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Principal Component Artificial Neural Network Calibration Models for Simultaneous Spectrophotometric Estimation of Phenobarbitone and Phenytoin Sodium in Tablets

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Abstract

Simultaneous estimation of all drug components in a multicomponent pharmaceutical dosage form with artificial neural networks calibration models using UV spectrophotometry has been reported as a simple alternative to using separate models for each component. A novel approach for calibration using computed spectral dataset derived from three spectra of each component has been described. Spectra of Phenobarbitone and Phenytoin sodium were recorded at several concentrations within their linear range and used to compute the calibration mixture between wavelengths 220 to 260 nm at an interval of 1 nm. Principal component back-propagation neural networks trained by Levenberg-Marquardt algorithm were used for building and optimizing calibration models using MATLAB® Neural Network Toolbox. Neural network models were compared to principal component regression model. The calibration model was thoroughly evaluated at several concentration levels using spectra obtained for 95 synthetic binary mixtures prepared using orthogonal designs. The optimized model showed sufficient robustness even when the calibration sets were constructed from different set of pure spectra of components. Although the components showed significant spectral overlap, the model could accurately estimate the drugs, with satisfactory precision and accuracy, in tablet dosage with no interference from excipients as indicated by the recovery study results.

Key words: Principal components, artificial neural networks, UV spectrophotometry, Phenobarbitone, Phenytoin sodium.

Introduction

Simultaneous determination of components in a multicomponent drug formulation could be difficult task, especially when characteristics of these components from analytical point resemble closely in addition to the presence of other pharmaceutical excipients. In recent past, multivariate chemometric methods for analysis of multicomponent systems have been reported in international journals mostly due to the advent of fast and affordable computers and rapid scanning spectrophotometers controlled by computer software.

Artificial Neural Networks (ANNs) are a data processing system consisting of a large number of simple, highly interconnected processing elements inspired by the biological system and designed to simulate neurological processing ability of human brain. Theoretical background information on ANNs can be found elsewhere.[1–3] Applications of ANNs in the field of chemistry and pharmacy have been reviewed.[4–12]

Computationally, ANN is an approach for handling multivariate and multi-response data and hence suitable for modeling, i.e. a search for an analytical function that will give a specified n-variable output for any m-variable input.[9] Unlike standard modeling techniques where the mathematical function is required to be known in advance, ANN models do not require knowledge of the mathematical function in advance and are called 'soft models', i.e. the models are able to represent the experimental behavior of the system when the exact description is missing or too complex.[13] ANNs adapt to any relation between input and output data on the basis of their supervised training. The characteristics that make ANN systems different from traditional computing are: learning by example, distributed associative memory, fault tolerance and pattern recognition.[13] The flexibility of ANNs and their ability to maintain their performance even in the presence of significant amounts of noise in the input data are highly desirable[2, 7] since perfectly linear and noise free data sets are seldom

available in practice, thus making it suitable for multivariate calibration modeling. There are reports on the application of ANNs for mixture analysis[14–18] though most of them employ separate networks for estimation of each component and calibration involving synthetic binary mixtures for calibration.

The current research work evaluates the performance characteristics of Principal component Artificial neural network (PC-ANN) model trained by Levenberg-Marquardt algorithm[19] against the Principal component regression (PCR) model for an anticonvulsant combination of Phenobarbitone 30 mg (PBT) and Phenytoin sodium 100 mg (PTN) available in India. The use of computed spectral datasets has been demonstrated and compared with the normal practice of using spectra of synthetic mixtures for the calibration models. A method for routine pharmaceutical quality control of this tablet dosage form by multivariate calibration based on soft modeling using principal component based back-propagation neural network has been presented.

Experimental

Chemicals and reagents: Analytical reagent grade NaOH was used to prepare 0.01M NaOH solution in distilled water which then served as a solvent for making the stock solutions and all further dilutions of PBT, PTN, their standard combinations and the tablet powder. Class A volumetric glassware such as pipettes and volumetric flasks were used for the purpose of making dilutions.

