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Simultaneous spectrophotometric determination of paracetamol and para-aminophenol in pharmaceutical dosage forms using two novel multivariate standard addition methods based on net analyte signal and rank annihilation factor analysis

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Recently we proposed two multivariate standard addition methods using net analyte signal concept and rank annihilation factor analysis, which we named SANAS and SARAF, respectively. These methods could model both indirect sample matrix effects and direct interference effects of the coexisting analytes. Here, the potential of these methods for simultaneous determination of paracetamol and para-aminophenol in the synthetic mixtures and pharmaceutical products was examined. It was found that both models could predict the concentration of analytes in synthetic mixtures with relative errors of prediction lower than 7%. Moreover, the employed methods exhibited the recoveries in the range of 93.11 and 108.67 for analysis of the drugs in the real matrices. From SANAS plots, the figures of merit including sensitivity, selectivity and limit of detection were calculated as 0.143 μg/ml, 0.587 and 0.018 μg/ml for paracetamol and 0.128 μg/ml, 0.547 and 0.020 μg/ml for para-aminophenol, respectively.

Supporting information may be found in the online version of this article.

Keywords: multivariate standard addition; paracetamol; para-aminophenol; net analyte signal; simultaneous determination

Introduction

Paracetamol (PAR, N-acetyl-p-aminophenol or acetaminophen) is an effective and safe analgesic agent to moderate pain; it is used worldwide to reduce fever, cough, cold, headache, and migraine headache and also to moderate pain associated with gastric symptoms.[1,2] It is a synthetic non-opiate derivative of para-aminophenol (PAP) and in inappropriate storage conditions, such as high temperatures and acidic or basic media, it could be hydrolyzed to PAP. The presence of PAP with PAR as an impurity or a synthetic intermediate has been detected and reported to have nephrotoxicity and teratogenic effects.[3,4] According to the European pharmacopoeias, the presence of PAP in PAR preparations is limited to a low value of 0.005%.[5] Depending on the storage condition, however, the amount of PAR converted to PAP varies and in some instances is higher than the permissible level.[5] Since PAP shows both environmental and biochemical risks, determination of its trace amounts in pharmaceutical preparations is of special importance.[6]

PAR is available in different dosage forms of tablets, capsules, drops, suspensions, elixirs, and suppositories and so the simultaneous determination of PAR and PAP in acetaminophen formulations can be very important in drug quality control. The determination of PAR and PAP individually or in the presence of other compounds in drug products has been reported using numerous methods such as elecrochemical methods,[7–9] spectrophotometry,[10,6] chemiluminescence,[11,12] and liquid chromatography.[13,14] In addition, simultaneous determination of PAR and PAP or determination of PAP in PAR formulations have been the subject of some researches employing spectrophotometry[15] liquid chromatography[13,14] and electroanalytical techniques.[16]

In simultaneous determination of several coexisting analytes, especially when spectroscopic methods are employed, peak overlapping. The limitation of poor selectivity of some analytical sensors in calibration models has been compensated by applying the first-order multivariate methods.[17] Another serious problem in calibration could occur when the sensitivity of sensors varies with the changing of the matrix in the sample, i.e. matrix effect, which can be diminished using standard addition method (SAM). Generalized standard addition method (GSAM)[17,18] is a combination of multivariate calibration methods with standard addition method to handle the interference effects of both sample matrix and coexisting species. Very recently, we proposed two new multivariate standard addition methods based on net analyte signal concept (named SANAS) and rank annihilation factor analysis (named SARAF).[19] SANAS process the signals

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of a multichannel instrument and results in a linear calibration graph similar to that which is obtained in the conventional standard addition method. Thus, it reduces the dimension of the multivariate signals and visualizes them as a univariate plot. The obtained standard addition plots are used to quantify the amount of analyte(s) in the unknown samples and to calculate the figures of merit of the employed analytical procedure as well. On the other hand, SARAF, which works based on the annihilating the contribution of the analyte of interest from the total signal, produces prediction results that are more robust to the outliers.[19]

In this work, we employed the SANAS and SARAF methods to develop a simple and efficient method for simultaneous determination of trace amounts of PAR and PAP in the synthetic mixtures as well as pharmaceutical dosage forms.

Theory

Standard addition method using net analyte signal (SANAS)

Net analyte signal (NAS) is a well known algorithm which has been applied to different branches of chemometrics like calibration,[20–22] wavelength selection and outlier detection,[23] figures of merit calculation,[20,24] the development of new spectral processing methods,[25–27] and hard-soft modelling methods.[28,29] NAS for an analyte is defined as the part of its spectrum that is orthogonal to the spectral space of the other sample components.[30] The theory of NAS, the base of SANAS, can be found elsewhere in detail;[31,32] a brief description of the NAS theory is presented here and will be continued with the SANAS algorithm.

