

UV DETERMINATION OF EPINEPHRINE, URIC ACID, AND ACETAMINOPHEN IN PHARMACEUTICAL FORMULATIONS AND SOME HUMAN BODY FLUIDS USING MULTIVARIATE CALIBRATION**Hanieh Montaseri^{a,*}, Habibollah Khajehsharifi^a and Saeed Yousefinejad^b**^aDepartment of Chemistry, Yasouj University, 75914-353, Yasouj, Iran^bInstitute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

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In this work, a spectrophotometric methodology was applied in order to determine epinephrine (EP), uric acid (UA), and acetaminophen (AC) in pharmaceutical formulations and spiked human serum, plasma, and urine by using a multivariate approach. Multivariate calibration methods such as partial least squares (PLS) methods and its derivatives were used to obtain a model for simultaneous determination of EP, UA and AC with good figures of merit and mixture design was in the range of 1.8–35.3, 1.7–16.8, and 1.5–12.1 $\mu\text{g mL}^{-1}$. The 2nd derivative PLS showed recoveries of 95.3–103.3, 93.3–104.0, and 94.0–105.5 $\mu\text{g mL}^{-1}$ for EP, UA, and AC, respectively.

Keyword: ternary mixture; simultaneous determination; multivariate analysis.

INTRODUCTION

Epinephrine (EP), known as adrenaline, is a hormone that belongs to the family of excitatory chemical neurotransmitters,¹ and it mainly exists in the cationic form in biological fluids.^{1,2} EP plays a very important role in the function of the central nervous system (CNS), renal, hormonal, and cardiovascular system,³ as well as increases heart rate, contracts blood vessels, and dilates air passage, and it participates in the fight-or-flight response of the sympathetic nervous system.⁴ Accurate and selective measurements of catecholamines such as dopamine, epinephrine, norepinephrine (NEP), and serotonin (SE) in biological samples are important for both clinical diagnoses and pathological studies of some diseases.⁵

Uric acid or urate (UA) is produced by xanthine oxidase from xanthine and hypoxanthine, which in turn are produced from purine. UA is more toxic to tissue than either xanthine or hypoxanthine and is released in hypoxic conditions.⁶ In humans and higher primates, UA is the final oxidation (breakdown) product of purine (guanine and adenine) metabolism and is excreted in urine. Over half the antioxidant capacity of blood plasma comes from UA,⁷ and about 70% of daily UA disposal occurs via the kidneys; moreover, in 5%-25% of humans, impaired renal (kidney) excretion leads to hyperuricaemia.⁸ Abnormal levels of UA concentration leads to several diseases, such as hyperuricaemia, gout,^{9,10} leukemia, pneumonia,⁹ and Lesch-Nyhan syndrome.¹¹ Note that the normal concentration of UA in the blood is 120–450 $\mu\text{mol L}^{-1}$ and in urine about 2 $\mu\text{mol L}^{-1}$.¹²⁻¹⁴

Paracetamol or acetaminophen (AC) is an acylated aromatic amide that was first introduced by Von Mering in 1893 as an antipyretic/analgesic medicine. AC unlike other common analgesics, such as aspirin and ibuprofen, has relatively little anti-inflammatory activity. As a result, it is not considered to be a non-steroidal anti-inflammatory drug (NSAID). Acute overdoses of paracetamol can cause potentially fatal liver damage, and in some individuals, a normal dose can cause the same, but the risk can be heightened by alcohol consumption. Recent studies suggest that excessive doses and/or excessively high plasma concentrations of AC may be associated with hepatotoxicity.¹⁵⁻¹⁸ Therefore, understanding the pharmacokinetic properties of AC in humans can be valuable for preventing clinical toxicity.

