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Simultaneous Determination of Binary Mixture of Estradiol and Progesterone Using Different Spectrophotometric Methods

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Abstract:

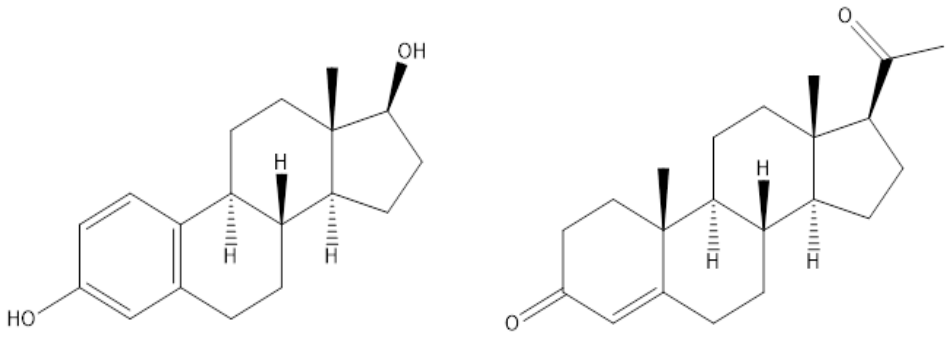
Four rapid, accurate and very simple derivative spectrophotometric techniques were developed for the quantitative determination of binary mixtures of estradiol (E2) and progesterone (PRG) formulated as a capsule. Method I is the first derivative zero-crossing technique, derivative amplitudes were detected at the zero-crossing wavelength of 239.27 and 292.51 nm for the quantification of estradiol and 249.19 nm for Progesterone. Method II is ratio subtraction, progesterone was determined at λmax 240 nm after subtraction of interference exerted by estradiol. Method III is modified amplitude subtraction, which was established using derivative spectroscopy and mathematical manipulations. Method IIII is the absorbance ratio technique, absorbance of both medicines was measured at two wavelengths λ1= 260, iso -absorptive point and λ2=240, λmax of progesterone. The Q equations were used to calculate the final concentrations. The calibration curve is linear from 5.0–140 and 2.0–32.0 µg/ml for estradiol and progesterone respectively. The proposed techniques' selectivity was tested using synthetic combinations created in the lab and assessed using the standard addition method. Using one-way ANOVA, the outputs of the proposed ways were compared, and the result showed no significant differences between the proposed techniques.

Keywords: Absorbance ratio method, Derivative spectrophotometry, Estradiol, Progesterone, Ratio subtraction.

Introduction:

Steroid hormones are synthesized from cholesterol, and many are of great clinical importance. As illustrated in Fig.1a, E2 has a chemical formula $C_{18}H_{24}O_2$. E2 is mostly created in the ovary, although minor amounts are also produced in the adrenal cortex and testis[1]. Estradiol, the most common type of estrogen, was once thought to be both male and female sex hormone. Estradiol, which is generated from the ovaries, is the most powerful natural estrogen[2,3]. The ovaries are the primary source of E2 in premenopausal female [4,5]. Progesterone (PRG) is a steroid hormone that regulates women's generative processes. It has a chemical formula of $(C_{21}H_{30}O_2)$[6]. Female reproductive functions are regulated by progesterone. During the luteal phase, or the second half of the menstrual cycle, PRG is formed largely by the corpus luteum in the ovaries. Progesterone is well formed in the adrenal glands to a lesser level during pregnancy[7, 8]. Progesterone is involved in every stage of human pregnancy. It has a key role in tissues other than the reproductive system, such as the mammary gland in the preparation for lactation, the circulatory system, the central nervous system, and bones [9, 10].

In recent years, analytical methods ultraviolet spectrophotometric method[6,11], derivative spectrophotometry DS [12-14], HPLC [12,15], IGC/MS stands for isotope dilution–gas chromatography/mass spectrometry[16], LCTMS[17], and GC-MS/MS [4,18] for estradiol and progesterone levels in human serum or plasma samples, as well as a pharmaceutical formulation, have been created.



(a) **(b)**

Figure 1. Chemical structure of (a) Estradiol and (b) Progesterone

This study aims to develop new and very simple techniques for resolving overlap spectra in their mixtures and pharmaceutical formulations without the need for separation. To measure E2 and PRG simultaneously in medicinal formulation, four distinct spectrophotometric techniques were used. The techniques are illustrated with mathematical explanations. The techniques do not require any complicated equipment or programming, and they are straightforward, accurate, and exact.

Theoretical Background

Derivative Spectrophotometry (DS)

By differentiating the sample's absorbance concerning to wavelength (λ), DS transforms a normal spectrum (basic, zero-order spectrum) into its 1st, 2nd, or higher derivative spectra. Derivative spectra offer a more distinctive shape in comparison to the parent shape; new maxima and minima appear as well as places where derivative spectra cross the X-axis. When the height of an analyte's derivative peak is measured at wavelengths where the spectra of other components are zeroed, the measured amplitude is related to the analyte's concentration. This method involves quantifying the amplitude of the derivative spectrum of a mixture at different wavelengths to identify the presence of numerous components in a combination. The "zero-crossing technique" is the name given to this method of quantitative determination [19-22.]

Ratio Subtraction Method (RSM)

If you have a mixture of 2 drugs, X (PRG) and Y (E2) with overlapping spectra, and the spectrum of Y is extended more than X, the determination of X can be done by dividing the spectrum of the mixture by a certain concentration of Y as a divisor (Y') [19] represent in Eq. 1

$\frac{X+Y}{Y'}$ …….. 1

The division in Eq. 1 will give a new curve that represents in Eq.2

$\frac{X+Y}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'}$ + Constant …… 2

Where X: concentration of PRG, Y: concentration of E2 and Y': concentration of E2 (µg/ml) as a divisor

If we subtraction of the absorbance values of these constants in Eq.2 the new curve obtained represent by Eq.3

$\frac{X}{Y'}$ + Constant – constant = $\frac{X}{Y'}$ ……..…… 3

Then multiply Eq.3 by advisor(Y'), we obtain the Eq.4 as original curve of X

$\frac{X}{Y'} \times Y' = X$ …… 4

The constant can be determined directly from Eq. 1 by the straight line that is parallel to the wavelength axis in the region where Y is extended [23,24.]

Modified Amplitude Subtraction Method (MAS)

When having a mixture of two drugs, X (PRG) and Y(E2), and the first derivative spectra of both drugs show overlapped peaks at λ1, while only the first derivative spectra of Y show a peak at λ2.

Two calibration curves are constructed relating the peak amplitude of Y at λ1 and λ2 verses the corresponding concentration of Y, and one calibration curve constructed relating the peak amplitude of X at λ1 verses the corresponding concentration of X (by first derivative for example)

$P_y = a+b_y$ at λ1 …… 5

$P_y = a+b_y$ at λ2 …… 6

$P_X = a+b_x$ at λ1 ……7

Where P_y: absorbance of Y (E2), a: absorptivity and y: concentration of y (E2) at λ1and λ2. P_X: absorbance of X (PRG), X: concentration of X (PRG) at λ1, b: slop.

The mixture is scanned, and the first derivative spectrum of the mixture is determined. We obtain the peak amplitude of mixture at λ1 (P_{mix1}) and λ2 (P_{mix2}).

By substitute P_{mix2} in Eq.6, so the concentration of Y can be calculated.

