Int J Pharm Bio Sci 2012 July; 3(3): (P) 532 - 542

Research Article Analytical Chemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

SIMULTANEOUS DETERMINATION OF METOPROLOL AND PROPRANOLOL USING CHEMOMETRIC-ASSISTED SPECTROPHOTOMETRY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Resolution of binary mixtures of metoprolol and propranolol without sample pre-treatment has been successfully achieved, using three different methods. The first method was based on application of Vierodt's method that involves measurements of the absorbances at 222.6 and 213.4 nm for metoprolol and propranolol. Calibration graphs were established in the range of 1-70 µg/mL and 0.5-30 µg/mLfor metoprolol and propranolol, respectively. The second method describes the use of multivariate spectophotometric calibration by using partial least squares (PLS) analysis of UV spectral data. In the third method, high performance liquid chromatography (HPLC) was performed by using reversed phase column and a mobile phase composed of 0.01 mol/LNaH_2PO_4 (adjusted to pH 3.0 with phosphoric acid) - methanol - acetonitril (45:45:10, v:v:v). The proposed methods were validated and results obtained by adopting the three methods were statistically analyzed.

KEYWORDS: **Chemometric method, HPLC,Metoprolol, PLS,Propranolol,Vierodt's method.**

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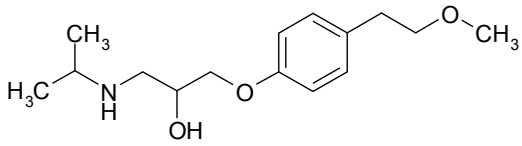
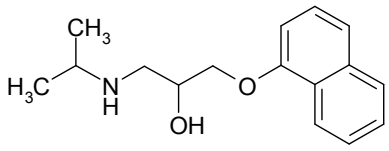
INTRODUCTION

β-Blockers (or *β*-adrenergic antagonists) are a group of drugs widely used in the treatment of cardiovascular diseases, namely, arterial hypertension, cardiac arrhythmias, and angina pectoris as well as other types of pathologies such as anxiety or glaucoma[1,2]. The International Olympic Committee prohibits the use of these drugs in several sports because they reduce heart rate and tremor and improve performance in sports that are not physiologically challenging but require accuracy, e.g. shooting[3]. *β*-Blockers were reported to be exceptionally toxic and most of them acted in a narrow therapeutic range[4,5]. A screening method has therefore a practical interest in diverse areas including forensic, toxicology and doping control.Different methods have been developed for the determination of *β*-Blockers including spectrophotometric[6,7], colorimetric[8,9], TLC[10], GC[11,12] and HPLC[13,14] methods. The assay procedures of these drugs in pure form and in pharmaceutical preparations listed in USP[16] and BP[17] are described potentiometric titration, spectrophotometric, and chromatographic methods. A simple and accurate UV-spectrophotometric method can be highly useful for the routine analysis of bulk and formulations.The most commonly prescribed *β*-blockers include metoprolol and propranolol[15]. The structural formulas of these drugs are illustrated below:



Metoprolol



Propranolol

Figure 1
Chemical structures of metoprolol and propranolol.

If a sample contains two absorbing drugs each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method)[18,19].The required informationsare:the absorptivities of metoprolol at λ_1 and λ_2 , a_{x1} and a_{x2}, respectively; the absorptivities of propranolol at λ_1 and λ_2 , a_{y1} and a_{y2}, respectively; the absorbance of the sample at λ_1 and λ_2 , A_1 and A_2 respectively. Let c_x and c_y be the concentrations of metoprolol and propranolol in the sample respectively. Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbances of metoprolol and propranolol.

At λ_1

$$A_1 = a_{x1}bc_x + a_{y1}bc_y \qquad (1)$$

At λ_2

s

$$A_2 = a_{x2}bc_x + a_{y2}bc_y \qquad (2)$$

For the measurements in 1 cm cells, b =1
On rearranging equation (2) and substituting for c_y in equation (1) gives

$$C_x = \frac{A_1 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (3)$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (4)$$

As well as, multivariate calibration is a useful tool in analysis of multicomponent mixtures because it allows the rapid and simultaneous determination of each component in the mixture, with minimum sample preparation, reasonable accuracy and precision and without the need of lengthy separations. With the aid of modern instrumentation to acquire and digitize spectral information and powerful computers to process large amounts of data, chemometric methods[20,21,22,23] based on factor analysis and artificial intelligence, including principal component regression (PCR), partial least squares (PLS) and artificial neural networks (ANN), have found increasing applications for multicomponent determination[24,25,26,27].