Spectroscopy is a simple analytical method for quantitative analysis, but the method is not selective and requires that analytes in a given sample have different spectrum with low overlap. Complex spectra can be resolved by simultaneous determinations using derivative techniques and chemometric methods, which avoids time-consuming separation. Derivative techniques have proven to be very useful in the resolution of binary and ternary mixtures, whereas multivariate calibration has been found to be the method of choice for more complex mixtures.¹⁹ Moreover, chemometric-assisted spectrophotometry is a combination of chemometrics and analytical chemistry that increases the signal-to-noise (S/N) ratio, improves selectivity of determination, optimizes experimental condition, increases analytical operation efficiency, and provides much more scientific information than conventional spectrophotometry.²⁰⁻²²

Chemometric algorithms for performing regression, including partial least squares (PLS) and principal component regression (PCR), are designed to avoid problems associated with noise and correlations in the data. Because the regression algorithms are based on factor analysis, the entire group of known measurements is considered simultaneously, and information about correlations among the variables is automatically built into the calibration model.²³ The first derivative PLS is commonly used to eliminate baseline offset variations within a set of spectra and the 2nd derivative PLS eliminates both the offset and slope. Irrespective of the order, both derivatives lead to an increased spectral resolution at the expense of a decreased signal-to-noise ratio.^{24,25} These methods allow for calibration to be performed by ignoring the concentration of all other components except the analyte of interest, and they do not require any derivatization, prior separation, or sample pretreatment.

In the present work, original PLS and its derivatives have been used to develop sensitive and selective procedures for the simultaneous determination of ternary mixtures composed of EP, UA, and AC. Note that AC can be considered as a narcotic drug that can be prescribed to all patients. In some cases, patients having high concentrations of UA in their bloodstream may suffer from an infarction that requires an EP injection. Catecholamine drugs are used to treat hypertension, bronchial asthma, and organic heart disease, and they are used in cardiac surgery and myocardial infarction.²⁶ Therefore, simultaneous determination of EP, UA, and AC using a simple procedure could be useful in clinical practice.

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EXPERIMENTAL

Materials

Apparatus and software

Electronic absorption measurements were carried out on a Jasco v-570 spectrophotometer (slit width of 1.0 nm, scan rate of 2000 cm/min) using 1.00 cm quartz cells. The measurements for pH were performed by a Metrohm 692 pH meter using a combined electrode. All spectra were digitized and stored at wavelengths from 220 to 320 nm in steps of 1 nm, and then transferred in TXT format to a Pentium 4, 2.4 GHz computer using MATLAB software, version 7.6.0 (The Math Works). PLS, 1st, and 2nd derivate PLS were conducted in the PLS Tool box (Eigenvector Company, version 2.1). Derivative spectra were obtained with a quadratic Savitzky–Golay algorithm with 11 convolution points.

Reagents and solutions

All chemicals used were of analytical reagent grade, and throughout the experiments, doubly distilled water was used. UA and EP were purchased from Merck, New Jersey, America, while AC and trichloroacetic acid were supplied from Darou Pakhsh, Tehran, Iran and Fluka, Buchs, Switzerland, respectively. The AC tablets and EP ampoule were purchased from GlaxoSmithKline, Dungarvan Ltd., Ireland and Darou Pakhsh Tehran, Iran, respectively. The stock solutions of UA and EP were prepared by addition of 2–3 drops of 1.0 mol L⁻¹ KOH (Merck) to appropriate volumes of doubly distilled water. AC was also prepared in doubly distilled water (without addition of KOH). An aqueous buffer solution (pH = 7.0, 0.1 mol L⁻¹) was prepared by weighing and dissolving adequate amounts of KH₂PO₄ in doubly distilled water, and the pH was adjusted using NaOH (Merck, New Jersey, America). The serum, plasma, and urine samples were prepared by spiking serum, plasma, and urine from humans with appropriate amounts from the stock solutions of EP, UA, and AC.

Procedure

Single component calibration

To determine the linear dynamic range (LDR) for each component, different volumes of stock solution (0.01 mol L⁻¹) of each component were added to 10 mL volumetric flask and diluted with a buffer solution (pH = 7.0). The electronic absorption spectra were recorded over the range of 220–320 nm. Maximum absorption of EP, UA, and AC were at the wavelength of 283, 292, and 243 nm, respectively. The LDR for each component was obtained by plotting absorbance versus concentration (Table 1).