$P_{mix2} = a+by$ (at λ2) conc. of y …….. 8

Then the calculated concentration of Y in Eq. 8 is substituted in Eq.5 to determine the peak amplitude of Y in the mixture at λ1

P_y in the mixture = a+b(conc. of y) ……9

Where P_{mix1} absorbance of mixture of [X (PRG) + Y (E2)] at λ 1

$P_{mix2:}$ absorbance of mixture of [X (PRG) + Y (E2)] at λ 2

Where Py in the mixture: absorbance of y (E2) in the mixture, y: concentration of y (E2)

Subtracting calculate concentration of Y in Eq.8 by P_{mix1}

P_{mix1}–(P_y in the mix) = Px in the mixture ……..10

Where P_{mix1}: absorbance of mixture of [X (PRG) + Y (E2)] at λ 1, P_y in the mixture: absorbance of y (E2) in the mixture and Px in the mixture: absorbance of X (PRG) in the mixture

By subsuming Eq.10 in Eq.7 the concentration of drug X (PRG) can be determined

Px in the mixture = a+bx **……**11

Where Px: absorbance of X (PRG) in the mixture, a: absorptivity, b: slop, X: concentration of X (PRG) in the mixture[25].

Q-Absorbance Ratio Spectra Method

For this strategy, regardless of concentration or path length, the ratio of absorbance at any two wavelengths for a chemical that obeys Beer's law is a constant number. The technique necessitates absorbance measurements at two λmax, one of which is the maximum absorptivity of one of the components λ2 and another is the iso-absorptive point, which is a wavelength with same absorptivity of the two components λ1.

Mathematical equations can be used to compute the concentrations of each component.

$C_X = (Q_M - Qy/Q_X - Qy) \times A/a_1$ …….. 12

$C_y = (Q_M - Q_X/Qy - Qx) \times A/a_2$ ……. 13

Where C_x and C_y are the x and y concentrations, A is the sample's absorbance at iso-absorptive λmax, and a_1 and a_2 are the x and y absorptivity at iso-absorptive λmax, respectively.

$Q_M = A_2 / A_1$ ……..14

$Q_X = a\,x_2 / a\,x_1$ ……..……..15

$Q_y = ay_2/ay_1$ ……16

Where, A_1 and A_2 were absorbance of combination at λiso and λ max

ax_1 and ay_1 are absorptivities of x and y at λ iso

ax_2 and ay_2 are absorptivities of x and y at λ max[26-28]

Materials and Methods

A Shimadzu UV-V twin beam spectrophotometer (demonstrate UV 1800, Japanese) with a fixed 1nm bandwidth and a 1 cm quartz cell was used for spectrophotometric measurements, with a laptop attached to the double beam spectrophotometer to record normal spectrum. All computations were performed by Matlab 6.5 and Microsoft Excel. ANOVA was performed using SPSS.

All of the reagents used in this investigation are analytical grade.

Preparation Standard Stock Solutions of E2 (200 µg /ml) and PRG (100 µg /ml) (Sigma Aldrich): were made by dissolving 0.0200 g of E2 and 0.0100 g of PRG in absolute ethanol, diluting to 100 ml in a volumetric flask, and daily working solutions were made by diluting stock solutions with absolute ethanol[4,29]

Synthetic sample simulating to capsule was prepared in laboratory, consisted of 1.0 mg of E2 and 100 mg of PRG with excipients 45 mg of lactose, 9.0 mg of microcrystalline cellulose and 5.0 mg polyethylene glycol) per one capsul[30,31]

Absolute ethanol was used as a blank to capture the normal spectra of the two components throughout range of wavelength 200-400 nm.

Construction of Calibration Graphs

Serial dilutions of (5.0–140) µg E2 and (2.0–32.0) µg PRG are correctly transferred from the standard working solution into two different sets of 10.0 ml volumetric flasks, then brought up to volume with absolute ethanol. From 200 to 400 nm, the spectra of the created standard solutions are scanned and saved in the laptop.

The First Derivative Spectrophotometric Technique (1D)

Aliquots of 1.0 mL from E2 and PRG standard working solution, equal to 200 µg/ml and 100 µg/ml for both drugs, were separately transferred into two 10.0 mL volumetric flasks, and then the volume was completed with absolute ethanol. The spectra of these two solutions were scanned against a blank solution of absolute ethanol and their absorptions were computed. The concentration of each drug was quantified by drawing a calibration graph with the drug concentrations as abscissa and dA/dλ as ordinate at the determined zero crossing point of the other. The first derivative spectra were recorded under certain selected instrumental parameters such as, Δλ, scaling factor and wavelength range. After that the working wavelengths of the two drugs, at the zero crossing points were recorded [32].

Ratio Subtraction Method (RSM)

Standard working solutions were precisely measured and aliquots ranging from (2.0-32.0 µg) PRG were put into 10.0 mL volumetric flasks. The

prepared standard solutions' spectra were scanned after adding absolute ethanol to the final volume. A calibration graph was used to generate the regression formula, which linked the absorbance of PRG normal spectra at max 240 nm to the proper concentration. The regression equation is obtained by constructing a calibration chart comparing the absorbance of normal spectra of E2 at 281.40 nm, where PRG display no interference, to the appropriate concentrations.

Modified amplitude subtraction method (MAS)

Standard working solutions containing (5.0-140 µg) E2 and (2.0-32.0 µg) PRG were meticulously measured and deposited individually into 10.0 ml volumetric flasks. Absolute ethanol was used to finish the volume. Each solution was scanned throughout a certain λmax range. The 1D of each solution was then calculated and recorded. Three calibration graphs were created: one matching PRG's peak values at 243.66 nm to its corresponding concentration, and the other two linking E2's peak amplitudes at 243.66 nm and 292.51 nm to their respective concentrations. Three regression equations were calculated.

Q-Absorbance Ratio Spectra Method

Different aliquots corresponding to (5.0–140) µg E2 and (2.0–32.0) µg PRG were correctly transfer based on their standard solutions into two distinct sets of 10.0ml volumetric flasks and volumetric flasks were filled to volume with absolute ethanol. Absolute ethanol was used as a blank to record the normal spectra from 200 - 400nm of these solutions. The absorbance values for both medications at the indicated wavelengths were computed using the absorbance values observed at 262nm, iso-absorptive point (λiso) and λ2 =240nm (λmax of PRG).

Analysis of Synthetic Pharmaceutical Dosage Forms

Five capsules of E2 and PRG were powdered and mixed well. Correctly weighed amount of the powdered capsules equal to 160 mg of one capsule was dissolved in 100 ml absolute ethanol with the aid of sonication and filtered to make stock solution. A viable solution filtrate was diluted with absolute ethanol to a concentration of 15.0 µg in a 10.0 ml volumetric flask, and the methods for linearity or laboratory generated mixtures of each technique were followed. The procedures' validity was tested by injecting known amounts of standard drug powders into the pharmaceutical dose forms (standard addition technique). After implementing the offered procedures, the recovery of the extra standards was estimated.

