Simultaneous quantitative analysis of a three-drug combination using synchronous fluorescence spectroscopy and chemometrics

Mandakini A. Shinde and Ottoor Divya*

Department of Chemistry, Savitribai Phule Pune University, Ganeshkhind Road, Pune 411 007, India

Establishing precise and accurate analytical methods for multicomponent analysis using time- and cost-effective ways are highly advantageous. Here, synchronous fluorescence spectroscopic method is coupled with chemometric tools in order to achieve simultaneous quantitative analysis of a three-component system consisting of metoprolol, propranolol and amiloride. Chemometric methods such as principal component regression and partial least squares regression were applied to the fluorescence data. Root mean square error values, correlation coefficient and limit of detection were used to judge the potential of the model for prediction. The proposed method worked well for simultaneous analysis of the three-drug system in the presence of strong serum albumin signals.

Keywords: Three-drug system, partial least squares regression, principal component regression, synchronous fluorescence spectroscopy.

NOWADAYS, drugs have become an essential factor in human life and researchers are involved in different studies related to drug resistance, determination of drug doses, drug designing, drug impurity profiling, pharmacology and pharmacokinetics, etc.[1–7]. Assessment of pharmacokinetic properties of drugs mainly depends on drugs concentration over time. Evaluation of adherence, absorptivity and bioavailability is also a cause or motive for drug analysis. Thus, studies on drug–DNA interaction, drug–micelle interaction, drug–serum albumin binding mechanism, simultaneous drug determinations and drug–metal ion interactions are extremely significant[8–12]. Drug combination therapies are frequently used nowadays because they help cure diseases easily and reduce drug resistance[13,14]. Hypertension is a major health concern related to blood pressure and cardiovascular illness. Pharmacotherapy studies of hypertension showed that combined treatment is more useful than single-drug therapy, as the therapeutic effect of the applied drug is enhanced by the combined treatment[15]. Complex agents that are used to treat hypertension include diuretics, β-blockers, calcium channel blockers, ACE inhibitors, angiotensin receptor antagonists, etc.[16]. Amiloride is a weak diuretic which increases the excretion of sodium by blocking the sodium channel in the distal renal tubule and reduces loss of potassium ions. Propranolol and metoprolol are non-selective β-blockers which are used for angina pectoris, cardiac arrest, etc. Due to their additive effect, diuretics and β-blockers are given in combination to control arterial pressure[16–18].

Various techniques such as UV–Vis spectrophotometry, high pressure liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), GC–MS, differential pulse polarography, cyclic voltammetry (CV), capillary electrophoresis, nuclear magnetic resonance (NMR), etc. have been used for drug analysis[19–27]. Fluorescence spectroscopic method is also widely used nowadays for drug analysis as most of the drugs are fluorescent in nature. The advantages such as high sensitivity (lower detection limits), selectivity and low cost make the fluorescent technique a better option for routine drug analysis compared to other techniques. There is a linear response between concentration of analyte species and fluorescence intensity, which makes it a more advantageous tool, as the chemometric methods can be employed directly for developing the calibration model.

In the present study, multivariate chemometric methods are applied for the quantitative simultaneous determination of ternary mixtures of drugs using spectrofluorimetry. Spectral profiles of multicomponent system are highly complex and the data output is very large. A quantitative analysis of each species is possible only when multivariate chemometric methods are applied on their spectral data. Thus, an attempt is made to develop a method for ternary drug analysis which will have lower detection limit and high precision. The developed model will be used for the prediction of drugs in pharmaceutical preparations and serum samples.

Amiloride hydrochloride (AMI), metoprolol tartrate (MET) and propranolol hydrochloride (PRO) in pure form were purchased from Sigma Aldrich. Tablets containing MET (Metolar 25), PRO (Ciplar-10) and AMI (Biduret) were purchased from local pharmacies. All solvents used throughout this study were of spectroscopic reagent grade. Triply distilled water (TDW) was used for the study.

Fluorescence spectra were recorded on Jasco spectrofluorimeter (150 W Xenon lamp as source). Spectral scanning was made in the wavelength range 280–450 nm. Excitation and emission monochromator slit widths were adjusted at 5 nm. Spectra were recorded at 1 nm interval with scan speed of 1000 nm/min. All multivariate calibration methods were performed on PLS toolbox 7.5 software and SOLO+MIA-731.