Table 1. Parameters for Univariate calibration curves

Analyte	λ_{\max}	LDR ($\mu\text{g mL}^{-1}$)	Regression co-efficient (R^2)	D.L ($\mu\text{g mL}^{-1}$)
AC	243	1.5–12.1	0.997	1.2×10^{-2}
UA	292	1.7–16.8	0.998	4.5×10^{-3}
EP	283	1.8–35.3	0.999	2.0×10^{-2}

Standard calibration and prediction sets

A mixture designed for three components was used for the calibration set to provide good prediction in the PLS approach with 36 samples as shown in Table 2. 7 mixtures was randomly designed as prediction set and their concentrations were chosen in the range of calibration concentration but were not identity to the concentration of calibration mixtures. On the other hand, the maximum absorbance of the standard solutions should not exceed the maximum absorbance

reading of the instrument (i.e., should not be overloaded). The standard solutions (calibration and prediction sets) were prepared in 10.0 mL volumetric flasks by adding appropriate amounts of each stock solution and diluted using a buffer solution (pH = 7.0). Finally, the absorption spectra of the solutions were recorded between 220 and 320 nm against a blank buffer.

Table 2. Concentration data of mixtures that used in the calibration set for the determination of EP, UA and AC

Mixtures	EP ($\mu\text{g mL}^{-1}$)	UA ($\mu\text{g mL}^{-1}$)	AC ($\mu\text{g mL}^{-1}$)
1	1.8	1.7	12.1
2	1.8	3.9	10.6
3	1.8	6.0	9.1
4	1.8	8.2	7.6
5	1.8	10.3	6.0
6	1.8	12.5	4.5
7	1.8	14.7	3.0
8	1.8	16.8	1.5
9	6.6	14.7	1.5
10	11.4	12.5	1.5
11	16.1	10.3	1.5
12	21.0	8.2	1.5
13	25.7	6.0	1.5
14	30.5	3.9	1.5
15	30.5	1.7	1.5
16	30.5	1.7	3.0
17	30.5	1.7	4.5
18	25.7	1.7	6.0
19	21.0	1.7	7.6
20	16.1	1.7	9.1
21	11.4	1.7	1.5
22	6.6	16.8	3.0
23	1.8	16.8	1.5
24	6.6	16.8	1.5
25	11.4	16.8	3.0
26	16.1	14.7	4.5
27	21.0	14.7	6.0
28	25.7	14.7	7.6
29	30.5	12.5	1.5
30	35.3	12.5	3.0
31	6.6	12.5	4.5
32	11.4	10.3	6.0
33	16.1	10.3	7.6
34	21.0	10.3	9.1
35	25.7	8.2	10.6
36	30.5	8.2	12.1

Sample preparation

Serum and plasma sample

The serum or plasma samples were homogenized. For the deproteinization, 1 mL of 24% w/v trichloroacetic acid was added to 1 mL of serum or plasma, and after 15 min, the resultant mixture was centrifuged at 3000 rpm.²⁷ The pH of the supernatant solution was adjusted to pH of 7.0 using an NaOH solution. Subsequently, appropriate amount of the EP, UA, and AC stock solutions were added to 0.5 mL of the prepared serum or plasma, and then filled to the final volume (10 mL) with a buffer solution to obtain the desired concentration. Electronic absorption spectra were recorded in the range of 220–320 nm.

Urine sample

Urine samples were diluted 1:3 with doubly distilled water. Cell debris and particulate matter were removed from the urine using low-speed centrifugation (for 5 min at 1500 rpm).²⁸ Then, NaOH solution was added to the supernatant until its final pH value was equal to 7.0. Moreover, appropriate amounts from EP, UA, and AC

stock solutions were added to 0.5 mL of the final prepared urine, and the volume was made up to 10 mL. Electronic absorption spectrum of the solution was recorded in the range of 220–320 nm.

EP ampoule sample

The EP injection solution (specified content of EP is 1.0 mg mL^{-1}) was diluted to 250 mL by adding doubly distilled water. Different capacities of diluted solutions were transferred into a series of 10 mL volumetric flasks and diluted with a buffer solution.²⁹ The standard addition method was used for analyzing the EP sample and the electronic absorption spectrum of the solution was recorded in the range of 220–320 nm.

Acetaminophen tablet sample

Ten tablets of AC were powdered by a mortar, and a weight equivalent to that of one tablet (0.5933 g) was dissolved in 50 mL of buffer solution. After 30 min of stirring and 15 min standing in the dark, the solutions were filtered in a 50 mL volumetric flask. Then, 1 mL of the solution was poured into a 25 mL volumetric flask and diluted with a buffer solution.³⁰ The standard addition method was used for analyzing the AC sample, and the electronic absorption spectrum of the solution was recorded in the range of 220–320 nm.

