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SPECTRUM SUBTRACTION METHOD FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL AND ORPHENADRINE CITRATE IN THEIR COMBINED PHARMACEUTICAL DOSAGE FORMS

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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 their pharmaceutical formulation. Spectrum subtraction technique has been used in simultaneous determination of both drugs without prior separation. Spectrum subtraction method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits. Advantages and disadvantages of spectrum subtraction technique were discussed and statistical comparison between the proposed method and the reference one was also performed.

Introduction

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

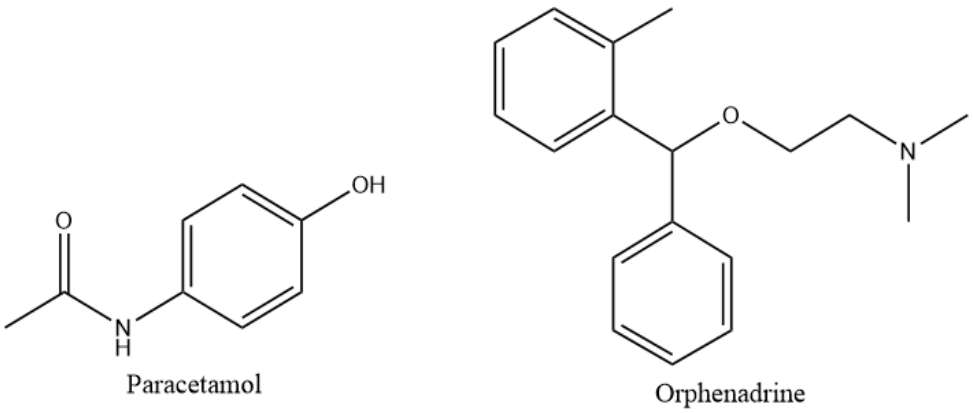

Fig. 1: Chemical structures of Paracetamol (PAR) and Orphenadrine citrate (ORP).

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, there is no reported method for the determination of this drug mixture using spectrum subtraction technique. As such, the aim of this 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 UV-visible spectrophotometer model V-630 (Japan), connected to an ACER compatible computer with spectra manager II software was used. The spectral slit width was 2 nm and it could scan at speed up to 8000 nm/min. All the measurements were carried out in 1 cm quartz cell over 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 Spectrum Subtraction method

The method relies on subtracting the spectrum of Y from the spectrum of the mixture (X + Y), therefore we can obtain the zero absorption spectrum of X again. This can be summarized as the following:

(X + Y) – Y = X

The concentration of X is calculated from the corresponding regression equation obtained by plotting the absorbance values of the zero order absorption spectra of X at its λmax against the corresponding concentrations. Zero absorption spectra of PAR & CAF can be recovered from their mixture through spectrum subtraction of CAF and PAR, respectively (Fig. 2). Zero absorption spectra of CAF and PAR are shown in (Fig. 3).

