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The simultaneous measurement of quaternary mixture in over-the-counter cold medications using sequential spectrophotometric resolution approach enhanced with in-lab sample enrichment

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Abstract

A sequential spectrophotometric resolution technique (SSRT) was developed in this study without the use of systematic separation procedures to determine drug of a quaternary combination; caffeine (CAF), pseudoephedrine (PSE), doxylamine succinate (DOX), and paracetamol (PAR). Their presence in a tablet with a gap ratio of 3:3:1:150, respectively, and their overlapping spectra with low absorptivities make their resolution and determination impossible without prior separation. successive ratio subtraction technique (SRST) and constant multiplication method were used to solve these problems. Furthermore, an in-lab sample enrichment technique was applied to increase minor components concentration and consequently their absorbanses (CAF, PSE, and DOX). The D^0 absorption spectra were generated by successive ratios followed by subtraction and multiplication of the constants. The maximum absorbances of the drugs tested, namely (CAF, PSE, DOX and PAR) were measured at wavelengths of 272.0, 257.0, 260.0, and 248.0 nm, respectively. The limits of detection (LOD) and limits of quantification (LOQ) were 0.021, 0.124, 0.186, 0.137 and 0.070, 0.414, 0.621, 0.456 (µg/mL), respectively. The linearitiy ranges (µg/mL) were 1.0–22.0, 1.0–24.0, 10.0–90.0 and 1.0–15.0 for CAF, PSE, DOX, and PAR, respectively. The International Conference on Harmonization (ICH) guidelines were applied for method validation, and the results obtained were within the limited parameters. The finding results were compared to official and/or published analytical methods to determine the procedure's reliability. It was noted that there was no actual difference in accuracy and precision between both meyhods. The proposed technique is sensitive, selective and economic;so it can be applied to the simultaneous analysis of these drugs in their commercial tablets and/or in quality-control laboratories.

Keywords Caffeine, Pseudoephedrine hydrochloride, Doxylamine succinate, Paracetamol, Successive ratio subtraction, Constant multiplication

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Introduction

Caffeine (CAF, Fig. 1a) is (1, 3, 7-trimethylxanthine). It can be present in coca nuts, coffee grounds, tea, and cocoa beans. Its pharmacological action is central nervous system stimulant, respiratory muscle relaxant, stimulate gastric acid secretion and diuretic [1]. It is also used in beverages as a flavoring agent. Many methods were reported for CAF assay such as spectrophotometric [2–8] and separation methods [7–16].

Pseudoephedrine hydrochloride (PSE) [(1S, 2S)-2-methylamino-1-phenylpropan-1-ol hydrochloride] shown in (Fig. 1b). It has sympathomimetic effect causing vasoconstriction and nasal airways congestion [17, 18]. CAF is a component of pharmaceuticals that include stimulants, painkillers, cold cures, weight-loss products, bronchial and cardiac stimulants, and also medications for the treatment of acne and other skin issues. PSE can be taken alone or in combination with other NSAIDs such as ibuprofen or aspirin, antihistamines, guaifenesin, dextromethorphan, and/or paracetamol. Several methods were used for its determination either alone or in combination with others including different spectrophotometric methods [19] and separation techniques [20–26], also by potentiometric method [27].

Doxylamine succinate (DOX); (Fig. 1c) is antihistaminic drug used for the treatment of allergy, hay fever, and common cold [28]. DOX is co-administered with Vitamin B6 (pyridoxine) to decrease morning sickness specialy during pregnancy [29]. Many spectrophotometric methods [30–33]and separation techniques [34–40] were reported for its analysis.

Paracetamol (PAR), (acetaminophen) is [N-(4-hydroxyphenyl) acetamide] (Fig. 1d). PAR has analgesic and antipyretic effect in numerous cold and flu remedies. Several methods are used for its assessment comprising: titrimetric [41], spectroscopic [42–46] and separation methods [47–50]. Many methods were reported for component with low absorbitivitiy [51], drug mixture with ratio variation and minor componants such as Fourier transform infrared spectroscopy (FTIR) combined with chemometric techniques [52].

To date, and to the best of our knowledge, no analytical methods are reported for the quantification of CAF, PSE, DOX and PAR, simultaneously, in their mixtures. These four drugs show severe spectra overlapping which hinders UV-spectrophotometric analysis in addition to the critical ratio in the pharmaceutical preparations and the presence of minor components. It is a challenge to resolve spectral overlapping of multi component mixtures without prior separation. However, in recent years, it has been proposed to use successive and progressive spectrophotometric resolution to examine ternary mixtures that partially or completely overlap where enrichment of a minor component in a combination may not affect the concentrations of major component(s) in higher ratio and reach their quantification limit.

In this study, in Lab sample enrichment technique was investigated for quantitative analysis of quaternion mixture by spiking the lowest concentration components

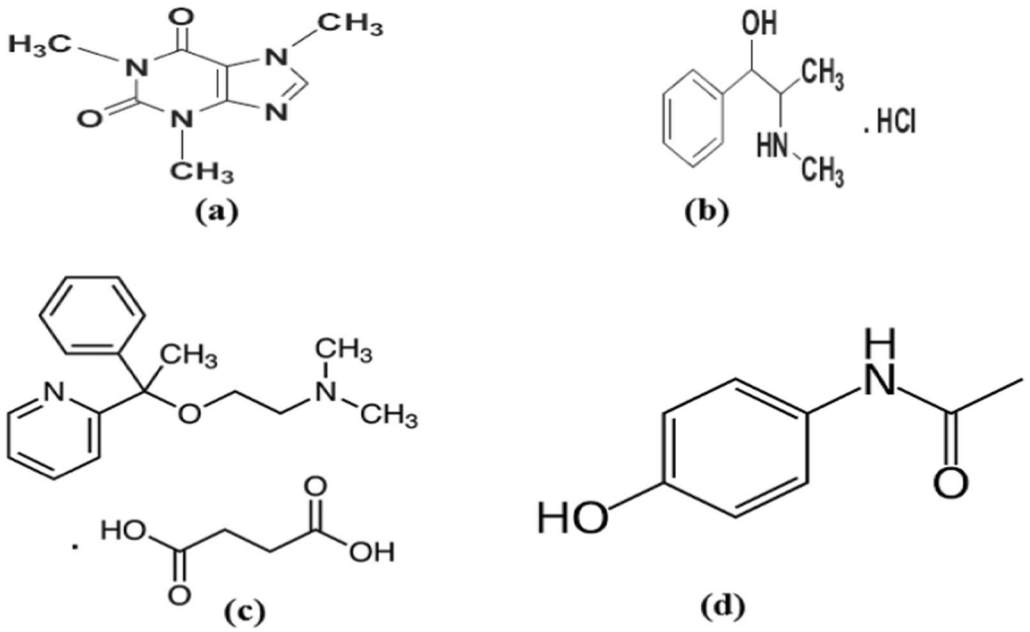

Fig. 1 The chemical structure of (**a**) CAF, (**b**) PSE, (**c**) DOX, (**d**) PAR

with known concentration of its pure form. Cafamol extra tablets containing CAF, PSE, DOX and PAR in critical ratio (3:3:1:150) is widely used in the local market for relieving all symptoms in common cold.