RESULT AND DISCUSSION

Electronic absorption spectra

The electronic absorption spectra of EP, UA, and AC are shown in Figure 1. Mixtures of EP, UA and AC are not feasible using conventional calibration procedure without prior separation because these compounds generate broad overlapping spectra. Therefore, multivariate calibration was conducted for ternary mixtures of EP, UA, and AC. Spectra were recorded in the region between 220 and 320 nm (1.0 nm steps) owing to overloading of the absorption values when there is a mixture of compounds and a low order of LDR. Note that the validation, artificial and unknown samples were analyzed using the same procedure.

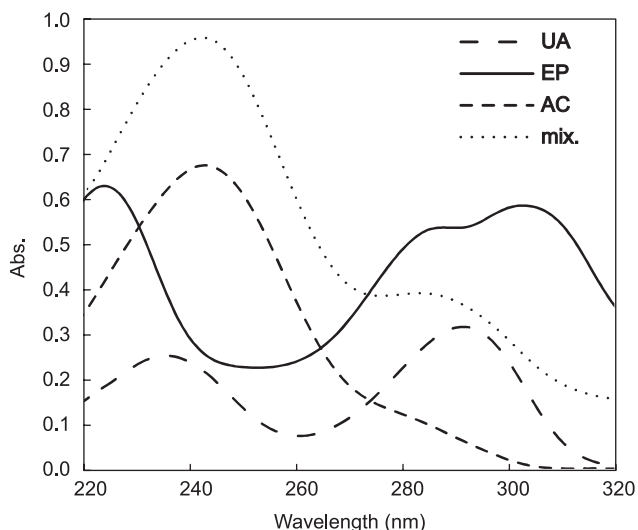


Figure 1. Absorbance spectra of Epinephrine (12.0 mg L^{-1}), Uric Acid (12.0 mg L^{-1}), Acetaminophen (12.0 mg L^{-1}) and their mixture

Univariate estimation of linear dynamic range of analytes

Optimal measurement conditions were established for simultaneous determination using the univariate method that estimates the

linear dynamic range of each analyte. Individual calibration curves, absorbance versus analyte concentration, were constructed with several points (and 3 replicates for each point). The linear regression equation was $A = 0.0633C_{AC} - 0.005$ ($R^2 = 0.997$) for the AC calibration graph in the range of $1.5\text{--}12.1 \text{ } \mu\text{g mL}^{-1}$, while the linear regression equations were $A = 0.042C_{UA} - 0.025$ ($R^2 = 0.998$) and $A = 0.015C_{EP} - 0.0043$ ($R^2 = 0.999$) for the concentration ranges of $1.7\text{--}16.8$ (UA) and $1.8\text{--}35.3 \text{ } \mu\text{g mL}^{-1}$ (EP), respectively.

Multivariate calibration and prediction

Multivariate calibration methods require a suitable experimental design for the standard calibration set to be a good predictor. In this study, the mixture design was used for experimental design and original PLS, and 1st and 2nd derivate PLS models were constructed for calibration data sets. A calibration set included 36 calibration samples containing $1.8\text{--}35.3$, $1.7\text{--}16.8$, and $1.5\text{--}12.1 \text{ } \mu\text{g mL}^{-1}$ of EP, UA, and AC, respectively, (Table 2) and seven prepared mixtures were not consistent with the previous set were used as an independent test (Table 3). It is noted that the concentration of analytes in the calibration samples must be orthogonal to give the most information from the analytical system. The multivariate figures of merit (LOD, LOQ, and sensitivity) for three components were calculated using net analyte signal (NAS) calculation,^{31–33} and are shown in Table 4.

Selection of the optimum number of factors for original PLS, 1st, and 2nd derivate PLS

The correct number of loading vectors to be used for modeling the data was determined using a cross validation calculation for all the samples in the training set. It was performed to calculate the prediction residual error sum of squares (PRESS), which is defined in the following Equation (1).

