SIMULTANEOUS DETERMINATION OF PARACETAMOL, ORPHENADRINE CITRATE, AND CAFFEINE TERNARY MIXTURE BY DIFFERENT SPECTROPHOTOMETRIC METHODS **

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The resolving power of spectrophotometric assisted mathematical techniques was demonstrated for the simultaneous determination of the challenging ternary mixture of paracetamol (PAR), orphenadrine citrate (Or.cit) and caffeine (CAF), where PAR, which has the highest absorptivity, is formulated in a very high concentration compared with Or.cit and CAF. Simultaneous area ratio subtraction (SARS), derivative spectrophotometry (D), constant multiplication (CM), and dual wavelength resolution techniques (DW) were able to resolve the three drugs. In SARS, PAR concentration was determined directly at 304 nm in the division spectra using PAR normalized spectrum as a divisor. After subtraction of the constant and multiplying by PAR normalized spectrum; CAF and Or.cit can be determined by first and fourth derivative methods, respectively, from the division spectra. Also, Or.cit and CAF can be obtained by the DW method and CM method, respectively, upon the division of the obtained ratio spectra by CAF spectrum. The proposed methods were validated with good accuracy and precision over the concentration ranges of 20–90, 3–40 and 1–20 μg/mL for PAR, Or.cit, and CAF, respectively. Specificity of the proposed methods was assessed by analyzing laboratory prepared mixtures containing different ratios of the cited drugs. The proposed methods were applied successfully for the determination of PAR, Or.cit, and CAF in their dosage form without any interference.

Keywords: *simultaneous area ratio subtraction method, derivative spectrophotometry, constant multiplication method, dual wavelength technique, paracetamol, orphenadrine citrate, caffeine.*

ОДНОВРЕМЕННОЕ ОПРЕДЕЛЕНИЕ ПАРАЦЕТАМОЛА, ЦИТРАТА ОРФЕНАДРИНА И КОФЕИНА В ТРОЙНОЙ СМЕСИ РАЗЛИЧНЫМИ СПЕКТРОФОТОМЕТРИЧЕСКИМИ МЕТОДАМИ

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Разрешающая способность математических методов обработки спектрофотометрических данных продемонстрирована на примере одновременного определения сложной тройной смеси парацетамола (PAR), орфенадрина цитрата (Or.cit) и кофеина (CAF), в которой PAR, имеющий самую высокую абсорбционную способность, содержится в очень высокой концентрации по сравнению с Or.cit и CAF. Для разрешения указанных трех препаратов использованы метод одновременного вычитания отношения площадей (SARS), производная спектрофотометрия (D), метод умножения на константу (CM) и метод разрешения с двумя длинами волн (DW). В SARS методе концентрация PAR определялась непосредственно на длине волны 304 нм в спектрах деления с использованием нормированного спектра PAR в качестве делителя. После вычитания константы и умножения на нор-

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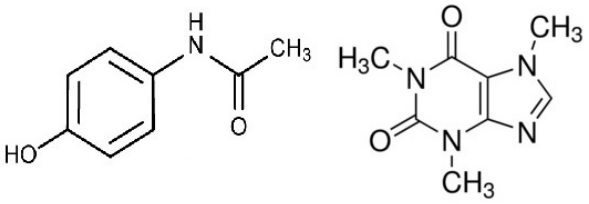
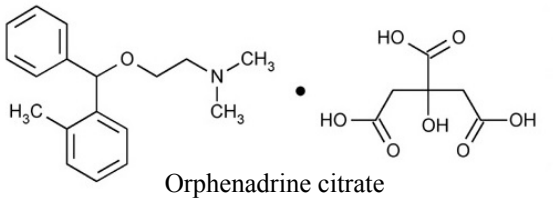
мализованный спектр PAR содержание CAF и Or.cit можно определить из спектров деления методами первой и четвертой производных. Кроме того, Or.cit и CAF могут быть найдены методами DW и CM после деления спектров отношения на спектр CAF. Предложенные методы обладают хорошей точностью и достоверностью в диапазонах концентраций 20–90, 3–40 и 1–20 мкг/мл для PAR, Or.cit и CAF соответственно. Специфичность предлагаемых методов оценена путем анализа лабораторно приготовленных смесей, содержащих различные соотношения препаратов. Методы успешно применены для определения PAR, Or.cit и CAF в лекарственных формах. Взаимовлияния субстанций не выявлено.

