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RATIO SUBTRACTION COUPLED WITH EXTENDED RATIO 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 pharmaceutical formulation. Ratio Subtraction coupled with Extended Ratio Subtraction has been used in simultaneous determination of both drugs without prior separation. Ratio Subtraction coupled with Extended Ratio 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 Ratio Subtraction coupled with Extended Ratio Subtraction were discussed and statistical comparison between the proposed method and the reference method was performed.

KEYWORDS: Spectrophotometric; Paracetamol; Orphenadrine citrate; Ratio Subtraction; Extended Ratio Subtraction; 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]

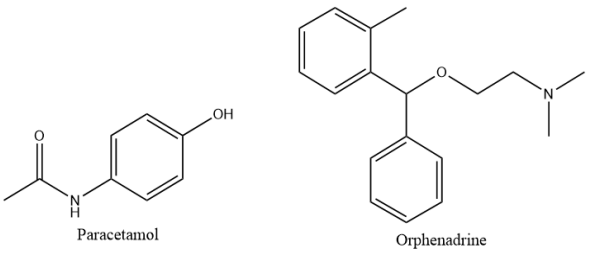

Fig. 1: Chemical structures of paracetamol (PAR) and orphenadrine (ORP).

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 Ratio Subtraction coupled with Extended Ratio 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 (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 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 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 Ratio subtraction method

Scanning of Zero order absorption spectra of the solutions which have been prepared were accomplished in the range of 200-400 nm for PAR & ORP against 90% methanol as blank (Fig. 2). Measurements were carried out at 220 nm for ORP in which the calibration curve was then constructed and the determination of the drug concentration then the regression equations were computed.

For application in mixtures and tablets, this method involve three steps in which 10 ug PAR was used as a divisor then subtraction of the constant value in plateau region (at 300 nm) and lastly multiplication of the resulted spectrum with the same divisor which was used first (10 ug PAR).

For extended ratio subtraction method

Scanning of Zero order absorption spectra of the solutions which have been prepared were accomplished in the range 200-400 nm for PAR & ORP against 90% methanol as blank (Fig. 2). Measurements were carried out at 248 nm for PAR, respectively in which the calibration curve was then constructed and the determination of the drug concentration then the regression equations were computed.

For application in mixtures and tablets, the obtained spectrum of ORP was divided by a known concentration of ORP' (5 µg/ml) as divisor to get the constant (ORP/ORP') in the plateau region (300 nm). The previously scanned zero order absorption spectra of the laboratory prepared mixtures (ORP + PAR) were then divided by the same divisor ORP' then subtraction of the constant from the obtained curve followed by its multiplication with the divisor ORP', the original spectra of PAR were obtained.

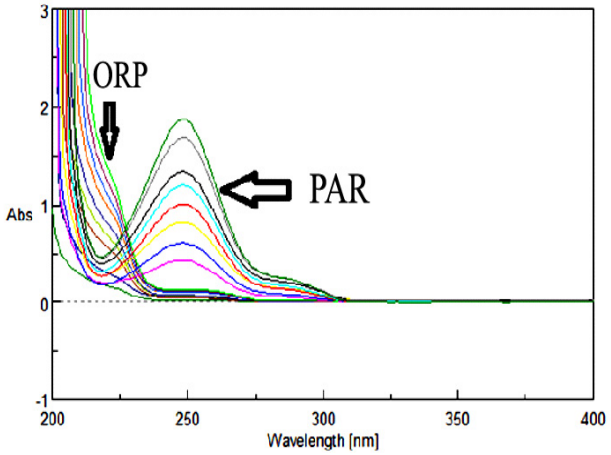

Fig. 2: Zero absorption spectra of PAR overlaid with zero absorption spectra of ORP.

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 100µg/mL PAR and 11.11µg/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 (Fig. 2). 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]

Ratio Subtraction coupled with Extended Ratio Subtraction method

248 and 220 nm 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 4-22 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 100.07% ± 1.22 for PAR and 99.43% ± 0.88 for ORP. The specificity of the method demonstrated accepted values with 99.07% ± 1.25 for PAR and 99.98% ± 1.52 for ORP. The results are detailed in Table 1.

Ratio Subtraction coupled with Extended Ratio Subtraction is an accurate method which depends on Zero regression equation for the determination of both drugs. On the other hand, it has two limitations; which are the need for more processing to determine the values of Ratio Subtraction coupled with Extended Ratio Subtraction in addition to requiring more time for performing this extra processing on each mixture.

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 Ratio Subtraction coupled with Extended Ratio Subtraction spectrophotometric 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)	99.43 ± 0.88	100.07 ± 1.22
Precision (±%RSD)		
Repeatability	98.74 ± 0.57	99.11 ± 0.12
Intermediate precision	99.82 ± 0.15	99.81 ± 0.48
Specificity (Mean ± SD)	99.98 ± 1.52	99.07 ± 1.25

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 Ratio Subtraction coupled with Extended Ratio Subtraction assay method.

	Ratio Subtraction coupled with Extended Ratio Subtraction 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	99.22	100.44	5.40	5	102.07	98.69
		5.60	98.57	99.03		5.60	101.26	101.01
		6	101.17	98.83		6	99.02	100.52
Mean			99.65	99.43			100.78	100.07
SD			1.36	0.88			1.58	1.22

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 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	.859	1	.859	.355	.583
		Within Groups	9.683	4	2.421		
		Total	10.542	5			
	ORP	Between Groups	.317	1	.317	.143	.724
		Within Groups	8.857	4	2.214		
		Total	9.175	5			

CONCLUSION

Ratio Subtraction coupled with Extended Ratio Subtraction 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 method, it was concluded that Ratio Subtraction coupled with Extended Ratio Subtraction method is very accurate but it requires extra processing and more time. Statistical comparison revealed that there is no observed significant difference between the proposed method and the reference one.