Results and Discussion:

For analytical research, spectrophotometric approaches such as DS, ratio spectra spectrophotometry, and other chemometric methods are relevant. Quality control and drug analysis are common uses for them. They are also chosen over other analytical techniques such as GC, LC-MS, and LC-NMR, which are more sophisticated and time-consuming than spectrophotometric procedures. Furthermore, they necessitate preceding extraction and separation operations, resulting in a cost and time increase. Spectrophotometric techniques are commonly used because they are simple to use, quick, accurate and inexpensive. As a result, spectrophotometric resolution of multicomponent combinations with overlapping spectra is a fascinating topic in analytical [20,33-36]

First Derivative Zero-crossing Method (1D)

Estradiol (E2) has a UV absorption spectrum that overlaps with progesterone's range (PRG). The zero-order absorption spectra of E2 and PRG, as well as their mixture, is shown in Fig. 2.0, with absolute ethanol serving as a reagent blank. There is a significant overlap of the spectrum. As a corollary, direct absorbance measurements will not be able to determine PRG during the occurrence of E2 or vice versa. Resolving mixtures using traditional spectrophotometry is difficult. As a result, derivative spectrophotometry can provide a satisfactory solution to this problem. As shown in Fig. 3, first derivative spectrum of E_2 solution undergoes zero absorption at wavelength 249.19nm, while PRG solution has absorption at this point. From this point of view zero-cross technique is used for determination of PRG in the presence of E_2 in the solution. On the other hand, PRG solution has zero absorption at wavelength 239.27nm and 292.51 nm, while E_2 solution has absorption at this point. So, E_2 has been determined in the presence of PRG in the solution.

Table1. demonstrates the results of statistical data of the calibration graphs using 1D spectrophotometry for simultaneous quantification of each E2 and PRG in binary mixture , Fig.4 shows 1D ratio spectra of (2.0 -32) µg/ml of PRG and (5.0 -140)µg/ml of E2.

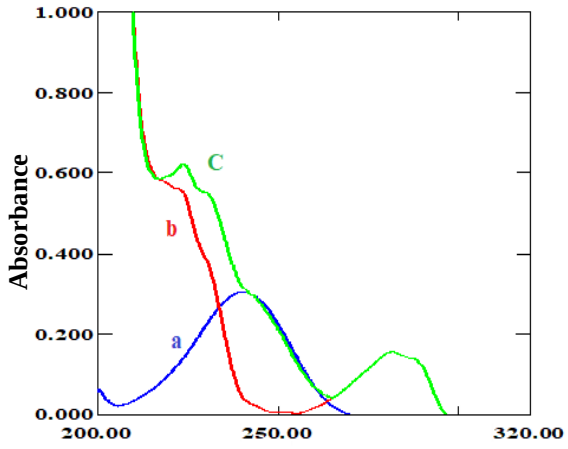


Figure 2. Zero-order spectra of (a) 8.0 µg/mL of PRG, 20 µg/mL of E2 and (C) their mixture in absolute ethanol

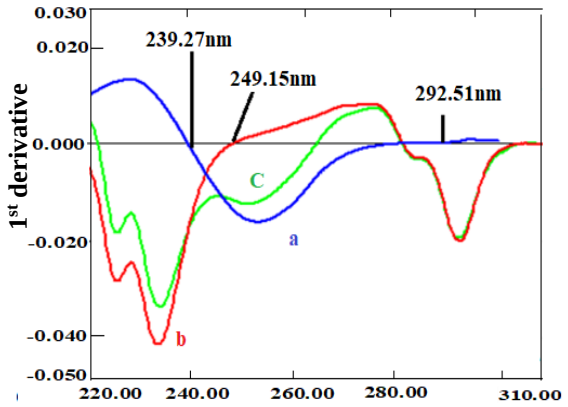


Figure 3. First derivative spectra of 20.0 µg /ml of E2, 8.0 µg/ml of PRG, and their combination in absolute ethanol.

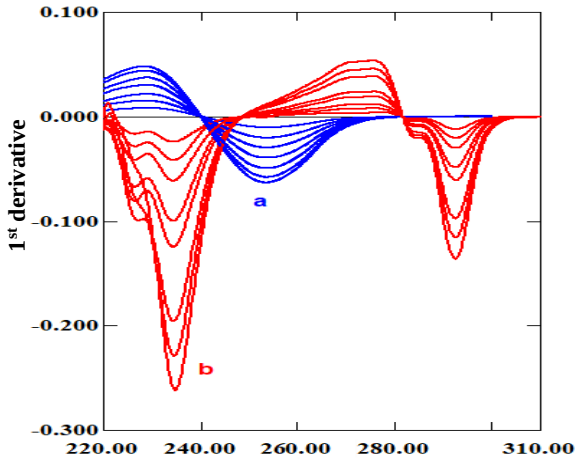


Figure 4. First derivative spectra of (a) (2.0-32) µg/ml **PRG and (b) (5.0-140) µg/ml E2**

Ratio Subtraction Method (RSM)

The RSM starts with a scan of the normal spectra of PRG standard solutions prepared in absolute ethanol, then confirms the linearity between absorbance at the chosen λmax of 240 nm and related to PRG concentration [19,30]. The determination of PRG (X) can be achieved by scanning the zero order absorption spectra of the laboratory prepared mixtures (PRG and E2), dividing them by a carefully chosen concentration (50 µg /ml) of standard E2 (Y' = divisor) producing a new ratio spectra (X/Y') + constant as illustrated in Fig. 5. This was followed by subtraction of the absorbance values of these constants (Y / Y') in plateau region 268 - 300 nm as shown in Fig.6, followed by multiplication of the obtained spectra by (Y') the divisor as shown in Fig.7. Finally, the original spectrum of PRG (X) was obtained and used for direct determination of PRG(X) at 240nm and the concentration calculated from the Eq.17

$P_{PRG} = 0.0382\ x - 0.0156$ 17

Where x is the concentration of PRG in µg/ml and P is the peak values of the normal spectrum of PRG at 240 nm. Though, to determine E2 alone, a calibration graph is created by connecting the absorbance of ^{0}D E2 spectra at 281.40 nm, wherever PRG illustrated no absorbance Fig. 2, concentration of E2(Y) can be calculated in Eq. 18

$P_{E2} = 0.0079\ x + 0.0306$ 18

Where x is the E2 concentration in µg/ml, and P is the peak value of E2's normal spectrum at 281.40 nm. Table.1, displays the statistical results from the quantified calibration curves for simultaneous PRG and E2 evaluation using the ratio subtraction.

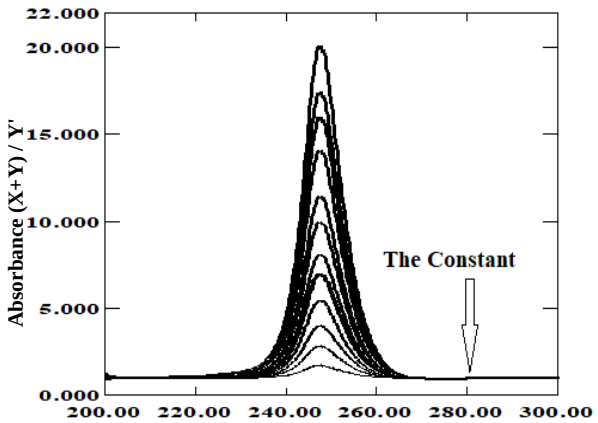


Figure 5. Ratio spectra of laboratory generated combinations of PRG (X) and E2 (Y) using 50 µg/ml of E2 (Y') as a divisor and absolute ethanol as a blank.