In the present work, we describe selective, valid, sensitive method for the analysis of CAF, PSE and DOX, simultaneously as minor components as well as PAR (a major component) in their dosage form by applying sequential spectrophotometric resolution technique (SSRT) augmented with enrichment by spiking the lab samples with the lowest concentration components.

Other than a few co-formulated prescription drugs that are either poorly absorbed or present in small amounts and therefore are outside of their quantification limits. This technique is successfully applied to combined drugs in dosage forms containing challengeable ratios within the quantitative limit. When these components are greatly enhanced in samples, their combined spectrophotometric signals increase, which reduces the deviation from Beer's law that happens when a component's contribution is low [53]. Following mixture analysis, in lab-made mixtures, pure standards of the mild constituent(s) in known amounts are spiked. To increase the concentration of the minor components and yield a spectrum of augmented components with concentrations within the quantitative limit. The same techniques were used to assess the additional concentration. The linear regression equation of the suggested technique was used at the designated spectrum and the actual added concentrations of the proposed drug were determined. Finally, difference between enhanced and added ones were calculated. Also, the concentration of the stated drugs in the combination is determined.

Successive spectrophotometric resolution technique (SSRT) [54–56] depends on utilizing original mathematical techniques. So, the constants found in the spectral analysis were done to clarify spectral overlapping of multicomponent dose forms. Ratio subtraction has recently been employed as a resolution method where stepwise elimination was used to remove interference from one or more components in the mixture [57–60]. In this study, we used the SSRT to solve the challenges of overlapping spectra with low absorptivity and significant ratio variation. Following is an explanation of the theory:

We might separate out the components of W, X, Y, and Z using sequential ratio subtractions if Z renewed further than Y, Y was, in turn, more prolonged than X, and X is more extended than W. W was then established. Therefore, W might be determined by consecutive ratio subtraction using the mixture's spectrum as a divisor (Z') and a specific concentration of Z and the other components can be determined by the same way. This can be summarized as follows:

$$(\mathbf{W}+\mathbf{X}+\mathbf{X}+\mathbf{Z})/\mathbf{Z}' = (\mathbf{W}/\mathbf{Z}') + (\mathbf{X}/\mathbf{Z}') + (\mathbf{X}/\mathbf{Z}') + (\mathrm{Z}/\mathbf{Z}') \quad (1)$$

$$(\mathbf{W}+\mathbf{X}+\mathbf{Y}+\mathbf{Z})/\mathbf{Z}' = (\mathbf{W}+\mathbf{X}+\mathbf{Y})/\mathbf{Z}' + \mathbf{Constant} \quad (2)$$

Directly from the (W + X + Y + Z)/Z' spectrum, the constant could be found by following the straight line that operated parallel to the wavelength axis in the region where Z was extended.

Constant multiplication method [58–60] had been devised as a new strategy for obtaining the first component (Z), in which Z could be calculated by multiplying Z' divisor by the previously obtained constant Z / Z', so we could obtain the D^0 curve of Z. The following could serve as a summary:

$$\mathbf{Z} = \mathbf{Z}/\mathbf{Z}' \cdot \mathbf{Z}' \quad (3)$$

The concentration of Z was calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of Z at its λ_{max} against the corresponding concentrations).

If we subtracted the measured value of the constant from the ratio spectrum Eq. (2), then multiplied the new spectrum by Z' divisor; we obtain the spectrum of W + X + Y as tertiary mixture.

This could be summarized in the following equations: ***(W+X+Y)/Z'. Z'+* constant−constant = *(W+X+Y)/Z'. Z'= W+X+Y.*** then devise the new spectrum by Y′ devisor

$$(\mathbf{W}+\mathbf{X}+\mathbf{Y})/\mathbf{Y}' = (\mathbf{W}/\mathbf{Y}') + (\mathbf{X}/\mathbf{Y}') + (\mathbf{Y}/\mathbf{Y}') \quad (4)$$

$$(\mathbf{W}+\mathbf{X}+\mathbf{Y})/\mathbf{Y}' = (\mathbf{W}+\mathbf{X})/\mathbf{Y}' + \mathbf{Constant} \quad (5)$$

By multiplying the constant by ***Y'*** devisor, compound Y will be obtained. Furthermore, It was noted that, the D^0 curve of ***Y***. This could be summarized as follows ***Y = Y/Y'. Y'*** therefore we could obtain the D^0 curve of Y. The concentration of Y was calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of Y at its λ_{max} against the corresponding concentration.

If we subtracted the measured value of the constant from the ratio spectrum Eq. (5), then multiplied the new spectrum by ***Y'***, we obtain the spectrum of W + X as binary mixture. This could be summarized in the following equations:

$$(\mathbf{W}+\mathbf{X})/\mathbf{Y}' + \mathbf{Constant}-\mathbf{Constant} \quad (6)$$

$$(\mathbf{W}+\mathbf{X})/\mathbf{Y}' \cdot \mathbf{Y}' = \mathbf{W}+\mathbf{X} \quad (7)$$

The obtained spectrum was successively divided by X as a divisor (X′). The constant could be determined directly from the (W + X)/X′ spectrum by the straight line that was parallel to the wavelength axis in the region where X was extended.

$$(\mathbf{W}+\mathbf{X})/\mathbf{X}' = (\mathrm{W}/\mathbf{X}') + (\mathbf{X}/\mathbf{X}') \quad (8)$$

For obtaining the third component (X), by multiplication of X′ divisor by the previously obtained constant X/ X′, therefore we could obtain the D^0 curve of X. This could be summarized as follows:

$$\mathbf{X} = \mathbf{X}/\mathbf{X}' \cdot \mathbf{X}' \quad (9)$$

The concentration of X was calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of X at its λ_{max} against the corresponding concentrations). If we subtracted the measured value of the constant X / X′ from the ratio spectrum Eq. (8), then multiplied the spectrum by X′, we obtain D^0 curve of W. This could be summarized in the following equations:

$$(\mathbf{W}/\mathbf{X}') + \mathbf{Constant} - \mathbf{Constant} = \mathbf{W}/\mathbf{X}' \quad (10)$$

$$\mathbf{W}/\mathbf{X}' \cdot \mathbf{X}' = \mathbf{W} \quad (11)$$

The aim of this work was to utilize SSRT augmented with in-Lab enrichment technique as a robust method for simultaneous quantification of CAF, PSE, DOX and PAR in quaternary mixture.