REFERENCES

1. Yehia AM, Abd El-Rahman MK. (Application of normalized spectra in resolving a challenging Orphenadrine and Paracetamol binary mixture). *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 2015; 138: 21–30. DOI: 10.1016/j.saa.2014.11.025.
2. F.A. B, R.L. B, S. B. (A clinical and pharmacologic review of skeletal muscle relaxants for musculoskeletal conditions). *American Journal of Therapeutics*, 2005; 12(2): 151–171.
3. SratthaphutT L, Ruangwises N. (Determination of Paracetamol and Orphenadrine Citrate in Pharmaceutical Tablets by Modeling of Spectrophotometric Data Using Partial Least-Squares and Artificial Neural Networks). *Yakugaku Zasshi*, 2007; 127(10): 1723–1729. DOI: 10.1248/yakushi.127.1723.
4. H.S A, B.M G, Raikar PP. (Validated Method for the Simultaneous Estimation of Orphenadrine Citrate and Paracetamol in Tablets by Simultaneous Equation Method). *International Journal of ChemTech Research*, 2018; 11(No. 07): 45–55. DOI: 10.20902/ijctr.2018.110706.
5. Nejem L, Antakli S, Bagdashe H. (Spectrophotometric determination of paracetamol and orphenadrine citrate in tablet). *Asian Journal of Chemistry*, 2013; 25(2): 1079–1082.
6. Sharaf El-Din MK, Abuirjeie MA, Abdel-Hay MH. (Simultaneous Determination of Acetaminophen with Orphenadrine Citrate, Ibuprofen or Chlorzoxazone in Combined Dosage forms by Zero-Crossing Derivative Spectrophotometry). *Analytical Letters*, 1991; 24(12): 2187–2206. DOI: 10.1080/00032719108053044.
7. Sebaiy MM, El-adl SM, Mattar AA. (Different

techniques for overlapped UV spectra resolution of some co-administered drugs with paracetamol in their combined pharmaceutical dosage forms). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020; 224: 117429. DOI: 10.1016/j.saa.2019.117429.

8. Alfeen MA, Elias B, Al-Ahmad Y. (Simultaneous Determination of Orphenadrine Citrate and Paracetamol in Tablets by using RP- HPLC Coupled with UV Detection). *Chemistry and Materials Research*, 2017; 9(6): 28–35.
9. Samson Israel D, Krishna Chaitanya K, Gowri Sankar D, Vijayalakshmi A. (Method development and validation for simultaneous determination a multiple drug dosage form of Paracetamol, Orphenedrine, Ibuprofen by RP-HPLC.). *Journal of Global Trends in Pharmaceutical Sciences*, 2013; 4(3): 1153–1162.
10. Nazir A, Naseer Y, Raza S, Shahid R. (Development & Validation of Analytical Method Used for Simultaneous Determination of Paracetamol, Caffeine and Orphenadrine Citrate By Hplc, in Pharmaceutical). *Science International*, 2016; 28(6): 5215–5218.
11. Sultana N, Arayne MS, Ali SN, Zuberi MH. (Simultaneous Determination of Paracetamol and Orphenadrine Citrate in Dosage Formulations and in Human Serum by RP-HPLC). *Medicinal Chemistry Research*, 2012; 21(9): 2443–2448. DOI: 10.1007/s00044-011-9772-4.
12. Koppala Srinivasarao;, Parsharamulu Rayam; (TLC-spectrodensitometric and microemulsion RP-HPLC chromatographic methods for determination of orphenadrine and paracetamol). *Analytical Chemistry an Indian Journal*, 2013; 13(1): 69–76.
13. Eisele APP, Clausen DN, Tarley CRT, Dall'Antonia LH, Sartori ER. (Simultaneous Square-Wave Voltammetric Determination of Paracetamol, Caffeine and Orphenadrine in Pharmaceutical Formulations Using a Cathodically Pretreated Boron-Doped Diamond Electrode). *Electroanalysis*, 2013; 25(7): 1734–1741. DOI: 10.1002/elan.201300137.
14. Lotfy HM, Tawakkol SM, Fahmy NM, Shehata MA. (Successive spectrophotometric resolution as a novel technique for the analysis of ternary mixtures of pharmaceuticals). *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 2014; 121: 313–323. DOI: 10.1016/j.saa.2013.10.090.
15. Moussa BA, Mahrouse MA, Fawzy MG. (Different resolution techniques for management of overlapped spectra: Application for the determination of novel co-formulated hypoglycemic drugs in their combined pharmaceutical dosage form). *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 2018; 205: 235–242. DOI: 10.1016/j.saa.2018.06.072.
16. Lotfy HM, Mohamed D, Mowaka S. (A comparative study of smart spectrophotometric methods for simultaneous determination of sitagliptin phosphate and metformin hydrochloride in their binary mixture). *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 2015; 149: 441–451. DOI: 10.1016/j.saa.2015.04.076.
17. ICH. (Validation of Analytical Procedure: Text and Methodology Q2 (R1)). *ICH Steering Comittee*, 2005; 1994(October 1994): 13. DOI: http://www.ich.org/fileadmin/Public_ Web_Site/ICH_Products/ Guidelines/Quality /Q2_R1/Step4/Q2_R1__Guideline.pdf.