Instruments and software: UV absorption measurements were carried out on PerkinElmer Lambda 25 double beam spectrophotometer controlled by UVWINLAB software version 2.85.04, using matched 1.00 cm quartz cells. All weights were measured on an electronic balance with 0.01 mg sensitivity. Spectra of all the solutions were recorded against a blank solution containing no analytes, between 215 to 300 nm and saved in ASCII format. Matlab® version 6.1 was employed for building Principal component Levenberg-Marquardt back-propagation neural networks (PC-ANN). PCR regression model was also employed on the same data using custom built functions for the MATLAB in order to provide for a comparative evaluation of the performance of the PC-ANN. All computations were carried out on a desktop computer with a Pentium 4, 1.6 GHz processor and 256 MB RAM.

Preparation of standard solutions: Standard solutions of pure PBT and PTN were made at different concentration levels ranging from 2 to 10 mg L^{-1} and 6 to 21 mg L^{-1} respectively for the purpose of linearity determination and to design the calibration data matrix from their spectra. The analytical levels of 4 and 13.33 mg L^{-1} were chosen for PBT and PTN respectively. The absorbance spectra, around the analytical level chosen for the two standards, are shown in Figure 1.

Calibration data: Since the absorbances were additive linearly in the desired range and no serious baseline problems or interactions were found and since majority of chemometric techniques for regression and calibration do assume linear additivity, the process described below was adopted in the design of calibration data set for training the PC-ANN. Three spectra of each component at three different concentration (low, medium and high) levels were employed in all possible combinations to provide a fair simulation of calibration data set with some degree of experimental variation. A full factorial design was employed to obtain 56 training pairs from each spectral pair resulting in a total of 504 training pairs (56×9) representing the mixture space evenly with target concentrations that are orthogonal. A total of 504 training pairs thus obtained, constituting the complete calibration set, were used to train the PC-ANN and for the calibration of the PCR model. All the target concentrations in the calibration set were then standardized (to a mean of 0 and standard deviation of 1). Spectral region between 220 and 260 nm was chosen on the basis of visual inspection of the spectra. Absorbance values at every 1 nm interval in the selected spectral region served as the input values for the model.

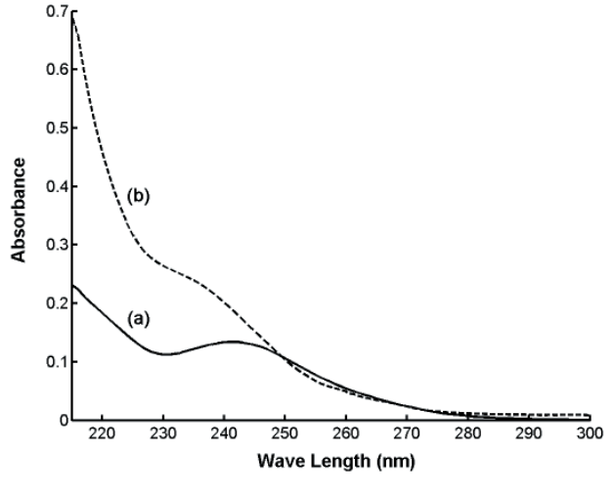

Figure 1. UV Spectra of Phenobarbitone and Phenytoin sodium. Overlain spectra of PBT (a) at concentration of 3.44 mg/L and PTN (b) at concentration of 10.63 mg/L in 0.01M NaOH.

Validation data: Randomized validation data sets were used for the internal validation and terminating the training of the PC-ANN at an optimum point to prevent over-fitting and retain generalization ability of the network. Validation data set of the same size was also designed from three different pairs of spectra of PBT and PTN standards out of which at least two pairs were different from that used in the calibration dataset.

Synthetic binary mixtures for model evaluation: The preparation of synthetic binary mixtures was spread over 9 different days from fresh stock solutions of pure PBT and PTN, each day by separate weighing, in 0.01 M sodium hydroxide. Standard mixtures of the components were prepared with the concentrations lying within the known linear absorbance-concentration range by dissolving varying proportions of PBT and PTN stock solutions; the concentration of PBT varied between 50 to 180 % of the test level concentration while that of PTN varied between 50 to 150 % of its analytical level concentration. The concentrations of components were selected to span the mixture space fairly evenly, as shown in Figure 2.

