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Net analyte signal-based methods for the simultaneous determination of paracetamol, propyphenazone and caffeine by UV spectrophotometry

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Three different net analyte signal-based (NAS) methods - NAP CLS, HLA/GO and HLA XS were successfully applied for the determination of ternary mixtures of paracetamol (PAR), propyphenazone (PRO) and caffeine (CAF). The methods used absorbance UV-spectral data for resolving this complex system with overlapping drug spectra. A reduced five-level orthogonal design was used for the formation of a calibration set including the three compounds. The chemometric models were tested on an external validation dataset with concentrations within the calibration range. All proposed chemometric algorithms could be successfully applied for the determination of the above compounds in the pharmaceutical tablet formulation *Saridon*®. The obtained results showed that the NAS performance was similar compared to partial least-squares method (PLS-1). In addition, the use of the net analyte signal concept allowed the calculations of the analytical figures of merit. A moving window wavelength selection strategy was used, which significantly reduced the number of factors and improved the analytical recoveries.

Keywords: Drugs, Spectral overlap, NAS, NAP CLS, HLA/GO, HLA/XS, Wavelength selection

NTRODUCTION

The area of spectroscopy is one of the most promising fields in pharmaceutical analysis. Processing of the measured spectra is therefore needed to extract information about the components of interest. Chemometrics contribute to the processing of the spectra by delivering algorithms to find differences between spectra (classification) [1-8] or to link spectra to concentrations of compounds (calibration) [9,10]. In recent years the resolution of highly overlapped spectra has advanced dramatically due to the development of robust numerical methods.

The application of multivariate calibration techniques on spectral data offers the great advantage of speeding up complex systems resolution. Among the various chemometric approaches applied for multicomponent analysis, classical least squares (CLS), principal component regression (PCR), partial least squares regression (PLS) have been successfully adopted in many quantitative assays of pharmaceutical formulations [11, 12]. Hybrid linear analysis (HLA) is a relatively new linear algorithm, which combines the advantages of knowing pure component spectra (like CLS) with the modeling advantage of ignoring all other species (e.g., PLS) [13]. Two variants of HLA algorithms were introduced which did not require the pure spectrum to be known. Finally hybrid linear analysis developed by Xu & Schechter called HLA/XS [14], hybrid linear analysis called HLA/GO and net analyte pre-processing combined with classical least squares (NAP/CLS) developed by Goicoechea & Olivieri [15] were applied for resolving multicomponent pharmaceutical mixtures [16].

In the case of pharmaceutical analysis, either full-factorial or central composite designs are often employed for calibration. The preparations studied require designs other than full-factorial ones, which would imply too many calibration mixtures. Brereton's experimental plans are a suitable strategy to reduce calibration samples [17].

Multicomponent drug formulations are a great challenge for analytical chemists to develop reliable and easy methods for simultaneous estimation which do not require individual calculations for every single component [18]. The pharmaceutical multidrug formulations, in which one dominant drug is combined with other related drugs, are designated to enhance the pharmacological effects of each substance and to cover a wider medical treatment. One such popular combination, containing paracetamol as a dominant drug and

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propyphenazone and caffeine in smaller quantities is named *Saridon*®. *Saridon*® is manufactured by Bayer and it is a widely prescribed combination for pain relief in Eastern Europe.

Paracetamol (PAR) (4-hydroxyacetanilide) is a synthetic drug, derived from p-aminophenol. It is a widely used over-the-counter analgesic and antipyretic agent, which has no antiinflammatory properties. Therefore, it helps to prevent headache and other pain and is a major ingredient in numerous cold and flu remedies [19].

Propyphenazone (PRO) (3-dimethyl-1-phenyl-3-pyrazolin-5-one) is derived from pyrazolone. It is an analgesic, antipyretic and anti-inflammatory agent [20]. It is a non-steroidal antiinflammatory drug (NSAID) incorporated together with paracetamol into many over-the-counter analgesic combinations.

Caffeine (CAF) (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) is a xanthine substance used as a psychotropic stimulant drug [21]. Caffeine is also known to synergistically increase the analgesic effect of paracetamol and propyphenazone [22, 23], providing relief for symptoms like headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, migraine, toothache and menstrual pain.

