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APPLICATION OF MULTIVARIATE CALIBRATION AND DERIVATIVE ANALYSIS IN THE SPECTROPHOTOMETRIC DETERMINATION OF SOME ASCORBIC ACID PHARMACEUTICAL MIXTURES

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تم تطبيق طريقتين طيفيتين مدعومتين بالحسابات الإحصائية لتقدير حمض الأسكوربيك (فيتامين ج) سواء منفردا أو مخلوطا مع أي من حمض الأسيتيل ساليسيليك (الأسبرين) أو الساليسيلاميد أو الدايبيرون (النوفالجين). والطريقتان هما: التحليل التعييري متعدد المتغير والتحليل بالمشتقة الأولى. وقد أظهر المنحني الطيفي تداخلا معتبرا بين منحنى الأسكوربيك وأي من المنحنيات الثلاث. وكان معدل الاسترجاع كبيرا في كلتي الطريقتين حيث تراوح بين ٩٨,٥٢% و ١٠٠,٦٣%. وتم تحديد الأطوال الموجية لقياس المركبات الدوائية في وجود بعضها البعض باستخدام طريقة المشتقة الأولى. وتم تطبيق الطريقتين على العديد من المستحضرات الصيدلية المتوفرة بالسوق المحلية المصرية. وكذلك مقارنة نتائج الطرق المقترحة بالطرق الدستورية ؛ حيث ثبت توافقا كبيرا بينهما.

Two chemometric assisted spectrophotometric methods; multivariate calibration and zero-crossing first derivative analysis were applied for the determination of ascorbic acid (vitamin C) in its single pharmaceutical formulations and its simultaneous determination in mixtures with either acetyl salicylic acid (aspirin), salicylamide or dipyrone (novalgin). The components of the three mixtures show a considerable degree of spectral overlapping. Resolution of the binary mixtures under investigation has been accomplished by using classical least squares (CLS) or by using the first derivative analysis. Good recoveries were obtained by both methods. In the CLS determintions, the recoveries for the first mixture were 98.76±1.24 and 98.52±1.48 for ascorbic acid and acetyl salicylic acid respectively. In the second mixture, the recoveries were 100.38± 0.38 and 100.63±0.63 for ascorbic acid and salicylamide respectively. In the third mixture, the recoveries were 100.48±0.48 and 98.53± 1.47 for ascorbic acid and dipyrone respectively. On application of the first derivative method, the recoveries were 97.76±2.24 and 98.60±1.40 for ascorbic acid and acetyl salicylic acid mixtures; 102.60± 2.60 and 100.38± 0.38 for ascorbic acid and salicylamide; 98.53±1.47 and 99.28± 0.72 for ascorbic acid and dipyrone mixtures. Wavelengthes are given at which ascorbic acid and either of the three combined drugs were simultaneously determined by the first derivative spectrophotometry. The mixtures were simultaneously determined in many commercial dosage forms with high accuracy without interference from the commonly encountered excipients. Good agreement was found between results obtained with the two suggested methods and those obtained by the reported pharmacopoeial methods.

INTRODUCTION

Spectrophotometric methods, even after introduction of other instrument-based procedures, continue to be of interest because of the ease in accessibility and the quick applicability to the routine analysis. The great advent and wide spread of the laboratory computer has allowed analytical chemists to use the chemometric methods for facile and accurate determinations. In pharmaceutical analysis, chemometrics have increasingly wide applications,[1-5] especially on analyzing formulations of bi- or multi-component mixtures. Multivariate calibration methods, as chemometric methods applied to spectral data, are currently widely used for pharmaceutical analysis.[6-11] Classical least squares (CLS)

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analysis is one of the simplest multivariate methods that can be performed with easily accessible statistical software. On the other hand, derivative spectrophotometry is a fast, simple and rapid technique that is useful for determining drugs in multicomponent systems or single component formulations in the presence of interfering excipients.[12] It enhances the qualitative features and thus increases the fingerprinting utility for selective identification and quantification of pharmaceutical compounds.[13]

In the present work, the possibility of determining ascorbic acid was investigated both in single formulations and in the simultaneous analysis of combined formulations of ascorbic acid either with acetylsalicylic acid, salicylamide or dipyrone in certain vitamin C- analgesic binary mixtures were investigated. The suggested methods are based on the spectrophotometric measurements in the general range of 210-350 nm together with either multivariate calibration or first derivative analysis.

The importance of ascorbic acid (vitamin C) to the human body is widely acknowledged through the globe. It is an essential vitamin occuring in different concentrations in a variety of natural samples. It is also added to several pharmaceutical products as an essential ingredient or in conjunction with other ingredients such as the studied analgesics. It is also used as a stabilizer for vitamin B-complex and as an antioxidant. It has many desirable effects and it is widely used in the treatment of certain diseases such as scurvy, common cold, anaemia, haemorrhagic disorders, wound healing and infertility. It is also essential for the development and regeneration of muscles, bones, teeth and skin.[14] The increasing use of pharmaceuticals and other natural samples containing vitamin C calls chemists to develop increasing numbers of methods for its analysis. Since spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, a great number of these methods have been proposed, reported and reviewed.[14-32]

Acetyl salicylic acid (aspirin), a commonly used well-known analgesic drug, was previously determined spectrophotometrically by many methods.[33-49] Chemometric methods such as partial least square multivariate calibration,[33] second derivative,[34-36] third derivative[37] and isosbestic point of derivative absorption curves[38] were also applied. In combination with vitamin C, aspirin was simultaneously spectrophotometrically determined.[39-42] In addition, many spectrophotometric methods applying the direct UV measurements were also reported.[43-49]

Spectrophotometric determination of the other two commonly used analgesics; salicylamide[50-54] and dipyrone[55-65] were also reported. Compound products of vitamin C and dipyrone were determined.[64] Two component mixtures of ascorbic acid and dipyrone were previously assayed by first derivative, second derivative, delta absorption, first derivative of the delta absorption, and second derivative of the delta absorption spectrophotometric methods applying the zero-crossing techniques of measurements.[65]

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were carried out on a computerized UV-1601 PC, UV-visible Shimadzu spectrophotometer (Tokyo-Japan), using 1.00 cm quartz cells. The obtained spectral data were saved in PC Shimadzu program and the subsequent statistical manipulation was performed by transferring the spectral data to Microsoft excel 2000 program and processing them with the standard curve fit package and matrix calculations.