$$PRESS = \sum (C_i - \hat{C}_i) \quad (1)$$

where C_i is the reference concentration for the i^{th} sample and \hat{C}_i represents the estimated concentration. One sample is eliminated at a time in the cross validation model. PLS calibration was performed on 35 calibration spectra, after which the concentration of the sample (not included in the calibration process) was evaluated. This process was repeated 36 times and each sample was not included in one of the calibration runs. The concentration of each sample was then predicted and compared with the known concentration of the three components in the reference sample.³⁴ One reasonable choice for the optimum number of factors would be the number that results in the minimum PRESS. Since there are a finite number of samples in the training set, in many cases, the minimum PRESS value causes over fitting for unknown samples that were not included in the model. A solution to this problem was suggested by Haaland et al. in which the PRESS values for all previous factors were compared with the PRESS value at the minimum.³⁵

The F-statistical test can be used to determine the significance of PRESS values greater than the minimum. The maximum number of factors used to calculate the optimum PRESS were selected, and the optimum number of factors obtained by the PLS model on original and derivative data are summarized in Table 5. In all cases (PLS and derivatives), the number of optimum factors were selected according to PRESS values and their F-ratio probabilities using plots of PRESS vs. number of factors (these plots are not shown).

Statistical parameters

Generally, to evaluate the predictive ability of a multivariate

Table 3. Added and found results of the synthetic of EP, UA and AC

Original PLS									
Mix.	Found ($\mu\text{g mL}^{-1}$)			% Recovery					
	EP	UA	AC	EP	UA	AC	EP	UA	AC
1	10.0	15.0	1.8	10.8	15.6	1.9	105.6	104.2	105.6
2	26.0	14.3	3.6	21.7	13.8	3.7	83.5	96.4	102.1
3	6.0	12.1	5.0	7.3	12.4	5.1	121.5	102.5	101.0
4	3.6	8.8	6.8	4.3	8.8	6.7	119.4	100.0	99.0
5	20.0	6.5	8.4	17.6	6.1	8.1	88.3	94.5	96.5
6	15.0	4.5	10.1	13.0	3.6	9.8	97.0	81.2	97.0
7	34.0	2.0	11.0	30.5	1.8	11.0	90.0	92.0	100.2
1 st derivate PLS									
Mix.	Add ($\mu\text{g mL}^{-1}$)			Found ($\mu\text{g mL}^{-1}$)			% Recovery		
	EP	UA	AC	EP	UA	AC	EP	UA	AC
1	10.0	15.0	1.8	10.8	15.9	1.9	106.9	106.0	105.5
2	26.0	14.3	3.6	25.4	14.5	3.7	97.7	101.4	102.8
3	6.0	12.1	5.0	6.2	12.6	5.0	103.3	104.1	100.0
4	3.6	8.8	6.8	3.4	9.1	6.6	94.4	103.4	97.1
5	20.0	6.5	8.4	19.7	6.7	8.6	98.5	103.1	102.3
6	15.0	4.5	10.1	15.2	4.6	9.8	101.3	102.2	97.0
7	34.0	2.0	11.0	33.8	1.9	10.8	99.4	95.0	98.2
2 nd derivate PLS									
Mix.	Add ($\mu\text{g mL}^{-1}$)			Found ($\mu\text{g mL}^{-1}$)			% Recovery		
	EP	UA	AC	EP	UA	AC	EP	UA	AC
1	10.0	15.0	1.8	9.6	15.6	1.9	96.0	104.0	105.5
2	26.0	14.3	3.6	25.3	14.2	3.5	97.3	99.3	97.2
3	6.0	12.1	5.0	6.2	12.3	4.8	103.3	101.7	96.0
4	3.6	8.8	6.8	3.7	8.8	6.6	102.8	100.0	97.1
5	20.0	6.5	8.4	19.3	6.3	7.9	96.5	96.9	94.0
6	15.0	4.5	10.1	14.3	4.2	9.8	95.3	93.3	97.0
7	34.0	2.0	11.0	33.1	1.9	10.6	97.1	95.0	96.4

Table 4. Multivariate figures of merit[†] for AC, UA and EP

Analyte	SEN ($\mu\text{g mL}^{-1}$)	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
AC	0.187	0.064	0.214
UA	0.051	0.235	0.783
EP	0.015	0.780	2.599

[†] SEN (sensitivity), LOD (limit of detection), LOQ (limit of quantitation)

Table 5. Statistical parameters of the optimized models based on PLS and its derivatives

Method	Analyte	NPC	PRESS	RMSEP	%RSEP
PLS	EP	4.0	1.08	0.39	5.45
	UA	6.0	2.99	0.65	6.45
	AC	8.0	4.44	0.25	1.46
1 st derivate	EP	5.0	4.56	0.81	12.10
	UA	5.0	1.45	0.46	4.34
	AC	7.0	9.48	0.37	2.31
2 nd derivate	EP	6.0	3.54	0.71	10.50
	UA	4.0	1.34	0.44	4.28
	AC	5.0	5.93	0.29	1.76

calibration model, the root mean square error of prediction (RMSEP) and relative standard error of prediction (%RSEP) are used.