It is found that the combination of the above mentioned drugs is also very effective in controlling fever originating from bacterial or viral infection. It is usually available in a tablet dosage form as a single unit dose with drug concentrations in varying proportions, but in *Saridon*® the content is: 250 mg of paracetamol, 150 mg of propyphenazone and 50 mg of caffeine.

Literature survey revealed several analytical methods reported for the determination of ternary combinations of PAR, PRO and CAF. Separation techniques such as HPLC and HPTLC have been reported for the analysis of this drug mixture in pharmaceutical dosage forms [24-26]. Vidal *et al.* carried out spectrophotometric determination by means of a single flow-through UV multiparameter sensor [27]. Derivative ratio zero-crossing spectrophotometric method of the three analytes was also demonstrated by Dink *et al.* [24]. The same authors proposed UV chemometric determination of this ternary mixture in pharmaceutical preparations [28]. De Luca *et al.* applied chemometric methods not only for absorbance but in derivative spectra for better resolving of the above drugs with a novel mathematical procedure including wavelength selection [29].

In the work of Gergov *et al.* [30] on the same drug mixture and *Saridon*® formulation the predictability of different PLS algorithms was investigated. This motivated us to further extend our work and to investigate the quantitative power of NAS-based methods and to compare them with PLS. NAS multivariate models may benefit from suitable wavelength selection which avoids heavily overlapped spectral regions. A sensor selection approach based on moving spectral windows strategy was applied in this work.

The goal of the present study was to compare different NAS calibration approaches (NAP CLS, HLA/XS, HLA/GO) and PLS1. Thus, an optimal chemometric methodology could be offered to solve the significant problems of modeling and analysis of multicomponent pharmaceutical systems.

EXPERIMENTAL

Reagents and solutions

Paracetamol, propyphenazone and caffeine were purchased from Sigma–Aldrich. All stock solutions were prepared by dissolving 100 mg of the corresponding compounds in 1 L of water. 22 mixtures, containing 0 – 24 mg L^{-1} of PAR, 0 – 20 mg L^{-1} of PRO and 0 – 20 mg L^{-1} of CAF in possible compositions were prepared from the stock solutions.

Apparatus and software

Absorption spectra were recorded over the λ range of 190 – 820 nm using a quartz cuvette of 1.0 cm optical path, by a HP8452A diode-array UV–VIS spectrophotometer.

Data were handled using MATLAB® software (ver. 7.0.1). Multivariate calculations by PLS1 and HLA were performed using MVC1 program which is available on the Internet and performed under MATLAB environment (MATLAB, ver. 7.0.1) or by our MATLAB codes which are available by request [31]. Before running MVC1, the spectral data for calibration, validation and real samples were saved in special format that is compatible with MVC1. MVC1 has the ability to display graphical presentations related to calibration tools.

Sample solutions

Pharmaceutical formulations were assayed by weighing the content of five tablets and crushing them into fine powder. An amount exactly corresponding to the average tablet weight was suspended in water and made up to a volume of 1000 mL. The suspension was sonicated for 10 min and then filtered through a PTFE 0.45 μm membrane filter. Samples for analysis were obtained after serial dilution 1:25 of this filtrate with water and analyzed.

Design of the experiment - calibration and validation sets

A calibration design set of 22 samples was used based on five levels, which was coded between -2 and +2 for each compound in the mixture. The levels were related to the concentrations of the compounds [17]. The same calibration design was used in our previous study [30]. The concentrations of the calibration set solutions were prepared within the linear range of the calibration graph. The design had a value of $r_{12} = 0.0$, so the two concentration vectors were orthogonal to one another. The difference vector [1 3 2 0] and cyclical generator -2, -1, 2, 1 were used in the calibration design matrix. The construction of multilevel calibration designs has been described in the literature [17]. A validation set was prepared in order to test the performance of the models. The chosen concentration levels of the three solutes were within the ranges selected for the calibration set.

Theoretical background

HLA method. Hybrid linear analysis (HLA) is a relatively new linear algorithm, which can be used when data for the considered pure analyte are available [13]. The main idea of HLA is to obtain a limited number of factors of a data matrix in which the contribution of the analyte of interest has been removed, and is therefore based on net analyte signal (NAS) calculation. The net analyte signal (NAS) for analyte *k* (*r*k*) is given by the following equation:

$$r^*k = [I - R_{-k}(R_{-k})^+]r = P_{NAS,kr} \quad (1)$$

where *R* is *JxJ* orthogonal projection matrix which projects a given vector onto the NAS space, *r* is the spectrum of a given sample, *I* is *JxJ* unitary matrix, R_{-k} is *JxI* a column space spanned by the spectra of all other analytes except k, $(R_{-k})^+$ is the pseudo-inverse of R_{-k} usually computed by singular value decomposition using *A* factors.

