

**Q ABSORBANCE METHOD FOR SIMULTANEOUS DETERMINATION OF
PARACETAMOL AND ORPHENADRINE CITRATE IN THEIR COMBINED
PHARMACEUTICAL DOSAGE FORMS**Mahmoud M. Sebaiy^{1*} and Amr A. Mattar²¹Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt.²Pharmaceutical Medicinal Chemistry Department, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo 11829, Egypt.

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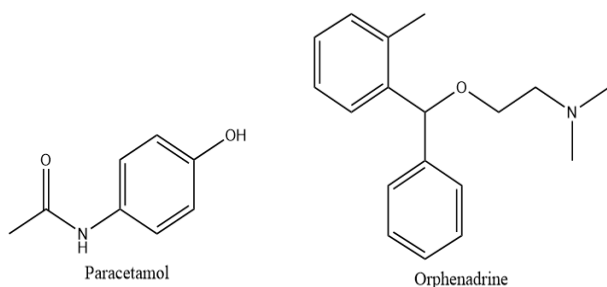
ABSTRACT

A simple, specific, accurate and precise spectrophotometric method was settled for simultaneous determination of paracetamol and orphenadrine citrate in their pure form and in pharmaceutical formulation. Q Absorbance has been used in simultaneous determination of both drugs without prior separation. Q Absorbance method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits. Advantages and disadvantages of Q Absorbance were discussed and statistical comparison between the proposed method and the reference method was performed.

KEYWORDS: Spectrophotometric; Paracetamol; Orphenadrine citrate; Q Absorbance; ICH guidelines.**INTRODUCTION**

Paracetamol (PAR); N-(4-Hydroxyphenyl)acetamide (Fig. 1) is related to a non-steroidal anti-inflammatory drugs (NSAID) which acts centrally and peripherally for treatment of non-inflammatory conditions in patients with gastric symptoms.^[1]

Orphenadrine citrate (ORP); (±)-N,N-Dimethyl-2-[(o-methyl-a-phenylbenzyl)oxy]ethylamine citrate (Fig. 1) is a skeletal muscle relaxant which acts centrally by depressing a specific neurons in the nervous system so that impulses of the somatic nerves can't be generated.^[1] The combination of non-steroidal anti-inflammatory drug and a skeletal muscle relaxant is better than single agents alone.^[2] Orphenadrine citrate can be used in combination with paracetamol as it prolongs and increases its antinociceptive effect.^[1]

**Fig. 1: Chemical structures of paracetamol (PAR) and orphenadrine (ORP).**

The literature revealed that several methods have been carried out for the analysis of PAR and ORP in their mixture form or in their combination with other drugs. PAR & ORP were determined by spectrophotometric methods^[1,3-7], HPLC methods^[8-11], TLC and microemulsion HPLC method^[12], and square wave voltammetric method.^[13]

To the best of our knowledge, there is no reported method for the determination of this drug mixture using Q Absorbance technique. As such, the aim of work is to develop a spectrophotometric method which is accurate, fast and non-complicated for determination of PAR & ORP combination without the interference of their additives or their excipients in pharmaceutical formulations.

EXPERIMENTAL**Apparatus**

JASCO dual beam (Japan) UV-visible spectrophotometer model V-630, connected to an ACER compatible computer with spectra manager II software was used. The spectral slit width is 2 nm at speed up can be increased up to 8000 nm/min. All the measurements have been carried out in 1 cm quartz cell. The wavelength ranges were 200 – 400 nm at room temperature. Also, PASW statistics 18[®] software program was used for statistical analysis.

MATERIALS AND REAGENTS

Pure standards

PAR and ORP were obtained as a gift from Egyptian International Pharmaceutical Industries Co. (EIPICO), located in 10th of Ramadan city, Egypt. Their purity was reported to be 99.50% and 99.70%, respectively.

Pharmaceutical formulations

Orphenadrine plus[®] tablets were obtained from the market (label claim: Orphenadrine citrate 50 mg and Paracetamol 450 mg) manufactured by Alexandria Co., Egypt.

Solvents

HPLC grade Methanol was obtained from LiChrosolv, Merck KGaA, 64271 Darmstadt Germany. All of measurements were carried out by using 90% Methanol (HPLC grade methanol: Distilled water 9:1).

Standard solutions

PAR and ORP stock standard solutions of 1 mg/mL were prepared in 90% methanol. PAR working standard solutions of 40 µg/mL were prepared in 90% methanol while ORP working standard solutions of 50 µg/mL were prepared by dilution from the stock solution with 90% methanol.

Laboratory prepared mixtures

Solutions of different ratios of PAR & ORP were prepared by transferring accurate aliquots from their standard solutions to 10 mL volumetric flasks and then diluting with 90% methanol.

Procedures

Construction of calibration curves

For PAR: Working solutions equivalent to (4-22 µg/mL) were prepared by adding aliquots (1, 1.50, 2, 2.50, 3, 3.50, 4, 4.50, 5, 5.50 mL) of PAR working standard solution (40 µg/mL) to a series of 10 mL volumetric flasks and diluting with 90% methanol.