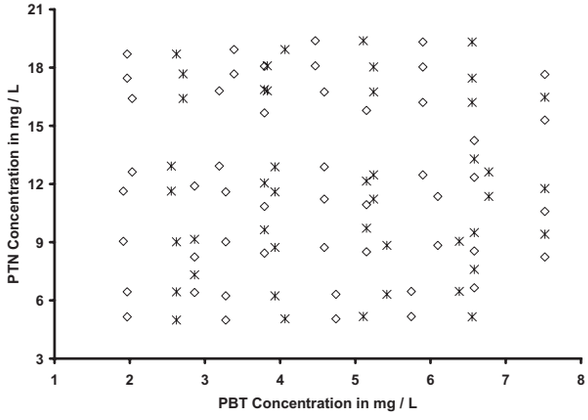

Figure 2. Synthetic binary mixture design for testing the neural networks. Each point represents a mixture at the respective concentration of the components. The mixtures have been split in two groups R1 (◊) and R2 (×). The design ensures that the model is thoroughly validated in a well distributed concentration space especially with regard to chosen analytical level.

Analysis of tablet dosage form: For the analysis of the active components of the anticonvulsant tablet (Phenytal, PBT 30 mg and PTN 100 mg, Intas Pharmaceuticals, India, Batch No: C014), twenty tablets were accurately weighed, carefully powdered and mixed. Tablet powder corresponding to the equivalent of 45 mg of PTN was dissolved in 0.01M sodium hydroxide solution by sonication for 5 min and made up to 100 ml. The solution was centrifuged and 3 ml of supernatant was diluted to 100 mL. Three replicate dilutions were made from each stock solution, repeating the entire process for a total of 5 weights of the tablet powder.

For accuracy studies, by recovery, the same tablet powder was used in amounts corresponding to the equivalent of 30 mg of PTN (in order to enable spiking up to desired levels). The powder was then spiked with a known quantity of pure PBT and PTN and dissolved in 0.01M sodium hydroxide by sonication and made up to 100 ml with the same solvent. The solution was then centrifuged and 3 ml of supernatant was diluted to 100 ml. A total of five powder samples were spiked to different levels in the range of 80 to 120%, each in three dilution replicates.

PC-ANN model: Principal component analysis was carried out by employing custom developed functions in MATLAB using the inbuilt Eigen value decomposition function ('eig') to obtain the latent (Eigen) vectors and the corresponding Eigen values. The scores obtained by projecting the standardized absorbance values on to these Eigen vectors were used as inputs. PC-ANNs had an input layer with neurons corresponding to the number of principal components chosen for the calibration set employed, variable number of neurons in the hidden layer and two neurons in the output layer corresponding to the two components of interest. The input and output layer nodes had a linear transfer function while only the hidden layer nodes had sigmoid transfer function for the PC-ANN decided on the basis of our earlier studies on neural models.[15–16] Optimization of network, to achieve generalization of the model and avoid over-fitting, was done starting with 2 neurons in the hidden layer and gradually increasing the number till no significant improvement in performance (<2 % in mean %REP) in the network was achieved. PC-ANNs were trained according to Levenberg-Marquardt[19] algorithm available in the neural network toolbox of MATLAB through the 'trainlm' function. The training was terminated when the validation performance as estimated by the mean square error (MSE), for a validation dataset, increased continually for more than 10 epochs since the last time it decreased.

PCR model: The PCR model also had the same inputs and targets as the PC-ANN model and was built and tested using custom developed function in MATLAB for multi linear regression. Leave one out cross validation was employed to determine the optimum number of principal components to be employed for input to the model.

Evaluation of models: All trained PC-ANNs with different configurations and the PCR model were evaluated for their modeling capability by testing with the spectral data obtained from the synthetic binary mixture designs as described above. RMSE, %REP and Residual standard deviation were evaluated for each component of the mixture and the mean %REP representing the combined error for the entire mixture was used to perform multiple comparisons between models and choose the optimum performing model.

Tablet analysis: Spectra recorded from the tablet solutions were analyzed by the chosen optimum PC-ANN model and the concentrations predicted for each solution were used for calculation of the tablet content. Similarly PBT and PTN concentrations in the solutions prepared for recovery study were also obtained from the respective spectra and percentage recovery was calculated to determine the accuracy of the method.

