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Partial least squares method for simultaneous spectrophotometric determination of uracil and 5-fluorouracil in spiked biological samples

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Abstract

A quantitative spectroscopic method using multivariate data models based upon ultraviolet spectroscopy, is proposed for the simultaneous analysis of binary mixtures of uracil (UR) and 5-fluorouracil (5-FU). By multivariate calibration methods, such as partial least squares (PLS), it is possible to obtain a model adjusted to the concentration values of the mixtures used in the calibration range. In this study, the calibration model is based on absorption spectra in the 220–314 nm range for 25 different mixtures of UR and 5-FU. PLS was used for the construction of the calibration sets containing UR and 5-FU in the concentration range of 1.12-28.02 and 1.30-38.76 (in µg mL-1), respectively. Prediction error sum of squares (PRESS) was 0.5172 and 3.8191 and the root mean standard error of prediction (RMSEP) was 0.2936 and 0.7978 for UR and 5-FU, respectively. This procedure allows the simultaneous determination of UR and 5-FU in synthetic and spiked real samples.

Keywords*:* Simultaneous determination; Partial least squares, Uracil; 5-fluorouracil; Biological samples

1. Introduction

The deoxyribonucleic acid (DNA) consists of nucleobases (adenine, guanine, cytosine, thymine and in the case of RNA, uracil), deoxyribose and phosphate [1]. DNA integrity and function depend on processes that either exclude or remove the normal RNA base uracil (UR). UR can be used for drug delivery and as a pharmaceutical. When elemental fluorine is reacted with UR, 5-fluorouracil (5-FU) is produced. 5-FU is an anti-cancer drug (antimetabolite) used to masquerade as UR during type nucleic acid replication process. Because 5-FU is similar in shape to, but does not perform the same chemistry as UR the drug inhibits RNA replication enzymes, by eliminating RNA synthesis and stopping the growth of cancerous cells. UR's use in the body is to help carry out the synthesis of many enzymes necessary for cell function through bonding with riboses and phosphates [2]. UR serves as allosteric regulator and coenzyme for reactions in the human body and in plants [3]. The presence of UR is an indication of lactic acid bacteria contamination in the fruit [4]. UR derivatives containing a diazine ring are used in pesticides [5]. Fluorinated pyrimidines and related nucleosides have a significant anti-cancer activity. 5-FU is one of the most active anti-cancer drugs, clinically useful in the treatment of solid tumours arising from the gastrointestinal, breast, head, some skin cancers, yet it causes significant unpredictable and often serious toxicity. In the liver, the catabolic clearance of 5-FU is mediated by a series of enzymes that are normally responsible for the breakdown of pyrimidines like UR [6, 7].

Analytical methods have been described in the literature for the determination of UR and 5-FU, including gas chromatography (GC) [8], high performance liquid chromatography (HPLC) [9-11], ultra-performance liquid chromatography (UPLC) [12], capillary electrophoresis (CE) [13,14]. There are not simple and fast. They need expensive instrument and extra pure solvents and reagents.

Quantitative chemometrics methods, such as partial least squares (PLS) have been applied to multivariate chemical data for the analysis of mixtures with overlapping spectra [15-18]. PLS is a linear regression method that forms components as new independent variables in a regression model [19-24]. The theoretical basis of PLS regression is available in several references [25]. The basics of PLS regression is to suggest that after the composition of X and Y matrices into two new score and loading matrices using singular value decomposition or principal component analysis, it should be maximized the covariance between score vector in X-space and a score vector in Y-space or

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equivalently to maximize the size of the loading vector in Y-space derived from the score of the vector in X-space. In addition, several multicomponent determinations based on the application of these methods to spectrophotometric data have been reported [26-30].

To the best of our knowledge, this is the first report on the simultaneous spectrophotometric determination of UR and 5-FU in the chemical literature. The aim of this work is to propose partial least squares method to resolve two mixtures of UR and 5-FU in synthetic and real samples.

2. EXPERIMENTAL

2.1. Instrumentation and software

A jasco V-530 (UV-Vis spectrophotometer) diode array spectrophotometer controlled by Jasco computer and equipped with a 1 cm path-length quartz cell was used for UV-Vis spectra acquisition. Data acquisition between 220-314 nm was performed with the UV-Vis chem. A metrohm 744 pH-meter supplied with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 4.0 and 7.0. The data were treated in an AMD 2000 XP (256Mb ram) micro computer using MATLAB software, version 7.1(The Math Works). PLS calculates was carried out in the "PLS-toolbox" version 2.0(Eigenvectors company).