Chemicals

Pharmaceutical grade ascorbic acid (Egyptian Co for Chemicals and Pharmaceuticals, S.A.E., Egypt), acetyl salicylic acid (Chemical Industries Development, CID, Giza, Egypt), salicyamide (Chemical Industries Development, CID, Giza, Egypt) and dipyrone (Memphis, Cairo, Egypt) were used as working standards after confirming their purity and compliance with the pharmaceutical requirements. All solvents and working reagents used throughout this work were analytical grade.

Dosage forms

The following investigated formulations were obtained from the local Egyptian market:

A- Ascorbic acid binary mixtures

1- Aspocid-C effervescent tablets (Chemical Industries Development, CID, Giza, Egypt); Labeled to contain 250 mg ascorbic acid and 500 mg acetyl salicylic acid.
2- Cidal-C tablets (Chemical Industries Development, CID, Giza, Egypt); labeled to contain 50 mg ascorbic acid and 500 mg salicylamide.
3- Cevagine ampoules (Memphis, Egypt) labeled to contain 1g ascorbic acid and 1g dipyrone.

B- Ascorbic acid formulations

- Cevarol tablets (Memphis, Egypt); labeled to contain 500 mg ascorbic acid.
- Vitacid C effervescent tablets (Chemical Industries Development, CID, Giza, Egypt); labeled to contain 1g ascorbic acid.
- Ascorbovit effervescent sachs (Pharco, Egypt); labeled to contain 1g ascorbic acid.
- Cevarol ampoules (Memphis, Egypt); labeled to contain 1000 mg ascorbic acid.
- Cevilene drops (El-Kahira, Egypt); labeled to contain 100 mg/ml ascorbic acid.
- C-vit drops (Lotas Pharma, Egypt); labeled to contain 100 mg/ml ascorbic acid.

Preparation of standards

Ascorbic acid and acetyl salicylic acid, salicylamide or dipyrone mixtures were prepared by dissolving an accurately weighed amount (100 mg) of each drug in 0.1 N HCl, then diluting quantitatively with the same solvent solution to obtain the appropriate dilution for each drug according to its linear calibration range.

Preparation of samples

1- Tablets

Twenty tablets were accurately weighed and finely powdered. An accurately weighed amount of the powder equivalent to one tablet was transferred to 100-ml volumetric flask and diluted to about 80 ml with 0.1N HCl. The mixture was sonicated well for about 10 minutes, diluted to the mark with the same solvent and filtered. The first portion of the filtrate was discarded. The clear solution obtained was used as stock sample solution. Suitable aliquots of the stock solution were quantitatively diluted with 0.1 N HCl to obtain the suitable working sample solution for UV-measurements.

2- Effervescent tablets and sacks

An effervescent tablet or the content of one sach is dissolved in about 200 ml of distilled water in a 250-ml beaker. When all effervescence ceased, the solution is transfered quantitatively to a 250-ml volumetric flask and completed to the mark with distilled water. This solution was used as a stock sample solution and suitable aliquots of it were quantitatively diluted with 0.1 N HCl to obtain the suitable working sample solutions for UV-measurements.

3- Drops and Ampoules

The content of one ampoule or an accurately measured volume of the drops equivalent to 200 mg of ascorbic acid is transferred quantitatively to a 100-ml volumetric flask and diluted to the mark with 0.1 N HCl. This solution was used as a stock sample solution and suitable aliquots of it were quantitatively diluted with 0.1 N HCl to obtain the suitable working sample solutions for UV-measurements.

Standard solutions for multivariate calibration and derivative analysis

In order to obtain the calibration matrix for applying CLS & first derivative analysis, five solutions of each of the pure components; ascorbic acid, acetyl salicylic acid, salicylamide and dipyrone were prepared with concentrations in the range of 5-25, 5-25, 8-40 and 8-80 µg/ml respectively. These ranges were previously verified to obey Beer's law for each of the studied drugs in 0.1 N HCl. The absorption data in the range of 210-350 nm (digitized every 1.0 nm) were subjected to least squares analysis in order to obtain the calibration K matrix (see below) and to first derivative analysis in order to determine the zero crossing wavelengths. Laboratory prepared mixtures were then prepared by mixing known amounts of ascorbic acid with either acetyl salicylic acid, salicylamide, or dipyrone in different varied proportions in 0.1 N HCl (Tables 1-3), in order to verify the precision of the method for analysis of such mixtures and matching the purchased formulations with those having comparable concentrations.