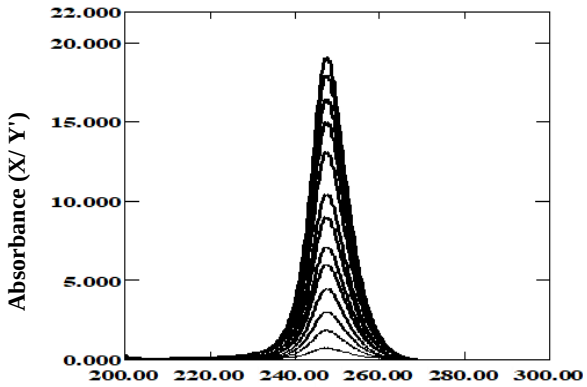


Figure 6. Ratio spectra of laboratory generated combinations of PRG (X) and E2 (Y) using 50 µg/ml of E2 (Y') as a divisor and absolute ethanol as a blank after subtracting of the constant.

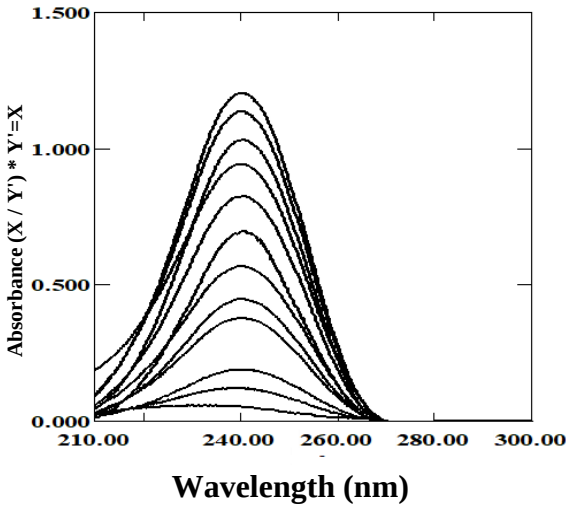


Figure7. Normal absorption spectra of PRG gained by RSM for the analysis of laboratory generated combinations after multiplication by 50 µg/ml of E2 divisor.

Modified Amplitude Subtraction Method (MAS)

At wavelength 243.66 nm, the first derivative spectra of E2 and PRG overlap, but PRG makes no contribution at wavelength 292.51 nm, as seen in Fig.8. A calibration curve exhibiting a linear connection between the peak values of E2 at 243.66 nm and 292.51 nm was generated in the range of 5.0-140 µg/ml, also PRG has calibration curve at 243.66 nm in the range of 2.0 -32 µg/ml and the regression equations was discovered to be

$P_{E2 \text{ at } 292.51nm} = 0.001\,x + 0.0005$ …..…… 19

$P_{E2 \text{ at } 243.66nm} = 0.0003x + 0.0007$ ……..20

$P_{PRG \text{ at } 243.66nm} = 0.0007x + 0.0003$…….21

Where $P_{E2 \text{ at } 292.51nm}$: absorbance of mixture (E2+PRG) at λ2=292.51nm, X: concentration of E2, 0.0005: absorptivity and 0.001: slop.

$P_{E2 \text{ at } 243.66nm}$: absorbance of mixture (E2+PRG) at λ1=243.66nm, X: concentration of E2, 0.0007: absorptivity and 0.0003: slop.

$P_{PRG \text{ at } 243.66nm}$: absorbance of mixture (E2+PRG) at λ1=243.66nm, X: concentration of PRG, 0.0003: absorptivity and 0.0007: slop.

The concentration of E2 may be simply estimated using the obtained regression Eq. 19 after scanning the mixture and detecting the peak value at 292.51 nm. Using the acquired regression equation and the computed E2 concentration, the peak amplitude of E2 at 243.66 nm may be determined in Eq.20. Finally, by subtraction the hypothesized peak values of E2 at 243.66 nm from the measured peak values of the mixture at the same λmax, the peak values of PRG can be derived. The concentration of PRG may be estimated using the Eq.21 that represents a linear correlation between the peak values first derivative spectra of PRG at 243.66 nm from 2.0-32.0 µg/ml. Table1 illustrates the results of statistical data of the calibration graphs using modified amplitude subtraction method for simultaneous quantification each of E2 and PRG in binary mixture

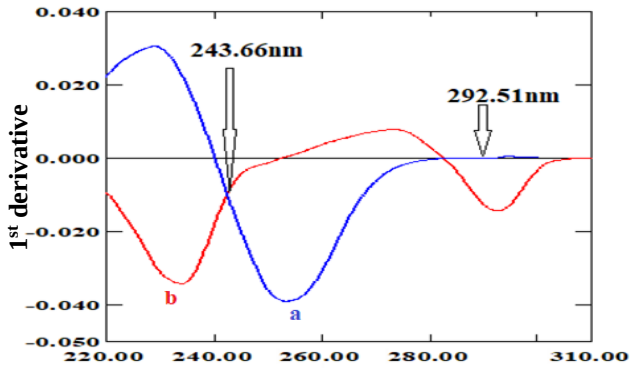


Figure8. First derivative spectra of (a) 20.0 µg /ml PRG and (b) 20.0 µg/ ml of E2

Q-Absorbance Ratio Spectra Method

Fig. 9 shows the iso-absorptive point at which both drugs have the same absorbance value and λ max for PRG. Absorbance of each drug was measured twice; at the iso-absorptive point and at λ max for PRG, then calibration for each drug alone was performed and specific absorptivities of both drugs were determined. Relative concentration of the two drugs in the mixture was calculated by applying the Eq.22 and 23.[37-39]

$Cx = [(QM-Qy)/(Qx-Qy)] \times A1/ax1$ ……..22

$Cy = [(QM-Qx)/(Qy-Qx)] \times A1/ay1$ ……23

Where $QM = A_2$ (absorbance of the sample at 240 nm)/ A_1 (absorbance of the sample at 262 nm), $Qx = ax_2$ (absorptivity of PRG at 240 nm)/ ax_1 (absorptivity of PRG at 262 nm), $Qy = ay_2$

(absorptivity of E2 at 240 nm) / ay_1 (absorptivity of E2 at 262 nm).

A1 and A2 are the absorbance of the mixture at 262 nm and 240 nm, respectively. ax_1 and ay_1 are the absorptivities of PRG and E2 at 262 nm, ax_2 and ay_2 are the absorptivities of PRG and E2 at 240 nm. Table 1. illustrates the results of statistical data of the calibration graphs using Q-absorbance ratio spectra technique for simultaneous quantification each of E2 and PRG in binary combination.

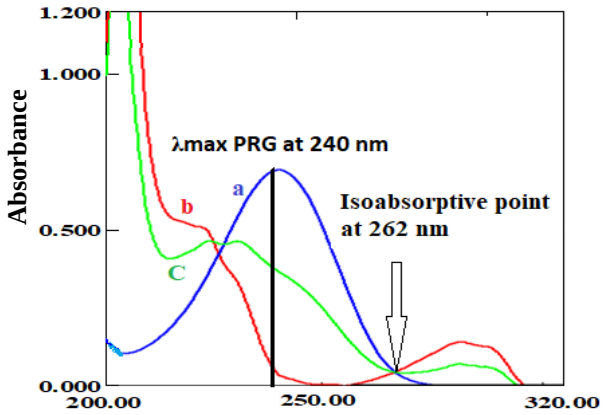


Figure 9. Normal spectra of (a) 20.0µg/ml PRG, (b) 20.0µg/ml E2 and (C) their mixture (10.0+10.0µg/ml) of each.

Table 1. The statistical parameters for quantification of binary mixture E2 and PRG using the proposed techniques.