For sample preparation, stock solutions of amiloride, metoprolol and propranolol of 10 ppm (10 μg/ml) were prepared separately by dissolving accurately weighed 0.001 g of each drug in methanol and diluting to volume with triply distilled water in 100 ml volumetric flask.

*For correspondence. (e-mail: divya@chem.unipune.ac.in)

Appropriate dilutions were made from stock solution, keeping working concentration range 0–240, 0–180 and 0–60 ng/ml of MET, AMI and PRO respectively. Thirty different ternary combinations were prepared out of which 20 samples were used as calibration dataset and 10 samples were used for validation purposes. The concentrations of MET, AMI and PRO employed in the calibration dataset and validation dataset are given in Tables S1 and S2 respectively (see supplementary material online).

For the analysis of drugs in pharmaceutical samples, stock solutions of amiloride, metoprolol and propranolol of 10 ppm were prepared separately as follows. Weight of the drug equivalent to 0.001 g of active ingredient was weighed and dissolved in methanol and diluted to volume with TDW in each 100 ml volumetric flask. Appropriate volume of stock solution was pipetted and added to 10 ml volumetric flask followed by dilution up to the mark to obtain a final concentration range 0–100, 20–50 and 0–140 ng/ml for MET, PRO and AMI respectively.

The evaluation of robustness of the method was carried out by performing drug analysis in the presence of serum. Stock solution of bovine serum albumin of 6600 ng/ml was prepared. The working concentration of serum was maintained constant (660 ng/ml). Drug mixture containing MET concentration from 60 to 240 ng/ml, PRO concentration from 20 to 60 ng/ml and AMI concentrations from 40 to 180 ng/ml was prepared in this serum concentration.

Multivariate calibration method comprises of two steps. In the first step, which is termed as calibration, an empirical model is built. The relationship between the data generated from a set of reference samples and the respective concentrations of their component(s) is generated in the form of a calibration model. This is followed by a second step called validation and prediction. Two commonly employed multivariate methods are principal component regression (PCR) and partial least squares regression (PLSR).

PCR and PLSR are factor-based methods, which perform data decomposition into spectral loadings and scores prior to model building using new variables[28,29]. In PCR, the data decomposition is done using only spectral information, whereas PLSR employs spectral and concentration data simultaneously. PCR is a two-step multivariate calibration method. In the first step, a principal component analysis (PCA) is performed which reduces the large number of variables to a much lesser number of principal components (PCs) that capture majority of variance in the data. Thus the measured variables (e.g. fluorescence intensity at different wavelengths) are converted into new ones (scores on latent variables). The second step of PCR involves a multiple linear regression (MLR) which is performed on the scores obtained in the PCA step[30].

PLSR is another quantitative spectral decomposition technique that generalizes and combines features from PCA and MLR and performs the data analysis in one step. In brief, PLSR decomposes both the spectral profile and concentration matrices into a product of two smaller matrices. In this process, it utilizes the variables included in the concentration matrix for spectral data decomposition. It involves a data compression step where the measured data are compressed to a smaller number of variables called 'scores' in a new coordinate system. The new coordinate axes are called latent variables (LVs) or PCs, and are linear combinations of the original variables[30,31].

Figure S1 *a* and *b* (see supplementary material online) shows the individual excitation and emission spectra of each component used for the study, i.e. metoprolol (MET), propranolol (PRO) and amiloride (AMI) in methanol solvent. λ_{ex} of the selected drugs, MET, PRO and AMI was found to be 275, 290 and 366 nm respectively. λ_{em} of MET and AMI was found to be at 302 and 417 nm respectively. In the case of PRO, a structured emission was observed with emission maximum at 327 and 337 nm. AMI excitation spectrum overlaps appreciably with MET and PRO emission spectra. PRO excitation spectrum and MET emission spectrum merge at many points. On careful analysis of these spectra it is evident that partial spectral overlap is present, which may result in fluorescence resonance energy transfer and quenching. Such a spectrum of drug combinations will not provide much information with respect to their quantitative analysis.