Data processing

Data were processed on an Intel Pentium III 750 MHz PC-compatible computer. The VISTA 6 version 6.4.3436-EWU (May 10, 2001) software was used for the Principal component applications. The parameters chosen to compare the different models were the relative root mean squared error (RRMSE) and the standard deviation of the model fitness (σ_{fit}):

$$RRMSE\ (\%) = 100 \times \sqrt{\frac{\sum(C_i - \hat{C}_i)^2}{\sum C_i^2}} \qquad (1)$$

Where $\hat{C}_i$ and C_i are the predicted and the real concentrations respectively, for the compound in the standard or sample solutions.

$$\sigma_{fit} = \sqrt{(A_{act} - A_{pred})^2 / n-2} \qquad (2)$$

Where n is the number of data points for the two component mixture, and $A= K_1 C_1 + K_2 C_2$, where K_1 and K_2 are the column vector of the individual component absorptivities.

RESULTS AND DISCUSSION

A- Multivariate linear regression method

The absorption spectra for the studied drugs are shown in Figure 1 (a, b, and c). As can be seen, a considerable degree of spectral overlapping occurs in the region from 210 to 320 nm. The degree of spectral overlapping can be conveniently given by $(D_i)^{0.5}$. Where D_i is the magnitude of dependency which can be calculated for a two components mixture from equation (3).

$$Di = \frac{(\Sigma\ k_1\ k_2^t)^2}{\Sigma\ k_1 k_1^t .\ \Sigma k_2 k_2^t} \qquad (3)$$

Where K_1 and K_2 are the *l x n* matrices of regression coefficients for compounds 1 & 2 respectively.

In case of the presently studied compounds, the spectra shown in Figure 1 (a, b, and c) lead to Di= 0.59 implying a 76.8% of spectral overlap for the first mixture (ascorbic acid and acetyl salicylic acid), Di= 0.68 implying a 82.5% of spectral overlap for the second mixture (ascorbic acid and salisylamide) and Di= 0.66 implying a 81.3% of spectral overlap for the third mixture (ascorbic acid and dipyrone) respectively.

Full-spectrum methods usually provide significant improvement in precision over methods restricted to a small number of wavelengths. A convenient method for resolving the mixtures, which can in principle be applied to the present case, is the least squares analysis. The simplest of them is the classical least squares (CLS). It should certainly be preferred when the selection of variables is simple, i.e. when some variables are rather selective for the compounds or characteristics being determined. The regression coefficients for different selected collinear wavelengths may have relatively little meaning for interpretation purposes, but the model performs well, both in calibration and in prediction, provided that the model possess linearity between response and concentration and the prediction is performed within the calibration domain. In addition, the baseline effects and noise are probably non-significant. Under these conditions, it is probably the method to be recommended and this is the case in the present work.

As mentioned previously in CLS a linear relationship between the absorbance and the component concentrations at each wavelength is assumed. Such relationship is given by equation (4).

$$A = C\ K + E \qquad (4)$$

Where A is the *m x n* matrix of calibration spectra, C is the *m x l* matrix of component concentrations, K is the *l x n* matrix of regression coefficients and E is the *m x n* matrix of spectral errors or residuals not fit by the model. By means of the calibration sample set, estimation of absorptivities is possible by solving for the matrix K according to the general least-squares solution indicated by equation (5).

$$K = (C^t .\ C)^{-1} .\ C^t .A \qquad (5)$$

Analysis is then based on the spectrum (A_{un}) of the unknown samples by:

$$C_{un} = A_{un}\ K^t\ (K .\ K^t)^{-1} \qquad (6)$$

Where C_{un} is the vector of sought-for concentrations.

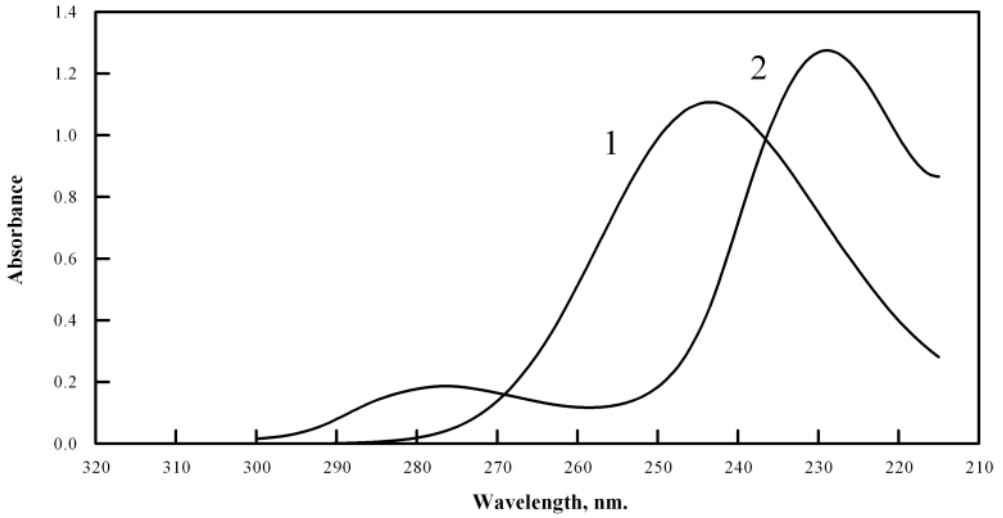

Fig. 1(a): Degree of overlapping of the absorption spectra of (1) ascoribc acid (25 µg/ml) and (2) acetyl salicylic acid (20 µg/ml), in 0.1 N HCl.