2.2. Chemicals

All the used chemicals were of analytical reagent grade. Trichloroaceticacid were purchased from Fluka, while UR and 5-FU were supplied from Merck. The stock solution of UR and 5-FU were prepared by dissolving them in KOH (Merck). A buffer solution (pH=7.0) was made of KH2PO4 and NaOH (Merck). Throughout the experiments, deionized water was used.

2.3. Procedure and sample preparation

Known amounts of the standard solutions were placed in a 10-mL volumetric flask and diluted to the final volume with deionized water (final pH = 7.0). Then they were used to set up the calibration set contained 25 standard solutions and the prediction set contained 6 standard solutions [31]. The linear dynamic range for each component was determined by regressing the absorbance at the corresponding λmax versus the analyte concentration. The concentration of UR and 5-FU ranged from between 1.12-28.02 and 1.30-38.76 µg mL^{-1}, respectively.

2.4. Serum and plasma samples

The serum and plasma samples were homogenized. For the deproteinization, 1 ml of 24% w/v trichloroacetic acid was added to 1 mL of serum and plasma. After 15 min, the resulted mixture was centrifuged for 15 min at 3000 rpm. Then, some of the NaOH solution was added to the supernatant solution to reach a final pH value of 7.0. Afterwards, an appropriate amount of the stock solution of UR and 5-FU was added to 0.5 ml of the finally prepared serum and plasma and then completed to the final volume (10 mL volumetric flask) with buffer solution to obtain the desired concentration in the linear range. The absorption spectrum was recorded in the range of 220-314 nm against a blank solution of universal serum and plasma [32].

2.5. Urine Sample

The Urine sample was diluted 1:3 with distilled water. Then, the cell debris and the particulate matter were removed from the urine using low-speed centrifugation (for 5 min at 1500 rpm). Afterwards, a certain amount of NaOH solution was added to the supernatant solution to reach a final pH value of 7.0. Also, appropriate amount of the stock solution of UR and 5-FU was added 0.5 ml of the finally prepared urine and completed to the final volume (10 ml volumetric flask) with a buffer solution to get the desired concentration in the linear range [33].

3. Results and discussion

3.1. Spectral behaviour

Figure 1 shows that the absorption spectra in the aqueous solutions of UR and 5-FU at pH 7.0. However pH 7.0 was chosen as the optimum pH for this work because both UR and 5-FU have maximum absorbance and minimum overlap at this pH (Figure 2).

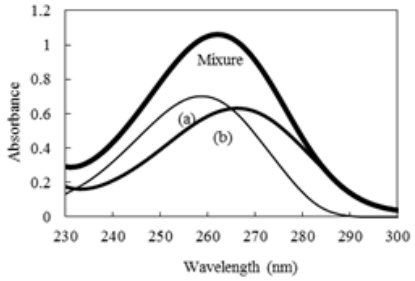

Figure 1. Absorption spectra of (a) UR (4.28 µg ml^{-1}), (b) 5-FU (5.32 µg ml^{-1}) and Mixture at pH 7.00 and temperature 298 k

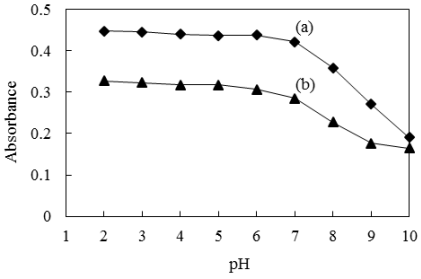

Figure 2. The effect of pH on the absorbance of: (a) UR (λmax = 258.3 nm, 1.12 µg ml^{-1}) and (b) 5-FU (λmax = 268 nm, 1.30 µg ml^{-1}).

3.2. Univariate calibration

Figure 3 shows the individual calibration curves were made with several points, as absorbance at λ_{max} versus analytes concentration in the range for UR and 5-FU. The λ_{max} used to produce the calibration curve, were 258.3 and 268 nm for UR and 5-FU, respectively. The regression line, line equations, and R^2 are also shown in Figure 3.