All these methods comprise two separate stages. In the first step, termed calibration, an empirical model is built, representing the relationship between the data generated from a set of reference samples and the respective concentrations of their component(s) of interest. This is followed by a second step called prediction, in which the calibration model is used to determine the concentration of the components in the unknowns from their spectral data.

PLS-1 is a tool for the resolution of mixtures, and in recent years it has been applied to optical as well as electrochemical and other signals[28,29,30].The basis of these methods along with their applications, have been reported in the literature[31,32,33,34]. PLS regression was originally developed by Wold[35,36].

The aim of this paper is to investigate the ability of Vierodt'sand PLS-1 methods to quantify a two-component mixture of metoprolol and propranolol. In addition, a HPLC method was developed for the assay of the components of the studied mixture. The proposed methods are simple and accurate. They resulted in a significant reduction in analysis time and proved to be suitable for routine determination of the two components of the studied mixture.

MATERIALS AND METHODS

Reagents, stock solutions and commercial tablets

Metoprolol tartrate and propranolol hydrochloride were obtained from Sigma (St. Louis, MO, U.S.A). Methanol and acetonitril (HPLC grade) were purchased from Fluka (Buchs SG, Switzerland). All other chemical and solvents were of analytical reagent grade. Deionized water was purified by a Milli-Q system (Millipore, Bedford, USA). Stock solutions of metoprolol and propranolol (1000 mg/L) were prepared in MeOH. Working solutions were prepared daily by dilution of the stock solutions with methanol. All the solutions were protected from light throughout the experiments.

Instrumentation

The apparatus used for the HPLC analysis was an Agilent 1200 series (Agilent Technologies, USA) with a multiple wavelength-UV detector. Chromatographic peaks were electronically integrated and recorded using Chem. Station Rev. A 10.01. The separated *β*-blockers were detected by UV/Vis detector. Separation was carried out by a KnauerEurospher 100-5 C18 column with particle size of 5 µm (150 mm × 4.6

mm I.D.).All spectrophotometric measurements were carried out with a Shimadzu 1601 PC (Japan) double beam spectrophotometerequipped with 1 cm quartz matched cell.Measurements of pHwere made with a Metrohm 632 pH-meter.PLS program wasmodeled using ParLeS v3.1 software.

Vierodt'smethod

To verify the governing Beer´s law, several standard solutions in the concentration range of 0-100 µg/mL and 0-50 µg/mL for metoprolol and propranolol respectively were prepared. All dilutions were scanned in the wavelength range of 200-350 nm. Metoprolol has λ_{max} of 222.6 nm while propranolol has λ_{max} at 213.4 nm (Figure 2). The absorbances of the resulting solutions were measured at 222.6 nm and 213.4 nm and calibration curves were plotted. The absorptivity coefficients of these drugs were determined using calibration curve equation (Table 1). Two equations were formed and concentrations of metoprololand propranolol were calculated.

Table 1
Absorptivity values for metoprolol and propranolol

	Absorptivityat 213.4 nm	**Absorptivityat 222.6 nm**
Metoprolol	14.42	20.55
Propranolol	143.33	116.61