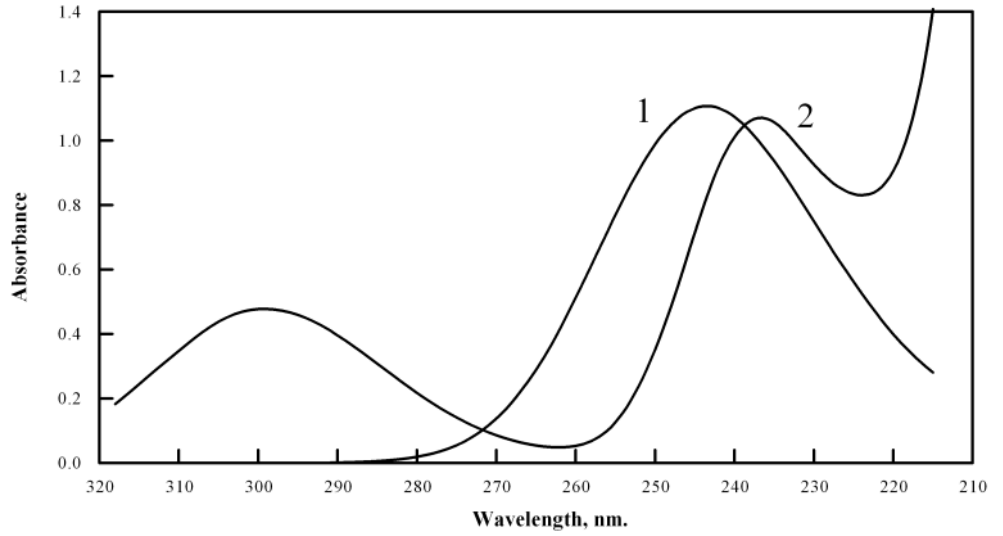

Fig. 1(b): Degree of overlapping of the absorption spectra of (1) ascoribc acid (25 µg/ml) and (2) salicylamide (20 µg/ml), in 0.1 N HCl.

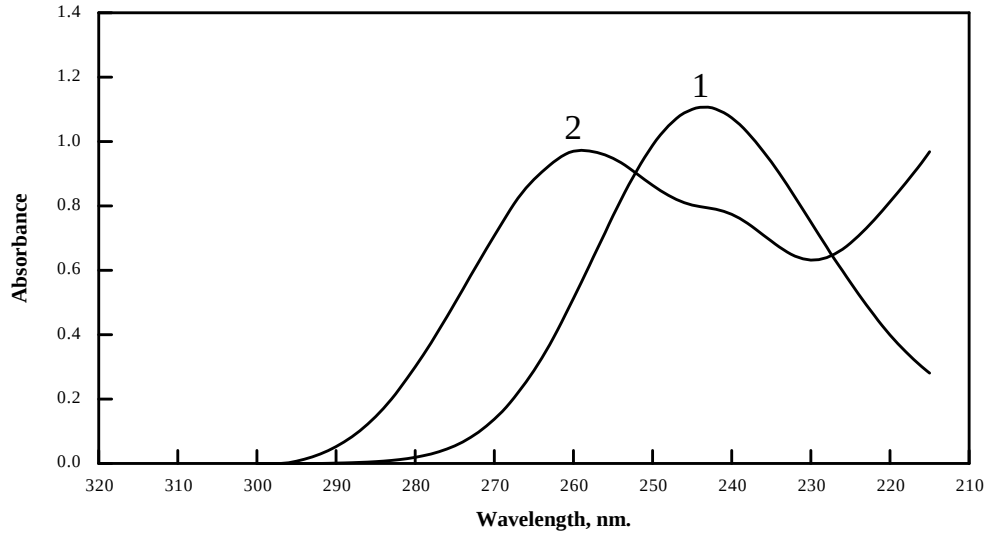

Fig. 1(c): Degree of overlapping of the absorption spectra of (1) ascoribc acid (25 µg/ml) and (2) dipyrone (40 µg/ml), in 0.1 N HCl.

Several mixtures, both synthetic and commercial dosage forms were subjected to the CLS analysis. Tables (1-3) summarize the results obtained for the suggested synthetic binary mixtures. As can be seen, the recoveries in all cases were satisfactory and the relative deviations between the estimated and true concentrations expressed by the relative root mean squared error (RRMSE) were found to be ranged from 0.01 to 2.56%, from 0.05 to 2.49% and from 0.48 to 2.57, for ascorbic-acetyl salicylic acid, ascorbic acid-salicylamide and ascorbic acid -dipyrone mixtures respectively.

B- First derivative method

For the determination of ascorbic acid, working standards containing 5 - 25 µg/ml were recorded over the 210-350 nm range against 0.1 N HCl as blank. The calibration curve was then constructed by plotting the measured amplitude of the maximum of the wavelength of 258.5 nm for ascorbic acid vs the corresponding concentration. For the simultaneous determination of ascorbic acid and acetyl salicylic acid; λ of 258.5 nm was used for determination of ascorbic acid and λ of 243.4 nm was used for determination of acetyl salicylic acid (Fig. 2). For the simultaneous determination of ascorbic acid and salicylamide; either λ of 236.6 nm or 262.2 nm was used for determination of ascorbic acid (λ of 262.2 nm being of higher sensitivity was chosen for our determination) and λ of 243.4 nm was used for determination of salicylamide (Fig. 3). For the simultaneous determination of ascorbic acid and dipyrone; λ of 258.5 nm was used for determination of ascorbic acid and λ of 287.4 nm was used for determination of dipyrone (noting that the amplitude of absorbance of dayroom at λ of 243.4 nm, the zero crossing of ascorbic acid, is relatively very low; therefore, λ of 287.4 nm was selected for our measurements where ascorbic acid gives zero amplitude while dipyrone gives a considerable amplitude as shown in Fig. 4). In comparison between the suggested method and the previously reported method [65] for the first derivative assay of ascorbic acid and dipyrone binary mixtures, different solvents and different wavelengths of measurements were applied. In that reported method, ascorbic acid was measured at 253 and 225 nm using 0.1M HCl as a solvent; while dipyrone was not determined in this solvent. On the other hand, dipyrone was measured at 205 nm in distilled water and at 283 or 245 nm in 0.1 M NaOH as a solvent.