Suppose that $\mathbf{r}$ is a multicomponent mixture spectrum and the goal is the extraction of kth analyte pure portion ($\mathbf{r_k}^*$) from this spectrum. If we have the rank annihilated matrix $\mathbf{R_{-k}}$, which is the matrix of spectra of all components in the mixture except the kth component, the orthogonal part of $\mathbf{r}$ on the $\mathbf{R_{-k}}$ space is $\mathbf{r_k}^*$:

$$r_k^* = (I - R_{-k}R_{-k}^+)r \quad (1)$$

where, $\mathbf{I}$ is an identity matrix and $\mathbf{R_{-k}}^+$ represents the pseudo inverse of $\mathbf{R_{-k}}$. So $(\mathbf{I} - \mathbf{R_{-k}}\mathbf{R_{-k}}^+)$ is a projection matrix to obtain orthogonal part of $\mathbf{r}$ that projects $\mathbf{r_k}$ onto the null space of the rows of $\mathbf{R_{-k}}$. Several approaches have also been proposed to obtain $\mathbf{R_{-k}}$ and to construct the projection matrix. In classical approach $\mathbf{R_{-k}}$ is simply considered as pure spectra of all coexisting components, except the analyte.[30]

If no information is available about pure spectra of the analytes, an inverse calibration approach should be applied. Lorber *et al*.,[20] Berger *et al*.,[21] Xu and Schechter,[22] Goicoechea and Olivieri,[31] and Ferre *et al*. [32] used different algorithms to construct $\mathbf{R_{-k}}$ matrix by an inverse approach. In the Lorber approach, the absorbance data matrix of calibration mixtures ($\mathbf{R}$) is reproduced by principal components analysis (PCA), or PLS using f significant principal components, yielding $\mathbf{R_{reb}}$. Then by a rank annihilation step in the f-dimensional space, the part of the original matrix spanned by the interferences could be found:

$$\mathbf{R}_{-k} = \mathbf{R}_{reb} - \alpha\hat{\mathbf{c}}_k\mathbf{r}_k^T \quad (2)$$

where, $\mathbf{r_k}$ is related to the pure spectrum vector of the kth analyte or ever a linear combination of the $\mathbf{R}$ rows, which is chosen in such a manner that includes a contribution from the spectrum of the kth analyte. Although any reasonable spectrum can be used for this purpose, it has recommended using a spectrum that contains maximal information on the analyte; therefore, the pure spectrum of the kth analyte is the best choice (if it was available). $\hat{\mathbf{c}}_k$ is the projection of the vector of kth analyte concentration, $\mathbf{c_k}$, onto the f-dimensional subspace and is calculated by $\hat{\mathbf{c}}_k = (\mathbf{R}_{reb}\mathbf{R}_{reb}^+)\mathbf{c}_k$.

The matrix $\mathbf{R_{-k}}$ could be used to construct projection matrix for extracting the net kth analyte signal from unknown mixture spectra ($\mathbf{r_u}$). Now, consider if the spectra of the unknown sample (i.e. a real sample) is affected by the matrix of the sample. Clearly, the indirect interferences or matrix effect will change the spectrum of sample (peak position and/or peak intensity) and so cause a significant difference between calibration and unknown (prediction) spectra. In this situation, the $\mathbf{R_{-k}}$ obtained from calibration set does not work for an unknown sample in the prediction step. SANAS is a solution to encounter matrix effect using net analyte signal calculations.[19] In a simple word, in SANAS, $\mathbf{R_{-k}}$ is constructed by a standard addition strategy.

Consider an unknown sample solution containing n signal-generating components. In a series of m similar volumetric flasks, an equal amount of this solution is added. One flask is left and to the remaining flasks are added different concentrations of the calibration standards. If the absorption spectra of these solutions (m solutions) are recorded in w wavelength, the absorbance data could be collected in a matrix $\mathbf{R_{sa}}$ (standard addition data), each row of which is the digitized absorbance spectrum of one flask. It should be noted that to one flask no standards were added and thus its corresponding spectrum in $\mathbf{R_{sa}}$ possesses information about only the unknown sample. This row vector is named as $\mathbf{r_u}$. By subtracting $\mathbf{r_u}$ from all rows of $\mathbf{R_{sa}}$, a matrix $\mathbf{R_{sm}}$ is obtained. This new matrix possesses spectral information of the added standards, to which the matrix effect is included. Hence, the subscript 'sm' is used to denote matrix-effect included standards.

Since the concentration of added standard of a given analyte in $\mathbf{R_{sm}}$ matrix is known, the $\mathbf{R_{sm}}$ could be used to construct $\mathbf{R_{-k}}$ for this analyte (k might be each of n analytes). It is interesting to note that to generate a SANAS model for a given analyte, no information about the concentration of the other analytes is necessary. The matrix $\mathbf{R_{sm}}$ is first subjected to PCA or PLS analysis and is reproduced by using the first f significant principal components to produce $\mathbf{R_{sm,reb}}$, and then is replaced by $\mathbf{R_{reb}}$ in Eqn 2 to calculated $\mathbf{R_{-k}}$,

Now, $\mathbf{R_{-k}}$ is available and the extraction of net signal of kth analyte from the original standard addition matrix ($\mathbf{R_{sa}}$) is straightforward (using Eqn 1). It's better to reconstruct $\mathbf{R_{sa}}$ also by first f significant principal components and then calculate the net part related to kth analyte.

$$\mathbf{R_{sa,k}}^* = (\mathbf{I} - \mathbf{R_{-k}}\mathbf{R_{-k}}^+)\mathbf{R_{sa,reb}} \quad (3)$$

where $\mathbf{R_{sa,reb}}$ is $\mathbf{R_{sa}}$ after reconstruct by f significant principal components and $\mathbf{R_{sa,k}}^*$ is the net signal of the kth analyte of interest that represent a direct relationship with the concentration of the kth analyte in standard addition samples (the summation of unknown and added standard concentrations). By plotting the Euclidean norm of the vectors of $\mathbf{R_{sa,k}}^*$ (i.e., $||\mathbf{r_{sa,k}}^*||$) *vs* the concentration of the added standards of the kth analyte, one can obtain a linear standard addition graph similar to a univariate standard addition plot. The extrapolation of this linear calibration graph to the concentration axes gives the negative sign of the kth analyte's concentration in the unknown mixture.