For ORP: Working solutions equivalent to (5-50 µg/mL) were prepared by adding aliquots (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL) of ORP working standard solution (50 µg/mL) to a series of 10 mL volumetric flasks and diluting with 90% methanol.

The absorption spectra were measured at room temperature over the wavelength (200-400 nm) for all measurements.

For Q Absorbance method

Q Absorbance method uses the ratio of absorbances at two selected wavelengths, one, which is an isoabsorptive point and other being the λ_{\max} of one of the two components. From the overlay spectra of the two drugs, it is evident that PAR and ORP show an isoabsorptive point at 211 nm and the second wavelength used is 248 nm, which is the λ_{\max} of PAR (Fig. 2). The absorbances at 211 nm (isoabsorptive point) and 248 nm (λ_{\max} of

PAR) were measured and absorptivities were calculated. The concentration of two drugs in the mixture can be calculated using following equations:

$$C_x = (Q_m - Q_y) \cdot A_1 / (Q_x - Q_y) \cdot a_{x1}$$

$$C_y = (Q_m - Q_x) \cdot A_1 / (Q_y - Q_x) \cdot a_{y1}$$

Where

$$Q_m = A_2 / A_1$$

$$Q_x = a_{x2} / a_{x1}$$

$$Q_y = a_{y2} / a_{y1}$$

A₂ = Absorbance of Mixture at 248 nm

A₁ = Absorbance of Mixture at 211 nm

a_{x1} = absorptivity of PAR at 211 nm

a_{y1} = absorptivity of ORP at 211 nm

a_{x2} = absorptivity of PAR at 248 nm

a_{y2} = absorptivity of ORP at 248 nm

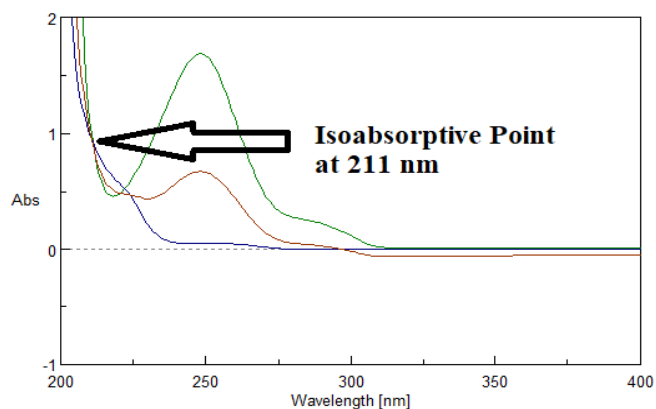


Fig. 2: Zero absorption spectrum of 20 µg/mL OPR overlaid with 20 µg/mL PAR and a mixture of 10 µg/mL ORP & 10 µg/mL PAR revealed that 211 nm is an isoabsorptive point.

Analysis of laboratory prepared mixtures

After preparation of different ratios of laboratory prepared mixtures, the spectra of these mixtures were measured and treated in the same way as described under the proposed methods.

Application to pharmaceutical formulation

10 Tablets of Orphenadrine plus[®] were weighed and crushed then an amount equivalent to 50 mg PAR and 5.55 mg ORP in each tablet was transferred into a 50 mL volumetric flask and diluted with 90% methanol as follow: First, 30 mL of 90% methanol were added and sonicated then dilution was carried out to the mark and filtered. Second, 10 mL of the dilution was transferred into a 100 mL volumetric flask to give a concentration equivalent to 100 µg/mL PAR and 11.11 µg/mL ORP. Third, any further dilutions were done in 10 mL volumetric flasks and treated in the same way as described under the proposed methods.

RESULTS AND DISCUSSION

Method Optimization

Two major problems were found during the analysis of PAR & ORP binary mixture; first, the overlapped spectra between the absorptivities of the drugs, and second, PAR, the major constituent in the dosage forms, had unfortunately high absorbance, while ORP the minor

component in the dosage forms, had low absorbance values (Fig. 3). As such, sample enrichment technique^[14] was used in which the concentration of the minor component ORP in its binary mixture was increased to facilitate its determination. This was done by the addition of fixed amount of standard ORP to each experiment when combined with PAR, then subtracting its concentration before calculating the claimed concentration of the drug. Sample enrichment technique was used to solve the same problem for analyzing other drug mixtures of different drug ratios.^[15,16]

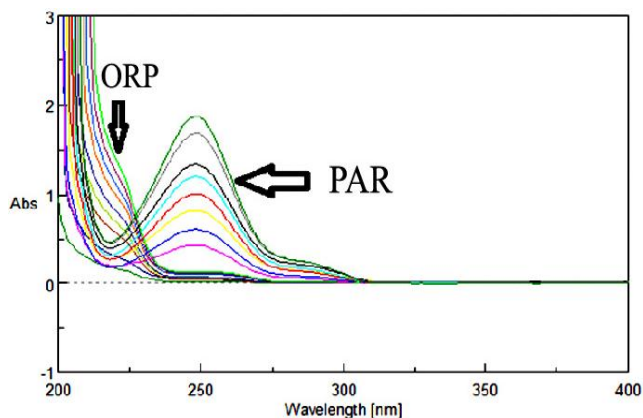


Fig. 3: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP.