Results and discussion

There are many pitfalls in the use of calibration models, perhaps the most serious being variability in instrument performance over time. Each instrument has different characteristics and on each day and even hour the response may vary. Therefore it is necessary to reform the calibration model on a regular basis, by running a standard set of samples, possibly on a weekly basis.[20] Like other regression methods, there are constraints concerning the number of samples, which at times may be limiting the development of an ANN model. The number of adjustable parameters (synaptic weights) is such that the calibration set is rapidly overfitted if too few training pairs are available leading to loss of generalization ability. Therefore, calibration sets of several hundred training pairs may often be necessary to get a representative distribution of the concentration across their range. This makes it expensive in time and resources to develop calibration mixtures physically in such large numbers which is rarely possible in routine laboratory studies and justifies our attempt to use mathematically constructed calibration data set from individual spectra of components. However, this approach cannot be applied in cases where significant non-linearity is exhibited.

The overlain absorption spectra in Figure 1 show strong spectral overlap, which complicates the determination of the individual drug concentrations from a spectrum of a mixture. When considered separately, concentrations between 2 to 10 mg L^{-1} for PBT and 6 to 21 mg L^{-1} for PTN were found to be linear, with r^2 of 0.9999 and 0.9998 for each, slopes of 0.0391 and 0.0647, intercepts of 0.0005 and –0.0081 and residual standard deviation about the regression line being 0.0008 and 0.0056 respectively. Several PC-ANN models were built by gradually increasing the number of hidden neurons till the performance as determined by mean relative error of prediction %REP failed to improve significantly (>2%).

$$Root\ \ Mean\ \ Square\ \ Error(RMSE) = \sqrt{\frac{1}{m}\Sigma_1^m (C_{act} - C_{pred})^2}$$

$$\%REP = \frac{100 \times RMSE}{Mean Concentration}$$

C_{act} is the desired target, C_{pred} is the output produced by the network for each input vector, m is the number of input vectors or samples. The PC-ANN model trained rapidly taking less than one minute and fewer than 300 epochs. Each model of PC-ANN was trained five times with random initialization of weights and mean %REP with test data set is used to perform ANOVA with Hsu's Multiple Comparisons with the best[21] to determine the optimum PC-ANN models. Based on these results, the final PC-ANN model had an input of 5 neurons, an output of 2 neurons, both having linear transfer function and a hidden layer of 3 neurons with sigmoid transfer function. The 5 inputs correspond to the scores on the principal components obtained for the standardized calibration data matrix.

The optimized PC-ANN model was validated for its robustness by training the network using three different calibration sets and monitoring sets. Both the PC-ANN and PCR models were evaluated for their prediction characteristics using the 180 spectra for synthetic binary mixtures, including replicates, after eliminating consistent outliers. The %REP of the models are presented in Table 1 while the regression characteristics of the predictions are listed in Table 2 and the regression plots are shown in Figure 3 and 4 for PC-ANN and PCR models respectively.

Table 1. Percentage relative error of prediction of calibration models.

Model	Calibration Data Set[a]	Test Data Set[b]	% REP		
			PBT	PTN	Mean
PC-ANN	S1	R1+R2	0.9453	0.8150	0.8802
PC-ANN	S2	R1+R2	0.9185	1.5336	1.2261
PC-ANN	S3	R1+R2	1.0742	0.8692	0.9717
PC-ANN	R1	R2	0.7283	0.5415	0.6349
PCR	S1	R1+R2	0.8713	0.7225	0.7969
PCR	S2	R1+R2	0.9049	1.5163	1.2106
PCR	S3	R1+R2	1.0759	0.8691	0.9725
PCR	R1	R2	0.6858	0.5395	0.6126

[a] S1, S2, S3 are calibration data sets used in the calibration of models which are evaluated by the entire set of binary synthetic mixtures. [b] R1 and R2 are binary synthetic mixtures as illustrated in Figure 2. When R1 is used for calibration R2 is used for evaluation.

Spectra obtained from 30 tablet solutions (including replicates) prepared from 5 different weighings as described in the experimental section were analyzed by the optimum PC-ANN model and the average content was calculated. The results are summarized in Table 3. The accuracy of the method for analysis of tablets was further investigated using the recovery studies as described in the experimental section. The mean percentage recovery and its relative standard deviation obtained by the PC-ANN and PCR models for both PBT and PTN closely agreed as indicated in Table 4 and 5. In all cases the PC-ANN model performance compared well with the PCR model, showing no statistically significant difference (p-value > 0.05) as determined by *t*-test.

The use of linear transfer functions in the output layer in the PC-ANN resulted in faster training and output that was comparable to that obtained by PCR

Table 2. Prediction parameters for optimum calibration models.