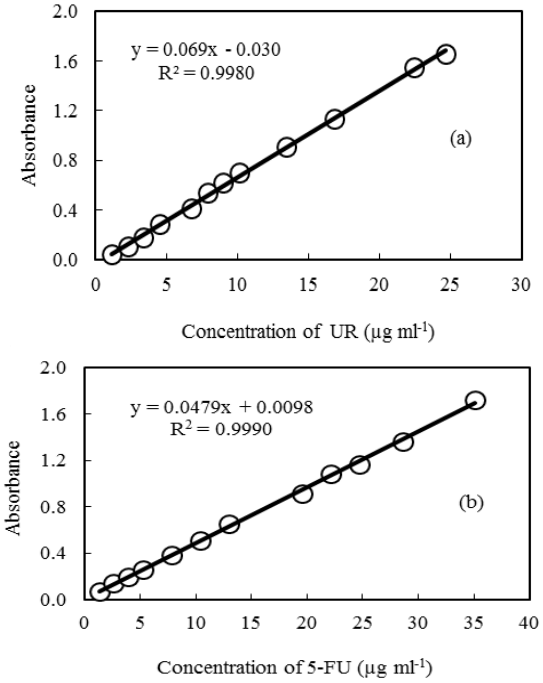

Figure 3. Analytical curve for univariate determination of (a) UR and (b) 5-FU.

3.3. Calibration and validation

The PLS methods are represented by two modifications, known as PLS-1 and PLS-2. The former performs the decomposition and regression for only one component at a time, whereas the latter calculates latent variables (optimum number of factors) based on all of the concentrations simultaneously so that only one calibration matrix is necessary. The ability of PLS calibration for resolving overlapped spectra was examined by selecting calibration and prediction sets. This paper reports the resolution of UR and 5-FU. Mixture analysis was carried out by application of the PLS-1 method to the conventional as well as the first-order derivative absorption spectra. Because of using PLS-1 modeling, for one to 10 latent variables (used in the PLS modeling), calculations were repeated for each component in prediction set sample solutions. Two sets of standard solutions were prepared. A set of standard samples was prepared according to mixture design. This leads to 25 samples for the calibration set. The concentration of UR and 5-FU was varied between 1.12-28.02 and 1.30-38.76 µg mL^{-1}, respectively. The composition of the calibration set is given in Table 1. For the prediction set, six mixtures that were not included in the calibration set were employed as an independent test. The results obtained by applying constructed PLS-1 for each action in the 6 prediction samples are summarized in Table 2. The results obtained show that PLS, as a full spectrum chemometric approach, gives accurate prediction results in the simultaneous determination of UR and 5-FU with high overlapping spectra.

Table 1. Concentration data of the calibration set for two-component system using mixture design

Mixture	UR (µg mL^{-1})	5-FU (µg mL^{-1})	Mixture	UR (µg mL^{-1})	5-FU (µg mL^{-1})
1	1.12	1.30	14	14.57	29.40
2	1.12	10.67	15	14.57	38.76
3	1.12	20.03	16	21.30	1.30
4	1.12	29.40	17	21.30	10.67
5	1.12	38.76	18	21.30	20.03
6	7.85	1.30	19	21.30	29.40
7	7.85	10.67	20	21.30	38.76
8	7.85	20.03	21	28.02	1.30
9	7.85	29.40	22	28.02	10.67
10	7.85	38.76	23	28.02	20.03
11	14.57	1.30	24	28.02	29.40
12	14.57	10.67	25	28.02	38.76
13	14.57	20.03			

Table 2. Actual and founded results of the synthetic mixture of UR and 5-FU by PLS

Mixture	UR			5-FU		
	Actual (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	Actual (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
1	26.90	27.52	102.32	5.20	5.51	105.97
2	23.54	23.55	100.03	11.71	12.29	104.95
3	20.18	20.12	99.73	15.61	16.01	102.57
4	12.33	12.58	102.00	26.02	24.52	94.22
5	8.97	9.19	102.44	29.92	29.00	96.93
6	5.04	5.17	102.56	32.52	32.87	101.09
RSEP (%)			1.6136			3.5916

3.4. Selection of the optimum number of factors

The optimum number of factors (latent variables) to be included in the model was determined by computing the prediction error sum of squares (PRESS) for cross-validated models using a high number of factors (half the number of total standard + 1), which is defined as follows:

$$PRESS = \sum_{i=1}^{n} (y_{pred} - y_{obs})^2 \qquad (1)$$

Where y_{pred} is the reference concentration for the *i*th sample and y_{obs} represents the estimated concentration. The cross-validation method employed was to remove only one sample at a time and then PLS calibrate the remaining standard spectra. By using this calibration the concentration of the sample left out was predicted. This process was repeated until each standard had been left out once.