Experimental

Apparatus

The measurments were done using UV- spectrophotometer (Jasco; Japan).

Experimental materials

Samples

The studied authentic samples; CAF, PSE, DOX, PAR were Kindly obtained from Amriya-Company, Egypt, with purity of 99.6 ± 0.3 and 100.1 ± 0.08, for CAF and PSE according to USP and BP methods [9, 62] respectively. For DOX 100.4 ± 0.2 and 99.7 ± 0.13 for PAR assured by the reported ones [34, 48].

Finished product

Cafamol Extra® tablets that contain 30.0 mg of both CAF, PSE, 3.0 mg of DOX and 450.0 mg PAR were obtained from local market.

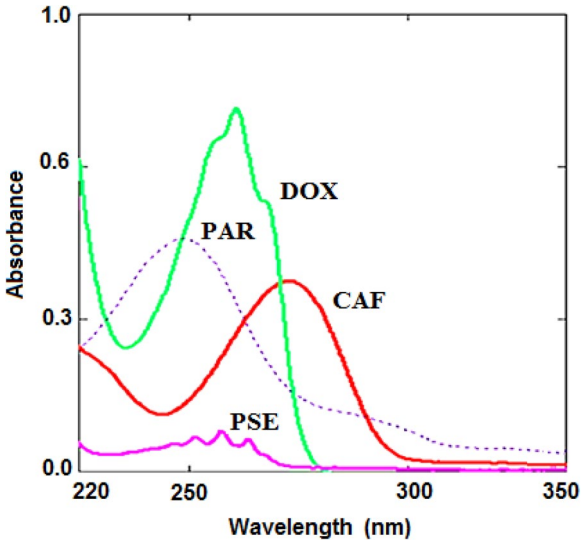

Fig. 2 Zero order- spectra for a quaternion mixture of CAF, PSE, DOX and PAR (2.0, 22.0, 20.0, and 1.0 µg/mL), respectively

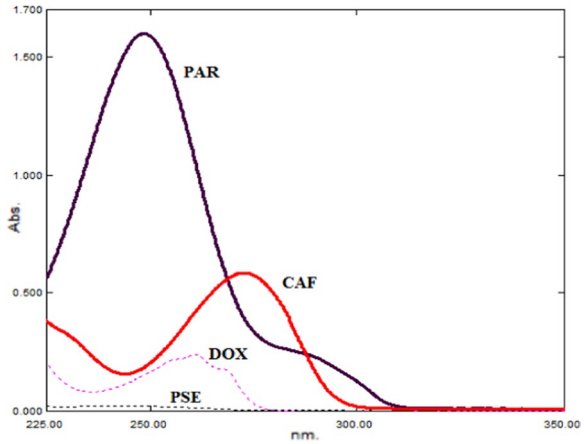

Fig. 3 Zero order- spectra for a quaternion mixture of CAF, PSE, DOX, and PAR (11.0, 20.1, 11.0, and 15.0 µg/mL), respectively after enrichment

Solvents

Methanol, grade-A was the product of Honeywell, Germany.

Standard solutions

To get (1000.0 µg/mL, stock solution), dissolving 100.0 mg of each CAF, PSE, DOX and PAR in 100.0 mL methanol.

Procedure

Spectral characteristics

Using methanol as a solvent, a series of standard solutions corresponding to 1.0–22.0 CAF, 1.0–24 PSE,

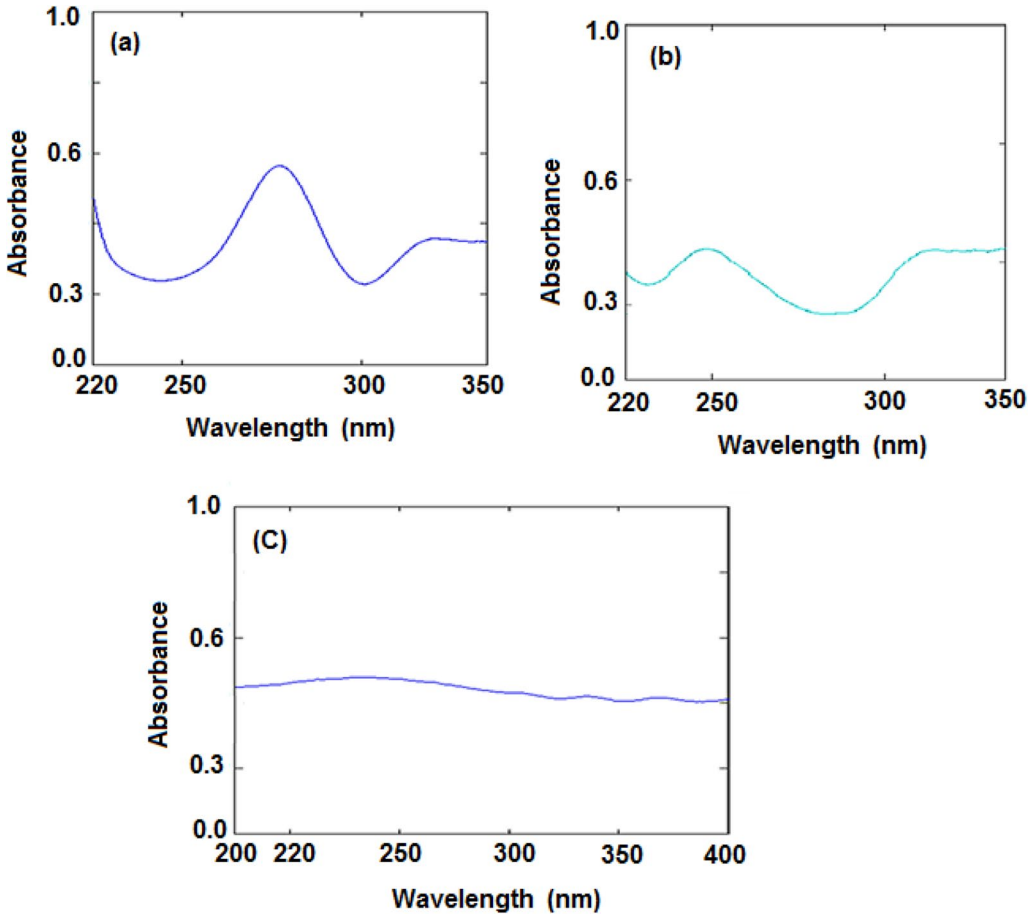

Fig. 4 Division spectra of lab-mixtures; (**a**) PAR (15 µg/mL), (**b**) CAF (20 µg/mL), and (**c**) DOX (50 µg/mL) as divisors, respectively

10.0–90.0 DOX, and 1.0–15.0 g/mL PAR were created from the stock standard solution. Then the UV range of the spectra was scanned.