Tables 1-3 summarize the results obtained by the first derivative method for the suggested synthetic binary mixtures. As can be seen, the recoveries in all cases were satisfactory and the relative deviations between the estimated and true concentrations expressed by the relative root mean squared error (RRMSE) were found to be ranged from 0.17 to 2.83%; from 0.28 to 2.60%; and from 0.18 to 2.78% for ascorbic acid- acetylsalicylic acid, ascorbic acid-salisylamide and ascorbic acid–dipyrone mixtures respectively.

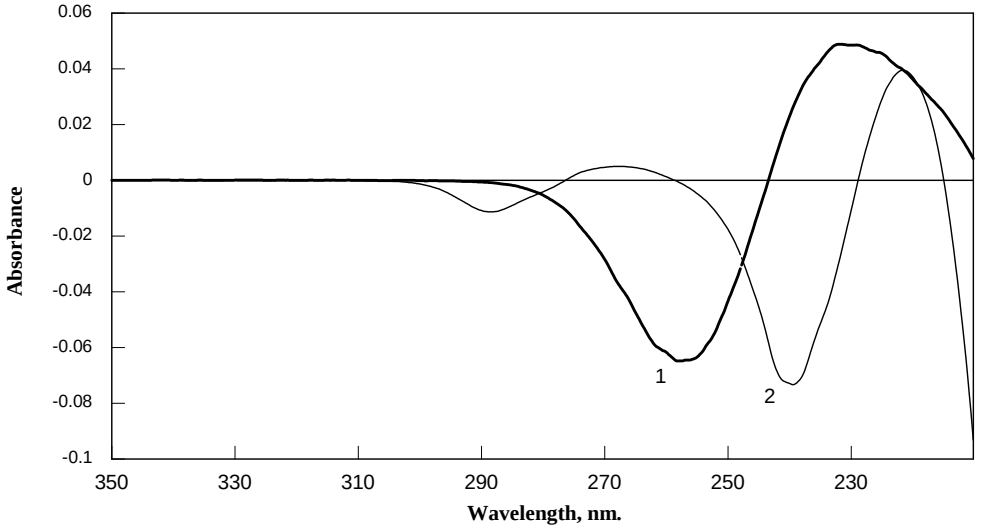

Fig. 2: First derivative absorption spectra of (1) ascorbic acid (25 µg/ml) and (2) acetylsalicylic acid (25 µg/ml) in 0.1 N HCl.

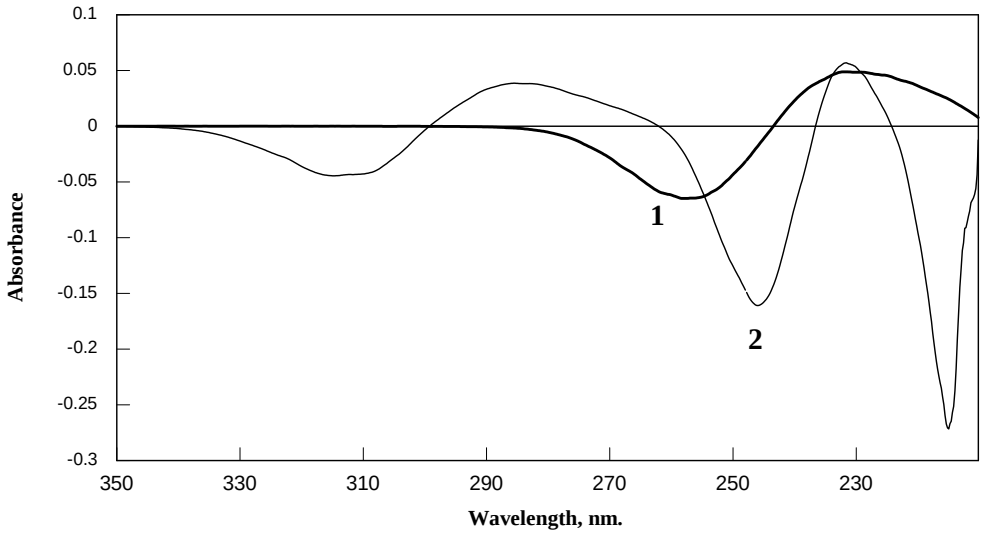

Fig. 3: First derivative absorption spectra of (1) ascorbic acid (25 µg/ml) and (2) salicylamide (40 µg/ml) in 0.1 N HCl.