Methods	Compounds	λmax (nm)	Linearity µg/mL	Regression equation	r^2	LOD µg/ml
1D	E2	239. 27	5.0-130	y= 0.0009x+0.0154	0.9997	1.33
1D	E2	292.51	5.0-130	y= 0.0009x+0.0057	0.9996	1.23
RSM	E2	281.40	5.0 – 140	y= 0.0079x + 0.0306	0.9999	1.58
MAS	E2	243.66	5.0-140	y= 0.0003x + 0.0007	0.9995	1.57
MAS	E2	292.51	5.0-140	y= 0.001x+0.0005	0.9996	1.37
Q-Abs.	E2	240	5.0-140	y= 0.0018x+0.0311	0.9995	1.44
Q-Abs.	E2	262	5.0-140	y= 0.0018x +0.0377	0.9996	1.49
1D	PRG	249.15	4.0-30.0	y= 0.0016x + 0.0004	0.9995	1.08
RSM	PRG	240	2.0 – 32.0	y= 0.0382x - 0.0151	0.9998	0.31
MAS	PRG	243.66	2.0-32.0	y= 0.0007x + 0.0003	0.9996	0.65
Q-Abs.	PRG	240	2.0-32.0	y= 0.00382x -0.0151	0.9998	0.31
Q-Abs.	PRG	262	2.0-32.0	y= 0.005x – 0.0007	0.9996	0.52

Accuracy and Precision

Under the optimum conditions, the accuracy and precision of the (1st derivative spectrophotometric, RSM , AMS and Q-Absorbance ratio spectra technique) techniques for simultaneous determination of E2 with PRG in binary mixture under linearity were studied depending upon the values of the error percentage (Error %) and relative standard deviation percentage (RSD %) for five replicated measurements of three different standard concentrations. The results of error studies for both drugs, are not greater than ±5.0 % which designates that the proposed methods have acceptable accuracy. Moreover, the RSD values for the determination of drugs are less than 4.44 %. The results are illustrated in Table. 2.

Table 2. Accuracy and Precision of the proposed techniques for simultaneous quantification of E2 and PRG in a binary combination.

Compounds	Technique of analysis	Concentration (µg /ml)	Error%	RSD%
E2	^{1}D 239.27nm	5.0	-2.22	4.44
		60	-2.96	1.41
		130	-1.37	0.76
	^{1}D 292.51 nm	5.0	+4.44	0.55
		60	-4.26	0.78
		130	-3.67	0.78
	^{0}D 281.40 nm	5.0	-3.79	1.25
		60	+1.20	0.10
		140	+0.11	0.30
	MAS at 243.66 nm	5.0	+3.31	0.80
		60	-1.66	0.52
		140	-4.28	0.23
	MAS at 292.51 nm	5.0	-2.0	4.43
		60	-0.83	3.26
		140	-1.78	0.33
	Q- Abs. at 240nm	5.0	-2.22	1.13
		60	-0.19	0.76
		140	-1.74	0.31
	Q- Abs. at 262nm	5.0	+2.22	0.18
		60	0.55	0.59
		140	-0.63	0.37
PRG	^{1}D 249.15 nm	2.0	-4.69	3.26
		15.0	+ 0.89	3.07
		30	-0.83	0.74
	RSM at 240 nm	2.0	+3.53	1.10
		18.0	-1.09	0.11
		32.0	-0.02	0.06
	MAS at 243.66 nm	2.0	+3.57	0.43
		18.0	+0.79	0.10
		32.0	+1.33	1.18
	Q- Abs. at 240 nm	2.0	+3.53	1.10
		18.0	-1.09	0.11
		32.0	-0.02	0.06
	Q- Abs. at 262 nm	2.0	+5.00	1.40
		18.0	-1.89	1.13
		32.0	-0.43	0.62

Study of Interferences

The influences of different compounds on the simultaneous determination of binary mixture E2 with PRG using four different spectrophotometric techniques were studied. Results indicated that the compounds under study with the mentioned concentration did not interfere, as shown in Table.3.

Table 3. T limit and error% of some interfering compounds on the simultaneous determination of binary mixture of E2 with PRG

Methods	Lactose T. limit 1400 µg/ml		Starch T. limit 800 µg/ml		Poly ethylene glycol T. limit 2000 µg/ml		Microcrystalline-cellulose T. limit 1000 µg/ml	
	E2 Error%	PRG Error%	E2 Error%	PRG Error%	E2 Error%	PRG Error%	E2 Error%	PRG Error%
1D	- 4.23	+ 4.67	- 4.79	- 4.93	+ 4.09	+ 3.93	not effected	not effected
RSM	+4.65	+ 4.39	+ 4.33	- 4.78	- 3.84	+4.77	not effected	+4.36
MAS	+ 4.71	+ 4.28	+ 4.91	+4.19	+ 4.66	- 4.83	not effected	- 4 .47
Q-Abs.	+ 4.75	+ 4.18	+ 4.81	+ 4.38	+ 4.27	+ 4.89	+ 4.92	+ 4.07

Application of Methods

The proposed techniques were applied successfully for simultaneous quantification of binary mixture of E2 with PRG in pharmaceutical synthetic mixture samples with the help of standard addition methods. The results of the application and recovery study are summarized in Table. 4.

Table 4. Determination of E2 and PRG in synthetic mixtures by the proposed techniques

Methods	taken (µg/ml)	Spiked(µg/ml)	E2 Found (µg/ml) ± SD[a]	Recovery %	PRG Found (µg/ml) ± SD[a]	Recovery %
1D	15	0	14.47± 0.071	99.52	14.32± 0.076	99.37
	15	5	19.5 ±0.058	99.48	19.47± 0.067	99.43
	15	10	25.50 ±0.116	99.21	24.21± 0.079	99.54
	15	15	29.50 ±0.075	99.56	30.25± 0.061	99.60
RSM	15	0	14.48± 0.213	98.61	14.79± 0.102	99.32
	15	5	20.68± 0.068	99.37	20.29± 0.153	99.51
	15	10	24.48± 0.081	99.35	25.26± 0.106	99.21
	15	15	96.67±0.103	99.32	30.50± 0.251	98.36
MAS	15	0	14.20±0.108	98.59	14.37± 0.086	100.76
	15	5	19.30± 0.082	99.17	19.51± 0.095	100.46
	15	10	24.60± 0.128	98.38	25.14± 0.075	99.61
	15	15	29.80± 0.101	99.32	30.43± 0.115	98.65
Q-Abs.	15	0	14.40± 0.076	98.95	14.28± 0.095	100.69
	15	5	19.34± 0.051	99.48	19.40±0.072	100.72
	15	10	24.22± 0.080	99.29	25.15± 0.072	99.44
	15	15	29.36± 0.159	99.18	30.34± 0.098	99.39

a) Average of three determination

Statistical Analysis

The E2 and PRG employed a one-way ANOVA test to compare the ratio subtraction modified amplitude subtraction and Q-absorbance ratio spectra approach with traditional derivative spectrophotometry (zero-crossing methodology). Snedecor's F-values were computed and compared to the conventional tabular value (P = 0.05) for this purpose. For each insecticide, the same computing procedures were used. Table. 5. demonstrates that in the analysis of variance, the calculated or experimental F values are less than the tabulated values, indicating that there were no significant differences between the four approaches.