This drawback of conventional fluorescence can be overcome by extending the dimensionality of fluorescence, i.e. by introducing an additional parameter $\Delta\lambda$. This technique, known as synchronous fluorescence spectroscopy (SFS) is widely used for multicomponent analysis. Wavelength range 280–450 nm was maintained in the synchronous fluorescence scanning because all components

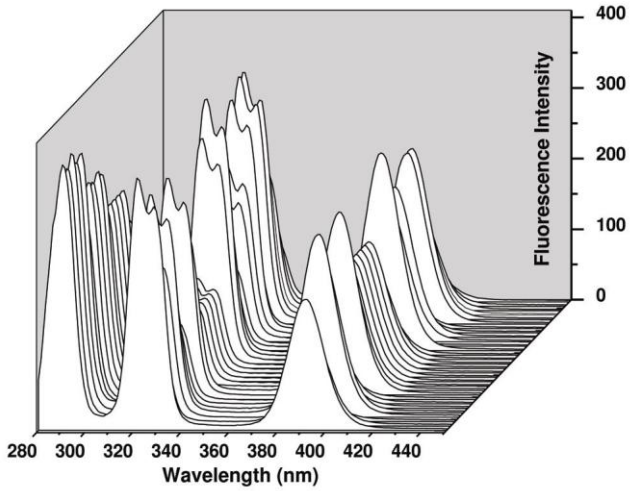

Figure 1. Synchronous fluorescence spectra of ternary mixture of drugs containing metoprolol, propranolol and amiloride in 3D view.

Table 1. Comparison of different preprocessing methods adopted for partial least square regression (PLSR) and principal component regression (PCR) methods for the calibration and validation of metoprolol (MET), propranolol (PRO) and amiloride (AMI) synthetic ternary mixtures

		NONE		AUTO		MNCN	
Component	Statistical parameters	PLSR	PCR	PLSR	PCR	PLSR	PCR
MET	R^2	0.998	0.999	0.997	0.998	0.998	0.999
	No. of factors	6	6	6	6	6	6
	RMSEC	2.224	2.318	3.072	2.643	2.160	2.210
	RMSECV	3.130	3.119	6.477	5.480	3.504	3.391
	RMSEP	3.770	3.506	4.869	4.548	2.606	2.545
PRO	R^2	0.991	0.989	0.993	0.991	0.992	0.983
	No. of factors	6	6	6	6	6	6
	RMSEC	1.754	1.973	0.632	1.684	1.503	1.408
	RMSECV	3.168	3.156	2.642	2.401	2.337	2.296
	RMSEP	1.856	1.724	2.929	1.835	1.655	1.576
AMI	R^2	0.992	0.992	0.978	0.981	0.992	0.992
	No. of factors	3	3	3	3	3	3
	RMSEC	3.543	3.543	6.714	6.215	3.515	3.516
	RMSECV	4.869	4.869	10.838	9.763	5.137	5.137
	RMSEP	5.936	5.936	8.814	8.339	4.045	4.678

show fluorescence signals in this range. Also, $\Delta\lambda$ of 10 nm was selected as it gave the fine-resolved spectra with a good spectral resolution. As the $\Delta\lambda$ value increases, the spectra get broadened and overlap of bands occurs.

Well-resolved spectra of drugs could be observed when samples were analysed using synchronous fluorescence spectroscopy (Figure 1). The spectral peaks at around 290, 330 and 390 nm correspond to MET, PRO and AMI respectively. Even though a separation of spectral features was achieved, spectral contribution of each component cannot provide quantification. To achieve this, multivariate computational methods such as PLSR and PCR were applied on the SFS data to extract quantitative information.

PLSR and PCR are the most successful multivariate calibration methods that can be applied to the whole spectrum; however, precision will be reduced if noisy data with scarcely informative wavelengths get included. Precision and thus the robustness of the model can be improved by discarding data at particularly noisy wavelengths. The wavelength range used in this experiment was from 280 to 450 nm (171 wavelengths). Using the spectral data of 20 samples calibration model was developed and validated. This model was applied to a prediction set which comprises of valid samples whose concentrations fits into the calibration range. Best predictions depend upon standard composition of calibration set and hence selection of concentration range is important.

Data organization is the first task for the application of multivariate methods on spectral information. **X** block contains spectral data obtained by SFS at different wavelength and **Y** block contains concentration values of sample components. Arrangement of **X** block data was done in the form of a matrix consisting of rows (samples) and columns (fluorescence intensity values at different wavelengths). Data in the matrix form (number of samples × fluorescence intensities at 171 wavelengths, i.e. 20 × 171) were loaded into PLS toolbox for further processing. **Y** block data matrix size was 20 × 3, i.e. number of samples × number of components. Spectra may also contain nonlinearities introduced by light scatter and noise. Preprocessing methods reduce the variability or undesired scatter in the data. Different pre-processing methods like autoscale, mean centring and smoothening were used on the data. Data were also analysed without any preprocessing method (none). A comparison was made among different preprocessing techniques employed, which is presented in Table 1.