Calibration graphs

The qualified solution's peaks were collected as can be seen in (Fig. 2). The calibration graphs were made for the following; CAF, PSE, DOX, and PAR at 272.0, 257.0, 260.0 and 248.0 nm, respectively.

Application of the proposed method in lab-mixtures

In a series of 10.0-mL volumetric flasks, serial dilutions of CAF, PSE, DOX, and PAR were precisely transferred from their working solutions, thoroughly mixed, and then each flask was enriched with (10.0, 10.0, and 20.0 µg/mL) of CAF, PSE, and DOX, respectively, and then filled to the mark volume with methanol. This procedure was done in order to create mixtures containing various ratios of the studied drugs. The spectra of this mixtures were scanned in range 200 to 400 nm as shown in (Fig. 3).

Afterward, these spectra were successively divided by the PAR spectrum at a concentration of 15.0 µg/mL, and the plateau area at (319.0–335.0 nm) was used to measure the amplitudes of the PAR/ PAR' (I) constants and subtract them (Fig. 4a).

The constant amplitudes were collected at (278.0–290.0 nm) region, preceded by multiplying of the spectrum of CAF (20.0 µg/mL).

The collected peaks were divided using standard DOX (50.0 µg/mL) as a divisor to determine the amplitudes of the constant of DOX/DOX′ (III) (Fig. 4c). The amplitudes of the constants were measured at (262.0–270.0 nm) region, then multiplied by the spectrum of the standard DOX (50.0 µg/mL) to obtain the PSE spectrum. The proposed drugs' zero order absorption spectra could be obtained by multiplying the values (I, II, III) by the zero order absorption spectra of standard PAR (15.0 µg/mL), CAF (20.0 µg/mL), and DOX (50.0 µg/mL), respectively.

The proposed method's matching regression equation at the appropriate wavelength was used to estimate the

actual added and augmented concentrations of the cited drug(s).

Application to the finished product

A quantity precisely equal to 30.0 mg of CAF, PSE, 3.0 mg of DOX, and 450.0 mg of PAR was weighed from ten Cafamol extra® tablets, finely powdered, transferred into beaker (100.0 mL). Afterthat 30.0 mL of the selected solvent (methanol) was used, ultrasonically processed for 15 min, and then filtered into a 100.0 mL volumetric flask. The solution was then finished to the mark with methanol. Next, wash the residue three times with 15.0 mL of methanol each time. To prepare a solution with 11.0 mg/mL of CAF, PSE, 20.1 mg/mL of DOX, and 15.0 mg/mL of PAR, accurately transfer an aliquot into a 10.0-mL calibrated flask. Next, spike the solution with standard solutions of 10.0, 10.0, and 20.0 mg/mL of CAF, PSE, and DOX from each of their stock solutions, respectively, and adding methanol to reach the desired volume. The method described under "Analysis of laboratory prepared mixtures" was used to determine each drug's dosage form as a tablet. Prior to using the previously mentioned methods, the dosage form was mixed with various known concentrations of pure standard CAF, PSE, DOX, and PAR using the standard addition technique. After calculating the drug concentrations, it was possible to successfully calculate the mean recoveries.

Results and discussion

Fastness and sensitivity are the main characters of spectrophotometric techniques. In this work we focused on applying a mathematical technique upon developing this method**.** The ratio of CAF, PSE, DOX and PAR in Cafamol extra tablets is critical ratio (3:3:1:150). To attain this ratio in their dosage form content, low CAF, PSE, and DOX concentrations above the range were required. simultaneous quantification of each drug concentration in zero order spectra was therefore very challenging; the enrichment technique (addition of the standers of minor components to their lab mixture) was therefore applied.

Each drug's absorptivity and spectral characteristics made it difficult to obey Beer's law. The dosage form ratios of CAF, PSE, DOX, and PAR in methanol were separately scanned and overlaid. According to Fig. 2, the spectra (zero ordering) of pure CAF, PSE, DOX, and PAR had a significant amount of overlap, making it difficult to determine all four simultaneously using standard spectrophotometric techniques. In the UV region, PAR was longer than the other components (200.0–400.0 nm). Also, CAF was more extended than DOX which is more extended than PSE.

It is obvious from previously reported methods for analysis of mixtures that there is no spectrophotometric method offered an opportunity to enable the quaternion mixture of the studied drugs to be analysed simultaneously, simply, accurately, precisely and economicaly. In the present study the developing lab-samples enrichment technique and successive ratio subtraction (SRS) spectrophotometric method were able to evaluate these drugs by resolving the overlapping spectra and low absorptivities and gap ratio, without previously separation steps. Also there no need to determine the standard added as the mathematical factorization (divition and subtraction) extract the D^0 curves, in addition that the absorbitivities and/or dervatization ratios are not required.

Successive ratio subtraction coupled with constant multiplication (SRS–CM)

The PAR was longer than CAF, which was longer than DOX, which was longer than PSE, according to the zero-order absorption spectrum (D^0) (Fig. 2). Researchers were able to calculate a constant from the straight line parallel to the wavelength axis at the extended part (PAR/PAR') by dividing the spectrum of the lab-mixture by a divisor spectrum of standard 15.0 µg/mL PAR at (319.0–335.0 nm) as showed in Fig. 4a. This constant was multiplied by the divisor spectrum (PAR') to produce the original PAR curve, and the absorbance at its maximum wavelength of 248.0 nm also was quantified (Fig. 5a).

A plateau region parallel to the wavelength axis was apparent in the extended range of the acquired division spectrum, and this allowed us to determine the constant (CAF/CAF') (278.0–290.0 nm) (Fig. 4b). This constant was multiplied by the (CAF') to create the original CAF curve (Fig. 5b), from which the absorbance at its maximum wavelength of 272.0 nm was determined. Their regression equations represented the linear relationship between the absorbance at 272.0 nm and the CAF concentrations, and the CAF concentration was determined by substituting them.