Q Absorbance method

211 and 248 nm absorbances were used for determination of PAR & ORP in presence of each other at the same wavelengths. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-22 $\mu\text{g/mL}$ for PAR and 5-50

$\mu\text{g/mL}$ for ORP with correlation coefficients of ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with $100.36\% \pm 1.08$ for PAR and $100.46\% \pm 0.80$ for ORP. The specificity of the methods demonstrated accepted values with $98.89\% \pm 0.92$ for PAR and $99.09\% \pm 0.67$ for ORP. The results are detailed in Table 1.

Q Absorbance is very easy and simple as it depends on zero absorption spectra without the need of extra processing. On the other hand, it has two limitations; which are the need for some specific calculations to determine the values of Q Absorbance in addition to requiring more time for performing the standard addition on each mixture.

Method validation

All methods were validated according to ICH guidelines.^[17] The linear regression data for the calibration curve showed good linear relationship (Table 1). The accuracy was calculated by analyzing the standard addition where satisfactory results were obtained as shown in Table 1.

The specificity of the method was calculated by assaying the laboratory prepared mixtures of PAR & ORP within the linearity range and good results were obtained (Table 1).

The intra- and inter-day precisions were calculated by the analysis of 3 different concentrations of the drugs 3 times on the same day and on 3 successive days (Table 1).

Table 1: Assay parameters and validation results obtained by applying Q Absorbance assay spectrophotometric method.

Mixture	PAR & ORP			
	ORP		PAR	
Method Parameters				
Wave length (nm)	211	248	211	248
Linearity range ($\mu\text{g/mL}$) (n=3)	5-50	5-50	4-22	4-22
Intercept	0.0427	0.0096	0.1526	0.0800
Slope	0.0485	0.0026	0.0528	0.0911
Correlation coefficient (r)	0.9994	0.9994	0.9990	0.9990
Accuracy (Mean \pm SD)	100.46 \pm 0.80	100.46 \pm 0.80	100.36 \pm 1.08	100.36 \pm 1.08
Precision ($\pm\%$RSD)				
Repeatability	101.81 \pm 0.73	101.25 \pm 0.55	101.07 \pm 0.94	99.71 \pm 0.86
Intermediate precision	99.73 \pm 0.88	98.61 \pm 0.29	100.05 \pm 0.92	99.55 \pm 0.99
Specificity (Mean \pm SD)	99.09 \pm 0.67	99.09 \pm 0.67	98.89 \pm 0.92	98.89 \pm 0.92

Application to Pharmaceutical Formulation

The proposed method was successfully applied for determination of PAR and ORP in their pharmaceutical formulation (Orphenadrine plus[®] tablets). The results were acceptable and with sufficient agreement with the labeled amounts. The standard addition technique was

applied and showed that no interference of the excipients was observed (Table 2).

Table 2: Analysis of the pharmaceutical preparation (Orphenadrine Plus® tablets) by applying Q Absorbance assay method.

	Q Absorbance assay							
	ORP				PAR			
			Recovery%				Recovery%	
	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added
0.60	5	98.21	99.12	5.40	5	98.25	101.38	
	5.60	99.87	101.04		5.60	100.90	100.11	
	6	98.40	100.93		6	98.85	99.89	
Mean		98.83	100.36			99.33	100.46	
SD		0.91	1.08			1.39	0.80	

Statistical Analysis

Statistical comparison of the proposed method with a reference one was performed through One-way ANOVA method by using PASW statistics 18® software program

in which there was no significant difference between the proposed method and the reference method[4] as shown in Table 3.

Table 3: Statistical comparison of the results obtained by the proposed method and the reference method using One-way ANOVA.

Tablets	Drugs		Sum of Squares	df	Mean Square	F	Sig.
Orphenadrine Plus® tablets	PAR	Between Groups	.721	1	.721	.337	.593
		Within Groups	8.553	4	2.138		
		Total	9.274	5			
	ORP	Between Groups	2.483	1	2.483	1.451	.295
		Within Groups	6.846	4	1.712		
		Total	9.330	5			

CONCLUSION

Q Absorbance method was successfully applied for the determination of paracetamol and orphenadrine in their binary mixtures and in their dosage form. The proposed method is simple, sensitive and accurate and could be used for routine analysis by using simple technology or instruments. By comparison with the previous reported methods, it was concluded that Q Absorbance method doesn't require extra processing but it needs more time and some calculations. Statistical comparison revealed that there is no observed significant difference between the proposed method and the reference one.

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