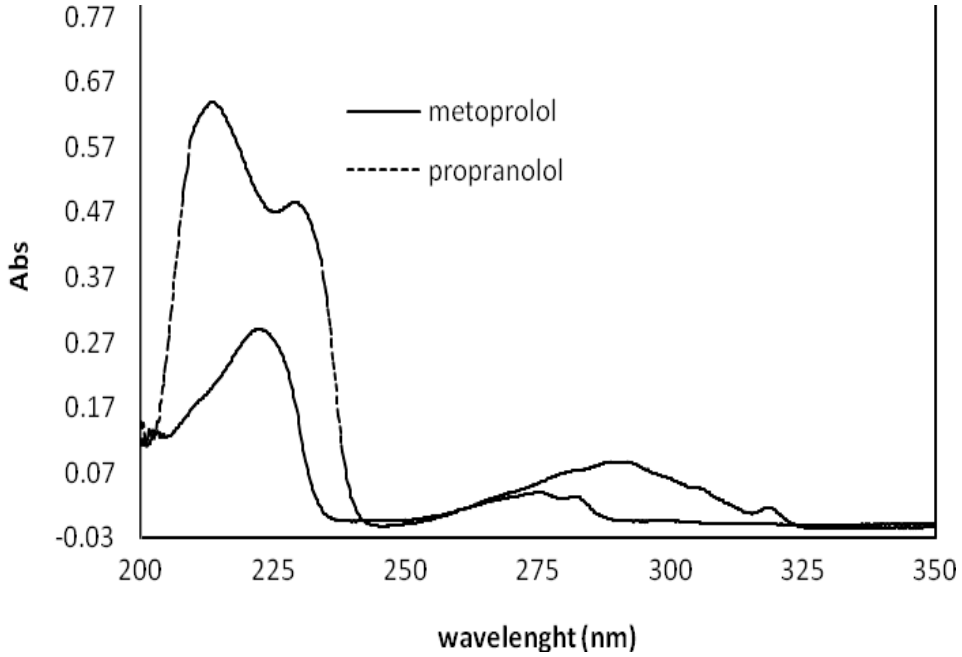


Figure2
Overlain spectra of metoprolol(10 µg/mL)and propranolol (5 µg/mL).

Multivariate method

A four-level factorial design was used to produce a calibration set of 16 samples[37,38,39](Table 2) andathree-level set was derived to produce a prediction set of 9 samples(Table 3). The UV absorption spectra were recorded over the wavelength range of 200–350 nm. The data points of the spectra were collected every 0.2 nm.

Table 2
Calibration set composition

Standard	Metoprolol(µg/mL)	Propranolol(µg/mL)
C_1	16	4
C_2	0	12
C_3	8	4
C_4	8	8
C_5	8	0
C_6	8	12
C_7	24	4
C_8	0	0
C_9	24	12
C_{10}	24	8
C_{11}	0	4
C_{12}	24	0
C_{13}	16	8
C_{14}	16	12
C_{15}	16	0
C_{16}	0	8

Table 3
Predictionset composition

Sample	Metoprolol (µg/mL)	Propranolol (µg/mL)
P_1	10	5
P_2	20	10
P_3	20	2
P_4	4	5
P_5	4	2
P_6	20	5
P_7	10	2
P_8	4	10
P_9	10	10

HPLC method

Series of working solutions of metoprolol and propranolol were prepared by the appropriate dilution of the stock solutions with methanol to reach the concentration ranges of 0.1-100 µg/mLfor metoprolol and 0.1-50 µg/mLfor propranolol.Triplicate 10 µL injections were made for each concentration using the following chromatographic conditions: Mobile phase consisting of 0.01 mol/LNaH_2PO_4 (adjusted to pH 3.0 with phosphoric acid)-methanol - acetonitril (45:45:10, v:v:v). The flow rate was 1.0 mL/min.Detector wavelength: 223nm for metoprolol and 213 nm for propranolol, Column temperature: 22°C. Peak area of each concentration was plotted against

the corresponding concentration to obtain the calibration graph.

Pharmaceutical dosage form

For 50 mg metoprolol and 10 mg propranolol tablets, 20 of each tablet mixed and weighed. After grinding, mixing and homogenizing of an accurately weighed 20 tablets from each pharmaceutical products, 1/20 of sample was used for analysis. Weighed sample was mixed with 40 mL methanol and the mixture was subjected to ultrasonication for 15 minutes,then it was diluted to 100 mL with methanol and mixed.

RESULTS AND DISCUSSION

Vierodt'smethod

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values are given in Table 4. Regression analysis of Beer's law evaluated(Table 6). LOD and LOQwere calculated according to ICH guidelines[40](Table 6) and reveals a very high sensitivity of the method.

