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Induced dual wavelength method for determination of paracetamol in its binary mixture with orphenadrine citrate

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Abstract
In this paper, an induced dual wavelength (IDW) spectrophotometric method was used to overcome the limitation reported by the traditional dual wavelength method for determination of paracetamol in its binary mixture with orphenadrine citrate. Dual wavelength method requires same absorbance at two different wavelengths (where the difference is zero), so it can be used for determination of orphenadrine but vice versa is inapplicable. As such, introducing the IDW method for determination of paracetamol in presence of orphenadrine citrate would be valuable. IDW method was validated according to ICH guidelines where accuracy, precision, repeatability and robustness were found to be within the accepted limits. Statistical comparison between the proposed method and the reference method was also performed.

Keywords: induced dual wavelength, spectrophotometric, paracetamol, orphenadrine citrate, 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]. ORP can be used in combination with PAR as it prolongs and increases its antinociceptive effect [1].

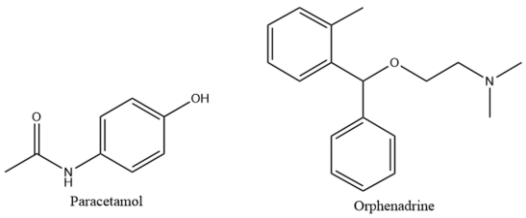

Fig 1: Chemical structures of pracetamol (PAR) and orphenadrine citrate (ORP).

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, induced dual wavelength (IDW) spectrophotometric method will be the first method to overcome the dual wavelength problem for determination of PAR in PAR & ORP mixture. Our previous published Dual wavelength method [7] was able to determine ORP in presence of PAR but couldn't be used for determination of PAR, that is why, IDW method was developed to shed the light on the importance of the mathematics in solving the spectral overlapping of related drugs.

Experimental
Apparatus
JASCO dual beam (Tokyo, Japan) UV-visible spectrophotometer model V-630, connected with an ACER compatible computer with spectra manager II software has been used. The instrument spectral slit width was 2 nm and it could scan at different speeds up to 8000 nm/min. All the measurements have been carried out in 1 cm quartz cell over a wavelength range of 200 – 400 nm at room temperature.

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 Induced dual wavelength (IDW) method

IDW was developed to determine PAR in presence of ORP. Absorbance was measured at 248 and 222 nm. IDW method was applied by calculating the equality factor for pure ORP at two selected wavelengths ($F = [A_{248}/A_{222}] = 0.111$). This factor equalizes the absorbance of the interfering substance (ORP) at the two selected wavelengths, while the absorbance of the component of interest (PAR) will be different. The difference in absorbance of the zero order spectra of the mixture at 248 nm and 222 nm, after multiplication of the later by F (at which the absorbance of ORP will equal zero) can be applied then to determine PAR through using the regression equation (Fig. 2).

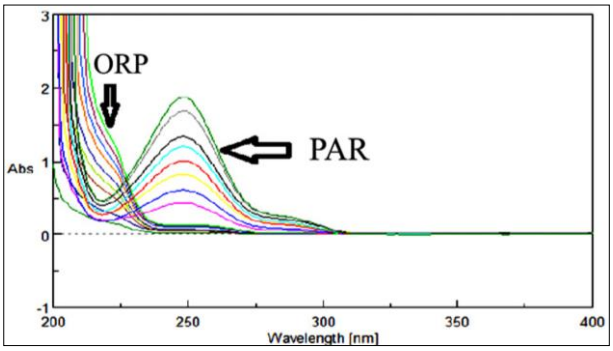

Fig 2: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP demonstrated that All wavelengths of ORP have different absorbances.

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. 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].

For IDW method

Absorbance was measured at 248 and 222 nm where PAR can be determined in presence of ORP. The calibration curves revealed accepted linear relationships between concentrations and the IDW in the range of 4-22 µg/mL for PAR with a correlation coefficient of 0.9991. The accuracy of the method showed accepted values within the limit of 99.67% ± 0.71. Also, the method specificity showed accepted values within the range 99.05% ± 0.84. The results are revealed in Table 1. IDW method is simple, accurate and easy method and can overcome the dual wavelength problem in determination of PAR.

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).

Application to Pharmaceutical Formulation

The proposed method was successfully applied for determination of PAR in its 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 1: Assay parameters and validation results obtained by applying the induced dual wavelength (IDW) spectrophotometric method.

Method Parameters	Determination of PAR in presence of ORP
Wave length (nm)	248-222
Linearity range (µg/mL) (n=3)	4-22
Intercept	0.0722
Slope	0.0881
Correlation coefficient (r)	0.9991
Accuracy (Mean ± SD)	99.67 ± 0.71
Precision (±%RSD)	
Repeatability	100.87 ± 0.42
Intermediate precision	101.02 ± 0.20
Specificity (Mean ± SD)	99.05 ± 0.84

Statistical Analysis

Statistical comparison of the proposed method 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 one [4] as shown in Table 3.

Table 2: Analysis of the pharmaceutical preparation (Orphenadrine Plus® tablets) by applying the induced dual wavelength (IDW) spectrophotometric methods.

	Dual wavelength method			
	PAR			
			Recovery%	
	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added
	0.60	5	98.88	98.87
		5.60	100.31	100.24
		6	100.98	99.89
Mean			100.06	99.67
SD			1.07	0.71

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

Tablets	Drugs		Sum of Squares	df	Mean Square	F	Sig.
Orphenadrine Plus® tablets	PAR	Between Groups	.001	1	.001	.001	.979
		Within Groups	6.993	4	1.748		
		Total	6.994	5			

Conclusion

Induced dual wavelength spectrophotometric method can be used to overcome the dual wavelength method problem for determination of paracetamol in its binary mixture with orphenadrine reported in our previous method. Calculation of the equality factor and the its use in the calculations equalizes different wavelengths to make the difference between them is zero. Statistical comparison showed that there was no significant difference between the proposed method and the reference method in determination of paracetamol in the used mixture.

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