HLA HS method. There are different alternative approaches, which can be used in order to estimate the R_{-k} matrix. One of them is proposed by Xu and Schechter [14]. Each spectrum from the calibration matrix is divided by its concentration, except for the zero concentrations, and the sum of the resulting spectra is calculated using the following equation (2):

$$s_{cal} = \frac{1}{I'}\sum_{i=1}^{I}\frac{r_{i,cal}}{c_{ik}} \quad (2)$$

where I' is the number of the calibration samples for which c_{ik} is different from 0.

The resulting mean spectrum is obtained from the spectral matrix using the following equation (3):

$$(R_{-k})_{i',j} = R_{i',j}/c_{ik} - s_{cal,j}^{\mathrm{T}} \quad (3)$$

The calibration spectra with $c_{ik} = 0$ are then added to the matrix from equation (3) and thus the desired matrix R_{-k} is obtained. The net sensitivity vector s_k* is calculated through projection of $s_{k;LS}$ on the NAS area, using least squares method approximation according to the following equation:

$$s_k^* = P_{NAS,k}\left[\frac{R^T c_k}{c_k^T c_k}\right] \quad (4)$$

The concentration of κ for the unknown sample is calculated from the *r* spectrum using the equation (5), which is a basic step in the prediction for the methods, based on net analyte signal (NAS) [6].

$$c_{un,k} = \frac{s_k^T P_{NAS,k} r}{s_k^T P_{NAS,k} s_k} = \frac{s_k^T P_{NAS,k}\ P_{NAS,k} r}{s_k^T\ P_{NAS,k}\ s_k} = \frac{(s_k^*)^T r_k^*}{\|s_k^*\|^2} \quad (5)$$

HLA GO method. The applied HLA GO [11] method in this research involves the use of mean (uncentred) calibration profile. At first it is obtained as:

$$\overline{r_{cal}} = \frac{1}{I}\sum_{i=1}^{I} r_{i,cal} \quad (6)$$

where $r_{i,cal}$ is the profile for the *i*-th calibration sample. Then the contribution of analyte *k* is subtracted from the data matrix *R* in the following way:

$$R_{-k} = R - \frac{c_k\ \bar{r}_{cal}^T}{\bar{c}_{k,cal}} \quad (7)$$

where $\bar{c}_{k,cal}$ is the mean (uncentred) calibration con-centration of analyte *k*. The calculation of net sensitivity (s_k^*) is then carried out according to the following equation:

$$s_k^* = P_{NAS,k}\left[\frac{\bar{r}_{cal}^T}{\bar{c}_{k,cal}}\right] \quad (8)$$

NAP CLS method. The last possible approach to estimate the net sensitivity vector s_k* and R_{-k} matrix includes approximation of $s_{k;LS}$ using least squares method for obtaining the R_{-k} matrix (equation 9) and s_k* (equation 4).

$$R_{-k} = R - c_k\ s_{k,LS} \quad (9)$$

This method is called NAP CLS [11] and includes the following steps: (1) preprocessing of the raw initial spectral matrix *R* through projection on the space, orthogonal to the space of all mixture components, except for the *k* analyte, which results in obtaining the net analyte signal matrix R_k^* and regression of the obtained matrix with the concentrations using classical CLS procedure.

Figures of merits for the analytical method. Selectivity, sensitivity, signal to noise ratio, LOD

and LOQ are among the valuable analytical information that can be obtained from NAS [32]. MVC1 contains a special sub-routine, based on net analyte signal concept for estimation of figures of merit for the analytical method [31].

Determination of number of factors(A). The optimal number of principal factors is essential in building multivariate models [33]. The prediction error decreases with the number of factors used until an optimal value is reached. Most of the information is usually in the first factors but it is not guaranteed that the useful information is exclusively reserved to these factors. Full crossvalidation is the most used validation method, in which one reference at a time is removed from the calibration set, after that the same sample is predicted by using the calibration built with the other references. Several tests have been proposed to select the number of PCs.