Figures of merit calculation using NAS

NAS is a convenient tool to characterize the figures of merit associated with the calibration models.[30,20] So NAS could be critical for a meaningful understanding of analytical performance and calculating parameters concepts like selectivity (SEL), sensitivity (SEN), limit of detection (LOD), and limit of quantitation (LOQ) in multivariate calibration. Replacing $\mathbf{R}_{sm,reb}$ by the $\mathbf{R}_{sa,reb}$ in Eqn 3 gives the net analyte signal of the calibration standards affected by the unknown matrix ($\mathbf{R}_{sm}{}^*$). The produced variation by the $\mathbf{R}_{sm}{}^*$ is directly related to the concentration of the analyte of interest in the calibration set:

$$R^*_{sm} = c_k s^*_k \tag{4}$$

where $s_k{}^*$ is the net sensitivity vector of the k^{th} analyte and multivariate sensitivity simply defined as the norm of $s_k{}^*$:

$$SEN_k = \left\| \mathbf{s}^*_k \right\| \tag{5}$$

The ratio of SEN_k to the norm of the pure spectrum of the k^{th} analyte, gives a measure of multivariate selectivity for the analyte of interest:

$$SEL_k = \frac{\left\| \mathbf{s}^*_k \right\|}{\left\| \mathbf{s}_k \right\|} \tag{6}$$

We can also calculate the LOD and LOQ, as other important figures of merit, using the following equations:[33]

$$LOD_k = \frac{3\,\|\varepsilon\|}{\left\| \mathbf{s}^*_k \right\|} \tag{7}$$

$$LOQ_k = \frac{10\,\|\varepsilon\|}{\left\| \mathbf{s}^*_k \right\|} \tag{8}$$

where ε is a measure of the instrumental noise at different working wavelengths. The value of $||\varepsilon||$ was estimated, in turn, by registering spectra for several blank samples, calculating the norm of the NAS for each blank ($||NAS_{blank}||$), and the corresponding standard deviation of all resulting $||NAS_{blank}||$. This standard deviation was taken as an approximation to $||\varepsilon||$.

Standard addition method using RAFA (SARAF)

In SARAF, an iterative rank reduction method is used to find the concentration of k^{th} analyte in the unknown sample ($c_{u,k}$).[19] In each standard addition sample, the concentration of k^{th} analyte (c_k) is the sum of unknown ($c_{u,k}$) and added standard ($c_{s,k}$) concentrations, $c_k = c_{u,k} + c_{s,k}$. As was discussed in previous section, $\mathbf{R_{sm}}$ and $\mathbf{C}_s$ are available and so the pure spectra of the analytes affected by the unknown's matrix ($\mathbf{S}_m$) could be obtained by a simple least square:

$$\mathbf{S}_m = \mathbf{C}_s{}^{+}\mathbf{R_{sm}} \tag{9}$$

Equation 10 shows how one can reduce the contribution of k^{th} analyte from $\mathbf{R_{sa,reb}}$

$$\mathbf{R_{-k}} = \mathbf{R_{sa,reb}} - (c_{u,k} + \mathbf{c}_{s,k})\mathbf{s}_{m,k}{}^{T} \tag{10}$$

where $\mathbf{c}_{s,k}$ is the concentration vector of the added standard of the k^{th} analyte to the unknown and $\mathbf{s}_{m,k}$ is the pure spectra vector of the k^{th} analyte (i.e. one row of $\mathbf{S_m}$). The matrix $\mathbf{R_{-k}}$ is calculated iteratively by changing in $c_{u,k}$. Only at a specified value of $c_{u,k}$ the rank of $\mathbf{R_{sa,reb}}$ is annihilated by one, and thus the rank of $\mathbf{R_{-k}}$ is lower than that of $\mathbf{R_{sa,reb}}$ in one unit. A common way to check whether the rank of $\mathbf{R_{sa,reb}}$ is reduced by one is calculating the number of significant (or systematic) principal components of $\mathbf{R_{-k}}$. Relative standard deviation (RSD) can be used as a criterion for determination of the number of principal components:[34]

$$RSD(f) = \sqrt{(\sum_{i=f}^{m} \lambda_i)/[w(m-f)]} \tag{11}$$

where λ_i is the i^{th} eigenvalue of $\mathbf{R}_{-k}$, f is the initial rank of $\mathbf{R_{sa,reb}}$ and w and m are the same as defined previously. At the correct value of c_{uk}, the rank of $\mathbf{R}_{-k}$ is $f-1$ and therefore its f^{th} RSD is at minimum value.

Experimental

Reagents

All chemicals were of analytical reagent grade. The stock solutions of PAR (Darou Pakhsh Co., Tehran, Iran) (2000 μg ml^{-1}) and PAP (Fluka, Buchs, Switzerland) (500 μg ml^{-1}) were prepared by dissolving suitable amounts of the compounds in doubly distilled water containing 0.01M HCl. The solutions were stored in the refrigerator and used to prepare the daily working solutions.