Table 4
Analytical parameters of the Vierodt'smethod

Parameters	Metoprolol	Propranolol
λ_{max}(nm)	222.6	213.4
Beer's Law limits (µg/mL)	1-70	0.5-30
Molar absorptivity (L/mol.cm)	14.1×10^3	42.4×10^3
Sandell sensitivity* (µg/cm^2/ 0.001 abs unit)	0.0487	0.0070

**** Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and b = 1 cm.***

Sandell's Sensitivity = Molecular weight / Molar Absorptivity

Multivariate method

Since not all wavelengths in the spectra carry the same quality of information and in order to ensure select each analyte'smost appropriate spectral working region and the number of factors to be used in PLS-1 method[41]. The predicted concentrations of the components (C_{pred}) in each sample were compared with the actual concentrations (C_{act})in the prediction samples and the root mean square error of cross validation (RMSECV) was calculated for each method as follows:

$$RMSECV = \sqrt{\sum (C_{pred} - C_{act})^2 / n} \qquad (5)$$

Where n is the number of training samples.

RMSECV indicates both the precision and accuracy of predictions[42]. The method developed by Haaland and Thomas[43] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that result in an insignificant difference between the corresponding RMSECV and the minimum RMSECV. The selection of the optimum number of factors was a very important pre-construction step because if the number of factors retained was more than required more noise would be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E.) of the prediction concentration[44]:

$$R.S.E.\ (\%) = \left(\frac{\sum_{j=1}^{N} (\tilde{C}_j - C_j)^2}{\sum_{j=1}^{N} (C_j)^2} \right)^{1/2} \times 100 \qquad (6)$$

Where N is the number of samples, $C_j$the concentration of thecomponent in the jth mixture and $\tilde{C}_j$ is the estimated concentration.

The total prediction error of *N* samples is calculatedas follows:

$$R.S.E._T\ (\%) = \left(\frac{\sum_{i=1}^{M} \sum_{j=1}^{N} (\tilde{C}_{ij} - C_{ij})^2}{\sum_{i=1}^{M} \sum_{j=1}^{N} (C_{ij})^2} \right)^{1/2} \times 100 \qquad (7)$$

The R.S.E.(%) of the predicted concentrations and other results are listed inTable 5.

Table 5
Statistical parameters for PLS-1 analysis of metoprolol and propranolol

Parameter	**Metoprolol**	**Propranolol**
Optimum spectral range (nm)	200–350	
Number of PLS Factors	3	
R.S.E. (%)	4.5	3.2
R.S.E.$_T$(%)	3.9	
Recovery (%)	100.21	101.02
Recovery$_T$(%)	100.51	

HPLC Method

HPLC method was applied for determination of metoprolol and propranolol. The specificity of the HPLC method is illustrated in Figure3 where complete separation of the two compounds was noticed. Characteristic parameters for regression equations of the HPLC method obtained are given in Table 6.

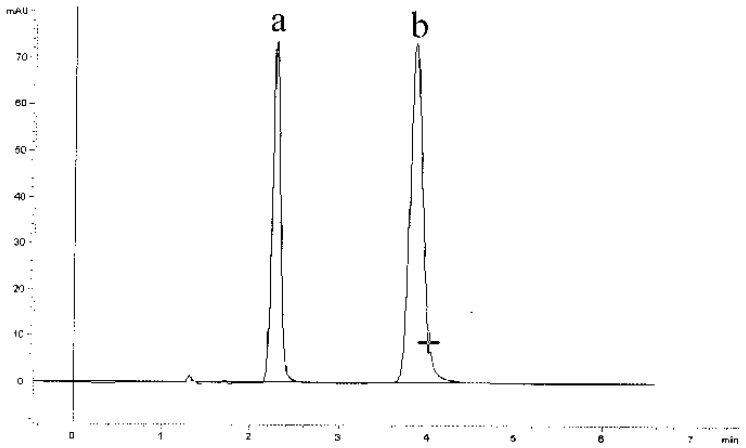


Figure3
HPLC chromatogram of 10 µL injection of mixture containing 50 µg/mLof metoprolol(a) and 10µg/mLof propranolol (b).