One reasonable choice for the optimum number of factors would be that number which yielded 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 has been suggested by Haaland et al [34, 35]. In which the PRESS values for all previous factors are compared with the PRESS value at the minimum. The F-statistical test can be used to determine the significance of PRESS values greater than the minimum. The maximum number of factors for UR and 5-FU used to calculate the optimum PRESS were selected 2 and 3 respectively. The optimum number of factors obtained by the application of PLS model is summarized in Table 3. In Figure 4 are shown the plots of PRESS against the number of factors with the PLS method for UR and 5-FU. The number of factors for the first PRESS values whose F-ratio probability drops below 0.75 was selected as the optimum.

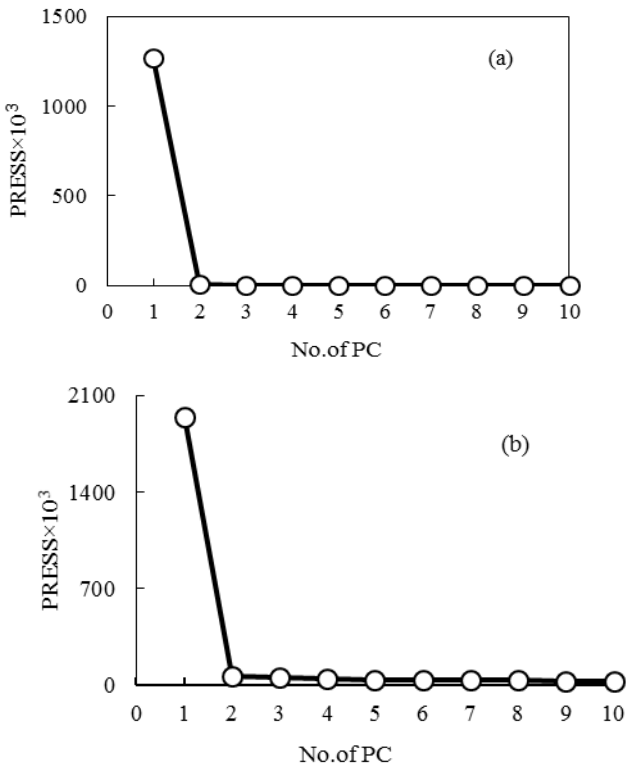

Figure 4. Plots of PRESS vs. No. of PC by PLS for (a)

Table 3. Statistical parameters of the optimized, using the PLS model for prediction set

Component	NPC	PRESS	RMSEP	RSEP (%)
UR	3	0.5172	0.2936	1.6136
5-FU	2	3.8191	0.7978	3.5916

3.5. Statistical parameters

To evaluate the predictive ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) were used (36).

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

$$RSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{obs})^2}{\sum(y_{obs})^2}} \times 100 \quad (3)$$

Where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed value of the concentration in the sample and n is the number of samples in the validation set. The values of RMSEP and RSEP (%) for UR and 5-FU are summarized in Table 3.

3.6. Determination of UR and 5-FU in synthetic mixtures

The predictive ability of the method was determined using 6 two-component mixtures of UR and 5-FU (their compositions are giving in Table 2). The results obtained by applying PLS algorithm to six synthetic samples are listed in Table 3 which also shows the recovery for prediction series of UR and 5-FU mixtures. As can be seen, the recovery was also acceptable. The plots of the predicted concentration versus actual values by the PLS method for UR and 5-FU are drawn in Figure 5.

3.7. Determination of UR and 5-FU in spiked real samples

To test the applicability and matrix interferences of the proposed method to the analysis of real samples, the method was applied in human serum, plasma and urine samples. The PLS method was applied for the determination of UR and 5-FU in serum, plasma and urine. The results are shown in Table 4. The good agreement between these results and known values indicates the successful applicability of the proposed procedure for simultaneous determination of UR and 5-FU in real samples.