Table 5. One way ANOVA a statistical study results of E2 and PRG in synthetic capsule obtained by applying four proposed techniques

		Sum of squares	Degree of freedom	Mean square	F	Sig
E2	Between groups	0.681225	3	0.227075	2.19511	0.14147
	Within groups	1.24135	12	0.103446		
	Total	1.922575	15			
PRG	Between groups	1.056867	3	0.352289	0.804496	0.52571
	Within groups	3.5032	8	0.4379		
	Total	4.560067	11			

Comparison with Other Methods

A comparison has been done between some of analytical variables obtained from the proposed methods for determination of estradiol and progesterone with other previous published methods [11-13] and the results summarized in Table. 6.

Table 6. Comparison of the proposed methods with some other Literature methods

			Estradiol			
Analytical parameter	**Zero crossing**	**RSM**	**MAS**	**Q-Abs.**	**Literature method [12]**	**Literature method [12]**
Linearity range (µg/ml)	5 -140	5 -140	5 -140	5 -140	0.5 – 8.0	0.23 – 7.5
LOD (µg/ml)	1.23	1.28	1.37	1.44	0.14	0.05
Recovery (%)	99.21-100.40	98.61-99.37	98.38-99.32	98.95-99.48	91.75 – 104.62	978.75– 106.57
RSD (%)	0.16	0.10	0.23	0.18	1.72	1.96
R^2	0.9997	0.9999	0.9996	0.9996	0.9967	0.9999
			Progesterone			
Analytical parameter	**Zero crossing**	**RSM**	**MAS**	**Q-Abs.**	**Literature method [12]**	Literature method [12]
Linearity range (µg/ml)	2 -32	2 -32	2 -32	2 -32	10 -22	0.6-1.4
LOD (µg/ml)	1.08	0.18	0.65	0.10	_____	_____
Recovery (%)	99.37-9.60	98.31-99.51	100.38-98.65	99.39-100.72	79.7 – 119.7	100.2– 101.8
RSD (%)	0.74	0.74	0.10	0.06	1.3	0.75
R^2	0.9995	0.9998	0.9996	0.9996	0.996	0.99986
Application	Pharmaceutical formulation	Pharmaceutical formulation	Pharmaceutical formulation	Pharmaceutical formulation	Pharmaceutical formulation, Analysis in oily injection	Pharmaceutical formulation, Analysis in oily injection

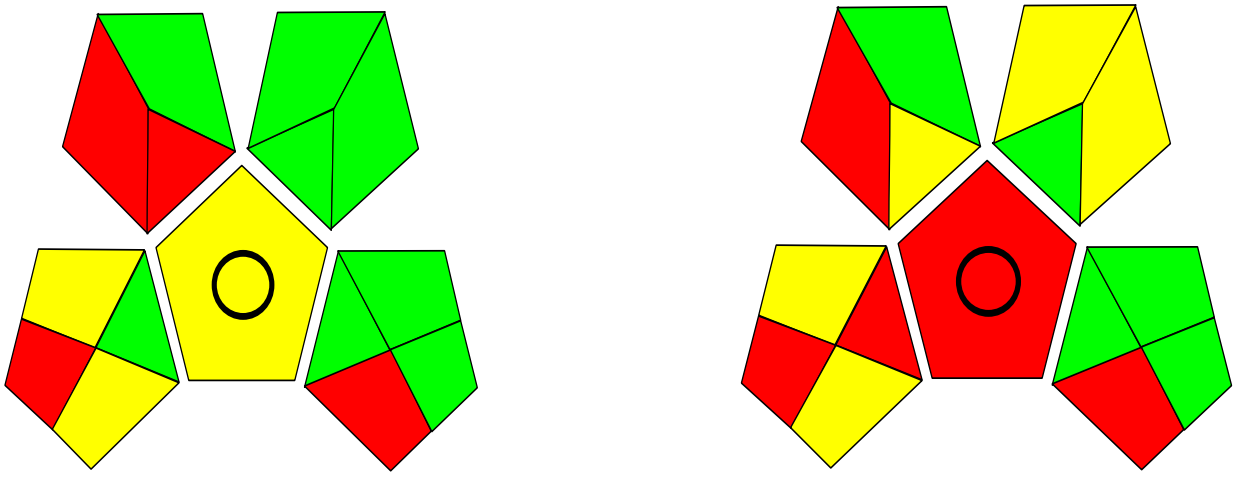
RSM: ratio subtraction method; MAS: Modified amplitude subtraction method; Q-Abs. : Q-Absorbance ratio spectra method; RSD%: percentage relative standard deviation

Greenness Assessment

The analyst's first aim is to develop an environmentally friendly technique that can be easily employed in routine analysis and has high efficiency and low cost. The goal of green analytical chemistry (GAC) is to develop an ecological approach that doesn't harm the environment or deal with dangerous compounds. Utilizing two analytical tools, the proposed methods' "greenness" was evaluated[40,41]

Green Analytical Procedure Index (GAPI) Approach

GAPI is a new tool to estimate the greenness of the overall method in the analytical process based on 15 different factors. According to the strength of the environmental influence, five pentagrams were created and coloured, each divided into three or four section. While yellow and red indicated medium and high environmental effects, respectively, green represented moderate environmental impact [40,42-44.] The proposed techniques showed eight green shaded sections, while the literature HPLC [15] technique demonstrated five green-shaded sections as shown in Fig.10.



(a) (b)

Figure 10. Analytical comparison between pictograms of (a) the proposed techniques and (b) other literature HPLC[15] ones for assessment of greenness

Analytical Eco-scale Approach

Analytical eco-scales are one of the greenness assessment tools that are skilled at assessing the efficacy of the technique and extracting measurable information about the method's environmental compatibility, taking into account the employed chemicals, instruments, and created waste. This tool relies on computing the analytical eco-scale value, which is obtained by assigning penalty points to all factors that hurt the environment, adding up all of these points, and then subtracting from a base of 100. The approach has a good green profile the higher the eco-scale value (greater than 85) that is obtained. Scores greater than 50 indicate competent green analysis, while scores less than 50 indicate insufficient green analysis [39,43,45-47] The result of eco-scale values for the proposed technique and literature HPLC method [15] are shown in Table. 7.

Table 7. Analytical comparison between eco-scale penalty points of the proposed techniques and other literature technique

Methods	Proposed methods	literature HPLC method [15]
	Reagents	
Ethanol	4	———
Methanol	———	6
Sodium hydroxide	———	1
Na_2HCO_3	———	0
Na CO_3	———	0
	Instrument	
Energy	0	2
Occupational hazards	———	———
Oven	———	2
Wastes	3	3
Total penalty points	5	14
Analytical eco-scale (Total score)	93	86

Conclusions:

The main task of this study is to find fast, accurate, sensitive and cost-effective spectrophotometric methods for the simultaneous quantification of E2 and PRG in pharmaceutical formulation. This research, employed the zero-crossing technique, RSM, MSM and Q-absorbance technique to identify a binary mixture of E2 and PRG in synthetic mixture samples. When employed to resolve a binary combination, these four approaches produced good results and did not need the use of complicated or costly gear. The efficacy of the recovered values ranged from 98.38 to 100.76percent with an RSD < 4.44 %. The impact of the proposed methods on the environment was assessed by the eco scale and green analytical procedure index (GAPI) tools, where the results take that proposed methods were the greenest than literature methods. Statistical studies shows that there is no discernible difference between the four strategies presented. Therefore, these four proposed approaches are typically suitable for the measurement of E2 and PRG.

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Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Salahaddin.

Authors' Contributions Statement:

D. K. A. carried out the experiment, wrote the manuscript, data analysis and performed the statistical analysis. Ch. M. R. designing, investigation, review. N. A. F. supervised the project.