The developed model, its validation and predictions were evaluated using parameters such as correlation coefficient and root mean square error (RMSE). RMSE is defined as follows

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (y_{\mathrm{pred}} - y_{\mathrm{ref}})^2}{n}}, \qquad (1)$$

where n is the number of samples used, and y_{pred} and y_{obs} are the predicted and reference concentration values respectively, of sample i in the calibration set (or validation set or prediction set). Root mean square error of calibration (RMSEC) is obtained during method development (y_{pred}: concentrations predicted by model during calibraion). Root mean square error of cross validation (RMSECV) is calculated when the model is cross-validated

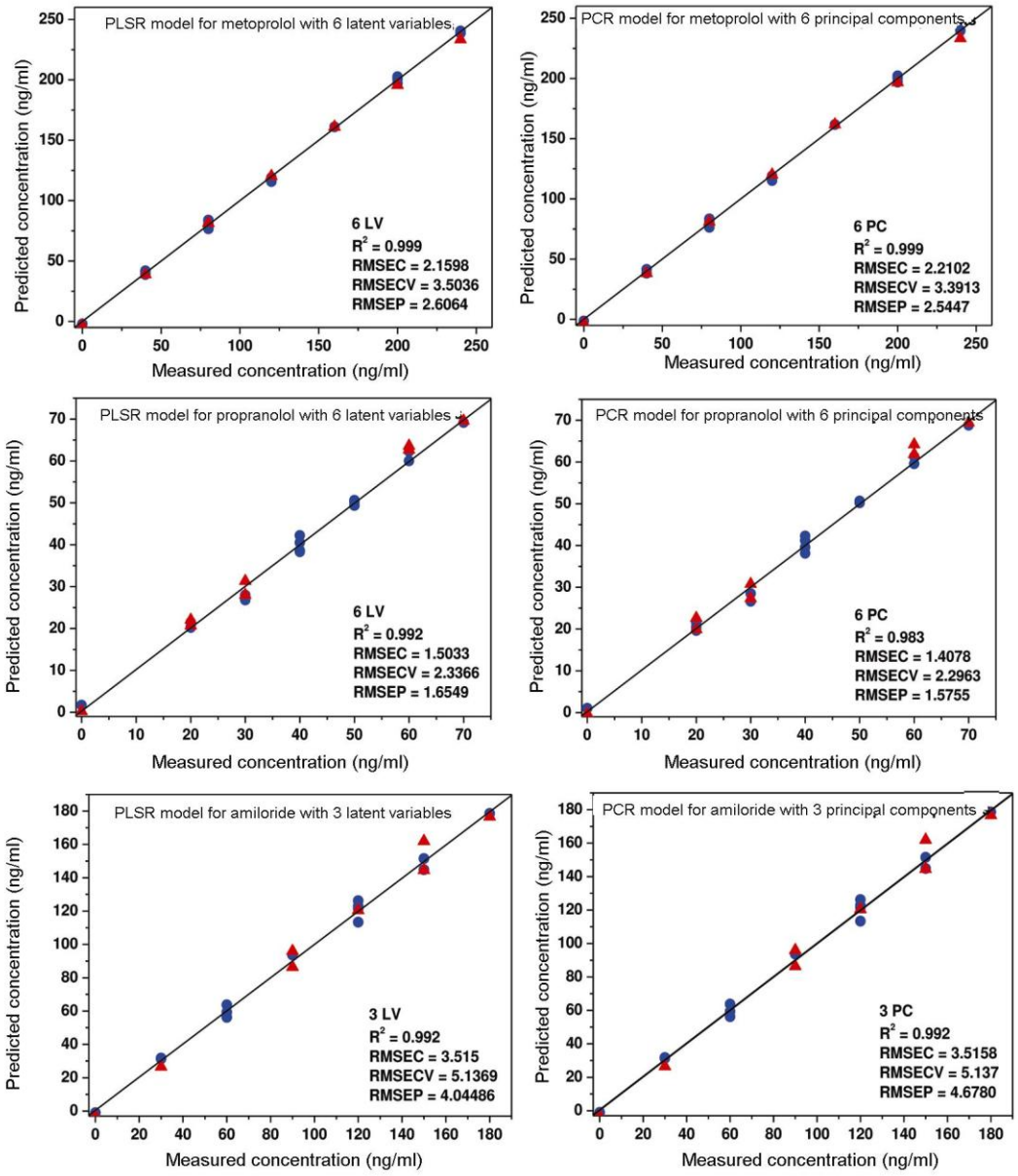

Figure 2. Measured versus predicted concentrations of MET, PRO and AMI in their synthetic mixtures (calibration dataset) based on cross validation by PLSR and PCR models (•, Calibration dataset; ▲, Prediction dataset).