Phosphate buffer solution (50 mM, pH 7.0) was prepared by mixing phosphoric acid (Fluka, Buchs, Switzerland) and NaOH solutions (Merck, Darmstadt, Germany) and adjusting the pH with a pH meter.

As real samples, the following pharmaceutical products were analyzed: Paracetamol-tablets 325 mg (Jalinous, Tehran, Iran), Paracetamol tablets 325 mg and 500 mg (Chemidarou, Tehran, Iran) and Paracetamol oral solution 120 mg/5ml (Behsa, Arak, Iran).

Instrumentation

Spectrophotometric measurements were performed with a diode array UV-VIS spectrophotometer (HP 8452A) equipped with a 10-mm quartz cell. Absorbance data were collected using UV-VIS Chem Station software for the instrument, and then transformed to a Pentium IV PC with Windows XP operating system for subsequent manipulation. All necessary programs were written in MATLAB (version 7, Math Works, Inc., Natick, Massachusetts) environment.

The pH of solutions was determined with an AZ 86502 pH-meter equipped with a combined glass electrode.

Experimental procedure

The calibration standard set was composed of nine randomly designed binary mixtures of PAR and PAP, where in the first sample the concentrations of both analytes were taken as zero (Table 1). These compositions were used for standard addition experiments. In the case of each unknown sample, to a series of nine 10.0-ml volumetric flasks containing a constant amount of unknown sample (synthetic or real samples), desired volumes of 2000 μg ml^{-1} of PAR and 500 μg ml^{-1} of PAP were added using a micro-syringe (to reach to the final concentrations indicated in Table 1). 6.0 ml of phosphate buffer (50 mM, pH 7.0) was added to each flask and the mixtures were then diluted to the mark with doubly distilled water. The absorption spectra of the resulting solutions were recorded in the wavelength range of

Table 1. Composition of added standards (PAR and PAP), to each of the synthetic and real unknown samples

No. sample	PAR (μg/ml)	PAP (μg/ml)
1	0	0
2	4	1
3	2	2.5
4	3	3.5
5	6	1.5
6	8	2
7	5	1
8	2	4
9	6	3

Table 2. The selectivity (SEL) and sensitivity (SEN) calculated for the PAR and PAP at different pH

	PAR		PAP	
pH	SEL	SEN	SEL	SEN
2	0.569	0.107	0.388	0.019
3	0.564	0.107	0.380	0.017
4	0.563	0.032	0.458	0.087
5	0.531	0.027	0.446	0.080
6	0.665	0.092	0.474	0.103
7	0.630	0.147	0.516	0.127
8	0.680	0.171	0.479	0.097
9	0.631	0.232	0.480	0.097
10	0.662	0.150	0.523	0.119

220–320 nm. The absorption spectrum of each sample, digitized in 2-nm intervals, was used as each row of the $\mathbf{R_{sa}}$ matrix. The first solution, containing no added standard, was selected as the unknown sample ($\mathbf{r_u}$). By subtracting $\mathbf{r_u}$ from every row of $\mathbf{R_{sa}}$, the matrix $\mathbf{R_{sm}}$ was obtained.

Real samples

The mean weight of Jalinous-325 mg, chemidarou-325 mg, and chemidarou-500 mg tablets were 0.3393 (±0.0041) g, 0.4292 (±0.0043) g and 0.5971 (±0.0047) g respectively. For analysis of each product, 10 tablets were ground and mixed. A weighted obtained powder amount equivalent to one-fifth of each tablet weight (i.e. 0.0679 g, 0.0858 g and 0.1194 g of Jalinous-325 mg, chemidarou-325 mg, and chemidarou-500 mg, respectively) were dissolved in doubly distilled water. Then, they were stirred for 5 min, filtered, and brought to volume in a 200.0-ml volumetric flask. The optimum stirring time was obtained by monitoring the changes in the light absorbance of the resulting solution as function of time (in the time interval of 1–11 min). It was found that after 5 min the absorbencies did not show considerable changes, which means that the dissolution is completed after 5 min of stirring. To prepare a standard addition set for analysis of the real samples, the above solution was diluted 10 times and 1.0 ml of the resulting solution was added to each one of the 10.0-ml flasks (9 flasks) and standard solution of PAR and PAP were added to them as explained previously.

To prepare the syrups sample, 0.5 ml of the acetaminophen syrups 120 mg/5ml (Behsa, Arak, Iran) was dissolved in doubly distilled water and was reached to the volume in a 200 ml volumetric flask. The resulting solution was used to prepare the standard addition set as was described for the tablet products.

Before analyzing the real samples, tablets and syrups were analyzed by a UV standard procedure based on the British Pharmacopoeia 1993 assay[35,36] and their paracetamol contents determined.

