Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2020.25.004176

Absorbance Subtraction Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in their Combined Pharmaceutical Dosage Forms

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ARTICLE INFO

Received: January 29, 2020

Published: February 04, 2020

Citation: Mahmoud M Sebaiy, Amr A Mattar. Absorbance Subtraction Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in their Combined Pharmaceutical Dosage Forms. Biomed J Sci & Tech Res 25(2)-2020. BJSTR. MS.ID.004176.

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. Absorbance Subtraction technique has been used in simultaneous determination of both drugs without prior separation. Absorbance 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 Absorbance Subtraction were discussed and statistical comparison between the proposed method and the reference one was also performed.

Keywords: Spectrophotometric; Paracetamol; Orphenadrine; Absorbance Subtraction; ICH Guidelines

Abbreviations: NSAID: Non-Steroidal Anti-Inflammatory Drugs; ORP: Orphenadrine Citrate; PAR: Paracetamol

Short Communication

Paracetamol (PAR); N-(4-Hydroxyphenyl)acetamide (Figure 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 (Figure 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].

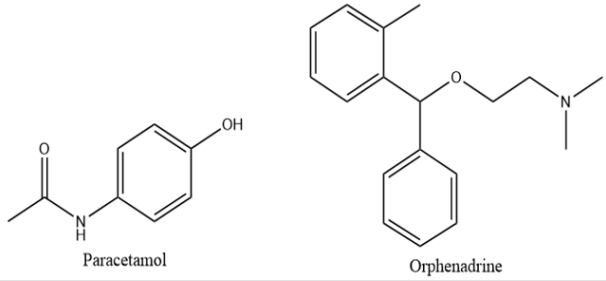

Figure 1: Chemical structures of paracetamol (PAR) and orphenadrine citrate (ORP).

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 Absorbance Subtraction 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 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 2nm and it could scan at speed up to 8000nm/min. All the measurements were carried out in 1 cm quartz cell over wavelength range of 200 - 400nm 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 50mg and Paracetamol 450mg) 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 1mg/mL were prepared in 90% methanol. PAR working standard solutions of 40µg/mL were prepared in 90% methanol while ORP working standard solutions of 50µg/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 10mL volumetric flasks and then diluting with 90% methanol.

Procedures

Construction of Calibration Curves

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

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

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

For Absorbance Subtraction Method

This method is based on the same principles of absorption factor method. If you have a mixture of two drugs X and Y having overlapped spectra intersect at isoabsorptive point (Figure 2) and Y is extended more than X, while X doesn't show any absorbance (A2) at another wavelength (λ2). In this method the isoabsorptive point (λiso) could be used for separate quantitative estimation of each X & Y in their mixture (X+Y). The determination can be done using mathematically calculated factor of one of these components. By simple manipulation step, we can get the absorbance value corresponding to X and Y, separately. So, the concentration of each component could be obtained via the isoabsorptive point regression equation without any need for a complementary method. The absorbance values corresponding to X and Y at λiso were calculated by using absorbance factor {Aiso / A2} which is a constant for pure Y representing the average of the ratio between the absorbance values of different concentrations of pure Y at λiso (Aiso) to those at λ2 (A2).

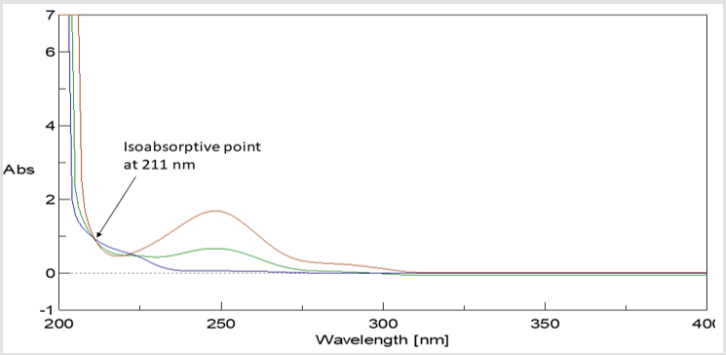

Figure 2: Zero absorption spectrum of 20µg/mL OPR overlaid with 20µg/mL PAR and a mixture of 10µg/mL ORP & 10µg/mL PAR revealed that 211nm is an isoabsorptive point and that ORP has no absorbance at 280nm.

Absorbance of Y in the mixture at λiso = (abs1/ abs2) × abs λ 2 (X+Y)

Absorbance of X in the mixture at λiso = abs λiso (X+Y)-((abs1/ abs2) × abs λ 2 (X+Y))

Where, abs1, abs2 is the absorbance of pure Y at λiso and λ 2; is called the absorbance factor and abs λiso (X + Y) and abs λ 2(X + Y) are the absorbance of the mixture at these wavelengths (λiso, λ 2). The concentration of each X or Y, separately, is calculated using the isoabsorptive point unified regression equation {obtained by plotting the absorbance values of the zero order curves of either X or Y at isoabsorptive point (λiso) against their corresponding concentrations X or Y respectively}.

