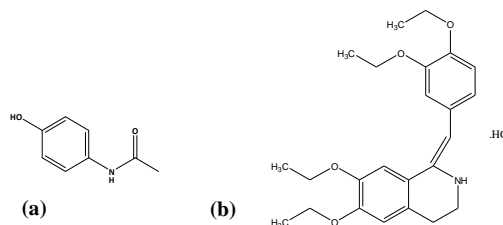


Figure 1. Structural formula of PAR (a) and DRH (b).

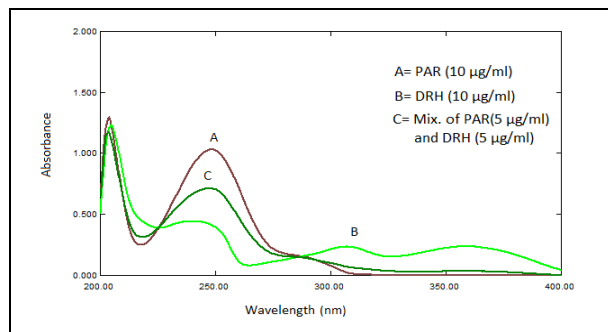


Figure 2. Absorption spectra of PAR, DRH and their mixture in methanol.

Ratio difference method²⁰⁻²²

In this method, absorption spectra of PAR were divided by that of DRH (8 µg/ml), as a divisor, to get the ratio spectra, as shown in Figure 3. The difference in peak amplitudes between 262 and 272 nm in the ratio spectra is proportional to the concentration of PAR without interference from DRH.

The method comprises two critical steps; the first is the choice of the divisor concentration and the selected divisor concentration should compromise between minimal noise and maximum sensitivity. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and good linearity is present at each wavelength individually.

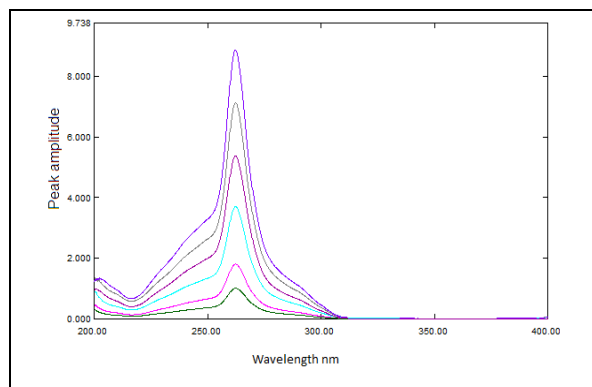


Figure 3. Ratio spectra of PAR at various concentrations (1,2,4,6,8,10 µg/ml) using 8 µg/ml of DRH as a divisor.

Ratio subtraction method²²⁻²³

In this method, the absorption spectra of binary mixtures of PAR and DRH were divided by that of DRH (8 µg/ml) to get the ratio spectra, as shown in Figure 4, then subtraction of the absorbance values in plateau region at 360 nm (the constant), as shown in Figure 5, followed by multiplication of the obtained spectra by the spectrum of the divisor, as shown in Figure 6 to get the final spectra. The peak amplitude at 248 nm in the final spectra is proportional to the concentration of PAR without interference from DRH.

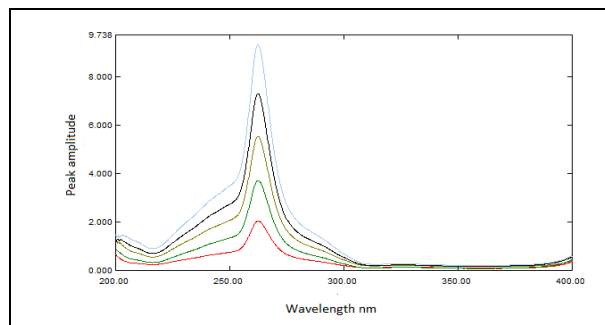


Figure 4. Ratio spectra of laboratory prepared mixtures of PAR and DRH using 8 µg/ml of DRH as a divisor.

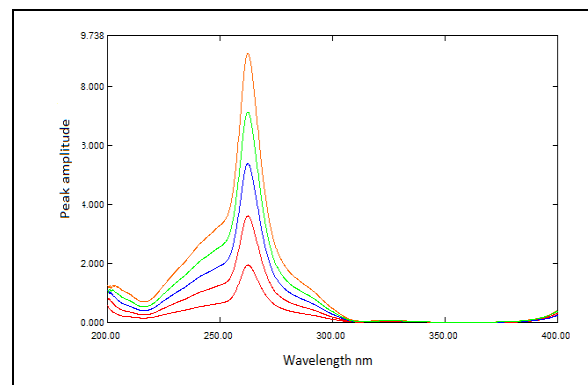


Figure 5. Ratio spectra of laboratory prepared mixtures of PAR and DRH after subtraction of the constant.