Results and discussion

Preliminary studies showed that UV absorbance spectra of the PAR and PAP are pH-dependent. Thus, it was essential to select an optimum pH condition, in which selectivity and sensitivity are in their maximum value for both analytes. To do so, the absorbance spectra of the individual analytes' solutions at a constant concentration were recorded in aqueous solutions of variable pH values between 2.0 and 10.0. The multivariate sensitivity (SEN) and selectivity (SEL) for each compound were used to quantitatively measure the effect of pH and find its optimal value. They were calculated according to the Lorber definition employing the NAS concept.[20,30,37] The results are collected in Table 2. As it could be seen, the sensitivity is dependent on pH more than selectivity. Clearly, the sensitivity of PAR and PAP is highest at pH = 9 and pH = 7, respectively. However, the selectivity of PAR in pH = 7 is similar to that of pH = 9 whereas the selectivity of PAP in pH = 7 is more than pH = 9. In addition, since the amount of PAP is much lower than that of PAR in real samples, it is better to have higher sensitivity for PAP. Thus, pH 7 was selected as optimum pH.

Individual and mixture spectra of PAR and PAP at pH 7 are shown in Figure 1. Whilst the absorbance spectra of PAR and PAP represent distinct peak maximum at 244 nm and 230 nm, respectively, the spectrum of the equimolar mixture of the analytes exhibited a single peak maxima, which is the result of high overlapping of the spectra of the studied drugs. Therefore, simultaneous spectrophotometric determination of PAR and PAP by conventional calibration methods is not feasible.

Analysis of synthetic binary mixtures

To investigate the ability of SANAS and SARAF for simultaneous determination of PAR and PAP before their application to real pharmaceuticals, some synthetic unknown samples of PAR and PAP were analyzed. Seven synthetic samples of different amounts

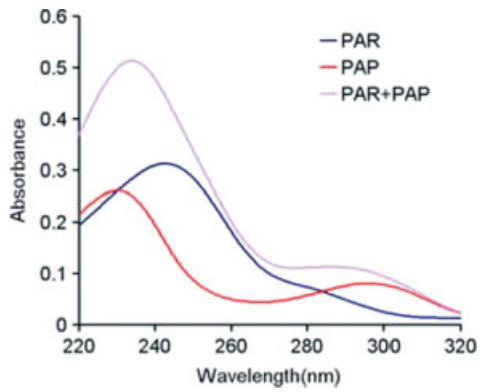

Figure 1. Absorbance spectra of the individual analytes' solution (each 4 μg/ml) and their equimolar mixture.

of PAR and PAP were prepared and a standard addition set was made for each of these samples (Table 3). Since in real samples, PAP is supposed to be present as an impurity in PAR formulations and its amount is very low in comparison with PAR, in most synthetic samples the concentration of PAP was selected lower than that of PAR. Preparation and analysis of each sample were repeated three times. The mean predicted values by SANAS are presented in the second block of Table 3. The relative error (RE%) of prediction is also given in this Table. The results are satisfactory good and almost all samples have not an RE bigger than 6%, except sample 3. The reported REs% for PAR is relatively lower than those of PAP. It might be because of the lower level of PAP compared with PAR.

The predicted concentrations depicted in Table 3 were based on the standard addition plots of SANAS. Such plots for analysis of synthetic unknown sample #1 of Table 3 are shown in Figure 2. As was noted in the theory section, these plots are obtained for each analyte by monitoring the changes in the ||NAS|| of each member of a standard addition solution as function of the concentration of the added standard. The plots of three repeated experiments are shown in Figure 2. Obviously, the plots are reproducible and the obtained standard addition graphs possess similar slopes and intercepts. The intersection of the plots with concentration axes could predict the concentration of the analytes in the unknown sample. In addition, the squared correlation coefficients of the shown calibration line are higher than 0.99, which confirm that there is a well-defined linear relationship between the ||NAS|| and the concentration of the added standards. Similar results were obtained for the other unknown samples. Details of analysis of unknown samples by SANAS are presented in Table S1 (supplementary information).

