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).

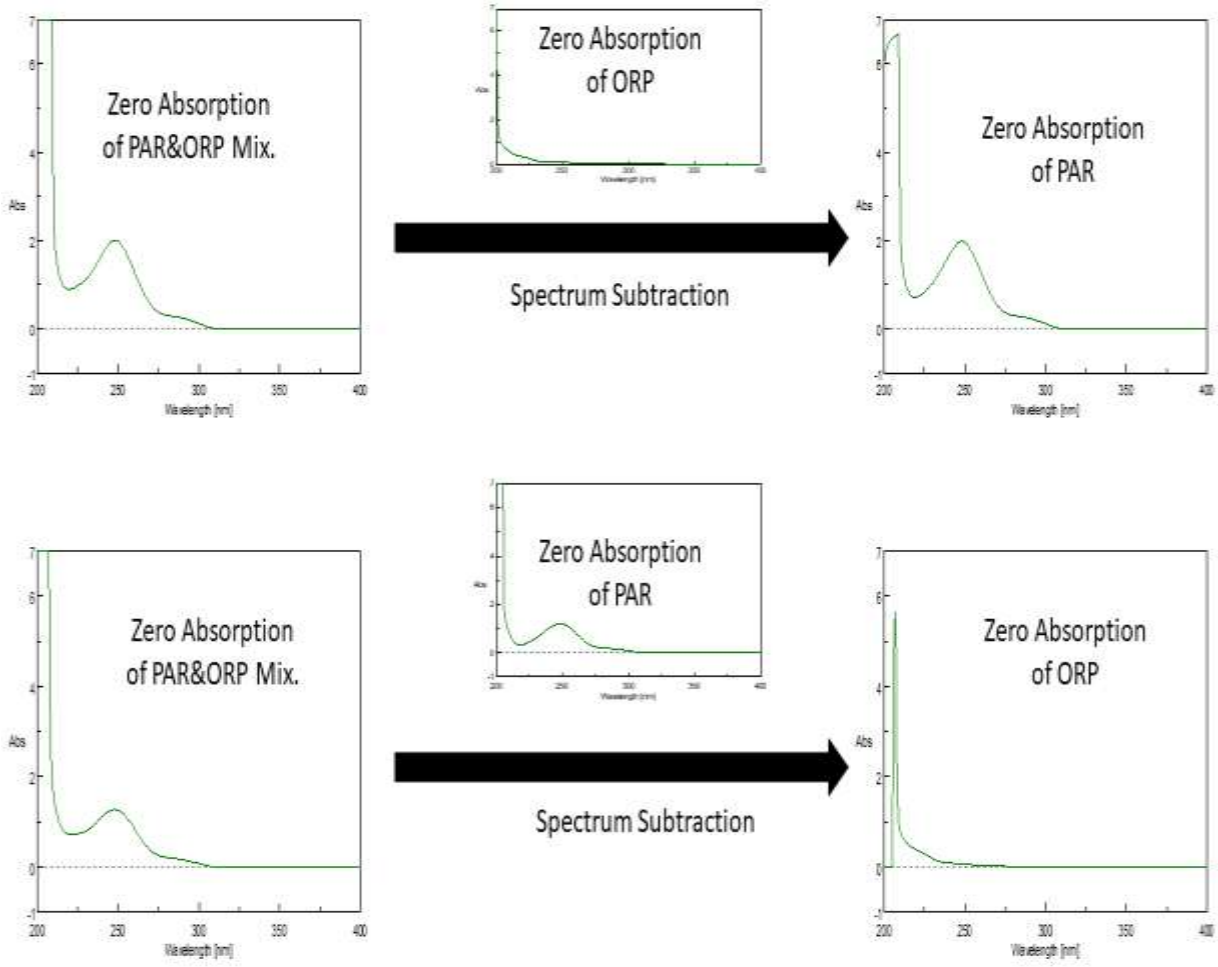

Fig. 2: Spectrum subtraction of ORP & PAR from their mixure resulting in absorption spectra of PAR and ORP, respectively.

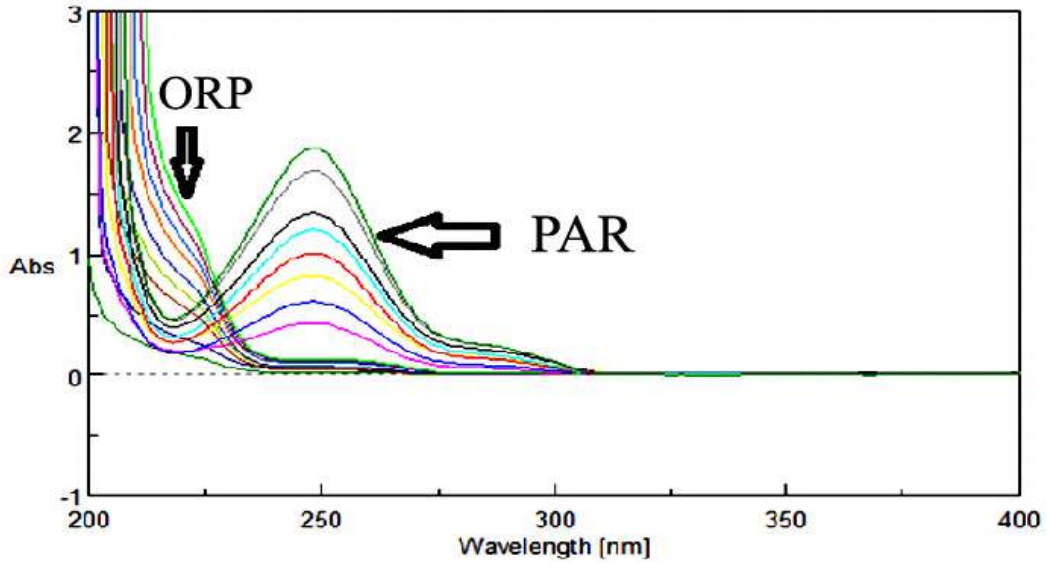

Fig. 3: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP.

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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