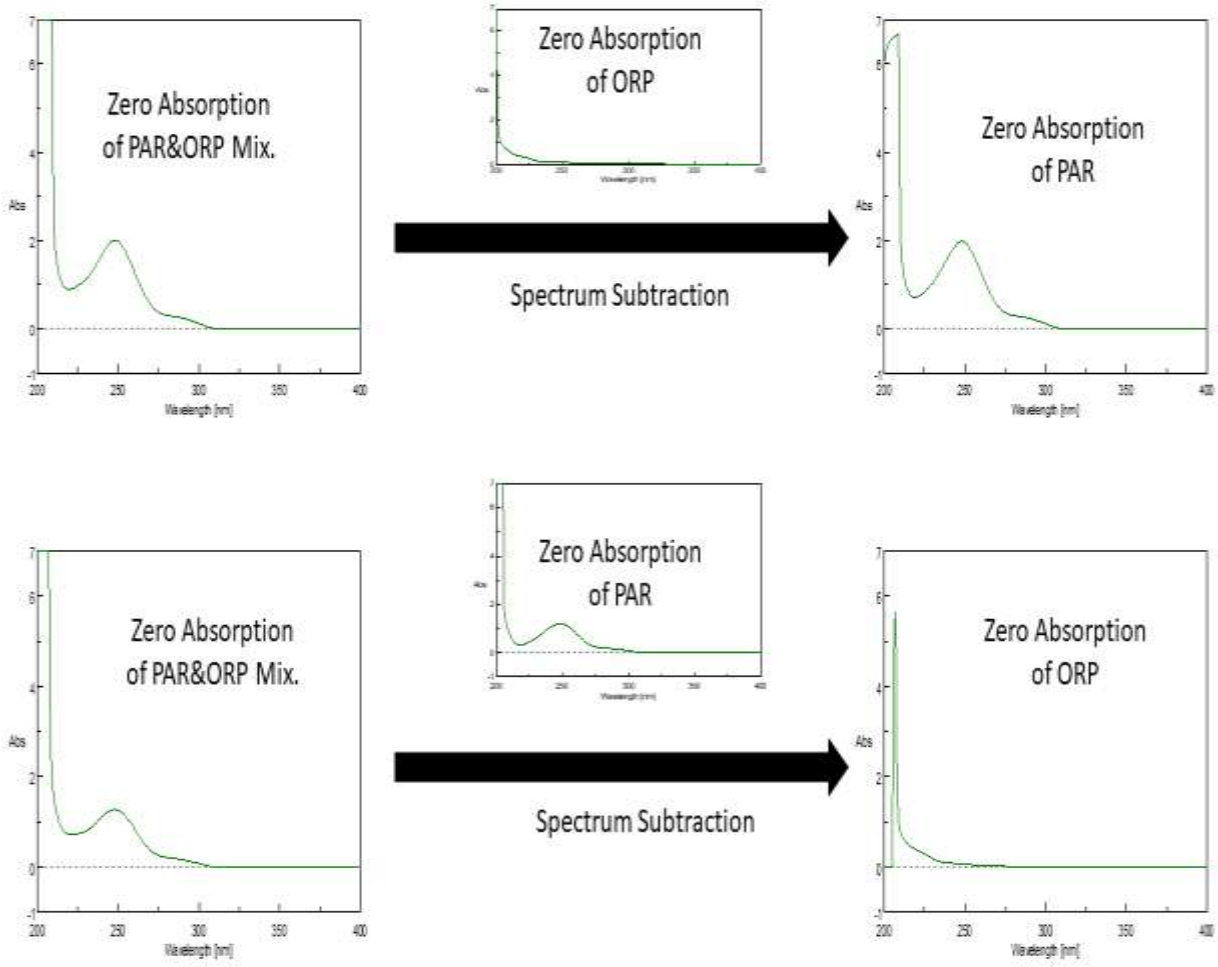

Fig. 2: Spectrum subtraction of ORP & PAR from their mixure resulting in absorption spectra of PAR and ORP, respectively.

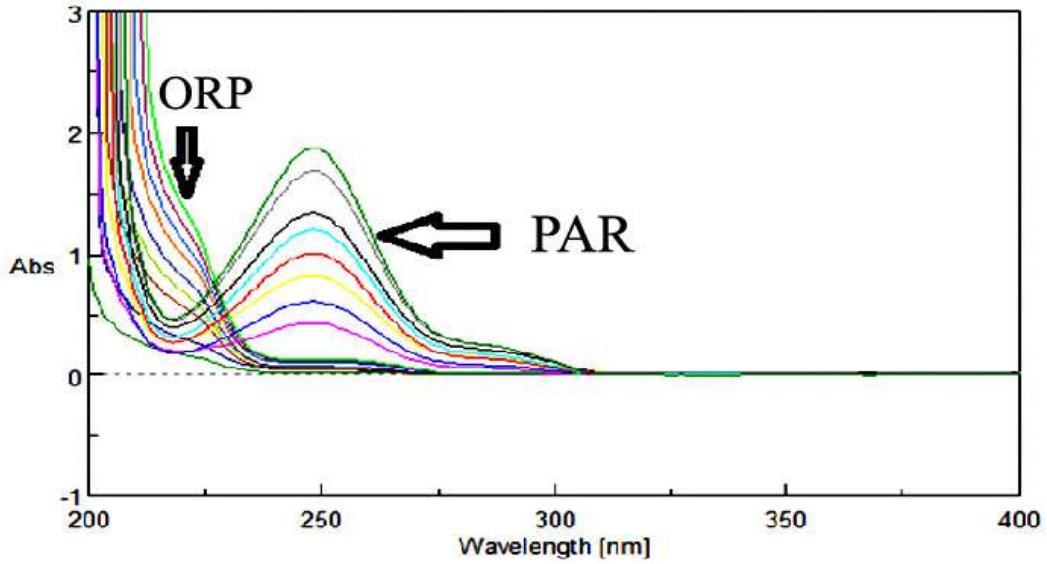

Fig. 3: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP.

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 method.

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 method.

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 before to solve the same problem for analyzing other drug mixtures of different drug ratios [15,16] .

Spectrum Subtraction method

248 and 220 nm absorbances were used for determination of PAR & ORP in presence of each other, respectively. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-22 µg/mL for PAR and 5-50 µg/mL for ORP with correlation coefficients of ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 100.02% ± 0.93 for PAR and 98.25% ± 0.14 for ORP. The specificity of the method demonstrated accepted values with 100.53% ± 1.71 for PAR and 99.63% ± 1.54 for ORP. The results are detailed in Table 1. Spectrum subtraction is very easy and simple as it depends on zero absorption spectra without the need of extra processing. It is having few steps to get the zero order spectra of the desired drug but it suffers from noise interference while acquiring the desired drug concentration by subtraction.

Method validation

The method was 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 Spectrum subtraction method.

Mixture	**PAR & ORP**	
Method Parameters	**ORP**	**PAR**
Wave length (nm)	220	248
Linearity range (µg/mL) (n=3)	5-50	4-22
Intercept	0.0304	0.0800
Slope	0.0274	0.0911
Correlation coefficient (r)	0.9996	0.9990
Accuracy (Mean ± SD)	100.02 ± 0.93	98.25 ± 0.14
Precision (±%RSD)		
Repeatability	98.74 ± 0.57	99.11 ± 0.12
Intermediate precision	99.82 ± 0.15	99.81 ± 0.48
Specificity (Mean ± SD)	99.63 ± 1.54	100.53 ± 1.71

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 proposed method.

	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	99.03	101.08	5.40	5	100.93	98.36
		5.60	100.19	99.60		5.60	101.54	98.10
		6	98.02	99.36		6	101.94	98.30
Mean			99.08	100.02			101.47	98.25
SD			1.09	0.93			0.51	0.14

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 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	3.125	1	3.125	2.400	.196
		Within Groups	5.209	4	1.302		
		Total	8.333	5			
	ORP	Between Groups	1.602	1	1.602	.848	.409
		Within Groups	7.554	4	1.888		
		Total	9.155	5			

Conclusion

Spectrum subtraction method was successfully applied for the determination of paracetamol and orphenadrine citrate 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 spectrum subtraction method is very simple and doesn't require extra processing. Statistical comparison revealed that there was no observed significant difference between the proposed method and the reference one.

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