Ключевые слова: *метод одновременного вычитания отношения площадей, производная спектрофотометрия, метод умножения на константу, метод двух длин волн, парацетамол, цитрат орфенадрина, кофеин.*

Introduction. Paracetamol (PAR) is chemically *N*-(4-hydroxyphenyl)acetamide [1]. It is used as an analgesic and antipyretic agent [2]. Orphenadrine citrate (Or.cit) is chemically (*RS*)-*N,N*-Dimethyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate [1]. It is a muscarinic antagonist used to relieve pain from muscle spasm [3]. Caffeine (CAF) is chemically 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione [1]:



Paracetamol Caffeine



Orphenadrine citrate

CAF is a stimulant drug and is the most widely used psychoactive drug in the world [4]. PAR, Or.cit, and CAF are combined together in a dosage form that is indicated for the symptomatic relief of mild to moderate pain of acute musculoskeletal disorders.

The combined ternary mixture in the dosage form contains one component in a very high concentration (PAR) when compared with Or.cit and CAF concentrations. It is usually difficult to use spectrophotometric measurements to determine such challenging mixtures particularly when the component of lower concentration has low absorptivity. One HPLC method [5] and one square-wave voltammetric method [6] were reported for the determination of PAR, Or.cit, and CAF in pharmaceutical formulations. However; we found no spectrophotometric methods to determine such a difficult mixture. Generally, HPLC is more specific than spectrophotometry, but spectrophotometric techniques are more simple and rapid. Therefore, the aim of this work was to develop and validate simple, accurate, and precise spectrophotometric methods to determine this challenging ternary mixture of PAR, Or.cit, and CAF.

Experimental. A Shimadzu dual beam (Kyoto, Japan) UV-visible spectrophotometer model UV-1650 PC connected to an hp 1020 laserjet printer was used. UVProbe 2.21 software was used. The spectral bandwidth was 2 nm, and scanning speed was 2800 nm/min with 0.1 nm interval.

Standard PAR and Or.cit (B.P. standards) were kindly supplied by EIPICO, Cairo, Egypt. Standard CAF certified to contain 99.7% was kindly supplied by GlaxoSmithKline, Cairo, Egypt. Muscerol extra® tablets (labeled to contain: 450 mg PAR, 35 mg Or.cit, and 15 mg CAF) manufactured by Pharmaline, Lebanon were purchased. Methanol of spectroscopic grade was purchased from Sigma Aldrich, Cairo, Egypt.

Stock standard solutions of 1 mg/mL were prepared for PAR, Or.cit, and CAF by weighing accurately 50.0 mg of each standard into three separate 50-mL volumetric flasks. The powders were dissolved and di-

luted to the volume with methanol. Working standard solutions of 200, 100, and 50 μg/mL in methanol were prepared for PAR, Or.cit, and CAF, respectively.

Aliquots of PAR, Or.cit, and CAF were transferred from their corresponding standard working solutions into a series of 10-mL volumetric flasks and made to the mark with methanol to prepare different ratios of the three drugs.

The zero-order (^0D) absorption spectra of PAR, Or.cit, and CAF (20, 30, and 10 μg/mL, respectively) were scanned against methanol as a blank.

Construction of calibration curves. Aliquots equivalent to 200–900 μg of PAR, 30–400 μg of Or.cit, and 10–200 μg of CAF was accurately transferred from their corresponding working standard solutions into separate series of 10-mL volumetric flasks and made to the mark with methanol. The zero order absorption spectra (^0D) of the three drugs were recorded against methanol as a blank. Normalized spectra were obtained for each PAR recorded spectrum, and the average of normalized spectra was computed. Division spectra of PAR were obtained by dividing its recorded spectra by the average normalized PAR spectrum.

Simultaneous area ratio subtraction (SARS). The constant plateau amplitudes at 304 nm were measured and plotted against the corresponding concentrations of PAR, and the regression parameters were computed. The constant was mathematically subtracted, and the obtained spectrum was multiplied by the average PAR normalized spectrum (divisor) so that the spectrum of Or.cit and CAF mixture was recovered.