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 50mg PAR and 5.55 mg ORP in each tablet was transferred into a 50mL volumetric flask and diluted with 90% methanol as follow: First, 30mL of 90% methanol were added and sonicated then dilution was carried out to the mark and filtered. Second, 10mL of the dilution was transferred into a 100mL volumetric flask to give a concentration equivalent to 100µg/mL PAR and 11.11µg/mL ORP. Third, any further dilutions were done in 10mL 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 (Figure 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].

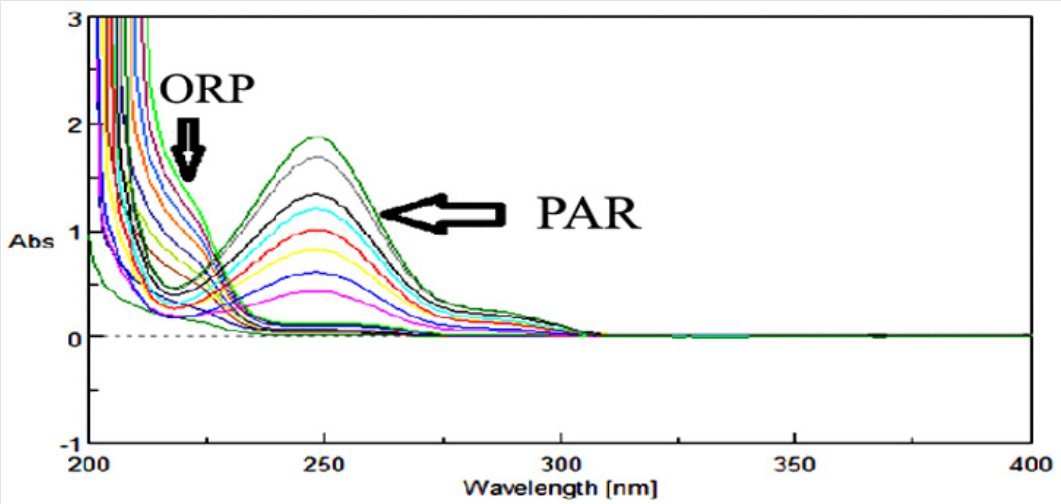

Figure 3: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP.

Absorbance Subtraction Method

211nm absorbances were used for determination of PAR & ORP in presence of each other. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-20µg/mL for PAR and 5-45µg/mL for ORP with correlation coefficients of ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 98.87% ± 0.97 for PAR and 99.47% ± 1.03 for ORP. The specificity of the method demonstrated accepted values with 100.17% ± 1.15 for PAR and 100.86% ± 1.51 for ORP. The results are detailed in Table 1. The only requirements of this method (AS) are the existence of isoabsorptive point of both components and the extension of the spectra of one component. The advantage of the absorbance subtraction method over the conventional isoabsorptive point one is that there is no need for another complementary spectrophotometric method to measure the concentration of one of the two components to get the second by subtraction. The disadvantage of AS method is the increased risk of error in calculating the absorbance factor in case of low concentrations of the extended component PAR or its low value of absorbance at extension region.

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

Method Parameters	ORP	PAR
Wave length (nm)	211	211
Linearity range (µg/mL) (n=3)	5-45	4-20
Intercept	0.0427	0.1963
Slope	0.0485	0.0390
Correlation coefficient (r)	0.9994	0.9994
Accuracy (Mean ± SD)	99.47 ± 1.03	98.87 ± 0.97
Precision (±%RSD)		
Repeatability	101.81 ± 0.73	99.78 ± 1.14
Intermediate precision	99.73 ± 0.88	99.55 ± 0.77
Specificity (Mean ± SD)	100.86 ± 1.51	100.17 ± 1.15

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).

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 Isoabsorptive assay 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	100.62	98.54	5.40	5	102.00	98.04
		5.60	101.64	99.29		5.60	98.81	99.92
		6	101.81	100.57		6	100.19	98.65
Mean			101.36	99.47			100.33	98.87
SD			0.64	1.03			1.60	0.96

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	.141	1	.141	.058	.822
		Within Groups	9.810	4	2.453		
		Total	9.951	5			
	ORP	Between Groups	2.319	1	2.319	1.540	.282
		Within Groups	6.024	4	1.506		
		Total	8.343	5			

Conclusion

Absorbance Subtraction method was successfully applied for the determination of paracetamol and orphenadrine citrate in their binary mixtures and in their dosage form. The advantage of the absorbance subtraction method over the conventional isoabsorptive point is that there is no need for another complementary spectrophotometric method to measure the concentration of one of the two components to get the second by subtraction. The disadvantage of the method is the increased risk of error in calculating the absorbance factor in case of low concentrations of the extended component PAR or its low value of absorbance at extension region. Statistical comparison revealed that there is no observed significant difference between the proposed method and the reference one.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2020.25.004176

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