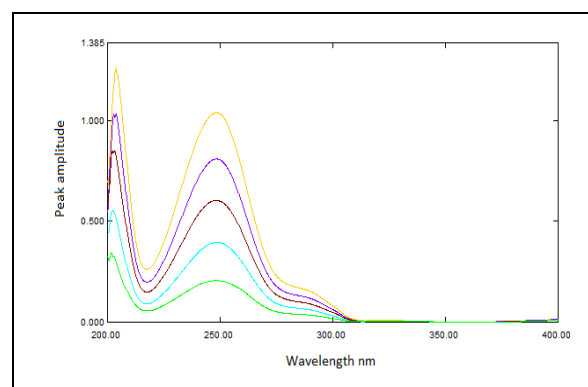


Figure 6. Final spectra of laboratory prepared mixture of PAR and DRH after multiplication by the divisor spectrum.

Mean centering of ratio spectra method²²

In this method, the obtained ratio spectra (from 200 to 300 nm) were mean centered and the mean centered values were measured at 262 nm to allow determination of PAR without interference from DRH.

Method validation²⁴

Linearity and range

Under the described experimental conditions, the calibration graphs for the methods were constructed

by plotting the response versus PAR concentrations in µg/ml. The regression plots were found to be linear over the range of 1-10 µg/ml for the three proposed methods as shown in Figures 8-10. Linearity ranges, intercepts, slopes and coefficients of determination for the calibration data were listed in Table 1.

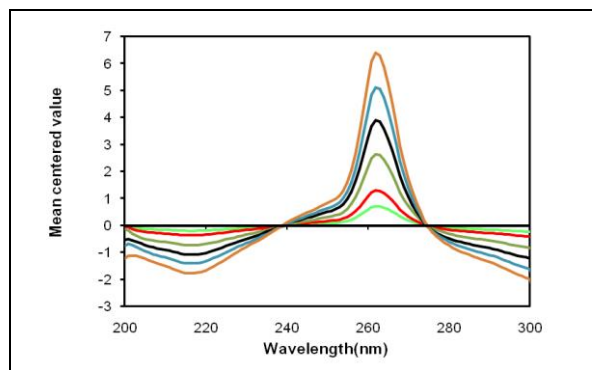


Figure 7. Mean centering of the ratio spectra of PAR at various concentrations (1, 2, 4, 6, 8 and 10 µg/ml) using 8 µg/ml of DRH as a divisor.

Limits of detection (LOD) and limits of quantification (LOQ)

LOD and LOQ were calculated according to ICH guidelines from the following equations:

$$LOD = 3.3 \sigma / S$$

$$LOQ = 10 \sigma / S$$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. The small values of LOD and LOQ, as listed in Table 1 indicate good sensitivity.

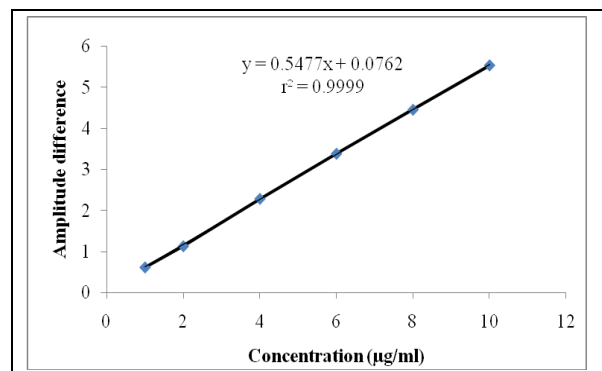


Figure 8. Calibration curve of paracetamol by ratio difference method.

Accuracy

Accuracy of the proposed methods was assessed using three different concentrations of pure samples of PAR covering the linearity range, each in triplicate, then accuracy as percent recovery (%R) was calculated. The good %R, listed in Table 1, confirms excellent accuracy.

Precision

Precision of the proposed methods was assessed using three different concentrations of pure samples of PAR covering the linearity range, each in triplicate, within one day for repeatability and in three successive days for intermediate precision, then precision as percent relative standard deviation (%RSD) was calculated. The small values of %RSD, listed in Table 1, indicate high precision of the methods.

Table 1. Assay parameters and validation data for determination of PAR by the proposed methods

Parameters	Ratio difference	Ratio subtraction	Mean centering
Wavelength (nm)	272-262	248	262
Linearity range (µg/ml)	1-10	1-10	1-10
Regression Equation*			
-Slope (b)	0.548	0.102	0.633
- Intercept (a)	0.076	0.010	0.076
Coefficient of determination (r ²)	0.9999	0.9999	0.9998
LOD (µg/ml)	0.135	0.128	0.148
LOQ (µg/ml)	0.409	0.389	0.447
Accuracy (% recovery)	100.00	101.08	99.79
Precision (%RSD):			
- Repeatability	1.051	1.069	1.565
- Intermediate precision	1.459	0.899	1.151

Precision

Precision of the proposed methods was assessed using three different concentrations of pure samples of PAR covering the linearity range, each in triplicate, within one day for repeatability and in three successive

days for intermediate precision, then precision as percent relative standard deviation (%RSD) was calculated. The small values of %RSD, listed in Table 1, indicate high precision of the methods.