Derivative spectrophotometry (D). For Or.cit, the fourth derivative of the recovered Or.cit and CAF spectra were obtained at $\Delta\lambda = 8$ nm and scaling factor 1000, and the amplitude of the fourth derivative peak was measured at 224.6 nm. For CAF, the first derivative of the recovered Or.cit and CAF spectra was obtained at $\Delta\lambda = 2$ nm and scaling factor 10 and the amplitude of the first derivative peak was measured at 287 nm. A Linear relationship between peak amplitudes of the fourth and first derivative of Or.cit and CAF against the corresponding concentrations of Or.cit and CAF, respectively was constructed and the regression equations were computed.

Constant multiplication (CM). The recovered Or.cit and CAF spectra were divided by the spectrum of 10 μg/mL CAF, and the obtained constant at 287 nm, where there is no contribution from Or.cit, was multiplied by the spectrum of 10 μg/mL CAF (divisor) to obtain the zero-order spectrum of CAF. A linear relationship between peak amplitudes at 272 nm (λ_{max}) against the corresponding concentrations of CAF was constructed, and the regression equation was computed.

Dual wavelength technique (DV). The absorbance difference (ΔA) of the recovered Or.cit and CAF spectra at 226 and 283 nm was calculated for Or.cit determination where the difference in CAF spectrum is zero. A calibration curve was constructed between this absorbance difference ($\Delta A_{226–283}$) against the corresponding concentration of Or.cit, and the regression equation was computed.

The absorption spectra of laboratory prepared mixtures were scanned and stored, and then the procedures were performed as described in "Construction of calibration curves" for the determination of PAR, Or.cit, and CAF.

Ten tablets of Muscerol extra® tablets were weighed and powdered. An accurately weighed amount of the powder equivalent to 450 mg PAR, 35 mg Or.cit, and 15 mg CAF was transferred into a 100-mL volumetric flask, 50 mL of methanol was added as a solvent, and the whole sonicated for 20 min. The volume was made to the mark with the same solvent and then filtered. Half a milliliter of the aliquot of the prepared solution was transferred into a 50-mL volumetric flask and made to the mark with methanol to obtain 45, 3.5, and 1.5 μg/mL PAR, Or.cit, and CAF, respectively.

Results and discussion. PAR, Or.cit, and CAF are formulated in a mixture with a large difference in the cited drug concentrations; the ratio of PAR, Or.cit, and CAF is 30:2.3:1, which makes this mixture one of the challenging mixtures in spectrophotometric determination of the three components. The major analyte (PAR) usually causes deviations from Beer's law during the determination of the minor analytes (Or.cit and CAF). The literature does not contain any spectrophotometric technique for resolving the spectral overlap in this mixture (Fig. 1). The application of normalized spectra is powerful in resolving such a challenging mixture. Normalized spectra are spectra representing the absorptivity of the component over a certain wavelength range, so they can tell us the applicable linear concentration range, at any specified wavelength. Figure 2 shows the normalized spectra of PAR in the concentration range from 20 to 90 μg/mL. The normalized PAR spectra are identical in their absorptivities at most of the wavelengths except in the range of 200–215 and 228–265 nm at all concentrations. The identical parts of spectra provide an excellent range for PAR determination in its high concentrations in a lot of wavelengths neglecting the nonlinear part from 200–215 and 228–265 nm.

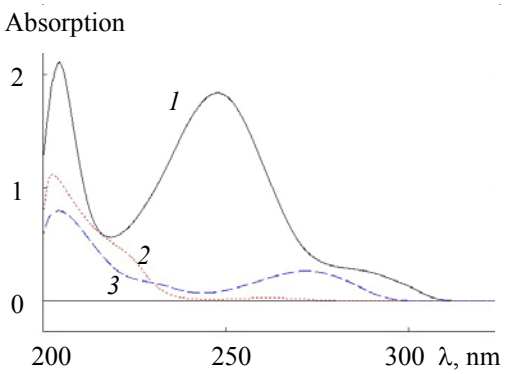


Fig. 1. Zero-order absorption spectra of 20 μg/mL PAR (1), 15 μg/mL Or.cit (2), and 5 μg/mL CAF (3).