using 'leave one out' method (y_{pred}: concentrations predicted by model using cross validation). In this method, regression model is calculated using $n - 1$ samples leaving out one sample at a time and predicting the concentration of the left-out sample. The prediction error of this process is calculated from the difference between the predicted and true values. This procedure is then repeated leaving out every sample in the calibration set once and the summed prediction error is calculated. When the model is applied to a new set of data it is possible to calculate a root mean square error of prediction (RMSEP), where y_{pred} is the concentrations predicted by the model for unknown samples[32].

RMSE and RMSECV values are comparatively lower if mean centring is adopted as a pre-processing method. Since the best and most appropriate results were obtained with mean-centring, it was applied to the data and further analysis was performed.

PLSR and PCR are factor-based techniques. Therefore, determination of the number of factors to be used in the calibration is a crucial step. Selection of the number of factors should be such that it should contain maximum analytical information. From the predicted residual error sum-of squares plot (PRESS plot), the number of factors can be selected. To select the number of factors, cross validation method was employed.

Table 2. Determination of MET–PRO–AMI in synthetic mixtures to check the validation of the developed model by PLSR method

Added concentration (ng/ml)			Found concentration (ng/ml)			Recovery (%)		
MET	PRO	AMI	MET	PRO	AMI	MET	PRO	AMI
40	–	150	38.83	–	157.31	97.08	–	104.87
80	–	150	81.41	–	144.21	101.76	–	96.14
–	20	–	–	21.04	–	–	105.2	–
120	70	90	120.24	69.57	85.17	100.2	99.39	94.63
160	–	–	161.02	–	–	100.64	–	–
160	20	120	161.02	20.67	120.81	100.64	103.35	100.67
200	60	30	195.67	62.66	28.42	97.84	104.43	94.73
–	30	–	–	28.02	–	–	93.4	–
–	60	180	–	63.6	176.35	–	106	97.97
240	30	90	233.39	31.31	96.07	97.25	104.37	106.74
Mean recovery (%)						99.34	102.31	99.39
RMSEP						2.606	1.655	4.045

Table 3. Determination of MET–PRO–AMI in pharmaceutical sample mixtures to check the validation of the developed model by PLSR method

Added concentration (ng/ml)			Found concentration (ng/ml)			Recovery (%)		
MET	PRO	AMI	MET	PRO	AMI	MET	PRO	AMI
0	20	140	–	19.37	137.92	–	96.86	98.51
0	40	60	–	42.39	63.79	–	105.98	106.33
90	30	0	85.89	28.64	–	95.43	95.47	–
60	50	0	61.21	52.64	–	102.02	105.28	–
70	10	110	70.83	9.71	112.13	101.19	97.07	101.93
100	30	140	103.81	32.08	139.75	103.70	106.95	99.82
20	20	30	19.75	20.94	29.86	98.74	104.71	99.54
Mean recovery (%)						100.22	101.76	101.23
RMSEP						2.167	1.702	2.30

The PRESS plot, where RMSECV values are plotted against the number of factors is shown in Figure S2 (see supplementary material online). The number of components which gives a minimum PRESS value is selected as the optimum number of components for model development. The optimum number of components required for the analysis of MET and PRO was found to be six by both PLSR and PCR methods. In case of AMI, optimum number of components was 3. The PRESS plot shows that the RMSECV value first decreases and then starts increasing when number of factors is 3 (Figure S2).

Figure 2 shows the calibration plot obtained using PLSR and PCR methods. Using the optimum number of factors, models were developed and the reference values of concentration were plotted against the concentration values predicted by the PCR and PLSR models. Both models predict the concentration well with linear fit and excellent correlation coefficients (0.983–0.999).

Cross validation was performed and was found to be good as evident from RMSECV values (Figure 2), which indicates the robustness of the model. RMSEC values obtained were 3.4, 2.3 and 5.1 ng/ml for MET, PRO and AMI respectively.