The root mean square error of prediction (RMSEP) was chosen to express the prediction error when PLS1 and HLA/GO procedures were applied. This parameter represents an estimate of the error when other samples are predicted with that model. The best prediction ability of the models is reached when the prediction error is at its lowest value.

$$SEP = \frac{1}{C}\left[\frac{\sum_{i=1}^{m}(C_{pred}-C_{act})^2}{m}\right]^{\frac{1}{2}} \quad (10)$$

where C_{act} indicates the actual concentration in the sample, C_{pred} is predicted concentration, C is the mean of real concentrations in the validation set and m is the number of samples in the prediction set.

Another important statistical parameter in evaluating the model quality is R^2. It represents an index of quality in fitting all data to a straight line and represents the fraction of total variance explained by the model. It is computed as:

$$R^2 = 1 - \frac{\sum_1^m(C_{act}-C_{pred})^2}{\sum_1^m(C_{act}-C)^2} \quad (11)$$

where C represents the mean of the true concentrations in the prediction set.

The last statistical parameter for evaluating the quality of validation is relative error of prediction:

$$REP(\%) = \frac{100}{C}\left[\frac{1}{I}\sum_1^m\left(C_{act}-C_{pred}\right)^2\right]^{\frac{1}{2}} \quad (12)$$

Softwares. The NAP/CLS, HLA/HS, HLA/GO and PLS1 algorithm was applied using the Toolbox MVC1 written for MATLAB [31], because these routines allow one to evaluate the figures of merit based of the NAS theory·

RESULTS AND DISCUSSION

Spectral overlapping and data preprocessing

The zero-order UV spectra of PAR, PRO and CAF in a concentration ratio equivalent to the content of the commercial pharmaceutical formulation in water are shown in Fig.1. The wavelength range from 190–300 (56 wav) was selected because of the absence of absorbance after 300 nm for all of the drugs. It is obvious that the studied analytes in combination could not be determined directly in this spectral region because of strong overlapping.

Regression model building and wavelength optimization

With the aim to extract the most significant analytical information from the spectral region of 56 wavelengths the NAP/CLS, HLA/HS, HLA/GO and PLS1 calibration models were firstly developed on data. Selection of the optimum number of factors to be used within the all proposed algorithms allows one to model the system with the optimum amount of information. In the present work, cross-validation has been used to select the optimum number of factors. The statistical parameters of model prediction for both chemometric methods are presented in Table 1. A comparative study of the statistical parameters of all chemometric approaches was undertaken.

The models were validated by an external validation set. The results from the calibration methods with net analyte signal were much better when the calibration was carried out in a selected spectral region instead of the whole spectrum. The results from applying the NAP CLS, HLA GO, HLA XS and PLS1 methods for calibration with and without spectral selection are presented in table 1. The spectral selection was carried out using moving window strategy on the absorption spectra in order to define the most informative spectral regions [34]. As shown, without the wavelength selection the factors number was much smaller for the NAS methods compared to PLS. The RMSEP and REP values for PAR and PRO were lower with the NAS methods in comparison with PLS, whereas CAF showed similar RMSEP and REP values with NAS and PLS methods. The slope and intercept values for PRO were unsatisfactory.