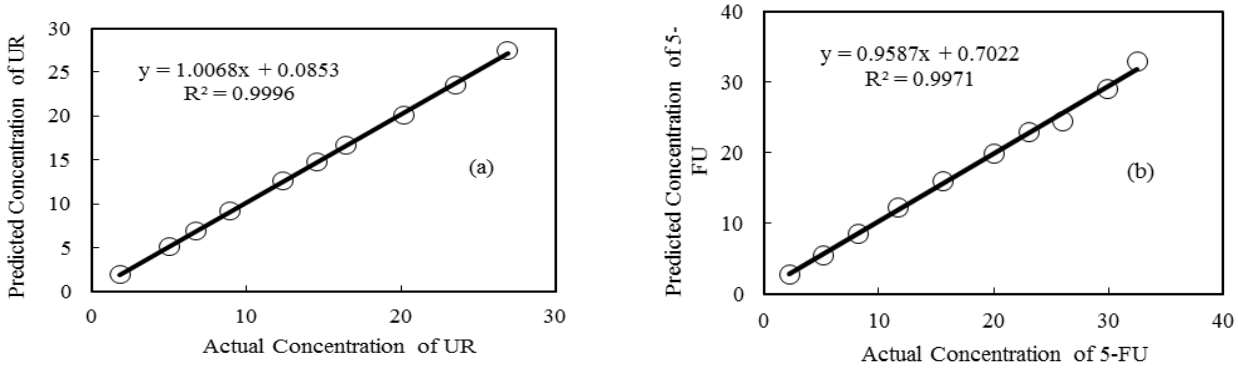

Figure 5. Plots of predicted concentration vs. actual concentration for (a) UR and (b) 5-FU (in μg ml^{-1})

Table 4. Recovery study of simultaneous determination of UR and 5-FU in spiked real samples by PLS

Serum	UR			5-FU		
	Actual (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	Actual (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
1	10.09	10.24	101.49	11.22	11.10	98.93
2	17.93	18.02	100.50	16.91	17.11	101.18
3	2.42	2.28	94.21	13.01	13.12	100.85
4	5.78	5.64	97.58	9.11	9.34	102.52
RSEP (%)			1.2231			1.3297

Table 4. (continue)

Plasma	UR			5-FU		
	Actual (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	Actual (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)
1	10.09	10.72	106.24	11.22	11.48	102.32
2	17.93	16.87	94.09	16.91	16.57	97.99
3	2.42	2.59	107.02	13.01	13.41	103.07
4	5.78	5.71	98.79	9.11	9.57	105.05
RSEP (%)			5.9514			2.8610

Table 4. (continue)

Urine	UR			5-FU		
	Actual (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	Actual (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)
1	10.09	10.00	99.11	11.22	11.27	100.45
2	17.93	17.66	98.49	16.91	16.27	96.22
3	2.42	2.62	108.26	13.01	13.15	101.08
4	5.78	5.84	101.04	9.11	9.01	98.90
RSEP (%)			1.6588			2.6152

4. Conclusion

The application of chemometric techniques such as PLS to ultraviolet spectroscopy is not particularly new but necessary in this work. The uracil and 5-fluorouracil mixture is an extremely difficult complex system due to the high spectral overlapping observed between the absorption spectra for their components. For overcoming the drawback of spectral interferences PLS multivariate calibration approaches have been applied. The good agreement clearly demonstrates the utility of this procedure for the simultaneous determination of uracil and 5-fluorouracil in human serum, plasma and urine samples without any primary chemical reaction or separation steps.

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روش حداقل مربعات جزئی برای اندازه گیری اسپکتروفوتومتری اوراسیل و ۵- فلئوراسیل در نمونه‌های بیولوژیکی افزوده شده

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چکیده:

یک روش اسپکتروسکوپی کمی برای تجزیه‌ی همزمان مخلوط دو جزئی اوراسیل (UR) و ۵- فلورواوراسیل (5-FU)، با استفاده از کالیبراسیون چند متغیره پیشنهاد شده است. با استفاده از روش های کالیبراسیون چند متغیره از قبیل کمترین مربعات جزئی (PLS) امکان دستیابی به طراحی یک مدل برای مقادیر غلظتی، از مخلوط مورد استفاده در محدوده ی کالیبراسیون وجود دارد. در این مطالعه؛ مدل کالیبراسیون بر روی طیف جذبی در محدوده ی ۳۱۴-۲۲۰ نانومتر اعمال شده است. PLS برای ترکیبی از سری کالیبراسیون شامل UR و 5-FU به ترتیب در محدوده ی غلظت ۲۸/۰۲-۱/۱۲ و ۳۸/۷۶-۱/۳۰ (میکروگرم بر میلی لیتر) استفاده شد. مجموع مربعات خطای پیشگویی (PRESS) به ترتیب برای UR و 5-FU ۰/۵۱۷۲ و ۳/۸۱۹۱ و مجذور میانگین خطای استاندارد پیشگویی (RMSEP) به ترتیب ۰/۲۹۳۶ و ۰/۷۹۷۸ بود. این روش اندازه‌گیری همزمان UR و 5-FU را در نمونه های سنتزی و واقعی فراهم می سازد.

کلمات کلیدی: اندازه گیری همزمان، کمترین مربعات جزئی، اوراسیل، ۵- فلورواوراسیل، نمونه‌های بیولوژیکی

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