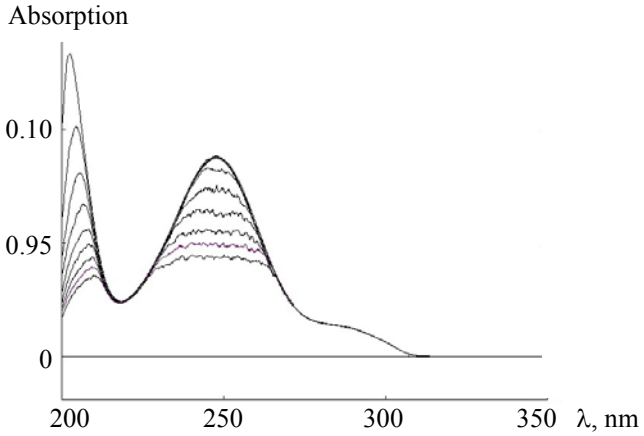


Fig. 2. Normalized spectra of different concentrations of PAR (20–90 μg/mL) in methanol.

Simultaneous area ratio subtraction (SARS) [7]. In the case of the extended spectrum of PAR, it can be directly determined from a calibration curve at the plateau generated by using the average normalized spectrum of PAR as a divisor because now the plateau is directly modulated by PAR concentration (Fig. 3). After that, the constant is mathematically subtracted and the spectrum of PAR was removed by multiplying the obtained spectrum by the divisor, and the rest is the recovered binary mixture of Or.cit and CAF. Figure 3 shows the PAR calibration at 304 nm with zero contribution from Or.cit and CAF. Different laboratory prepared mixtures were analyzed, and the PAR concentration was obtained at 304 nm by direct substitution by the plateau value in the corresponding regression equation (Fig. 4). Upon applying the SARS method, the recovered spectrum was due to a binary mixture of Or.cit and CAF, so different methods can be applied to the binary mixture for determination of both drugs.

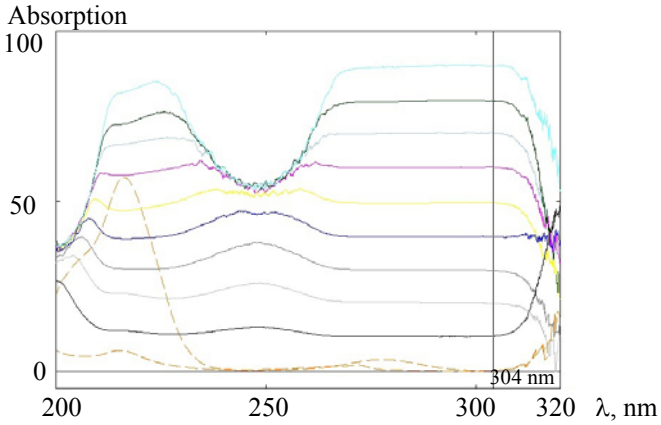


Fig. 3. Division spectra of PAR (20–90 μg/mL) using a normalized PAR spectrum as a divisor and calibration of PAR at 304 nm with zero Or.cit and CAF.

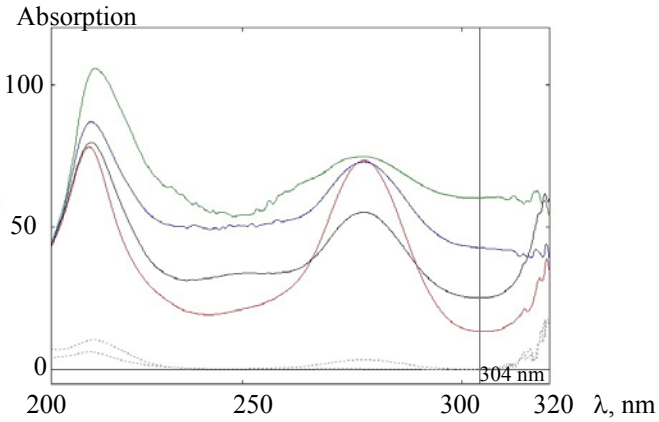


Fig. 4. Division spectra of the different laboratory prepared mixtures using normalized PAR spectrum as a divisor with zero contribution from Or.cit and CAF at 304 nm.