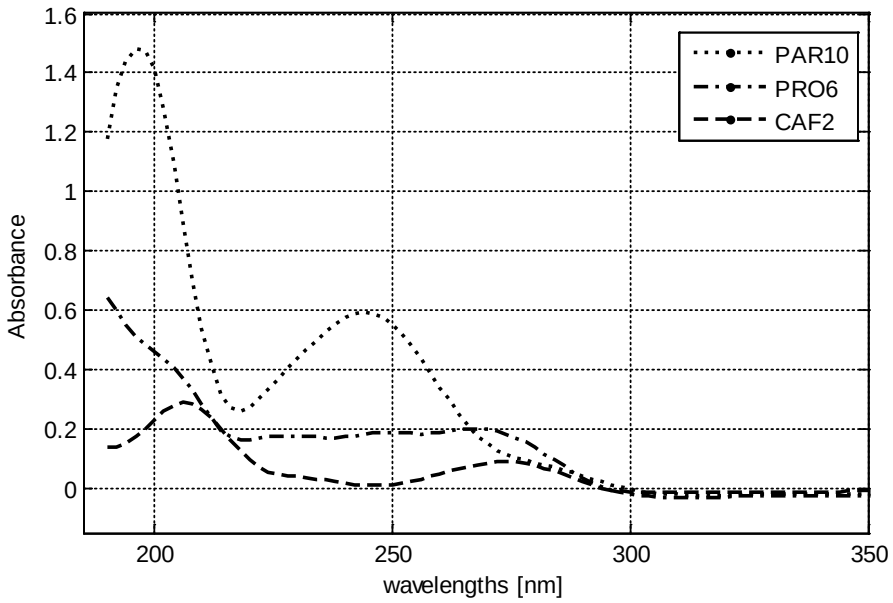

Fig.1. Absorbance spectra in water of paracetamol (10 μg mL^{-1}), propyphenazone (6 μg mL^{-1}) and caffeine (2 μg mL^{-1}).

After wavelength selection the number of the factors was significantly reduced. The obtained RMSEP values were much lower, especially for PRO and CAF. The estimated RMSEP and REP values were approximately the same and in some cases even better for the NAS calibration methods compared to PLS. For PRO and CAF the RMSEP and REP values were highly reduced after the spectral selection and were even lower for NAS than the PLS method. PRO showed the lowest values with HLA XS and CAF – with NAP CLS. Compared to the other two components, CAF had the lowest values for RMSEP and REP. After the wavelength selection PRO gave better values for the slope and the intercept.

Analytical figures of merit

Net-analyte signal (NAS) is a suitable method to characterize the analytical figures of merit related to the multivariate calibration during drugs quantification [35]. NAS calculations, in fact, could be applied for univariate, classical and inverse multivariate calibration [36]. For classical multivariate calibration, the basic equation that is needed to estimate figures of merit is:

$$s_k^* = [\, I - S_{-k}\, S_{-k}^{+}]\, s_k, \qquad (13)$$

where S is the matrix of sensitivities collected for the other solutes (other than the solute of interest), s_k is the sensitivity vector of the analyte, and s_k^* is the estimated net part of the k-th component that is orthogonal to the other constituents [37].

The best candidate for s_k is the pure spectrum of the analyte of interest. NAS is necessary to find meaningful parameters to assess the analytical performance of multivariate calibration like sensitivity (SEN), selectivity (SEL), limit of detection (LOD), limit of quantification (LOQ).

SEN was estimated from the net signal of analyte k (s_k^*) as $\|s_k^*\|$ [37]. SEL which measures the extent of spectral overlapping was estimated as $\|s_k^*\| / \|s_k\|$ [37]. LOD which gives the minimum detectable amount of the solute k was given as $3\|\varepsilon\| / \|s_k^*\|$ [35]. The minimum quantifiable amount of the solute was estimated as $10\|\varepsilon\| / \|s_k^*\|$ [35]. In the former two equations, $\|\varepsilon\|$ represents the instrumental noise which was estimated by recording five spectra of the blank over the range 190-300 nm (2 nm resolution). Then the norms of blank readings ($\|NAS_{blank}\|$) were estimated, and $\|\varepsilon\|$ was taken as the standard deviation of estimated norms [35]. Analytical sensitivity (γ) was given as:

$$\gamma = \frac{SEN}{\|\varepsilon\|}$$

The estimated figures of merit of the three drugs are presented in Table 2.

Sensitivity (SEN), selectivity (SEL), analytical sensitivity (γ), and limit of detection (LOD) were estimated using MVC1 toolbox, containing a special sub-routine based on net analyte signal concept for estimation of figures of merit for the analytical method**.** Estimated FOM for PAR, PRO and CAF were determined with all algorithms and used to compare analytical methods. The obtained results are given in Table 3.

Table 1. Comparison of validation parameters of NAP CLS, HLA GO, HLA XS and PLS1 methods before and after wavelength selection for the three components