This constant (CAF/CAF') was subtracted to yield the CAF D^0 spectrum. D0 spectra of DOX and PSE were obtained by dividing this result by the spectrum of the divisor. It was feasible to obtain the longer D0 spectrum of DOX by using the regular DOX′ (50.0 µg/mL) D^0 spectrum as a divisor. The ensuing division spectrum showed a plateau region at the expanded region parallel to the wavelength axis, from which the constant DOX/DOX′ (262.0–270.0 nm) could be derived (Fig. 4c).

This constant was multiplied by (DOX′) to get the DOX original curve (Fig. 5c), from which the absorbance at its maximum wavelength of 260.0 nm was calculated. The concentration of DOX was determined by swapping

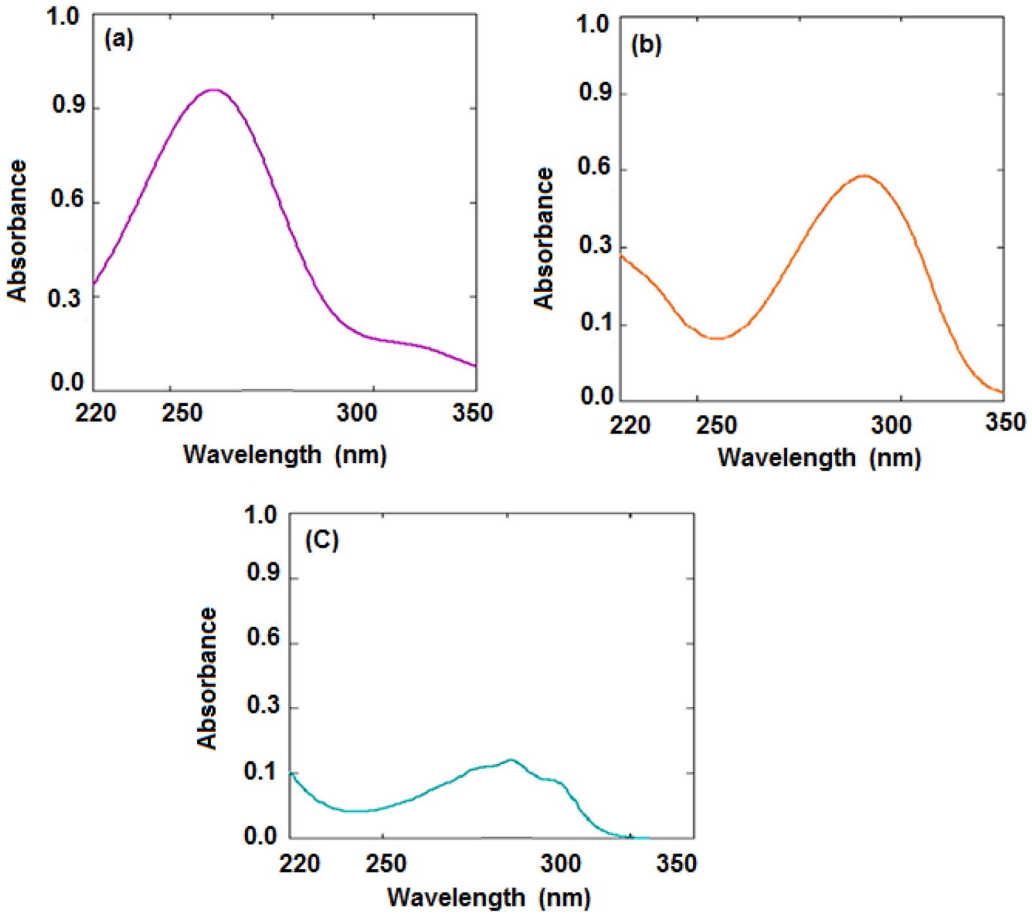

Fig. 5 Zero-order spectra of (**a**) PAR (15.0 µg/mL), (**b**) CAF (11.0 µg/mL), and (**c**) DOX (20.1 µg/mL)

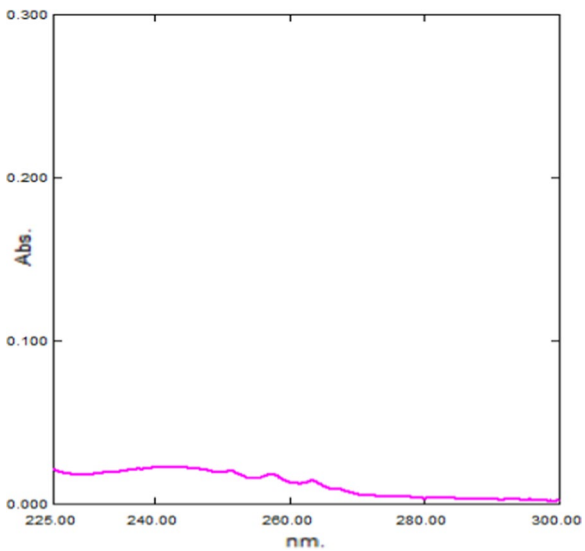

Fig. 6 Zero-order spectra of PSE (11.0 µg/mL) after subtraction and multiplication by a divisor DOX (50.0 µg/mL)

the linear relation between both the concentrations of DOX and the absorbance at the maximum wavelength, 260.0 nm, in their linear regression. Zero-order spectrum of the initial PSE curve was calculated (Fig. 6). Then, using zero order absorption spectra at its maximum wavelength of 257.0 nm, measuring the absorbance.

Method optimization

It's essential to optimize the numerous variables that may have an impact on the method.

Effect of solvent

Different solvents were tested like; acetonitrile, distilled water, and methanol.It was noted that methanol was the suitable solvent for dissolving the examined drugs.