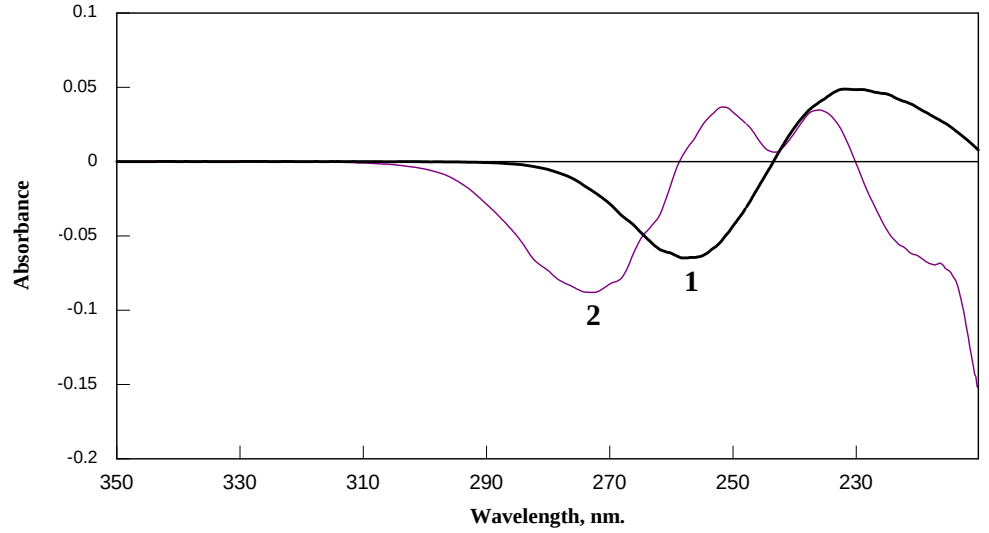

Fig. 4: First derivative absorption spectra of (1) ascorbic acid (25 µg/ml) and (2) dipyrone (80 µg/ml) in 0.1 N HCl.

C- Comparison of the two suggested methods

Figures 5-8 showed the actual and predicted amounts of the studied drugs given by the least squares regression of the spectral data that obtained experimentally in the range of 210-350 nm as well as that by the first derivative analysis. Comparison of the proposed two methods vs the official methods for the determination of the analysed compounds indicates good agreement and high accuracy and precision as obseved by the t and F values (Table 4).

Table 1: Results obtained by applying CLS and zero-crossing first derivative to binary synthetic mixtures of ascorbic acid and acetyl salicylic acid (aspirin) in 0.1 M hydrochloric acid solutions.

Mixture	Components	Actual ($\mu g.ml^{-1}$)	Found (CLS) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)	Found (1^{st} derivatives) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)
(1)	Ascorbic acid	25.00	25.171	100.69	0.69	25.242	100.97	0.97
	Aspirin	5.00	5.040	100.80	0.80	4.955	99.10	0.90
(2)	Ascorbic acid	20.00	20.182	100.91	0.91	19.686	98.43	1.57
	Aspirin	10.00	9.999	99.99	0.01	9.717	97.17	2.83
(3)	Ascorbic acid	15.00	15.384	102.56	2.56	15.280	101.87	1.87
	Aspirin	15.00	15.347	102.31	2.31	15.340	102.27	2.27
(4)	Ascorbic acid	10.00	10.057	101.57	1.57	9.983	99.83	0.17
	Aspirin	20.00	20.228	101.15	1.15	20.222	101.11	1.11
(5)	Ascorbic acid	5.00	5.081	101.64	1.64	4.901	99.02	0.98
	Aspirin	25.00	24.915	99.66	0.34	24.494	97.98	2.02
Aspocid-C Effervescent Tablets	Ascorbic acid	10.00	9.876	98.76	1.24	9.776	97.76	2.24
	Aspirin	20.00	19.710	98.52	1.48	19.72	98.60	1.40

Table 2: Results obtained by applying CLS and zero-crossing first derivatives to binary synthetic mixtures of ascorbic acid and salicylamide in 0.1 M hydrochloric acid solutions.

Mixture	Component	Actual ($\mu g.ml^{-1}$)	Found (CLS) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)	Found (1^{st} derivatives) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)
(1)	Ascorbic acid	25.00	25.013	100.05	0.05	25.068	100.97	0.97
	Salicylamide	4.00	4.014	100.35	0.35	3.951	98.78	1.23
(2)	Ascorbic acid	20.00	20.365	101.83	1.83	20.714	98.43	1.57
	Salicylamide	8.00	8.073	100.91	0.91	7.878	99.73	0.28
(3)	Ascorbic acid	15.00	14.951	99.67	0.33	14.920	99.47	0.53
	Salicylamide	12.00	12.183	101.53	1.53	11.932	99.42	0.58
(4)	Ascorbic acid	10.00	10.041	100.41	0.41	10.034	100.34	0.34
	Salicylamide	16.00	16.399	102.49	2.49	16.296	101.11	1.11
(5)	Ascorbic acid	5.00	4.931	98.62	1.38	4.897	97.94	2.06
	Salicylamide	20.00	20.113	100.56	0.56	19.703	97.98	2.02
Cidal-C Tablets	Ascorbic acid	4.00	4.015	100.38	0.38	4.104	102.60	2.60
	Salicylamide	40.00	40.252	100.63	0.63	40.152	100.38	0.38

Table 3: Results obtained by applying CLS and zero-crossing first derivatives to binary synthetic mixtures of ascorbic acid and dipyrone (novalgin) in 0.1 M hydrochloric acid solutions.

Mixture	Component	Actual ($\mu g.ml^{-1}$)	Found (CLS) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)	Found (1^{st} derivatives) ($\mu g.ml^{-1}$)	(%)	RRMSE (%)
(1)	Ascorbic acid	5.00	5.117	102.34	2.34	5.139	102.78	2.78
	Novalgin	48.00	48.543	101.131	1.13	49.140	102.38	2.38
(2)	Ascorbic acid	10.00	10.111	101.11	1.11	10.170	101.70	1.70
	Novalgin	40.00	40.231	100.58	0.58	40.070	100.18	0.18
(3)	Ascorbic acid	15.00	15.211	101.41	1.41	15.244	101.63	1.63
	Novalgin	32.00	32.333	101.04	1.04	32.790	102.47	2.47
(4)	Ascorbic acid	20.00	20.211	101.05	1.05	20.113	100.56	0.56
	Novalgin	24.00	24.223	100.93	0.93	24.480	102.00	2.00
(5)	Ascorbic acid	25.00	25.091	100.36	0.36	24.677	98.71	1.29
	Novalgin	16.00	16.412	102.57	2.57	16.270	101.69	1.69
Cevagen Ampoules	Ascorbic acid	32.00	32.154	100.48	0.48	31.530	98.53	1.47
	Novalgin	32.00	31.528	98.53	1.47	31.770	99.28	0.72