Component	Statistical parameters	Multivariate methods			
		NAP CLS		HLA GO	
PAR	Sensor range(nm)	190-300	250-268	190-300	250-268
	Factors	4	2	4	2
	RMSEP(μg mL–1)	0.1921	0.1636	0.2271	0.1550
	REP(%)	2.52	2.14	2.97	2.03
	R^2	0.9968	0.9977	0.9956	0.9979
	Slope	1.0504	0.9786	1.0603	0.9753
	Intercept	-0.2476	0.1850	-0.3007	0.1507
	Recovery(%) (RSD)	101.82(1.04)	100.28 (2.25)	102.2(0.88)	100.08(2.02)
		HLA XS		PLS	
	Sensor range(nm)	190-300	250-268	190-300	250-268
	Factors	4	2	8	3
	RMSEP(μg mL–1)	0.1625	0.1952	0.3160	0.1512
	REP(%)	2.13	2.56	4.14	1.98
	R^2	0.9977	0.9968	0.9916	0.9981
	Slope	1.0113	0.9827	0.9597	0.9701
	Intercept	-0.0651	0.2143	-0.0056	0.2172
	Recovery(%) (RSD)	100.93 (2.14)	101.14(2.51)	95.77(1.75)	99.80 (1.78)
PRO		NAP CLS		HLA GO	
	Sensor range(nm)	190-300	230-248	190-300	210-278
	Factors	6	2	7	5
	RMSEP(μg mL–1)	0.4822	0.2084	0.5203	0.4236
	REP(%)	8.84	3.82	9.54	7.77
	R^2	0.9453	0.9898	0.9363	0.9578
	Slope	0.9211	0.9381	0.8667	0.9162
	Intercept	-0.1249	0.0561	0.1110	0.6046
	Recovery(%) (RSD)	90.02(3.19)	95.60(1.28)	89.0(4.78)	104.4(7.69)
		HLA XS		PLS1	
	Sensor range(nm)	190-300	240-268	190-300	210-278
	Factors	9	2	10	6
	RMSEP(μg mL–1)	0.5150	0.1729	0.7082	0.2609
	REP(%)	9.44	3.17	12.98	4.78
	R^2	0.9376	0.9930	0.8819	0.9839
	Slope	0.8827	0.9737	0.7004	0.9110
	Intercept	0.1173	0.1076	0.9740	0.1497
	Recovery(%) (RSD)	89.75(9.34)	102.13(1.95)	90.08(8.60)	95.73(3.79)
CAF		NAP CLS		HLA GO	
	Sensor range(nm)	190-300	220-278	190-300	210-258
	Factors	6	5	6	5
	RMSEP(μg mL–1)	0.1467	0.0353	0.1471	0.0954
	REP(%)	4.42	1.01	4.43	2.87
	R^2	0.8597	0.9927	0.8592	0.9407
	Slope	0.9309	0.9288	0.9282	1.0277
	Intercept	0.0117	0.0346	0.0272	0.1054
	Recovery(%) (RSD)	93.93(8.20)	99.96(3.32)	94.69(8.5)	93.86(4.21)
		HLA XS		PLS1	
	Sensor range(nm)	190-300	210-258	190-300	210-258
	Factors	6	5	7	5
	RMSEP(μg mL–1)	0.1674	0.0608	0.1289	0.0441
	REP(%)	5.04	1.83	3.89	1.33
	R^2	0.8176	0.9759	0.8917	0.9873
	Slope	1.0823	0.9542	0.9205	0.9288
	Intercept	-0.2713	0.1312	0.0385	0.0346
	Recovery(%) (RSD)	88.93(8.41)	99.57(4.36)	94.91(7.13)	102.02(3.94)

Table 2. Figures of merits of PAR, PRO, CAF for NAP CLS and HLA GO methods.

Figure of merit	NAP CLS		HLA GO		HLA XS		PLS1	
				PAR				
Range(nm)	190-300	250-268	190-300	250-268	190-300	250-268	190-300	250-268
A	4	2	4	2	4	2	8	3
SEL	0.13	0.012	0.14	0.012	0.13	0.012	0.074	0.013
SEN[a]	0.064	0.0022	0.066	0.0022	0.065	0.0022	0.036	0.0023
γ	8.6	6.6	8.6	7.8	8.4	6.1	12	6.8
LOD[b]	0.35		0.35		0.36		0.33	0.41
				PRO				
Range(nm)	190-300	230-248	190-300	210-278	190-300	240-268	190-300	210-278
A	6	2	7	2	9	5	10	6
SEL	0.05	0.02	0.041	0.017	0.033	0.012	0.03	0.016
SEN[a]	0.024	0.0043	0.019	0.0042	0.016	0.0044	0.014	0.0063
γ	8	20	6.9	6.4	6.8	3.4	5.8	4.7
LOD[b]	0.39		0.46		0.46		0.30	0.36
				CAF				
Range(nm)	190-300	220-278	190-300	210-258	190-300	210-258	190-300	210-258
A	6	5	6	5	6	5	7	5
SEL	0.15	0.03	0.15	0.04	0.17	0.035	0.16	0.06
SEN[a]	0.063	0.0079	0.064	0.012	0.069	0.01	0.066	0.018
γ	21	17	20	9.1	22	7.7	22	11
LOD[b]	0.14		0.15		0.14		0.13	0.099

[a] SEN and analytical sensitivity (γ) measure the changes in response as a function of the concentration (mL μg–1).