Derivative spectrophotometry (D). Both Or.cit and CAF can be obtained using derivative spectrophotometry. The Or.cit concentration can be determined from the fourth derivative on the recovered spectrum using $\Delta\lambda = 8$ and scaling factor 1000 at 224.6 nm, which corresponds to a zero crossing of CAF (Fig. 5). Different laboratory prepared mixtures were analyzed, and the Or.cit concentration was accurately obtained by substitution in the corresponding regression equation (Fig. 6). The CAF concentration can be determined from the first derivative on the recovered spectrum using $\Delta\lambda = 2$ and scaling factor 10 at 287 nm, which corresponds to zero contribution from Or.cit (Fig. 7). Different laboratory prepared mixtures were analyzed, and the CAF concentration was accurately obtained by substitution in the corresponding regression equation (Fig. 8).

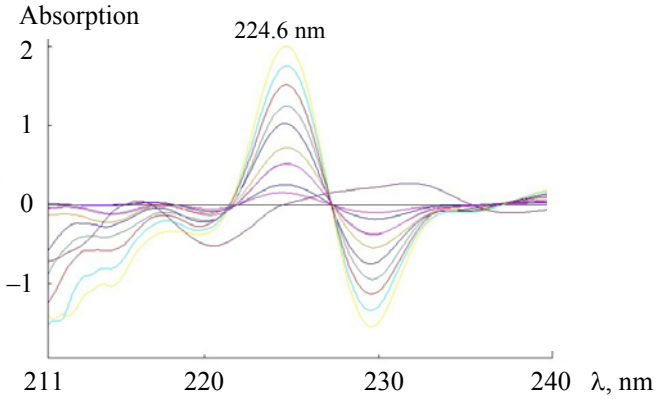


Fig. 5. Fourth derivative calibration of Or.cit (3–40 μg/mL) at 224.6 nm at zero crossing of CAF.

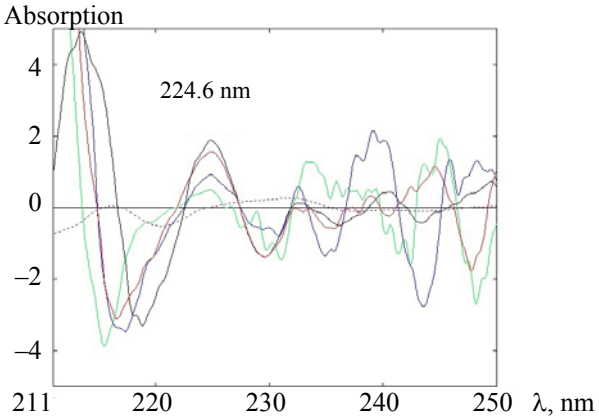


Fig. 6. Fourth derivative of the different recovered binary mixture of Or.cit and CAF at 224.6 nm at zero crossing of CAF.

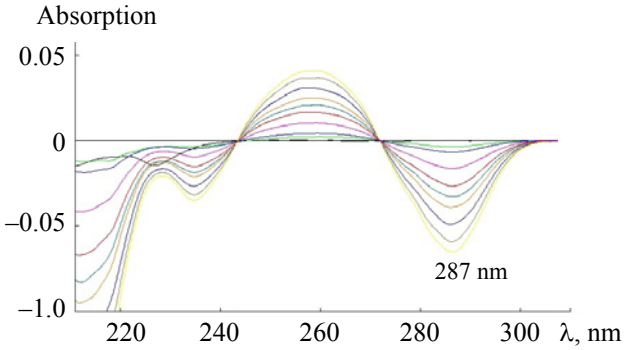


Fig. 7. First derivative calibration of CAF (1–20 μg/mL) at 287 nm at zero contribution from Or.cit.