References:

1. Soldin SJ, Soldin OP. Steroid hormone analysis by tandem mass spectrometry. Clin. chem. 2009;55(6):1061-6 https://doi.org/10.1373/clinchem.2007.100008
2.Michels KB, Binder N, Courant F, Franke AA, Osterhues A. Urinary excretion of sex steroid hormone metabolites after consumption of cow milk: a randomized crossover intervention trial. Amer. J

Clin.Nutr.2019;109(2):402-10. https://doi.org/10.1093/ajcn/nqy279
3. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. Curr. Top. deve. biol. 2017;125:109-46. https://doi.org/10.1016/bs.ctdb.2016.12.005
4. Ankarberg L C, Dahlgren J, Andersson MX. High-sensitivity quantification of serum androstenedione, testosterone, dihydrotestosterone, estrone and estradiol by gas chromatography–tandem mass spectrometry with sex-and puberty-specific reference intervals. J. ster. Bioch. Mole. biol. 2018;183:116-24. https://doi.org/10.1016/j.jsbmb.2018.06.005
5. Russell N, Grossmann M. Mechanisms in endocrinology: estradiol as a male hormone. Eur J Endocrinol.2019;181(1):R23-R43. https://doi.org/10.1530/EJE-18-1000
6. Najim SS. Spectrophotometric determination of progesterone and dopamine in breast cancer serum. U. Thi-Qar J. 2017; 12(1): 1-21
7. Honda L, Becerra HM, Richter P. Liquid chromatography–time-of-flight high-resolution mass spectrometry study and determination of the dansylated products of estrogens and their hydroxylated metabolites in water and wastewater. Anal Bioanal Chem. 2018; 410(30): 7909-19. https://doi.org/10.1007/s00216-018-1412-0
8. Desai R, Harwood DT, Handelsman DJ. Simultaneous measurement of 18 steroids in human and mouse serum by liquid chromatography–mass spectrometry without derivatization to profile the classical and alternate pathways of androgen synthesis and metabolism. Clin. Mass Spectrom. 2019; 11: 42-5 https://doi.org/10.1016/j.clinms.2018.12.0031.
9. Stanczyk FZ, Niu C, Azen C, Mirkin S, Amadio JM. Determination of estradiol and progesterone content in capsules and creams from compounding pharmacies. Menopause. 2019; 26(9): 966. doi:10.1097/GME.0000000000001356
10. Newman M, Pratt SM, Curran DA, Stanczyk FZ. Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC–MS/MS). BMC chem. 2019; 13(1): 1-12. https://doi.org/10.1186/s13065-019-0539-1
11. Attar AL, Redekar MP, Jadhav NR. Estimation of Progesterone in Oil based Injection by UV-Visible Spectroscopy. Curr Pharm Res. 2016; 6(2): 1825.
12. Aydoğmuş Z, Yılmaz EM, Yörüsün S, Akpınar S. Development of simultaneous derivative spectrophotometric and HPLC methods for determination of 17-beta-estradiol and drospirenone in combined dosage form. Int. Sch. Res. Not. 2015; 2015.
13. El-yazbi FA, Korany MA, Abdel-razak O, Elsayed MA. Derivative Spectrophotometric Analysis of Oestradiol Esters, Testosterone and Progesterone in Oily Injections. Anal Lett. 1985; 18(17): 2127-2142. https://doi.org/10.1080/00032718508067976
14. Toral MI, Soto C, Richter P, Tapai AE. Simultaneous determination of estradiol and medroxyprogesterone acetate in pharmaceutical formulations by second-derivative spectrophotometry. J AOAC Int. 2002;85(4):883-8. https://doi.org/10.1093/jaoac/85.4.883
15. Shishov A, Sviridov I, Timofeeva I, Chibisova N, Moskvin L, Bulatov A. An effervescence tablet-assisted switchable solvent-based microextraction: On-site preconcentration of steroid hormones in water samples followed by HPLC-UV determination. J Mole Liqu. 2017; 247: 246-53 https://doi.org/10.1016/j.molliq.2017.09.120.
16. Wu H, Ramsay C, Ozaeta P, Liu L, Aboleneen H. Serum estradiol quantified by isotope dilution–gas chromatography/mass spectrometry. Clin chem. 2002;48(2):364-6. https://doi.org/10.1093/clinchem/48.2.364
17. Wang Y, Zhang T, Zhao H, Zhou W, Zeng J, Zhang J, et al. Measurement of serum progesterone by isotope dilution liquid chromatography tandem mass spectrometry: a candidate reference method and its application to evaluating immunoassays. Anal bioanal chem. 2019; 411(11): 2363-71.
18. Arismendi D, Díaz K, Aguilera M N, Sepúlveda B, Richter P. Rotating-disk sorptive extraction for the determination of sex hormones and triclosan in urine by gas chromatography-mass spectrometry: Clean-up integrated steps and improved derivatization. MicrochemJ.2020;158:105149. https://doi.org/10.1016/j.microc.2020.105149
19. Asran A, Abdel Kader N, El-Ansary AL, Mohamed S. Simultaneous Quantification of Diaveridine and Sulfadimidine by Derivative and Ratio Derivative UV Spectroscopy. Egypt J Chem. 2020; 63(10): 3847-60 http://dx.doi.org/10.21608/ejchem.2020.22732.2353
20. Omer SA, Fakhre NA. Three different spectrophotometric methods for simultaneous determination of pyriproxyfen and chlorothalonil residues in cucumber and cabbage samples. J Spectro. 2019; 2019.https://doi.org/10.1155/2019/8241625
21. Murugan S, Vetrichelvan T. Absorbance Ratio and First Order Derivative Spectroscopic Methods for Simultaneous Determination of Sacubitril and Valsartan in Bulk and Tablet Dosage Form. Res J Pharm Techn. 2019; 12(11): 5251-4. http://dx.doi.org/10.5958/0974-360X.2019.00908.9
22. Redasani VK, Patel PR, Marathe DY, Chaudhari SR, Shirkhedkar AA, Surana SJ. A review on derivative uv-spectrophotometry analysis of drugs in pharmaceutical formulations and biological samples review. J Chin Chem Soc. 2018; 63(3): 4126-34. http://dx.doi.org/10.4067/s0717-97072018000304126
23. Bachri M, Masfria M, Syahputra S, Hafid H. Quantitative Estimation of Anti Hypertension Combination by Ratio Subtraction Spectrophotometry Method. Maced. J Medi Sci. 2019; 7(17): 2940. doi:10.3889/oamjms.2019.750
24. Sebaiy MM, Sobhy M, Mattar AA. Bivariate analysis and Ratio Subtraction coupled with extended ratio subtraction methods for simultaneous determination of Paracetamol and Diclofenac sodium in their combined pharmaceutical dosage forms. Chem. Res J. 2019; 4(6): 54-61