[b] Limit of detection (LOD) is the lowest concentration of an analyte that can be detected, but not necessarily quantified (μg mL–1).

Table 3. Assay results from application of NAP CLS, HLA XS, HLA GO and PLS on the pharmaceutical tablet Saridon®.

Method	Drug	A	Sensor range(nm)	Recovery(%) (RSD)
NAP CLS	PAR	4	190-300	98.41 (2.49)
		2	250-268	98.98 (3.13)
	PRO	6	190-300	88.49 (1.29)
		2	230-248	103.94 (0.99)
	CAF	6	190-300	89.44 (2.32)
		4	250-298	94.70 (2.73)
HLA XS	PAR	4	190-300	95.53 (2.15)
		2	250-268	99.54 (3.09)
	PRO	4	190-300	95.53 (2.15)
		2	250-268	99.54 (3.09)
	CAF	6	190-300	75.89 (1.37)
		4	220-298	99.14 (3.59)
HLA GO	PAR	4	190-300	98.90 (2.41)
		2	250-268	99.14 (3.12)
	PRO	7	190-300	84.96 (2.35)
		7	210-288	87.16 (0.74)
	CAF	6	190-300	88.51 (2.03)
		4	270-298	93.61 (2.85)
PLS	PAR	8	190-300	92.52 (2.36)
		3	250-268	98.71 (3.12)
	PRO	10	190-300	88.57 (1.19)
		6	210-278	104.92 (0.72)
	CAF	7	190-300	96.43 (2.43)
		6	260-288	96.43 (2.43)

Analysis of commercial formulations

Again, as shown, the number of factors was smaller for the NAS methods, compared to PLS. After the wavelength selection the estimated recoveries for all three components of the Saridon® tablet were much higher.

CONCLUSIONS

A comparative study on the application of multivariate calibration methods NAP/CLS, HLA/HS, HLA/GO and PLS1 for simultaneous determination of paracetamol (PAR), propyphenzone (PRO) and caffeine (CAF) has been

performed using data extracted from UV spectra. In general, comparable results were obtained after applying net-analyte signal methods compared with PLS-1 model using less factors for paracetamol and propyphenazone. Only for caffeine determination PLS1 model give slightly better results. As a conclusion after applying the studied methods for calibration – the most suitable model for the determination of PAR is HLA GO, whereas for the analysis of PRO and CAF it is better to use respectively HLA XS and NAP CLS.

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МЕТОДИ, ОСНОВАВАЩИ СЕ НА НЕТНИЯ СИГНАЛ НА АНАЛИТА ЗА ЕДНОВРЕМЕННО ОПРЕДЕЛЯНЕ НА ПАРАЦЕТАМОЛ, ПРОПИФЕНАЗОН И КАФЕИН ЧРЕЗ УВ СПЕКТРОФОТОМЕТРИЯ

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(Резюме)

Три различни метода, основаващи се на нетния сигнал на аналита - NAP CLS, HLA/GO и HLA XS, са приложени успешно за определяне на тройни смеси от парацетамол, пропифеназон и кафеин. Абсорбционни УВ спектрални данни са използвани за разделяне на тази сложна система с припокриващи се спектри на лекарствата. Редуциран ортогонален дизайн на пет нива е използван за формиране на калибрационна система, включваща трите съединения. Хемометричните модели са проверени с помощта на външна валидационна система от данни с концентрации в обсега на калибриране. Всички предложени хемометрични алгоритми са приложени успешно за определяне на горните съединения във фармацевтичната таблетна форма *Saridon*®. Получените резултати показват, че методът, основаващ се на нетния сигнал на аналита дава подобни резултати на тези с метода на частичните най-малки квадрати. В допълнение, използването на концепцията за нетния сигнал на аналита позволява да се изчислят аналитичните параметри. Използвана е стратегия на подвижен прозорец на дължината на вълната, която значително намалява броя на факторите и подобрява аналитичните добиви.