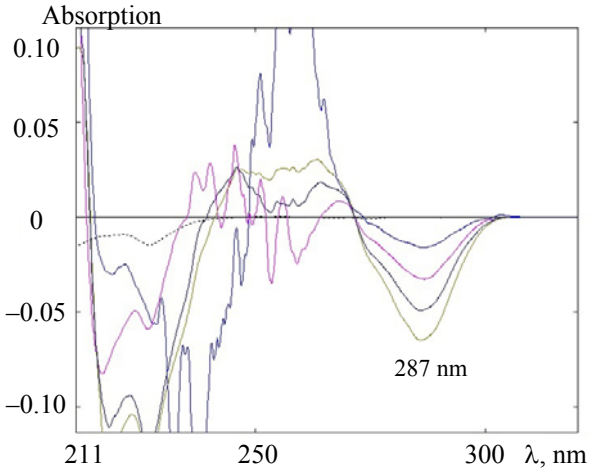


Fig. 8. First derivative of the different recovered binary mixture of Or.cit and CAF at 287 nm at zero contribution from Or.cit.

Constant multiplication (CM). For CAF determination, the spectrum of recovered binary mixture of Or.cit and CAF was divided by the spectrum of 10 μg/mL CAF, resulting in a ratio spectrum with a constant value at 287 nm, which corresponds to the extended part of CAF spectrum in the zero-order spectra (Fig. 9). The constant value was then multiplied by the spectrum of 10 μg/mL CAF "divisor" to recover the zero-order absorption spectrum of CAF. A calibration curve was constructed at CAF λ_{max} = 272 nm, and the CAF concentration was determined from the corresponding regression equation.

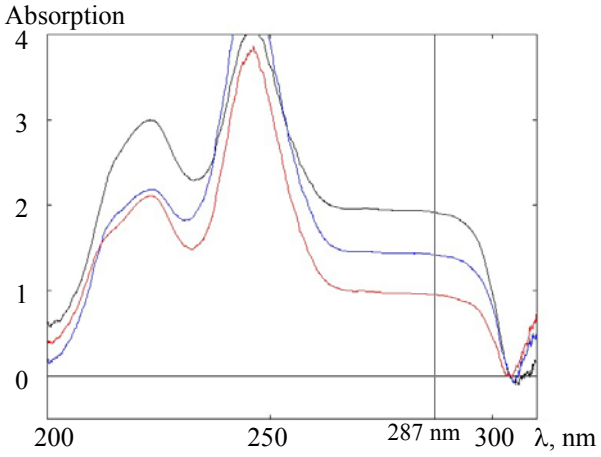


Fig. 9. Division spectra of the different recovered binary mixture of Or.cit and CAF by 10 μg/mL CAF spectrum showing the constant value at 287 nm for CAF determination by constant multiplication method.

Dual wavelength method (DW). For Or.cit determination, the dual wavelength method can be applied by selecting two wavelengths (at 226 and 283 nm), where the absorbance of the interfering analyte (CAF) is equal and there is a difference in the absorbance of Or.cit (Fig. 10). By calculating the absorbance difference (ΔA = 226–283) for the recovered Or.cit and CAF binary mixture spectrum, the ΔA is only related to Or.cit, while CAF is eliminated. The ΔA values are substituted in the corresponding regression equation relating the ΔA and Or.cit concentrations. The DW method is capable of determining the Or.cit concentration by applying mathematical steps on the zero-order absorption spectrum of the mixture without the need for a divisor or any manipulation steps.

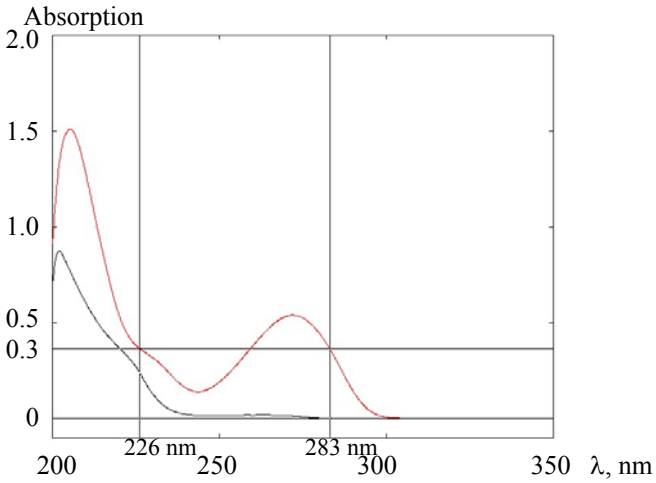


Fig. 10. Zero-order absorption spectra of 10 µg/mL Or.cit and 10 µg/mL CAF showing the same absorbance for CAF and different absorbance for Or.cit at 226 and 283 nm for Or.cit determination using dual wavelength method.