Table 6
Characteristic parameters for determination of metoprolol and propranolol by the proposed methods

Parameter	Metoprolol			Propranolol		
	Vierodt's	PLS-1	HPLC	Vierodt's	PLS-1	HPLC
Linearrange(µg/mL)	1-70	2-70	1-100	0.5-30	0.7-30	0.5-50
Slope	0.020	0.020	9.046	0.143	0.143	78.33
Intercept	0.006	0.006	9.24	-0.04	-0.04	15.48
r^2	0.9995	0.9995	0.9988	0.9993	0.9993	0.9997
LOQ (µg/mL)*	1	2	1	0.5	0.7	0.5
LOD (µg/mL)*	0.4	0.6	0.3	0.1	0.2	0.17
R.S.D(%)	0.90	0.78	0.65	0.77	0.69	0.53

****LOD and LOQ were estimated based on practical approaches to the proposed methods. For the Vierodt's method, they were estimated using linear regression method, where LOD = 3Sa/b and LOQ = 10Sa/b, Sa is the standard deviation of five reagent blank determinations and b is the slope of the calibration curve. For HPLC method LOD and LOQ were determined by using the signal-to-noise method[45], a signal-to-noise ratio (S/N) of three is used for estimating LOD and signal-to-noise ratio of ten is used for estimating LOQ. For calculation of the detection limits in PLS method, the absorbance changes of blank solution (five replicates) was recorded, i.e. a data matrix (D_{DL}) was constructed. The average signal of the data matrix, $\bar{v}$, and the standard deviation of the data matrix, s_{DL} , were calculated (both are vectors). The signal for calculating detection limit (v_{DL}) is defined as: $V_{DL} = \bar{v} + 3S_{DL}$. This signal (as a vector) was run by PLS regression as a prediction sample.***

In order to demonstrate the validity and applicability of the proposed methods, recovery tests were carried out by analyzing laboratory prepared mixtures of metoprolol and propranolol, with different ratios. Three laboratory-prepared mixtures containing different ratios of both drugs and three pharmaceutical dosage formswere prepared and analyzed with proposed mehods. The validity of the method was further assessed by applying the standard addition technique. Results are presented in Table 7.

Table 7
Determination of metoprolol and propranolol in laboratory prepared mixtures.

	Vierodt's	PLS	HPLC
Metoprolol	99.96±2.18	101.67±1.45	99.69±0.39
Propranolol	100.58±2.30	98.99±1.70	99.79±0.46

Table 8
Determination of metoprolol and propranolol in pharmaceutical dosage form.

Claimed amount taken (µg/mL)		Standard added (µg/mL)		Recovery of added standard (%) ± SD					
				Metoprolol			**Propranolol**		
Metoprolol	Propranolol	Metoprolol	Propranolol	Vierodt's	PLS	HPLC	Vierodt's	PLS	HPLC
50	10	40	8	95.49 ± 1.62	96.74 ± 1.45	99.51 ± 1.26	96.75 ± 1.50	98.20 ± 1.53	99.29 ± 1.61
50	10	50	10	96.33 ± 1.26	97.42 ± 1.23	98.69 ± 1.01	97.01 ± 1.97	97.03 ± 1.91	99.41 ± 1.29
50	10	60	12	95.91 ± 2.19	98.17± 1.81	99.15 ± 1.19	98.19± 2.26	97.81± 1.21	98.03 ± 0.78
Mean				95.91	97.44	99.12	97.32	97.68	98.91
SD				1.69	1.50	1.15	1.91	1.55	1.23

CONCLUSIONS

The Vierodt's,multivariate (PLS) and HPLC methods enable the quantitation of metoprolol and propranolol binary mixture with good accuracy and precision, either in laboratory prepared samples or in combined dosage forms. All the proposed procedures are simple, accurate, economical and rapid. The good recoveries obtained in all cases proved that the proposed methods could be applied efficiently for determination of metoprolol and propranolol binary mixture with quite satisfactory precision and could be easily used in quality control laboratory for their analysis.

REFERENCES

1 Bristow MR, ß-Adrenergic Receptor Blockade in Chronic Heart Failure. Circulation, 101 (5): 558-569 (2000).

2 Reiter MJ and Reiffel JA, Importance of beta blockade in the therapy of serious ventricular arrhythmias. Am J Cardiol, 82 (4): 9I-19I (1998).

3 International Olympic Committee (IOC), Medical Commission, List of Doping Classes and Methods, 1998.

4 Siren H, Saarinen M, Hainari S and Riekkola ML, Screening of β-blockers in human serum by ion-pair chromatography and their identification as methyl or acetyl derivatives by gas chromatography—mass spectrometry. J ChromatogrA, 632(1-2): 215-227 (1993).