25. El-Bagary RI, Mowaka S, Elkady EF, Attallah MA. Validated spectrophotometric methods for determination of weakly UV absorbed perindopril arginine in bulk and combined dosage form. Anal ChemLett.2016;6(6):766-82. https://doi.org/10.1080/22297928.2016.1250673
26. Tejashree KR, Dashetwar A, Kardile D, Jadhav A, Bhagat V, Shete R. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of metformin hydrochloride and voglibose in tablet dosage form. Res J Pharm Techn. 2021; 14(6):3179-83. http://dx.doi.org/10.52711/0974-360X.2021.00554
27. El-Abasawy N, Attia K, Abouserie A, El-Olemy A, Elsayed A. Different spectrophotometric methods applied for simultaneous analysis of binary mixture of formoterol and fluticasone: a comparative study. J Anal Pharm Res. 2018; 7(2): 160-5. https://www.researchgate.net/publication/328288227
28. Sebaiy MM, Mattar AA. Q Absorbance Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in Their Combined Pharmaceutical Dosage Forms. Eur. J Pharm Med Res. 2020;7:464-8.
29. Higashi T, Ito K, Narushima M, Sugiura T, Inagaki S, Min JZ, et al. Development and validation of stable-isotope dilution liquid chromatography–tandem mass spectrometric method for determination of salivary progesterone. Biomed Chromatogr. 2011; 25(11): 1175-80. https://doi.org/10.1002/bmc.1586
30. EL-Fatatry HM, Mabrouk MM, Hammad SF, Elsheikh SH. Different ratio spectra methods for simultaneous determination of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate. J Innov Pharm Bio Sci. 2016; 3(2): 166-81.
31 Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis. 2nd ed. Academic press imprint of Elsevier 2011.
32. Almas S. Derivative Uv Spectroscopic approaches in multicomponent analysis–A review. Int J Pharm Pharm Sci. 2019; 11(2): 1-11
33.Alsamarrai KF., Alsamarrai H.A.J. Spectrophotometric determination of Metronidazole and Metronidazole benzoate via first and second derivative order spectroscopy. Baghdad Sci J. 2017;14(2):320-7. https://doi.org/10.21123/bsj.2017.14.2.0320
34. Alaallah N.J, Dhahir SA, Ali HH. Determination of Sulfacetamide Sodium in Pure and Their Pharmaceutical Formulations by Using Cloud Point Extraction Method. Baghdad Sci J. 2021; 18(3): 0575. http://dx.doi.org/10.21123/bsj.2021.18.3.0575
35. Atole DM, Rajput HH. Ultraviolet spectroscopy and its pharmaceutical applications-a brief review. Asian J Pharm Clin Res. 2018; 11(2): 59-66. https://doi.org/10.22159/ajpcr.2018.v11i2.21361
36. Shukla SS, Nene R, Pandey W, Pandey RK. Development and validation of ratio spectra Dderivative spectrophotometric method for estimation of chlorpheniramine maleate and diphenhydramine hydrochloride. Int J pharm phytophamacol Res.2019; 9(6): 109-115.
37. Jawale P, Bhusari S, Wakte P. Development and validation of Q-Absorbance Ratio UV spectrophotometric Method for the simultaneous estimation of metformin and empagliflozin in bulk and pharmaceutical dosage form. Sci Technol Dev. 2022; 11(4): 0950-0707.
38. Qadir HK, Qader HA. Q-bsorbance ratio, derivative spectrophotometric and H-Ppoint standard addition methods for simultneouse determination of pridinol mesylate, and dicelofenac sodium inpharmaceutical formulation.JUOZ. 2021; 33(4): 43-60. http://dx.doi.org/10.21271/zjpas
39. Jawale A, Sachin SR, Chaudhari RY, Patil VR, Lokesh B. Development and validation of Q-Absorbance Ratio UV spectrophotometric Method for the simultaneous estimation of metformin and empagliflozin in bulk and pharmaceutical dosage form. Int J Nov Res Dev. 2022; 7(3): 2456-4184.
40. Płotka W J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta. 2018; 181: 204-9. https://doi.org/10.1016/j.talanta.2018.01.013
41. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White Analytical Chemistry: An approach to reconcile the principles of Green Analytical Chemistry and functionality. TrAC. 2021;138:116223. https://doi.org/10.1016/j.trac.2021.116223
42. Ferreira SS, Brito TA, Santana AP, Guimarães TG, Lamarca RS, Ferreira KC, et al. Greenness of procedures using NADES in the preparation of vegetal samples: Comparison of five green metrics. Talanta. 2022; 6: 100131. https://doi.org/10.1016/j.talo.2022.100131
43. Pena P F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Anal Chem. 2020; 92(14): 10076-82. http://dx.doi.org/10.1021/acs.analchem.0c01887
44. El-Masry AA, El-Wasseef DR, Eid M, Shehata IA, Zeid AM. Development of three ecological spectroscopic methods for analysis of betrixaban either alone or in mixture with lercanidipine: greenness assessment. R Soc Sci. 2022; 9(2): 211457.https://doi.org/10.1098/rsos.211457
45. Saleh SS, Lotfy HM, Tiris G, Erk N, Rostom Y. Analytical tools for greenness assessment of chromatographic approaches: Application to pharmaceutical combinations of Indapamide, Perindopril and Amlodipine. Microchem J. 2020; 159:105557. https://doi.org/10.1016/j.microc.2020.105557
46. Dias LD, Gonçalves KH, Queiroz JE, Vila Verde GM, Aquino GL. An eco-friendly and alternative method of forced degradation of fluoroquinolone drugs by microwave irradiation: a new application for analytical eco-scale. J. Micr. Powe. Elect. Ener. 2018;52(3):162-81. https://doi.org/10.1080/08327823.2018.1494470
47. Mohamed D, Fouad MM. Application of NEMI, Analytical Eco-Scale and GAPI tools for greenness assessment of three developed chromatographic methods for quantification of sulfadiazine and

trimethoprim in bovine meat and chicken muscles: Comparison to greenness profile of reported HPLC methods. Microchem. J. 2020;157:104873. https://doi.org/10.1016/j.microc.2020.104873

التقدير الاني للمزيج الثنائي للإ ستراديول و البروجسترون باستخدام طرق قياس الطيف الضوئي المختلفة

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الخلاصة:

أربع تقنيات طيفية مشتقة سريعة ودقيقة وبسيطة للغاية تم استخدامها من أجل التقدير الكمي للمزيج الثنائي من استراديول والبروجسترون المصنّعة على شكل كبسولة. الطريقة الأولى هي قياس الصفرى للمشتق الأول تم اكتشاف السعات المشتقة عند طول موجة عبور صفرى239.27 و292.51 نانوميتر لتقدير استراديول و 249.19 نانوميتر للبروجسترون. الطريقة الثانية هى الطرح النسبي يتم التقدير البروجسترون عند 240 نانوميتر بعد طرح التداخل الذى يمارسه استراديول. الطريقة الثالثة هى طرح السعة المعدلة تم انشاؤه بأستخدام التحليل الطيفى المشتق والتلاعب الرياضي. الطريقة الرابعة هي تقنية نسبة الأمتصاص تم قياس الأمتصاصية لكلا الدواءين عند طولين موجيين نقطة الأمتصاص متساوية λ1=260 ونقطة امتصاصλ2=240 لبروجسترون ويتم حساب التراكيز النهائية بواسطة معادلة Q. منحنى المعايرة خطي من 140 – 5 و 32 – 2 ميكرو غرام /مل لاستراديول وبروجسترون على التوالى. تم اختبار انتقائية التقنيات المقترحة بأستخدام توليفات تركيبة تم ا إنشاؤها فى المختبر وتم تقيمها بأستخدام طريقة الإضافة القياسية. بأستخدام ANOVAأحادى الأتجاه تمت مقارنة مخرجات الطرق المقترحة ولم تضهرالنتيجة أي فروق ذات دلالة احصائية بين التقنيات المقترحة.

الكلمات المفتاحية: طريقة النسبة الأمتصاص، القياس الطيفي المشتق، استراديول، بروجسترون، الطرح النسبي.