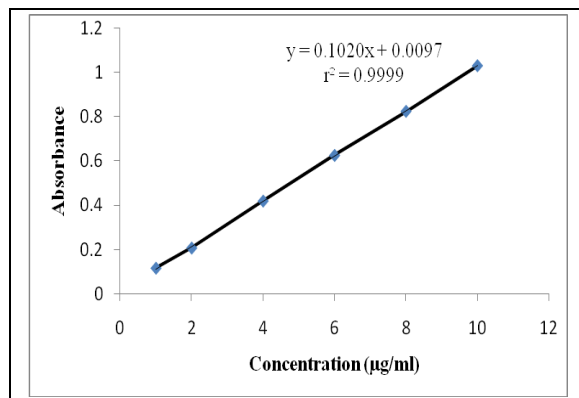


Figure (9): Calibration curve of paracetamol by ratio subtraction method.

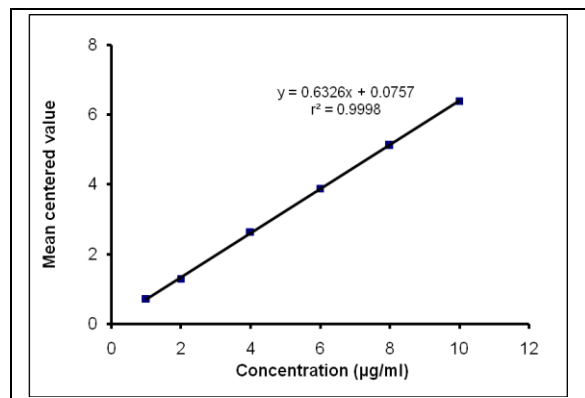


Figure 10. Calibration curve of paracetamol by mean centering method.

Table (2): Determination of PAR in laboratory prepared mixtures by the proposed methods

PAR (µg/ml)	DRH (µg/ml)	% Recovery of PAR		
		Ratio difference	Ratio subtraction	Mean centering
2	0.32	100.55	101.47	101.52
4	0.64	98.27	99.51	100.22
6	0.96	98.97	97.22	100.39
8	1.28	99.48	98.04	100.47
10	1.6	101.93	100.88	101.93
5	5	101.24	101.57	98.17
5	10	98.25	99.06	100.55
10	5	98.12	97.78	98.44
Mean ± %RSD		99.60±1.480	99.44±1.722	100.21±1.317

Selectivity

Selectivity of the proposed methods was assured by analyzing laboratory prepared mixtures of PAR and DRH at different ratios within the linearity range. Good recoveries of PAR indicate high selectivity of the proposed methods for determination of PAR in binary mixture with DRH as shown in Table 2.

Application to pharmaceutical preparation

The proposed methods were successfully applied for determination of PAR in Dropar® tablets

without interference from DRH or the additives present. Standard addition technique was applied and the good recoveries of the pure added amounts of PAR, indicates no matrix interference, as shown in Table 3. Statistical analysis of the results obtained by the proposed methods and the reported method² by applying t-test and F-test at 95% confidence level²⁵, revealed no significant difference between the proposed and reported methods, as shown in Table 4.

Table 3. Recovery study of PAR by standard addition technique using the proposed methods

Dropar®tablets (µg/ml)	Pure added (µg/ml)	% Recovery of pure added		
		Ratio difference	Ratio subtraction	Mean centering
2	2	98.00	97.57	99.35
	4	101.93	99.78	100.65
	6	100.48	99.03	99.68
	8	99.96	99.39	99.85
Mean		100.11	98.95	99.88
%RSD		1.404	0.845	0.553

Table 4. Determination of PAR in Dropar® tablets by the proposed and reported methods

Parameters	Ratio difference	Ratio subtraction	Mean centering	Reported method[2]
N	5	5	5	5
Mean %	99.95	100.05	100.26	99.64
%RSD	1.259	1.334	1.366	1.169
t	0.401 (2.306)	0.513 (2.306)	0.775 (2.306)	—
F	1.168 (6.388)	1.313 (6.388)	1.382 (6.388)	—

CONCLUSION

The proposed methods are simple, rapid, accurate and precise and can be used for the selective determination of paracetamol in presence of drotaverine hydrochloride in pure form and in pharmaceutical dosage forms.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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