Validation of the developed model was carried out using samples with known concentrations and the results obtained are presented in Tables 2 and S3 (see supplementary material online). From these data it is clear that the predicted values are in good agreement with the reference values, which is clear from low RMSEP values as well. RMSEP values obtained were 2.6, 1.6 and 4.0 ng/ml for MET, PRO and AMI respectively.

Recovery percentage was calculated for each of the drug components. The recovery range for MET was found to be 97–102% and 95–101% for PLSR and PCR methods respectively. In the case of PRO, the recovery range was 93–105% and 94–107% for PLSR and PCR methods respectively. In the case of AMI, the recovery range was 95–107% and 95–108% for PLSR and PCR methods respectively.

Limit of detection (LOD) was calculated and was found to be in the range 7–12 ng/ml (PLSR method: 7.59 ng/ml for MET, 5.27 ng/ml for PRO and 13.87 ng/ml for AMI; PCR method: 7.49 ng/ml for MET, 5.21 ng/ml for PRO and 13.87 ng/ml for AMI).

To check the applicability and robustness of the established models, these were used to predict drug

Table 4. Added and found concentrations of MET, AMI and PRO in serum with their recovery by PLSR method

Added concentration (ng/ml)			Found concentration (ng/ml)			Recovery (%)		
MET	PRO	AMI	MET	PRO	AMI	MET	PRO	AMI
60	30	70	57.05	32.05	66.89	95.09	106.83	95.56
100	50	120	102.62	50.92	127.37	102.62	101.85	106.14
100	40	160	97.44	42.29	164.91	97.44	105.73	103.07
130	60	90	128.86	62.13	87.18	99.12	103.55	96.87
130	60	100	129.64	57.11	105.49	99.72	95.18	105.49
190	50	40	190.49	48.18	38.39	100.26	96.36	95.97
230	20	80	230.06	20.95	76.83	100.03	104.77	96.04
240	50	180	236.99	50.24	174.82	98.75	100.49	97.12
Mean recovery (%)						98.88	101.85	99.53
RMSEP						2.024	1.854	4.551

concentration in pharmaceutical formulations and in serum samples. Recovery studies were also carried out to provide further support for the validity of the proposed methods.

For determination of drugs in pharmaceutical formulations, AMI, PRO and MET were purchased and dissolution was performed. From stock solutions suitable dilutions were made and various drug combinations were prepared and analysed. The results of the analysis are given in Tables 3 and S4 (see supplementary material online). Recovery range was found to be from 95% to 102% for MET, 95% to 107% for PRO and 98% to 106% for AMI by PLSR method. By PCR method, recovery range was found to be 96% to 106% for MET, 99% to 109% for PRO and 98% to 106% for AMI.

Let us now consider the determination of drugs in the presence of serum. Added and found concentrations of MET, PRO and AMI in serum with their recovery percentage are presented in Tables 4 and S5 (see supplementary material online).

Lower RMSEP values obtained for drugs in biological fluid indicate the good predictive abilities of the model in the presence of matrix effects (background serum albumin fluorescence signal) as well. The percentage recoveries were found to be 95–103% for MET, 95–107% for PRO and 95–106% for AMI by PLSR method. By PCR method, the percentage recoveries were 97–102 for MET, 95–106 for PRO and 95–106 for AMI.

In this study, an attempt was made to develop a reliable calibration model for the simultaneous analysis of a ternary mixture containing antihypertensive drugs, MET, PRO and AMI. Since the fluorescence spectral profiles of these three drugs are partially overlapping, this cannot be employed for quantitative analysis. Hence we have employed synchronous fluorescence spectroscopy which has a potential to analyse multifluorophoric samples. Since the fluorescence technique is sensitive (lower detection limits), the concentration range of analytes was maintained at ng/ml. PLSR and PCR calibration models were developed using the fluorescence spectral data and concentration data. The LOD obtained was from 7 to 12 ppb, which is found to be very low. Low RMSECV and RMSEP values obtained indicate the prediction ability of the calibration model, showing its approval for the application of the method to pharmaceutical formulations and serum. The method is simple, rapid, highly sensitive and easy to apply.