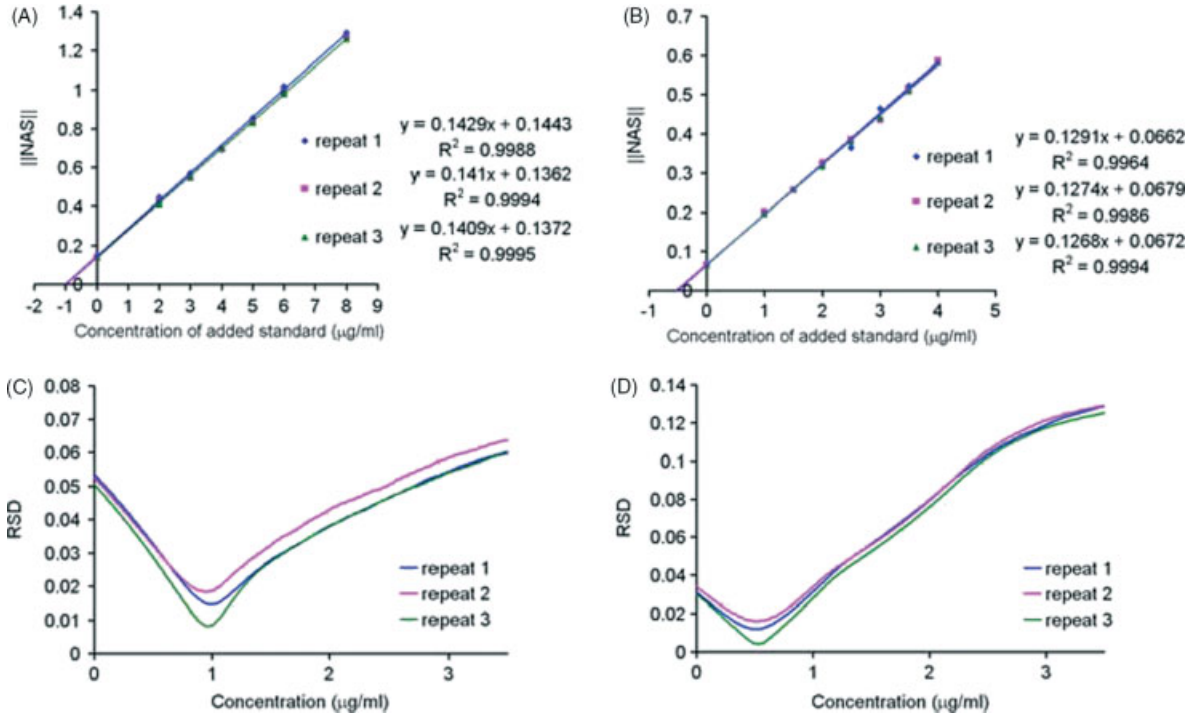

Figure 2. SANAS and SARAF plots of standard sample 1 (three replicate). **(A)** SANAS plot of PAR **(B)** SANAS plot of PAP (**C and D**) SARAF plots of PAR and PAP, respectively.

Table 3. Predicted concentration of PAR and PAP in seven synthetic samples by SANAS and SARAF and their corresponding relative errors of prediction (REP)

	SANAS						SARAF			
	Actual (µg/ml)		Predicted [a] (µg/ml)		REP (%)		Predicted (µg/ml)		REP (%)	
	PAR	PAP	PAR	PAP	PAR	PAP	PAR	PAP	PAR	PAP
Sample1	1.0	0.50	0.98 (±0.02)	0.53 (±0.01)	−2.00	6.00	0.97 (±0.03)	0.52 (±0.01)	−3.00	4.00
Sample2	2.0	0.50	1.97 (±0.03)	0.53 (±0.01)	−1.50	6.00	1.96 (±0.04)	0.53 (±0.01)	−2.00	6.00
Sample3	1.0	1.0	1.07 (±0.03)	0.94 (±0.04)	7.00	−6.00	1.05 (±0.03)	0.94 (±0.04)	5.00	−6.00
Sample4	4.0	1.0	3.93 (±0.07)	1.06 (±0.03)	−1.75	6.00	3.93 (±0.08)	1.06 (±0.03)	−1.75	6.00
Sample5	3.0	0.75	2.98 (±0.06)	0.77 (±0.03)	−0.67	2.67	2.98 (±0.06)	0.76 (±0.03)	−0.67	1.33
Sample6	6.0	0.50	5.88 (±0.01)	0.52 (±0.02)	−2.00	4.00	5.89 (±0.01)	0.52 (±0.02)	−1.83	4.00
Sample7	8.0	2.0	8.02 (±0.07)	2.06 (±0.05)	0.25	3.00	8.02 (±0.06)	2.05 (±0.07)	0.25	2.50

[a] Mean value (± standard deviation) (n = 3).

Table 4. Figures of merit for multivariate analysis of PAR and PAP

	SEN[a] (μg/ml)$^{-1}$	SEL[b]	LOD[c] (μg/ml)	LOQ[d] (μg/ml)
PAR	0.143	0.587	0.018	0.059
PAP	0.128	0.547	0.020	0.065

[a] Sensitivity.
[b] Selectivity.
[c] Limit of Detection.
[d] Limit of Quantitation.

As it was explained previously, an interesting application of SANAS is the possibility of the calculations of the figures of merit. They can be calculated based on the obtained standard addition plots using Eqns 5–8. The results are reported in Table 4. Obviously, sensitivity and selectivity of PAR is higher than PAP but LOD of PAP is a little larger than PAR.

SARAF was another multivariate standard addition method that was employed for simultaneous determination of the analytes of interest. As was explained previously, the basis of SARAF is to annihilate the rank of the analyte of interest iteratively according to Eqns 9–11. The RSDs (Eqn 11) that were obtained by iterative changes of concentration were plotted *vs* the iteratively changed concentration. The obtained SARAF plots are shown for synthetic sample #1 in the lower part of Figure 2. Clearly, the RSD reaches to minimum value at the concentrations, which are very close to the concentrations of analytes in the unknown sample. Although the SARAF plots of the repeated samples are not as close as those of SANAS, they exhibited high reproducibility for the predicted concentrations (they reach minimum values at very similar concentrations).

The mean values of the three replicate analyses by SARAF for each sample and their corresponding RE% are summarized in the third block of Table 3. As it can be seen, the absolute value of REs% obtained by SARAF for PAR in most cases are lower than 3% and for PAP are not bigger than 6%. These REs are similar to those obtained by SANAS and thus it is difficult to prefer one method over the other. The standard deviations of the predicted values of three repeated experiments by SARAF and SANAS (Table 3) are very small and also are very similar to each other. The details of predictions of PAR and PAP by SARAF in all synthetic samples (including repetitions) are included in Table S1.

The concentration range for PAR was 0.2–21.6 μg/ml and for PAP was 0.4–22.4 μg/ml. However, since the proposed method is a standard addition procedure, the total concentration of the analyte (i.e. the summation of the concentrations of the analyte (PAR or PAP) in the unknown sample and that of the added standard) should be in this range. According to the selected concentration of the added analytes for preparation of standard addition sets (Table 1), the higher concentration level that is work in our methods is 12.0 μg/ml for both analytes.