The divisor and its concentration

To find a balance between the choice of the divisor concentration was essential for achieving the good sensitivity and the least amount of noise. As divisors for the

Table 1 Different validated parameters of the present method

Parameters	CAF	PSE	DOX	PAR
Linearity range (µg/mL)	1.0–22.0	1.0–24.0	10.0–90.0	1.0–15.0
Slope	0.0528	0.0423	0.0125	0.1015
Intercept	0.0052	0.0918	0.0031	0.0809
LOD (µg/mL)	0.021	0.124	0.186	0.137
LOQ (µg/mL)	0.070	0.414	0.621	0.456
Correlation coefficient (*r*)	0.9999	0.9997	0.9998	0.9997
Accuracy (%R)[a]				
Mean ± SD	100.71 ± 0.851	99.21 ± 1.114	100.5 ± 0.772	99.88 ± 1.020
Precision (%RSD)[b]				
Repeatability	0. 938	0.911	0.613	0.508
Intermediate precision	0.953	0.917	0.745	0.553

[a] Nine determinations average

[b] Nine determinations precision

Table 2 Assay of CAF, PSE, DOX and PAR in lab-mixtures by the proposed analytical method

Lab mixture	Concentration (µg/mL)	In lab recovery*			
		CAF	PSE	DOX	PAR
A	11:11:20.1:15	100.02	99.98	98.23	100.54
B	12:12:21:10	100.68	99.58	98.68	101.01
C	14:14:22:8	99.21	101.52	100.23	100.35
D	16:16:23:6	98.87	100.04	100.12	98.78
Mean ± SD		99.70 ± 0.815	100.28 ± 0.85	99.32 ± 1.011	100.17 ± 0.967

*Each experiment repeated three times

Table 3 Standard addition technique of CAF, PSE, DOX and PAR in Cafamol Extra® tablets

Sample	CAF	PSE	DOX	PAR
Cafamol extra ® tablets (Found% ± S.D)*	98.21 ± 0.954	100.82 ± 0.0.748	99.22 ± 0.369	98.66 ± 0.528
Standard addition (Recovery % ± SD)**	100.32 ± 0.901	99.34 ± 0.712	99.64 ± 0.321	98.47 ± 0.257

*Each experiment repeated three times of three concentration of the tablets (20,40,60 mg)

**Means of nine determinations

quantification of the cited drugs in their pure form and/or laboratory-prepared mixtures, various concentrations of PAR (15.0, 10.0 and 5.0 µg/mL), CAF (20.0, 14.0 and 8.0 µg/mL), and DOX (70.0, 50.0 and 40.0 µg/mL) were tested.

Optimization of the constants

It was found that the best divisors that give the highest sensitivity, recovaries, minimum noise and repeatability are 15.0, 20.0, and 50.0 µg/mL for PAR, CAF and DOX, respectively.

Method validation

The validity of the sugggested analytical methods was prformed based on ICH/Q2 rules [61].

Linearity

A series of standard solutions were used to get calibration graphs and the linearity's were achieved as presented in Table 1.

Precision

Two level (intra and inter-day) precision were calculated by processing the various calibration graphs over

Table 4 Comparative study between the proposed and reported methods for CAF, PSE, DOX and PAR in their pure forms

	CAF		PSE		DOX		PAR	
	SSRT	*Official method [9]	SSRT	**Official method [62]	S,SRT	Reported method [34]	SSRT	Reported method [48]
Mean	100.26	100.02	100.19	100.33	100.03	99.74	100.31	99.98
SD	0.31	0.53	0.288	0.55	0.6	0.69	0.42	0.25
Variance	0.096	0.281	0.083	0.303	0.360	0.482	0.176	0.063
N	6	5	6	5	6	5	6	5
Student's t-test (1.833)	0.893		0.514		0.757		1.612	
F value (6.256)	2.923		3.647		1.323		0.354	

P = 0.05 Numbers between brackets are the tabulated ones

*Official RP-HPLC method for determination of CAF using mobile phase, acetonitrile: sodium acetate buffer (45:55 v/v) pumped at a flow rate of 1.0 mL/min through the column (C18; 150.0 mm × 4.6 mm, 5.0 mm). **Official RP-HPLC method for determination of PSE using mobile phase, Methanol: Triethylamine phosphoric acid solution (pH 6.8) (10:90 v/v) pumped at a flow rate of 0.6 mL/min through the column (C18; 150.0 mm × 3 mm, 5.0 mm) at 30 _C. ***Direct UV spectrophotometric method, measuring the absorbance in water at 244 nm.

the course of three different days, the proposed method's linearity was assessed; Small values % RSD, guaranteed elevated method precision; the outcomes are shown in Table 1.

Accuracy

Acceptable recovery percentage (%R) and SD-values ensure the method is accurate are summarized in Tables 1, 2. The results obtained suggested that the developed methods were accurate.

Detection and quantification limits; (LOQ &LOD)

Table 1 showed the resulted data of LOD and LOQ calculation.

Selectivity

Selectivity was evaluated at various mixtures that contained the investigated drugs in a variety of different ratios. The resulted data (% RSD and % recovery) were summarized in Table 3.

Statistical analysis

For the CAF, PSE [9, 62], and PAR [48] as well as the published method [34] for DOX, Comparative statistics were displayed in Table 4 between the findings data using suggested method and those that were acquired via approved methods. The calculated "t" and "F" values were lower than the theoretical values, demonstrating that there was no real difference between the accuracy and precision of the suggested protocol and those of the official or reported methods.

Conclusion

The proposed method utilizes SSRT augmented with in-lab enrichment techniques as a robust method for simultaneous quantification of CAF, PSE, DOX, and PAR in a quaternary mixture. This augmentation allows the determination of any mixtures containing components in critical ratios. These materials are included in samples to increase their concentrations, which improve their individual spectrophotometric signals and permit dedication. The recovery and reproducibility of the analysis results with an actual tablet sample, as well as a comparison between the mean contents of active substances in the tablets obtained using the suggested method and those obtained using the official analytical methods for CAF, PSE, and PAR, as well as the reported method for DOX, were used to determine the procedure's reliability.

Abbreviations

CAF	Caffeine
CM	Constant multiplication
D^0	Zero-order absorption spectrum
DOX	Doxylamine succinate
FTIR	Fourier transform infrared spectroscopy
ICH	International Conference on Harmonization
LOD	Limits of detection
LOQ	Limits of quantification
PAR	Paracetamol
PSE	Pseudoephedrine
RSD	Residual standard deviation
SSRT	Sequential spectrophotometric resolution technique
SRS	Successive ratio subtraction
SRS–CM	Successive ratio subtraction coupled with constant multiplication

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Author contributions
KMK: research idea conceptualization; supervised the study; data analysis; manuscript writing, revision, and editing; MSE: Writing—original draft, methodology, data curation, validation and investigation. AWM: Methodology. HAB and RMT: Writing—review and editing. All authors read and approved the final manuscript.

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The authors declare no competing interests.