Method validation. Validation was done according to ICH guidelines [8]. Linearity, selectivity, precision, and accuracy were determined with satisfactory results as shown in Tables 1 and 2. The developed methods were successfully applied for the determination of PAR, Or.cit, and CAF in Muscerol extra® tablets as shown in Table 3. Application of the standard addition technique revealed that there is no interference from the excipients.

TABLE 1. Analytical Parameters and Validation Results for the Determination of PAR, Or.cit, and CAF by the Proposed Spectrophotometric Methods

Parameter	PAR	Or.cit		CAF	
	SARS	4th derivative	DW	1st derivative	CM
Wavelength (nm)	304	224.6	226 and 283	287	272
Regression parameters					
Linearity range (µg/mL)	20.00 – 90.00	3.00 – 40.00		1.00 – 20.00	
Intercept	0.2128	–0.0008	0.0328	0.0036	0.007
Slope	0.9994	0.05	0.0198	0.0323	0.0528
Correlation Coefficient	0.9999	0.9997	0.9996	0.9999	0.9999
Accuracy (Mean±RSD)					
Low concentration[a]	100.60±0.16	100.87±0.52	99.87±0.63	99.48±1.04	99.54±0.37
Medium concentration[b]	100.51±0.08	99.98±0.35	99.74±0.44	100.24±1.03	100.73±0.64
High concentration[c]	100.27±0.07	100.13±0.20	99.88±0.24	100.60±0.72	99.00±0.40

Continue Table 1

Parameter	PAR	Or.cit		CAF	
	SARS	4th derivative	DW	1st derivative	CM
Precision (±%RSD)					
Repeatability[d]	±0.41	±0.59	±0.53	±0.71	±0.45
Intermediate Precision[e]	±0.49	±0.75	±1.07	±1.14	±0.96
Selectivity	101.06±0.43	100.22±0.78	98.30±0.75	99.41±0.58	98.55±0.85
LOD[f]	0.76 µg/mL	0.97 µg/ mL	1.00 µg/ mL	0.23 µg/ mL	0.22 µg/ mL
LOQ[f]	2.32 µg/ mL	2.94 µg/ mL	3.03 µg/ mL	0.71 µg/ mL	0.68 µg/ mL

[a] Accuracy low concentration (30, 8, and 3 µg/ mL) for PAR, Or.cit, and CAF, respectively.
[b] Accuracy medium concentration (45, 23, and 9 µg/ mL) for PAR, Or.cit, and CAF, respectively.
[c] Accuracy high concentration (75, 32, and 17 µg/ mL) for PAR, Or.cit, and CAF, respectively.
[d] Intraday precision (the %RSD of 3 different concentrations (25, 55, 85 µg/ mL for PAR, 12, 26, 38 µg/ mL Or.cit and 4,11,19 µg/ mL for CAF)/3 replicates each, within the same day).
[e] Interday precision (the %RSD of 3 different concentrations (25, 55, 85 µg/ mL for PAR, 12, 26, 38 µg/ mL Or.cit and 4,11,19 µg/ mL for CAF)/3 replicates each, repeated on 3 successive days).
[f] Calculated from equation [LOD =3.3 (S.D/S), LOQ = 10 (S. D/S); where S.D is the residual standard deviation of the slope and S is the slope for the proposed methods.