Model	Data Set	Phenobarbitone			Phenytoin sodium		
		Slope	Intercept	SD[a]	Slope	Intercept	SD[a]
PC-ANN	S1	0.997	0.022	0.0412	0.997	0.067	0.0924
PC-ANN	S2	0.999	–0.010	0.0393	0.99	0.15	0.1788
PC-ANN	S3	0.997	0.034	0.0431	0.998	0.035	0.1016
PC-ANN	R1	1.000	–0.014	0.0325	0.997	0.034	0.0641
PCR	S1	0.994	0.038	0.0377	0.997	0.044	0.0847
PCR	S2	0.998	–0.005	0.0388	0.99	0.144	0.1771
PCR	S3	0.997	0.036	0.0432	0.998	0.035	0.1017
PCR	R1	1.000	–0.290	0.0313	1.000	0.017	0.0619

[a] Residual standard deviation of the predictions by the model. The correlation coefficient was either 0.999 or 1.0 with all the models.

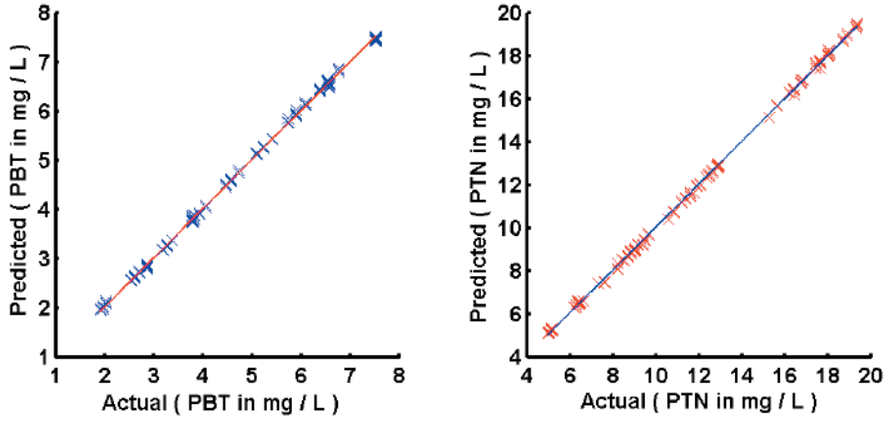

Figure 3. PC-ANN model prediction performances for PBT and PTN. The predicted concentrations versus actual concentrations in mg/L for each component by the optimum PC-ANN calibration model.

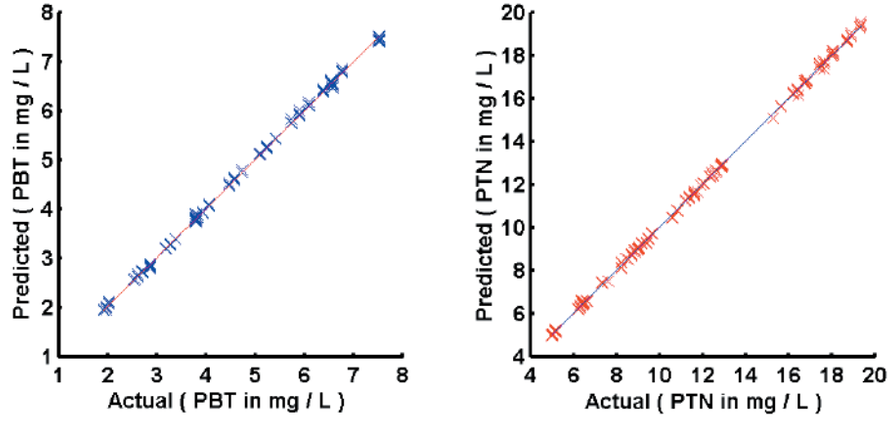

Figure 4. PCR model prediction performances for PBT and PTN. The predicted concentrations versus actual concentrations in mg/L for each component by the optimum PCR calibration model.

in problems such as this were the non-linearity could be insignificant thus confirming our earlier reported studies[16] based on simple back-propagation neural network on a different combination.

Table 3. Analysis of tablet samples by PC-ANN and PCR models.