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References

1. Nehlig A, Daval J, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev. 1992;17(2):139–70.
2. Alpdogǎn G, Karabina K, Sungur S. Derivative spectrophotometric determination of caffeine in some beverages. Turk J Chem. 2002;26(2):295–302.
3. Ferreyra CF, Ortiz CS. Simultaneous spectrophotometric determination of phenilpropanolamine HCL, caffeine and diazepam in tablets. J Pharm Biomed Anal. 2002;29(5):811–8.
4. Jones M, Thatcher RL. Spectrophotometric determination of aspirin, phenacetin, and caffeine in mixtures. Anal Chem. 1951;23(7):957–60.
5. Dinc E, Palabıyık I, Yurtsever F, Onur F. Simultaneous spectrophotometric determination of chlorphenoxamine hydrochloride and caffeine in a pharmaceutical preparation using first derivative of the ratio spectra and chemometric methods. J Pharm Biomed Anal. 2002;28(3–4):591–600.
6. Korany MA, Wahbi AM, Elsayed MA, Mandour S. First derivative spectrophotometric determination of certain drugs in two-component mixtures. Anal Lett. 1984;17(12):1373–89.
7. Xia Z. Simultaneous determination of caffeine, theophylline and theobromine in food samples by a kinetic spectrophotometric method. Food Chem. 2013;141(4):4087–93.
8. Clayton A, Thiers R. Direct spectrophotometric determination of salicylic acid, acetylsalicylic acid, salicylamide, caffeine, and phenacetin in tablets or powders. J Pharm Sci. 1966;55(4):404–7.
9. United States Pharmacopeia Commission. National Formulary, R.V., United States Pharmacopeal Convention, Vol. I & II. 2016.
10. Pickard CE, Hartley R, Lucock MD. A rapid HPLC method for monitoring plasma levels of caffeine and theophylline using solid phase extraction columns. Ann Clin Biochem. 1986;23(4):440–6.
11. Srdjenovic B, Djordjevic V. Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. J Chromatogr Sci. 2008;46(2):144–9.
12. Bispo M, Veloso M, Pinheiro H, Oliveira R, Reis J. Simultaneous determination of caffeine, theobromine, and theophylline by high performance liquid chromatography. J Chromatogr Sci. 2002;40(1):45–8.
13. Meyer A, Ngiruwonsanga T, Henze G. Determination of adenine, caffeine, theophylline and theobromine by HPLC with amperometric detection. Fresenius J Anal Chem. 1996;356(3–4):284–7.
14. Abourashed EA, Mossa JS. HPTLC determination of caffeine in stimulant herbal products and power drinks. J Pharm Biomed Anal. 2004;36(3):617–20.
15. Misra H, Mehta BK, Soni M. Study of extraction and HPTLC-UV method for estimation of caffeine in marketed tea (Camellia sinensis) granules. Int J Green Pharm. 2009;3(1):223–35.
16. Fekry RA, Kelani KM, Fayez YM, Tantawy MA. Comparative validated chromatographic methods for the simultaneous determination of caffeine, codeine, paracetamol along with the related compound" p-aminophenol" in tablets. JPC. 2022;35(1):51–9.
17. Karas S. The potential for drug interactions. Annal Emerg Med. 1981;10(12):627–30.
18. Shao IH, Wu C, Tseng H, Lin Y. Voiding dysfunction in patients with nasal congestion treated with Pseudoephedrine. Drug Des Dev Ther. 2016;10:2333–9.
19. Ivanovic D, Medenica M, Markovic S, Mandic G. Second-derivative spectrophotometric assay of Pseudoephedrine, Ibuprofen and Loratadine in pharmaceuticals. Arzneimittelforsch. 2000;50(11):1004–8.
20. Dong Y, Chen X, Chen Y, Hu Z. Separation and determination of Pseudoephedrine, Dextromethorphan, Diphenhydramine and Chlorpheniramine in cold medicines by nonaqueous capillary electrophoresis. J Pharm Biomed Anal. 2005;39(1):285–9.
21. Chen H, Chen X, Pu Q, Hu Z. Separation and determination of Ephedrine and Pseudoephedrine by combination of flow injection with capillary electrophoresis. J Chromatogr Sci. 2003;41(1):1–5.
22. Nalini CN, Kavitha K. Simultaneous determination of Pseudoephedrine hydrochloride and Cetrizine hydrochloride by reverse phase high performance liquid chromatography. Indian J Pharm Sci. 2006;68(1):95–7.
23. Haddad GM, Emara S, Mahmoud WMM. Development and validation of a stability-indicating RP-HPLC method for the determination of Paracetamol with Dantrolene or/and Cetirizine and Pseudoephedrine in two pharmaceutical dosage forms. Talanta. 2009;79(5):1360–7.
24. Kumudhavalli MV. Determination of Pseudoephedrine hydrochloride, Cetirizine dihydrochloride and Paracetamol uncoated tablet by RP-HPLC method. J Global Pharma Technol. 2010;2(4):1–5.
25. Rahul S, Sengar NPS, Mehta DP, Lodhi NS. A validated RP-HPLC method for determination of Guaifenesin and Pseudoephedrine HCl in tablet dosage form. Int J Pharm. 2012;2(2):317–21.
26. Makhija SN, Vavia PR. Stability indicating HPTLC method for the simultaneous determination of Pseudoephedrine and Cetirizine in pharmaceutical formulations. J Pharm Biomed Anal. 2001;25(3):663–7.
27. Farouk YB, Essam H, Saad EE, El-Sayed HZ, Kelani KM. Green potentiometric method for determination of triprolidine hydrochloride and pseudoephedrine hydrochloride in their different pharmaceutical matrices using liquid and solid contact gold electrodes. Anal Bioanal Electrochem. 2020;12(6):793–809.
28. Moffat AC, Osselton DM, Brian W, Jo W. Clark's analysis of drugs and poisons, 4th edn, vol. II. London: Pharmaceutical Press; 2011. p. 1309–11.
29. Sweetman SC. Martindale, the complete drug reference, 37th edn, vol. A. London: Pharmaceutical Press; 2011. p. 2140.
30. Pathak A, Rajput SJ. Simultaneous derivative spectrophotometric analysis of doxylamine succinate, pyridoxine hydrochloride and folic acid in combined dosage forms. Indian J Pharma Sci. 2008;70:513–7.
31. Nayak S, Kulkarini PV, Bhaskar V, Chavahan V. Development and validation of UV spectrophotometric method for simultaneous estimation of doxylamine succinate and Pyridoxine hydrochloride in bulk and tablet dosage form. Int J Pharm Pharm Sci. 2013;5:390–3.
32. Nataraj KS, Suvarna Y, Venkateswari G. Development and validation of method for simultaneous estimation of pyridoxine hydrochloride and