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (C_{\text{pred}} - C_{\text{obs}})^2}{n}} \quad (2)$$

$$\% \text{RSEP} = \sqrt{\frac{\sum_{i=1}^n (C_{\text{pred}} - C_{\text{obs}})^2}{\sum (C_{\text{obs}})^2}} \quad (3)$$

where C_{pred} is the predicted concentration in the sample, C_{obs} is the observed value of the concentration in the sample, and n is the number of samples in the validation set. The RMSEP and RSEP% values calculated for three components by original PLS, 1st, and 2nd derivate PLS methods are given in Table 5, which is the criterion of accuracy using actual (true) and predicted values, and the smallest values indicated better models. The comparison of the results obtained for original PLS, 1st, and 2nd derivate PLS models indicated that PRESS values for three components, especially EP, used in all models were close to each other; however, the 2nd derivate PLS was simpler than original PLS and 1st derivate PLS because of lower number of latent variables (especially for UA and EP). Plots of predicted concentration versus actual concentration for EP, UA, and AC by 2nd derivate PLS are shown in Figure 2. The correlation coefficients obtained for the 2nd derivate model was better than the original PLS and 1st derivate models. Thus, 2nd derivate PLS presents better prediction abilities when compared with other models.

Applications

Determination of EP, UA, and AC spiked in real samples

The three proposed methods were applied for the simultaneous determination of three analytes in real samples. Predicted values and recovery percentage obtained by 2nd derivate models for EP, UA, and AC in real samples are shown in Table 6. Recovery was quantified and no significant differences were observed between the amounts obtained from this method and the labeled amounts.

Analysis of pharmaceutical formulations

Two commercial pharmaceutical formulations were analyzed by original PLS, 1st, and 2nd derivate PLS, which are shown in Table 7, and then used to assess the reliability of the method. The 2nd derivate

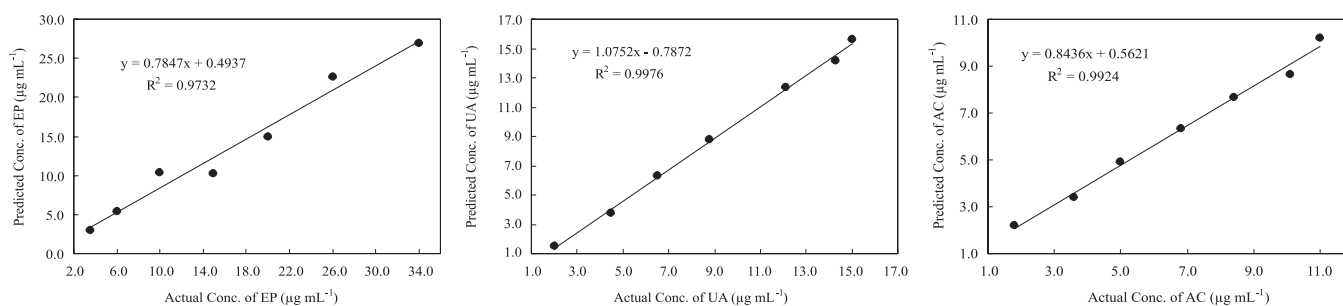


Figure 2. Plots of predicted concentration vs. actual concentration of EP, UA and AC by 2nd derivate PLS