5 Hemmersbach P and De la Torre R, Stimulants, narcotics and β-blockers: 25 years of development in analytical techniques for doping control. J Chromatogr B, 687 (1):221-238 (1996).

6 Ferraro MCF, Castellano PM, Kaufman TS, Chemometric determination of amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate in synthetic mixtures and pharmaceutical formulations. . J Pharm Biomed Anal, 34 (2):305-314 (2004).

7 Gölcü A, Dolaz M and Serin S, Spectrophotometric Determination of Propranolol as Cu(II), Ni(II) and Co(II) Dithiocarbamate Complexes. Turk J Chem, 25 (4): 485-490 (2001).

8. Salem H, Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations. J Pharm Biomed Anal, 29 (3): 527-538 (2002).

9 Gowda BG, ndirect Spectrophotometric Determination of Propranolol Hydrochloride and Piroxicam in Pure and Pharmaceutical Formulations. Anal Sci, 18 (6): 671-674 (2002).

10 Ruane RJ and Wilson ID, Ion-pair reversed-phase thin-layer chromatography of basic drugs using sulphonic acids.J Chromatogr, 441 (2): 355-360 (1988).

11 Angier MK, Lewis RJ, Chaturvedi AK and Canfield DV, Gas Chromatographic-Mass Spectrometric Differentiation of Atenolol, Metoprolol, Propranolol, and an Interfering Metabolite Product of Metoprolol.J Anal Toxicol, 29 (6): 517-521 (2005).

12 Kim KH, Lee JH, Ko MY, Hong SP and Youm JR, Chiral separation of beta-blockers after derivatization with (-)-alphamethoxy-alpha-(trifluoromethyl)phenylacetyl chloride by gas chromatography. Arch Pharm Res, 24 (5) 402-406 (2001).

13 Naidong W, Shou WZ, Addison T, Maleki S and Jiang XY, Liquid chromatography/tandem mass spectrometric bioanalysis using normal-phase columns with aqueous/organic mobile phases – a novel approach of eliminating evaporation and reconstitution steps in 96-well SPE. Rapid Commun Mass Spectrom, 16 (20): 1965-1975 (2002).

14 Albers S, Elshoff JP, Völker C, Richter A and Läer S, HPLC quantification of metoprolol with solidphase extraction for the drug monitoring of pediatric patients. Biomed Chromatogr, 19 (3): 202-207 (2005).

15 http://www.imshealth.com/portal/site/ims accessed on 12.2.2012.

16 The United States Pharmacopoeia, USP 24 NF 19, United States Pharmacopoeial Convention, Inc., 2000, pp. 756, 1428.

17 British Pharmacopoeia, Her Majesty's Stationary Office, London, 1998, pp. 624, 1700, 1103, 1904.

18 Mend HJ, Vogel's, "Textbook of Quantitative Chemical Analysis"; 6th Ed. Pearson education (Singapore) Pvt. Ltd., Indian branch, Delhi, 3-8, 630, (2003).

19 Beckett AH and Stenlake JB, Practical Pharmaceutical Chemistry, 4th ed., Part II. New Delhi: CBS Publisher and Distributors, 1997, p. 276.

20 Perez-Bendito D, Approaches to differential reaction-rate methods. Plenary lecture.Analyst, 115 (6): 689-697 (1990).

21 Crouch SR, Trends in kinetic methods of analysis. Anal ChimActa, 283 (1): 453-470 (1993).

22 Otto M, Chemometrics in kinetic analysis. Plenary lecture.Analyst, 115 (6): 685-688 (1990).

23 Cullen TF and Crouch SR, Multicomponent kinetic determinations using multivariate calibration techniques.MikrochimActa, 126 (1-2) 1-9 (1997).

24 Absalan G and Nekoeinia M, Simultaneous kinetic determination of Fe(II) and Fe(III) based on their reactions with NQT4S in micellar media by using PLS and PCR methods. Anal ChimActa, 531 (2): 293-298 (2005).

25 Ni Y, Qi Z and Kokot S, Simultaneous ultraviolet–spectrophotometric determination of sulfonamides by multivariate calibration approaches. ChemomIntell Lab Syst, 82 (2): 241-247 (2006).