TABLE 2. Determination of PAR, Or.cit, and CAF in Different Laboratory Prepared Mixtures by the Proposed Methods

Concentration (µg/mL)			% Recovery (n = 3)				
			PAR	Or.cit		CAF	
PAR	Or.cit	CAF	SARS	4th derivative	DW	1st derivative	CM
20.00	20.00	20.00	101.18	99.48	97.78	100.22	99.24
40.00	20.00	10.00	101.55	99.38	99.29	99.81	98.11
45.00*	3.50*	1.50*	100.55	101.03	98.41	99.90	97.22
60.00	30.00	5.00	100.98	100.85	99.19	99.32	97.73
15.00	40.00	15.00	100.63	99.29	97.25	99.15	98.74
20.00	20.00	10.00	101.39	100.78	98.54	98.89	98.48
25.00	15.00	15.00	101.57	101.04	98.38	99.57	99.12
45.00	7.00	3.00	100.60	99.94	97.55	98.45	99.75
Mean			101.06	100.22	98.30	99.41	98.55
SD			0.43	0.78	0.74	0.58	0.84
RSD			0.43	0.778	0.753	0.583	0.852

* The same concentration ratio as in the dosage form.

TABLE 3. Determination of PAR, Or.cit, and CAF in Muscerol Extra® Tablets* by the Proposed Spectrophotometric Methods and Application of Standard Addition Technique

% Assay	Standard addition Simultaneous area ratio subtraction				
	Taken (µg/mL)	Added (µg/mL)	Total found (µg/mL)	Standard found (µg/mL)	%Recovery of added (n = 3)
PAR by SARS 100.07±0.14	45.00	–	45.03	–	–
	45.00	5.00	49.99	4.96	99.17
	45.00	10.00	55.01	9.98	99.76
	45.00	15.00	60.03	15.00	100.02

Continue Table 3

% Assay	Mean±SD							99.65±0.44
Or.cit by 4th derivative 100.65±0.87 Or.cit by DW 100.34±0.83	Taken (µg/mL)	Added (µg/mL)	Fourth derivative method: Total found (µg/mL)	Fourth derivative method: Standard found (µg/mL)	Fourth derivative method: %Recovery of added (*n* = 3)	Dual wavelength method: Total found (µg/mL)	Dual wavelength method: Standard found (µg/mL)	Dual wavelength method: %Recovery of added (*n* = 3)
	3.50	–	3.52	–	–	3.51	–	–
	3.50	3.00	6.52	3.00	100.09	6.51	3.00	99.95
	3.50	3.50	7.03	3.51	100.27	7.00	3.49	99.62
	3.50	7.00	10.56	7.04	100.51	10.53	7.02	100.31
	Mean±RSD				100.29±0.21			99.96±0.35
CAF by 1st derivative 100.58±1.18 CAF by CM 100.59±0.72	Taken (µg/mL)	Added (µg/mL)	First derivative method: Total found (µg/mL)	First derivative method: Standard found (µg/mL)	First derivative method: %Recovery of added (*n* = 3)	Constant multiplication method: Total found (µg/mL)	Constant multiplication method: Standard found (µg/mL)	Constant multiplication method: %Recovery of added (*n* = 3)
	1.50	–	1.51	–	–	1.51	–	–
	1.50	1.00	2.51	1.00	99.98	2.51	1.00	100.26
	1.50	1.50	3.01	1.50	99.68	3.00	1.49	99.25
	1.50	3.00	4.51	2.99	99.82	4.54	3.03	100.97
	Mean±RSD				99.79±0.16			100.16±0.86

* B.N. 22.26 Labeled to contain: 450 mg PAR, 35 mg Or.cit and 15 mg CAF.

Conclusion. The use of normalized spectra is very important in resolving spectral overlap, especially for this challenging mixture with a large difference in the component concentrations. Simultaneous area ratio subtraction, which utilizes division spectra using PAR normalized spectra as a divisor, could directly determine PAR concentration in the mixture, while the resolution of the Or.cit and CAF spectra is achieved with simple manipulation steps performed on the division spectra. In order to determine the Or.cit and CAF concentrations, different methods were applied such as fourth and first derivatives, which use the recovered binary mixture spectrum directly without any mathematical manipulations. Also, the dual wavelength method developed for Or.cit determination involves simple mathematical calculations without any tedious software manipulations, in addition to the constant multiplication method for CAF determination using the value of the constant produced to recover the zero-order absorption spectrum of CAF. All the methods are selective, accurate and precise; therefore, they can be used for routine analysis of the previously mentioned drugs in quality control laboratories.

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