Table 6. Recoveries of EP, UA and AC in spiked real samples by 2nd derivate PLS

Urine samples		Added ($\mu\text{g mL}^{-1}$)			Found ($\mu\text{g mL}^{-1}$)			%Recovery		
Mixtures	EP	UA	AC	EP	UA	AC	EP	UA	AC	
U1	32.0	15.4	1.6	32.7	15.4	1.6	102.2	99.8	102.9	
U2	25.0	1.5	2.3	25.2	1.5	2.3	100.8	100.6	99.2	
U3	18.0	11.1	6.1	17.8	11.1	6.0	98.7	99.8	98.5	
U4	9.0	3.2	9.2	9.0	3.2	9.4	100.3	99.3	102.2	
U5	2.5	9.4	11.8	2.5	9.5	11.6	99.7	101.3	98.4	
Serum samples		Added ($\mu\text{g mL}^{-1}$)			Found ($\mu\text{g mL}^{-1}$)			%Recovery		
Mixtures	EP	UA	AC	EP	UA	AC	EP	UA	AC	
S1	32.0	15.4	1.6	32.2	15.3	1.6	100.7	99.3	101.5	
S2	25.0	1.5	2.3	25.7	1.5	2.3	102.6	102.5	101.4	
S3	18.0	11.1	6.1	18.3	11.2	6.0	101.6	100.7	99.0	
S4	9.0	3.2	9.2	9.0	3.2	9.3	100.1	101.2	100.9	
S5	2.5	9.4	11.8	2.5	9.5	11.6	100.8	100.7	98.6	
Plasma samples		Added ($\mu\text{g mL}^{-1}$)			Found ($\mu\text{g mL}^{-1}$)			%Recovery		
Mixtures	EP	UA	AC	EP	UA	AC	EP	UA	AC	
P1	32.0	15.4	1.6	32.0	15.2	1.6	100.1	98.6	100.5	
P2	25.0	1.5	2.3	25.3	1.5	2.4	101.0	98.3	103.0	
P3	18.0	11.1	6.1	18.0	11.1	6.2	99.8	100.3	101.6	
P4	9.0	3.2	9.2	9.2	3.2	9.4	102.6	98.9	102.2	
P5	2.5	9.4	11.8	2.5	9.2	11.6	100.1	98.0	98.5	

Table 7. Application of optimized models on the pharmaceuticals

EP ampoule	Add ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)			%Recovery \pm % RSD		
		Original PLS	1 st derivate	2 nd derivate	Original PLS	1 st derivate	2 nd derivate
1	3.0	2.9	2.8	2.98	96.7 \pm 1.2	99.0 \pm 1.7	99.4 \pm 1.5
2	10.5	10.2	10.0	10.8	97.1 \pm 4.2	94.6 \pm 4.8	102.9 \pm 1.7
3	26.6	25.8	28.0	26.4	97.0 \pm 0.7	105.2 \pm 1.8	99.2 \pm 3.4
4	32.4	31.5	30.5	31.8	97.2 \pm 3.9	94.0 \pm 1.2	98.1 \pm 2.7
AC tablet	Add ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)			%Recovery \pm % RSD		
		Original PLS	1 st derivate	2 nd derivate	Original PLS	1 st derivate	2 nd derivate
1	2.3	2.2	2.5	2.29	95.3 \pm 5.1	106.6 \pm 6.2	99.4 \pm 2.9
2	5.4	5.3	5.1	5.2	98.1 \pm 4.0	94.0 \pm 1.1	97.0 \pm 3.4
3	8.1	7.9	8.4	8.2	98.2 \pm 3.2	104.3 \pm 5.1	101.5 \pm 3.6
4	10.5	10.4	10.1	10.3	99.3 \pm 3.7	96.6 \pm 2.1	98.0 \pm 4.1

PLS was a better predictor of real samples than the original PLS and 1st derivate methods, as illustrated by comparison of relative standard deviation and recovery percentage for the three methods. Four replicate measurements were used to test the precision of the methods. The better R.S.D. values and recoveries were obtained with acceptable error for all assayed samples with 2nd derivate PLS. Consequently, 2nd derivate PLS performs better than other methods because the second derivate PLS method can improve the signal-to-noise ratio.

CONCLUSIONS

This research indicates that the 2nd derivate PLS is the best model for the simultaneous determination of ternary mixtures of EP, UA, and AC that cannot be resolved by ordinary spectrophotometric

methods. Ensuring methods are simple and precise, and they do not need prior concentration or extraction steps to prevent the loss of analytes. Furthermore, the method was successfully applied to the determination of analytes in real and pharmaceutical samples with excellent sensitivity and without the need for elaborate and tedious sample preparation steps. Satisfactory results demonstrate the utility of these procedures for the simultaneous determination of EP, UA, and AC in serum, plasma, and urine from patients using these medicines.

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