Analysis of real samples

Some Iranian brands of acetaminophen tablet and syrups were used as real samples. The composition of the standard addition solutions was the same as that used for analysis of the synthetic unknown solutions (Table 1). To investigate the accuracy of the proposed method, in addition to analysis of the real samples and comparison of the predicted values to the declared values by the companies, the real samples were spiked with two different levels of PAR and PAP and the percent of recoveries calculated. Thus, each real sample was analyzed three times: (1) analysis of the original real sample (Composition1); (2) analysis of the real sample spiked with some PAP (Composition2); and (3) analysis of the real sample spiked with both PAR and PAP (Composition3). Each composition was repeated three times. The results are represented in Table 5. The original sample (Composition1) was also analyzed by a UV spectrophotometric standard method according to British Pharmacopoeia 1993[35,36] and the results are included in Table 5.

As an example, the SANAS plot of real sample #4 of Table 5 (acetaminophen syrups, 120 mg/5 ml; Behsa, Arak) are presented in Figures 3A and 3B for PAR and PAP, respectively. As noted previously, three compositions were prepared using each real sample and preparation of each composition was prepared and analyzed three times. Figure 3 contains only one repeat of each composition. Interestingly, the slope of the standard addition lines is similar for real samples and the spiked ones. In addition, the intersection points of the plots with x-axes accurately determine the concentration of the analytes in the unknown samples.

SARAF plots of PAR and PAP in real sample #4 are shown in Figures 3C and 3D, respectively. Obviously, the minimum in RSD is observed at the concentrations where are close the concentration of analytes in the unknown samples. It is observed that when only PAP is added to the real sample, the minimum position of SARAF plot for PAR is not changed (compare Comp1 and Comp2 in Figure 3C). And for real sample without PAP, the SARAF plots do not exhibit a clear minimum (See Comp1 in Figure 3D).

The prediction results of real samples shown in Table 5 confirm the success of the employed multivariate standard addition methods for analysis of the real samples. Most of recoveries are in the range of 94–105% and there is not a significant difference between the prediction results for PAR and PAP. Special attention can be paid to the predicted values for PAP in the pharmaceutical products, in which PAP is absent according to the companies' claims. Table 5 shows that the predicted value of PAP in real samples by SARAF are zero or very close to zero (in the range of 0.003–0.007 μg/ml) whereas those obtained by SANAS are slightly higher (in the range of 0.028–0.077 μg/ml). Note that the predicted values of PAP in real samples by SARAF are lower than the LOD of the method. However, those obtained by SANAS are in the range of LOQ of the method.

The lower prediction error of real samples obtained by using of SARAF in some cases may be attributed to the fact that in SANAS, errors in $\mathbf{R_{sa}}$ are propagated to the NAS vectors of analyte k ($\mathbf{R_{sa,k}}^*$), which are subsequently propagated to the SANAS plot. It is clear that the slope and intercept of the SANAS plot are sensitive to the presence of outliers or the random errors. Hence, the values predicted by SANAS are more affected by random (or even systematic) errors with respect to SARAF. In other words, since SARAF did not use regression coefficients or regression line to determine the concentration of the unknown sample, it is less affected by the error propagation from the original data.[19] In addition, SARAF used lower numbers of calculation steps without the need to obtain a projection matrix as is used in SANAS (Eqns 1 and 3). It could also be noted that SARAF objective function is matrix rank and the rank annihilated matrix ($\mathbf{R_{-k}}$) is simply calculated by matrix subtraction (Eqn 5) and the random noises cannot significantly affect the rank determination by PCA.[38]

Table 5. Results of the analysis of PAR and PAP in pharmaceutical real sample using SANAS and SARAF

		SANAS						SARAF			
		Added (μg/ml)		Found [a] (μg/ml)		Mean Recovery		Found (μg/ml)		Mean Recovery	
		PAR	PAP	PAR	PAP	PAR	PAP	PAR	PAP	PAR	PAP
Jalinoos Tablet	comp1[b]	0.0[c]	0.0	3.214 ± (0.021)	0.028 ± (0.016)	–	–	3.214 ± (0.01)	0.000 ± (0.000)	–	–
(325 mg)	comp2	0.0	0.50	3.164 ± (0.023)	0.529 ± (0.005)	–	100.15	3.157 ± (0.021)	0.507 ± (0.006)	–	101.33
	comp3	1.0	0.50	4.248 ± (0.031)	0.522 ± (0.033)	103.44	98.87	4.213 ± (0.032)	0.493 ± (0.042)	100.03	98.67
Chemidarou Tablet	comp1	0.0[d]	0.0	3.253 ± (0.021)	0.074 ± (0.012)	–	–	3.287 ± (0.015)	0.003 ± (0.006)	–	–
(325 mg)	comp2	0.0	0.50	3.254 ± (0.021)	0.507 ± (0.011)	–	93.11	3.273 ± (0.049)	0.480 ± (0.010)	–	95.34
	comp3	2.0	1.0	5.239 ± (0.039)	1.007 ± (0.024)	99.32	96.57	5.243 ± (0.032)	0.983 ± (0.032)	97.82	98.00
Chemidarou Tablet	comp1	0.0[e]	0.0	4.858 ± (0.021)	0.041 ± (0.018)	–	–	4.887 ± (0.015)	0.007 ± (0.012)	–	–
(500 mg)	comp2	0.0	0.50	4.850 ± (0.038)	0.550 ± (0.018)	–	101.75	4.830 ± (0.026)	0.530 ± (0.040)	–	104.66
	comp3	1.0	1.0	5.864 ± (0.073)	1.091 ± (0.038)	100.59	104.99	5.830 ± (0.066)	1.060 ± (0.026)	94.30	105.33
Behsa Syrups	comp1	0.0[f]	0.0	6.271 ± (0.022)	0.077 ± (0.023)	–	–	6.280 ± (0.044)	0.000 ± (0.000)	–	–
(120 mg/5 ml)	comp2	0.0	0.50	6.250 ± (0.054)	0.567 ± (0.032)	–	97.97	6.253 ± (0.064)	0.543 ± (0.049)	–	108.67
	comp3	3.0	1.0	9.221 ± (0.063)	1.057 ± (0.054)	98.36	98.02	9.227 ± (0.075)	1.057 ± (0.050)	98.22	105.67