26 Ni Y, Qiu P and Kokot S, Simultaneous voltammetric determination of four carbamate pesticides with the use of chemometrics. Anal ChimActa, 537 (1-2): 321-330 (2005).

27 Chamsaz M, Safavi A and Fadaee J, Simultaneous kinetic-spectrophotometric determination of carbidopa, levodopa and methyldopa in the presence of citrate with the aid of multivariate calibration and artificial neural networks. Anal ChimActa, 603 (2): 140-146 (2007).

28 Navalon A, Blanc R, del Olmo M and Vilchez JL, Simultaneous determination of naproxen, salicylic acid and acetylsalicylic acid by spectrofluorimetry using partial least-squares (PLS) multivariate calibration Talanta, 48 (2) 469-475 (1999).

29 Guiberteau A, Galeano T, Espinosa-Mansilla A, de Alba PL and Salinas F, Abilities of differentiation and partial least squares methods in the analysis by differential pulse polarography Simultaneous determination of furazolidone and furaltadone. Anal ChimActa, 302 (1): 9-19 (1995).

30 Rupprecht M and Probst T, Employing multivariate calibration for the determination of radionuclides by inductively coupled plasma-mass spectrometry. Fresenius J Anal Chem, 359 (4-5): 442-445 (1997).

31 Martens H and Naes T, Ed. Multivariate Calibration, 1st. Edn, Wiley's publisher, Chichester: 85–95, (1989).

32 Thomas EV, A primer on multivariate calibration. Anal Chem, 66 (15): 795A-804A (1994).

33 Donachie A, Walmsley AD and Haswell SJ, Application and comparisons of chemometric techniques for calibration modelling using electrochemical/ICP-MS data for trace elements in UHQ water and humic acid matrices. Anal ChimActa, 378 (1-3): 235-243 (1999).

34 Collado MS, Mantovani VE, Goicoechea HC and Olivieri AC, Simultaneous spectrophotometric-multivariate calibration

determination of several components of ophthalmic solutions: phenylephrine, chloramphenicol, antipyrine, methylparaben and thimerosal. Talanta, 52 (5): 909-920 (2000).

35 Wold S, Martens H and Wold H, in The Multivariate Calibration Problem in Chemistry solved by PLS, Matrix Pencils (Lecture Notes in Mathematics), (A. Ruhe and Kagstrom B, Eds), Springer, Heidelberg, (1992).

36 Martens H and Naes T, Ed. Multivariate Calibration, 2nd. Edn, Wiley's publisher, Chichester: 116-165, (1992).

37 Lan WG, Wong MK, Chee KK and Sin YM, Orthogonal array design as a chemometric method for the optimization of analytical procedures. Part 3.Five-level design and its application in a polarographic reaction system for selenium determination.Analyst, 120 (2): 273-279 (1995).

38 Lan WG, Chee KK, Wong MK, Lee HK, Sin YM, Orthogonal array design as a chemometric method for the optimization of analytical procedures. Part 4.Mixed-level design and its application to the high-performance liquid chromatographic determination of polycyclic aromatic hydrocarbons.Analyst, 120 (2) 281-287 (1995).

39 Lan WG, Wong MK, Chen N, Sin YM, Orthogonal array design as a chemometric method for the optimization of analytical procedures. Part 5.Three-level design and its application in microwave dissolution of biological samples.Analyst, 120, (4): 1115-1124 (1995).

40 International Conference on Hormonisation: ICH Harmonised Tripartite Guidelines - Validation of Analytical Procedures, Federal Register 62, 1997, 27463.

41 Kramer R, Chemometric Techniques for Quantitative Analysis. *Partial Least-Squares*, Marcel Dekker Inc, New York. , 1998, pp. 121-130.

42 Beebe KR, Randy JP, Seasholtz MB, Chemometric: A Practical Guide, Wiley/ Interscience, NewYork, (1998).

43 Haaland DM and Thomas EV, Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal Chem, 60 (11): 1193-1202 (1988).

44 Otto M and Wegscheider W, Spectrophotometric multicomponent analysis applied to trace metal determinations. Anal Chem, 57 (1): 63-69 (1985).

45 ICH, Q2B In proceedings of The International Conference on Harmonization, Geneva (1993).