doxylamine succinate in tablet dosage form by first order derivative spectroscopy. Int J Pharm Pharm Sci. 2012;5(1):388–90.
33. Premkumara S, Karunakarana A, Murugesana V, Munu SJ, Jayaprakasha R, Murugesanb R. Validated UV-spectrophotometric method for the simultaneous estimation of pyridoxine hydrochloride and doxylamine succinate in bulk and in pharmaceutical dosage form. Adv J Chem A. 2019;2(3):245–55.
34. Ramadan H, Eltanany B, Zaazaa H, Eissa M. HPLC-DAD approach for determination of pyridoxine HCl and doxylamine succinate in pure and pharmaceutical dosage forms: a green stability-indicating assay method. Microchem J. 2021. https://doi.org/10.1016/j.microc.2021.106982.
35. Cesar PCP, Jardim ICSF. Simultaneous determination of clobutinol hydrochloride and doxylamine succinate from syrups by RP HPLC using a new stationary phase containing embedded urea polar groups. Braz J Pharm Sci. 2012;48:315–23.
36. Argekar A, Sawant J. Simultaneous determination of pyridoxine hydrochloride and doxylamine succinate from tablets by ion pair reversed-phase high-performance liquid chromatography (RP-HPLC). Drug Dev Ind Pharm. 1999;25(8):945–50.
37. Ravichandran S, Selvakumar S, Afreen, Banu N. RP-HPLC method development and validation for the doxylamine succinate and pyridoxine HCL in its pure and pharmaceutical tablet dosage form. Ind J Pharm Sci Res. 2018;8(1):35–43.
38. Pathak A, Rajput S. Simultaneous determination of ternary mixture of pyridoxine hydrochloride, doxylamine succinate and folic acid by a ratio spectra zero crossing, double divisor ratio spectra derivative and column HPLC methods. J AOAC Int. 2008;91(5):1059–69.
39. Argekar AP, Sawant JG. Simultaneous determination of pyridoxine hydrochloride and doxylamine succinate in tablets by HPTLC. J Liquid Chromtogr Rel Technol. 1999;22:2051–60.
40. Kohlhof KJ, Stump D, Zizzamia JA. Analysis of doxylamine in plasma by high-performance liquid chromatography. J Pharm Sci. 1983;72(8):961–2.
41. Pharmacopoeia, Ministry of health and family welfare, Government of India. 1996;2:350.
42. Knochen M, Javier G, Boaventura FR. Flow-injection spectrophotometric determination of paracetamol in tablets and oral solutions. J Pharm Biomed Anal. 2003;33(2):191–7.
43. Dinç E, Yucesoy C, Önur F. Simultaneous spectrophotometric determination of mefenamic acid and paracetamol in a pharmaceutical preparation using ratio spectra derivative spectrophotometry and chemometric methods. J Pharm Biomed Anal. 2002;28(6):1091–100.
44. Erk N, Bano E. Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC. J Pharm Biomed Anal. 2001;24(3):469–75.
45. Moreira A, Atvars T, Dias IL, Neto G, Zagatto EA, Kubota LT. Direct determination of paracetamol in powdered pharmaceutical samples by fluorescence spectroscopy. Anal Chim Acta. 2005;539(1):257–61.
46. Sebaiy MM, Sobhy M, Mattar AA. Different techniques for overlapped UV spectra resolution of some co-administered drugs with paracetamol in their combined pharmaceutical dosage forms. Spectrochim Acta A. 2020;224: 117429.
47. Sane R, Gadgil M. Simultaneous determination of paracetamol, chlorzoxazone, and nimesulide by HPTLC. JPC-Modern TLC. 2002;15(1):76–8.
48. Behera S, Ahmad F, Santra S, Banerjee S. UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Techn. 2012;3(6):2–6.
49. Bhimavarapu R, Chitra KP, Meda H, Kanikanti D. Forced degradation study of paracetamol in tablet formulation using RP-HPLC. Bull Pharm Res. 2011;1(3):13–7.
50. Sornchaithawatwong C, Vorrarat S, Nunthanavanit P. Simultaneous determination of paracetamol and its main degradation product in generic paracetamol tablets using reverse-phase HPLC. J Health Res. 2010;24(3):103–6.
51. Eid SM, Kelani KM, Badran OM, Rezk MR, Elghobashy MR. Surface enhanced infrared absorption spectroscopy (SEIRA) as a green analytical chemistry approach: coating of recycled aluminum TLC sheets with citrate capped silver. Anal Chim Acta. 2020;1117:60–73.
52. Kelani KM, Rezk MR, Monir HH, ElSherbiny MS, Eid SM. FTIR combined with chemometric tools (fingerprinting spectroscopy) in comparison to HPLC: which strategy offers more opportunities as a green analytical chemistry technique for pharmaceutical analysis. Anal Methods. 2020;12(48):5893–907.
53. Kenkel J. Analytical chemistry for technicians. 3. painos. ss. 216–218. CRC Press LLC, Boca Rato, Florida. 2003.
54. Lotfy HM, Tawakkol SM, Fahmy NM, Shehata MA. Successive spectrophotometric resolution as a novel technique for the analysis of ternary mixtures of pharmaceuticals. Spectrochim Acta A. 2014;121:313–23.
55. Lotfy HM, Hagazy MM. Comparative study of novel spectrophotometric methods manipulating ratio spectra: an application on pharmaceutical ternary mixture of omeprazole, tinidazole and clarithromycin. Spectrochim Acta A. 2012;96:259–70.
56. Lotfy HM, Hegazy MM. Simultaneous determination of some cholesterol-lowering drugs in their binary mixture by novel spectrophotometric methods. Spectrochim Acta A. 2013;113:107–14.
57. Lotfy HM, Saleh SS. Testing the purity of spectral profiles: finger-print resolution of complex matrices and extraction of absorbance signals. Spectrochim Acta A. 2018;205:160–9.
58. Lotfy HM, Saleh SS, El-Maraghy CM. Advanced approaches for the treatment and amplification of weak spectral signals produced by critical concentrations in white multicomponent systems. Spectrochim Acta A. 2020;224: 117339.
59. Lotfy HM, Saleh SS, Hassan NY, Salem H. Computation of geometric representation of novel spectrophotometric methods used for the analysis of minor components in pharmaceutical preparations. Spectrochim Acta A. 2015;151:628–43.
60. Saleh SS, Lotfy HM, Tiris G, Erk N, El-Naem OA. The power of High Impact Amplitude Manipulation (HIAM) technique for extracting the basic spectra of two Fixed-dose combinations (FDC)-Spectrophotometric purity analysis via spectral contrast angle. Spectrochim Acta A. 2022;273: 121036.
61. ICH Q2B, Note for guidance on validation of analytical methods methodology. International Conference on Harmonization, IFPMA, Geneva, 1997.
62. British Pharmacopoeia, Her Majestyis Stationery Office, London, 2003.

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