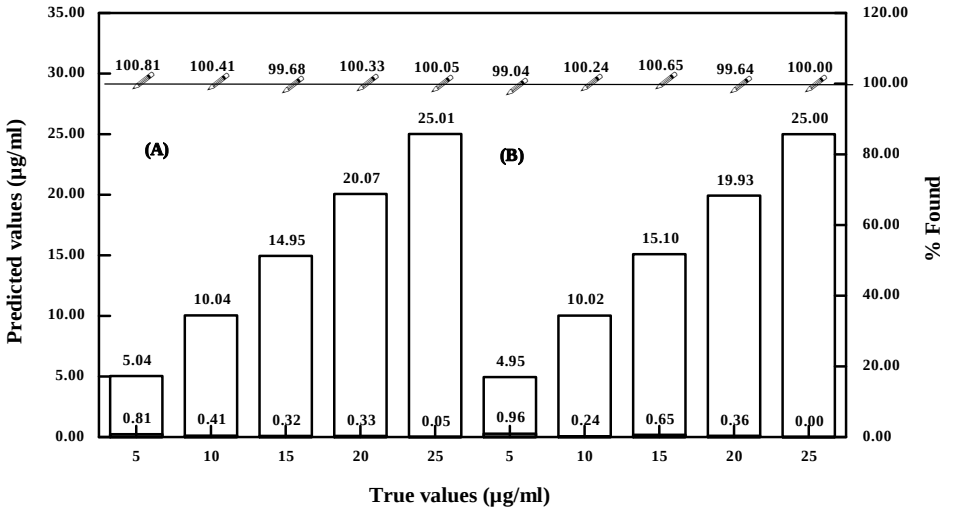

Fig. 5: Predicted concentrations of ascorbic acid ± errors (%) as calculated by: (A) MLR in the range of 200 to 300 nm and (B) 1st derivative at 258.8 nm.

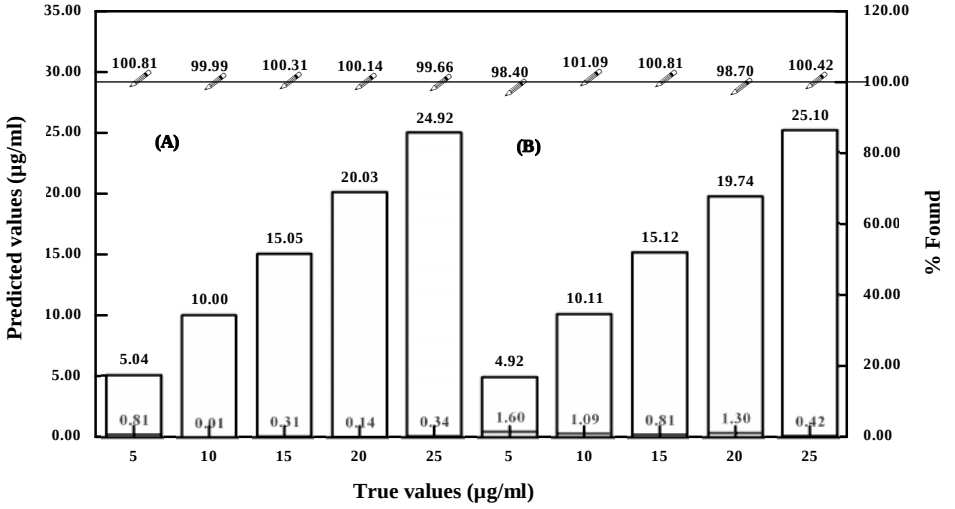

Fig. 6: Predicted concentrations of acetyl salicylic acid ± errors (%) as calculated by: (A) MLR in the range of 200 to 300 nm and (B) 1st derivative at 243.4 nm.

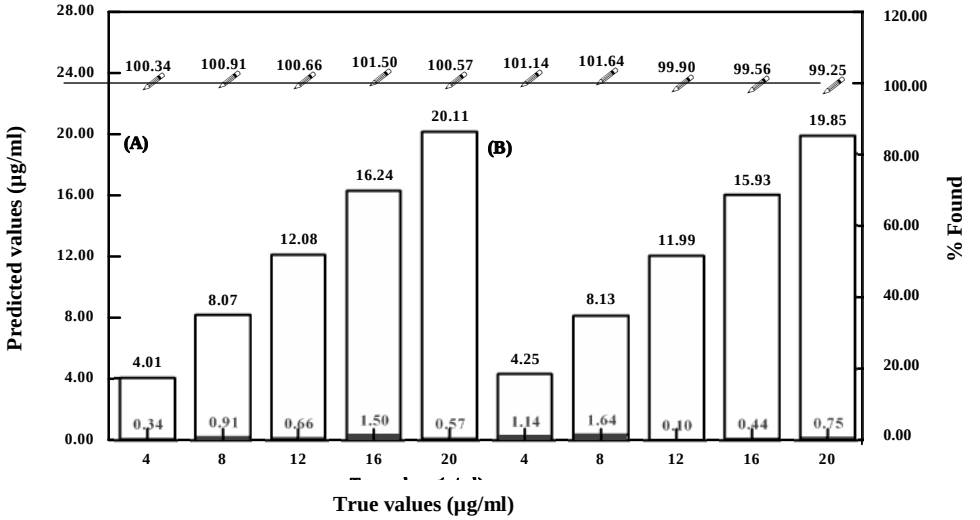

Fig. 7: Predicted concentrations of salicylamide ± errors (%) as calculated by: (A) MLR in the range of 200 to 320 nm and (B) 1st derivative at 243.4 nm.