1. Vogel, H. G., Maas, J. and Gebauer, A., *Drug Discovery and Evaluation: Methods in Clinical Pharmacology* (ed. Vogel, H. G.), Springer, Berlin, 2011, p. 564.
2. Prasad, E. M., Ibrahim, A. S., Lakshmi Devi, K., Ahmed, M. and Daddam, J. R., Drug designing and docking studies of breast cancer type 1 susceptibility protein (BRCA1) involved in breast cancer. *Int. J. Plant Anim. Environ. Sci.*, 2014, **4**, 752–763.
3. Suryaawanshi, S. B., Osman, H. A. and Nazeruddin, G. M., Computer aided drug designing and development of new nonsteroidal anti-inflammatory drugs considering mefenamic acid/diclofenac as a lead compound followed by their synthesis and evaluation. *Int. J. Pharm. Biol. Sci*., 2013, **4**, 436–443.
4. Pilaniya, K., Chandrawanshi, H. K., Pilaniya, U., Manchandani, P., Jain, P. and Singh, N., Recent trends in the impurity profile of pharmaceuticals. *J. Adv. Pharm. Technol. Res*., 2010, **1**, 302–310.
5. Bacchi S., Palumbo, P., Sponta, A. and Coppolino, M. F., Clinical pharmacology of nonsteroidal anti-Inflammatory drugs: a review. *Antiinflamm. Antiallergy Agents Med. Chem*., 2012, **11**, 52–64.
6. Falcao, A., Fuseau, E., Nunes, T., Almeida, L. and Soares-da-Silva, P., Pharmacokinetics, drug interactions and exposure–response relationship of eslicarbazepine acetate in adult patients with partial-onset seizures: population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses. *CNS Drugs*, 2012, **26**, 79–91.
7. Tuntland, T. *et al.*, Implementation of pharmacokinetic and pharmacodynamic strategies in early research phases of drug discovery and development at Novartis Institute of Biomedical Research. *Front. Pharmacol*., 2014, **5**, 174.
8. Williams, A. K., Dasilva, S. C., Bhatta, A., Rawal, B., Liu, M. and Korobkova, E. A., Determination of the drug–DNA binding modes using fluorescence-based assays. *Anal. Biochem*., 2012, **422**, 66–73.
9. Zhang, Q., Ni, Y. and Kokot, S., Competitive interactions of ionic surfactants with Salbutamol and bovine serum albumin: a molecular spectroscopy study with implications for Salbutamol in food analysis. *J. Agric. Food Chem*., 2013, **61**, 7730–7738.
10. Alam, K. D. *et al.*, *In vitro* binding chemistry of Amlodipine besyate and Atorvastatin calcium to serum albumin and their