[a] Mean value (± standard deviation) (n = 3).
[b] comp 1, comp 2 and comp 3 are three different composition of each real sample.
[c,d,e,f] : the concentration of PAR in these samples was 3.112 (±0.069), 3.198 (±0.027), 4.812 (±0.027) and 4.792 (±0.027) based on British Pharmacopoeia 1993 proceture.

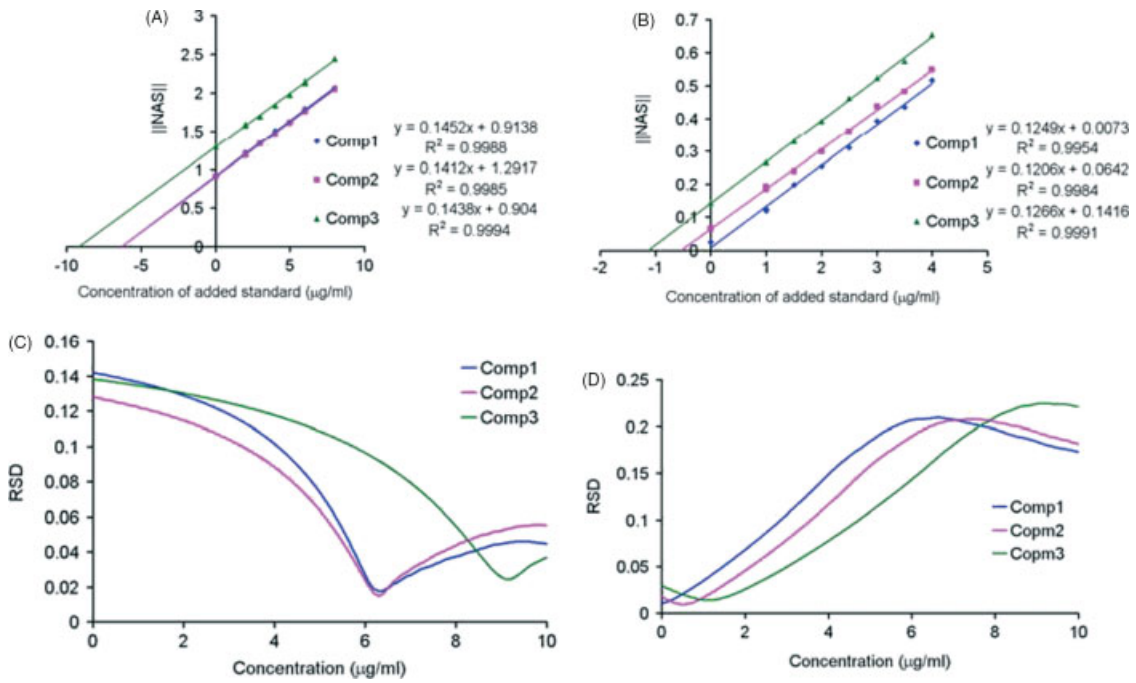

Figure 3. SANAS and SARAF plots of three different composition of real sample 4. **(A)** SANAS plot of PAR, **(B)** SANAS plot of PAP, **(C)** SARAF plot of PAR, **(D)** SARAF plot of PAP. Comp *i* shows the *i*th composition in the sample.

Conclusion

The contents of paracetamol and para-aminophenol, a usual drug and its problematic interference, were simultaneously determined using electronic absorption measurements, together with multivariate standard addition method based on net analyte signal calculations and rank annihilation factor analysis (SANAS and SARAF). Synthetic binary mixtures of combinations as well as commercial tablets and syrups were conveniently studied. Drug production steps such as reaction, crystallization, drying, solid dosage form manufacture, and tabletting were done in different conditions and the matrix effect of these different conditions could influence on the analyte spectrum. A big plus for standard addition methods is that they are not sensitive to matrix effect or indirect interferences. So these methods could be used to monitor the quality of paracetamol in different steps of drug manufacture that is applied at different temperatures, pH, or other conditions.

In comparison with simultaneous determination of PAR and PAP by electrochemical,[16] LC,[13,14] and kinetic sectrophotometric, the

present proposed spectrophotometric method is simpler and easy. On the other hand, the LOD of the present work is very close to that obtained by electrochemical method. [16]

Supporting information

Supporting information may be found in the online version of this article.

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