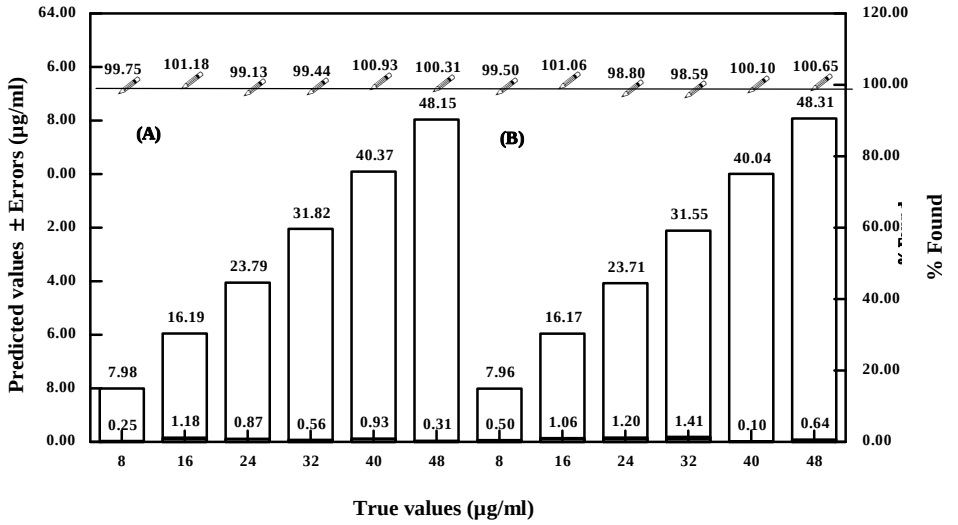

Fig. 8: Predicted concentrations of dipyrone (novalgin) ± errors (%) as calculated by: (A) MLR in the range of 200 to 300 nm and (B) 1st derivative at 287.4 nm.

Table 4: Analysis of the studied drugs in commercial dosage forms by the CLS, first derivative and reported methods*.

Dosage form	Drug	Claimed (mg)	Found % (± S.D.) CLS	Derivative	Reported**
Aspocid C eff. tablets	Ascorbic acid	250	98.76 (0.408) F = 4.010 t = 1.127	97.76 (0.817) F= 1.000 t = 0.943	98.53 (0.817)
	Acetyl salicylic acid	500	98.52 (0.817) F= 4.010 t = 0.146	98.60 (1.033) F= 6.410 t = 0.261	98.76 (0.408)
Cidal C tablets	Ascorbic acid	50	100.38 (0.817) F= 3.995 t = 0.612	102.60 (1.633) F= 1.000 t = 0.313	100.63 (1.633)
	Salicylamide	500	100.63 (1.633) F= 1.038 t = 2.939	100.38 (0.815) F= 3.869 t = 0.919	98.53 (1.603)
Cevagin ampoules	Ascorbic acid	1000	100.48 (1.630) F= 3.980 t = 2.939	98.53 (0.817) F= 1.000 t = 0.919	99.28 (0.817)
	Dipyron	1000	100.48 (1.630) F= 3.980 t = 2.939	98.53 (0.817) F= 1.000 t = 0.919	99.28 (0.817)
Cevarol tablets	Ascorbic acid	500	100.60 (0.589) F= 1.203 t = 1.732	100.70 (0.622) F= 1.079 t = 1.732	100.75 (0.646)
Vitacid C eff. tablets	Ascorbic acid	1000	100.73 (1.500) F= 5.375 t = 0.036	98.50 (1.000) F= 2.389 t = 2.893	100.76 (0.647)
Ascorbovit eff. sacks	Ascorbic acid	1000	101.00 (1.701) F= 8.340 t = 0.417	99.97 (0.967) F= 2.695 t = 1.009	100.60 (0.589)
Cevarol ampoules	Ascorbic acid	1000	99.21 (0.668) F= 1.018 t = 2.878	98.75 (0.957) F= 2.367 t = 2.733	100.71 (0.622)
Cevilene drops	Ascorbic acid	100/ml	98.50 (1.000) F= 2.250 t = 2.167	100.83 (1.040) F= 2.080 t = 1.020	100.73 (1.500)
C- vit drops	Ascorbic acid	100/ml	99.20 (0.668) F= 2.291 t = 2.271	98.75 (0.957) F= 1.116 t = 1.000	98.51 (1.011)

* Average of 4 determinations ± SD , Theoretical values at 95% confidence limit are t= 3.18 and F = 9.28.

** B.P 1998 p. 116 & p.120 for ascorbic acid and acetylsalicylic acid respectively; USP 25 p. 1548 for salicylamide, Reference 63 for dipyrone.

Conclusion

The contents of several synthetic mixtures and commercial dosage forms were simultaneously determined using CLS multivariate calibration analysis together with zero crossing first derivative spectrophotometric analysis. The good recoveries obtained in all cases as well as the reliable agreement with the reported procedures proved that, the proposed methods could be applied efficiently for determination of ascorbic acid either in single dosage forms or in binary pharmaceutical mixtures with acetyl salicylic acid, salicylamide or dipyrone with quite satisfactory precision. Advantages of the two proposed methods include; short analysis time, unnecessary sample pretreatment good accuracy and precision.

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