mutual effect to displace each other from the binding site. *Am. J. Drug Dis. Dev*., 2011, **1**, 220–230.
11. Samadi-Maybodi, A. and Hassani Nejad-Darzi, S. K., Simultaneous determination of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in pharmaceutical preparations using multivariate calibration. *Spectrochim. Acta A*, 2010, **75**, 1270–1274.
12. El-Gamel, N. E. A., The interactions of metal ions with nonsteroidal anti-inflammatory drugs (oxicams). *J. Coord. Chem*., 2009, **62**, 2239–2260.
13. Geyer, M., Antimalarial drug combination therapy: Report of a WHO Technical Consultation, World Health Organization, Geneva, 2001.
14. Parhi, P., Mohanty, C. and Sahoo, S., Nanotechnology-based combinational drug delivery: an emerging approach for cancer therapy. *Drug Discov. Today*, 2012, **17**, 1044–1052.
15. Skolnik, N. S., Beck, J. D. and Clark, M., Combination antihypertensive drugs: recommendations for use. *Am. Fam. Physician*, 2000, **61**, 3049–3056.
16. Stolarczyk, M., Maslanka, A., Apola, A. and Krzek, J., Analysis of hypotensive compounds occurring in complex agents. *Acta Pol. Pharm*., 2010, **67**, 441–454.
17. Hajian, R., Shams, N. and Rad, A., Application of H-Point standard addition method for simultaneous spectrophotometric determination of hydrochlorothiazide and propranolol. *J. Braz. Chem. Soc*., 2009, **20**, 860–865.
18. Narasimham, L. Y. S. and Barhate, V. D., Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms. *J. Chem. Metrol*., 2010, **4**, 1–20.
19. Sarafraz-Yazdi, A., Abedi, M. R., Eshaghi, Z. and Kakhki, J. F., Simultaneous determination of metoprolol and propranolol using chemometric assisted spectrophotometry and high performance liquid chromatography. *Int. J. Pharm. Biol. Sci*., 2012, **3**, 532–542.
20. Sampson, L., Wilson, B. and Hou, H. J. M., Gas chromatography-mass spectrometric analysis of forensic drug flunitrazepam upon exposure to UV irradiation. *J. Forensic Res*., 2013, **4**, 1–4.
21. Angier, M. K., Lewis R. J., Chaturvedi, A. K. and Canfield, D. V., Gas chromatographic mass spectrometric differentiation of atenolol, metoprolol, propranolol and an interfering metabolite product of metoprolol. *J. Anal. Toxicol*., 2005, **29**, 517–521.
22. Knoth, H., Petry, T. and Gartner, P., Differential pulse polarographic investigation of the antifungal drugs itraconazole, ketoconazole, fluconazole and voriconazole using a dropping mercury electrode. *Pharmazie*, 2012, **67**, 987–990.
23. Jain, R. and Sharma, R., Voltammetric quantification of anti-hepatitis drug adefovir in biological matrix and pharmaceutical formulation. *J. Pharm. Anal.*, 2012, **2**, 98–104.
24. Sikkander, M., Vedhi, C. and Manisankar, P., Cyclic voltammetric determination of 1, 4-Dihydro pyridine drugs using MWCNTs modified glassy carbon electrode. *Der Chem. Sin*., 2012, **3**, 413–420.
25. Suntornsuk, L., Capillary electrophoresis in pharmaceutical analysis: a survey on recent applications. *J. Chromatogr. Sci*., 2007, **45**, 559–577.
26. Srivastava, S. and Coutinho, E., Adrenergic antagonist propranolol as a novel, effective spermicide: an NMR study. *Int. J. Pharm. Pharm. Sci*., 2010, **2**, 196–200.
27. Holzgrabe, U., Deubner, R., Schollmayer, C. and Waibel, B., Quantitative NMR spectroscopy applications in drug analysis. *J. Pharm. Biomed. Anal*., 2005, **38**, 806–812.
28. Venkatesan, P., Dharuman, C. and Gunasekaran, S., A comparative study of principal component regression and partial least squares regression with application to FTIR diabetes data. *Indian J. Sci. Technol*., 2011, **4**, 740–746.
29. Darwish, H. W. and Backeit, A. H., Multivariate versus classical univariate calibration methods for spectrofluorimetric data: application to simultaneous determination of olmesartan medoxamil and amlodipine besylate in their combined dosage form. *J. Fluoresc*., 2013, **23**, 79–91.
30. Mevik, B. H. and Wehrens, R., The pls package: principal component and partial least squares regression in R. *J. Stat. Softw*., 2007, **18**, 1–23.
31. Lindberg, W., Persson J. A. and Wold, S., Partial least squares method for spectrofluorimetric analysis of mixtures of humic acid and ligninsulfonate. *Anal. Chem*., 1983, **55**, 643–648.
32. Kramer, R., *Chemometric Techniques for Quantitative Analysis*, Marcel Dekker, New York, 1998.

ACKNOWLEDGEMENT. This work is financially supported by SERB (DST), New Delhi (CS-212/2012).

Received 1 October 2014; revised accepted 10 December 2014

Numerical analyses of laboratory-modelled reinforced stone column

Yogendra Tandel[1,*], Mohsin Jamal[2], Chandresh Solanki[3] and Atul Desai[3]

[1]GIDC Degree Engineering College, Navsari 396 439, India
[2]U.V. Patel College of Engineering, Mehsana 384 012, India
[3]S.V. National Institute of Technology, Surat 395 007, India

A stone column develops its vertical load carrying capacity by the lateral pressure provided by the surrounding soil. In very soft clay ($C_u \leq 15$ kPa), the stone column may not derive its load carrying capacity. Sometimes the formation of stone column is doubtful. In such cases, the stone column may be wrapped with geosynthetic peripherally (circumferentially). Normally, reinforced stone columns are used for widely spread areas like air tank foundation and embankment in which they confined by surrounding the columns. The performance of a small group of reinforced stone columns is complex. This communication focuses on the numerical modelling of a small group of laboratory-modelled reinforced stone columns. The study is carried out considering parameters like area replacement ratio (ARR), stiffness of reinforcement material and reinforcement length. The performance of reinforced stone column group is discussed in terms of bearing ratio, (q/C_u)-settlement ratio, stress concentration factor and lateral deformation. The results of numerical analyses indicate that ARR and stiffness of geosynthetic are the governing parameters for enhancing the performance of reinforced stone column. The performance of partial reinforced stone column is close to that of a fully reinforced stone column.

*For correspondence. (e-mail: tandel.yogendra@gmail.com)