	PBT		PTN	
	PC-ANN	PCR	PC-ANN	PCR
Sample 1 (mg)	30.68	30.76	98.34	98.23
Sample 2 (mg)	30.53	30.61	100.83	100.73
Sample 3 (mg)	31.07	31.14	98.15	98.08
Sample 4 (mg)	29.99	30.09	99.29	99.24
Sample 5 (mg)	30.59	30.68	97.99	97.96
Mean Tablet content (mg)	30.57	30.66	98.92	98.85
Relative Std Deviation	1.26	1.24	1.19	1.18
Amount on the label (mg)	30.00	30.00	100.00	100.00
% of the reported content	101.91	102.19	98.92	98.85

Our elaborate study has confirmed the observations of Gemperline *et al.*[22], from a study with simulated data, who stated that 'Artificial neural networks having the appropriate architecture can be used to develop linear calibration models that perform as well as linear calibration models developed by PCR or PLS' and Despagne *et al.*[7] remarks that 'ANNs outperform linear methods for the strongly non-linear data set, which is not surprising, but their performance on slightly non-linear and linear data is comparable to the performance of linear methods such as PLS or PCR'.

Conclusions

The PC-ANN model developed in this study performed well in estimating both the components simultaneously when tested with spectra recorded on different days and exhibited ruggedness even when different sets of constructed calibration data were used in the model development as indicated by the prediction results. The results thus indicate that it may be redundant to train and optimize individual neural network models for each component in the mixture. Since it took less than 1 minute for training, the PC-ANN model can be quickly calibrated whenever the spectrophotometer performance characteristics alter using only three pairs of the spectra of the individual components, offering considerable advantage.

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Table 4. Recovery studies using the PC-ANN model.

Spiked Sample	Phenobarbitone (PBT)			Phenytoin sodium (PTN)		
	Actual (mg)	Found (mg)	% Recovery	Actual (mg)	Found (mg)	% Recovery
1	10.81	10.72	99.17	34.47	34.07	98.84
2	12.02	11.87	98.75	38.03	38.22	100.50
3	13.50	13.35	98.89	42.33	42.74	100.97
4	14.57	14.55	99.86	45.43	45.71	100.62
5	16.34	16.24	99.39	50.65	51.18	101.05
Mean			99.21			100.40
Relative Standard Deviation			0.44			0.90

Table 5. Recovery studies using the PCR model.

Spiked Sample	Phenobarbitone (PBT)			Phenytoin sodium (PTN)		
	Actual (mg)	Found (mg)	% Recovery	Actual (mg)	Found (mg)	% Recovery
1	10.84	10.87	100.28	34.45	34.25	99.42
2	12.04	11.94	99.17	38.01	38.30	100.76
3	13.53	13.39	98.97	42.31	42.73	100.99
4	14.59	14.56	99.79	45.41	45.66	100.55
5	16.36	16.24	99.27	50.63	51.03	100.79
Mean			99.50			100.50
Relative Standard Deviation			0.54			0.62

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Povzetek

Poročamo o hkratni določitvi vseh sestavin farmacevtskega pripravka z uporabo nevronskih mrež in UV spektrofotometrije ki je enostavna alternativa uporabi posameznih umeritvenih modelov za ločene sestavine. Opisan je nov pristop z uporabo podatkov pridobljenih iz po treh spektrov posamezne komponente: posneli smo absorpcijske spektre fenobarbitona in fenitoina pri več koncentracijah v linearnem območju. S pridobljenimi podatki smo izračunali spektre mešanic za umeritev v območju 220-260 nm v intervalu 1 nm. Za postavitev in optimizacijo kalibracijskega modela smo z uporabo programske opreme MATLAB® Neural Network Toolbox uporabili nevronske mreže z vzvratnim razširjanjem na podlagi glavnih komponent in Levenberg-Marquardtovega logaritma za učenje. Tako pridobljene modele smo primerjali z regresijskim modelom na podlagi glavnih komponent. Kalibracijski model smo natančno ovrednotili na več koncentracijskih nivojih z uporabo spektrov 95 sintetičnih binarnih mešanic pripravljenih na ortogonalen način. Optimizirani model izkazuje primerno robustnost, tudi kadar so kalibracijski seti iz različnih setov spektrov čistih komponent. Čeprav se spektri le-teh v precejšnji meri prekrivajo, lahko z modelom z zadovoljivo točnostjo in natančnostjo določimo analita v farmacevtskih pripravkih brez motenj, ki bi jih lahko predstavljale pomožne substance.