

Quantitative determination of naltrexone by attenuated total reflectance – FTIR spectrometry using partial least squares (PLS) wavelength selection

Mohammadreza Khanmohammadi ^{a,*}, Hamid Mobedi ^b, Elaheh Mobedi ^a, Kazem Kargosha ^c, Amir Bagheri Garmarudi ^a and Keyvan Ghasemi ^a

^a *Department of Chemistry, Faculty of Science, Imam Khomeini International University, Qazvin, Iran*

^b *Department of Novel Drug Delivery Systems, Iran Polymer and Petrochemical Institute, Tehran, Iran*

^c *Department of Chemistry, Chemistry and Chemical Engineering Research Institute, Tehran, Iran*

Abstract. A new method is presented for quantitative determination of naltrexone in aqueous solutions based up on the wavelength selection in mid-FTIR spectra using partial least squares (PLS) technique. The main aim is to find wavelengths which produce significant improvements in PLS prediction. PLS wavelength selection treatment is performed on the data obtained by attenuated total reflectance Fourier transform infrared spectrometry in 830–1800 cm^{-1} wavenumber range. No separation or extraction steps are required prior to determination procedure and a simple pretreatment is performed. Absorbance spectra were employed for measurements using a set of 7 aqueous standard solutions of naltrexone. The method is applicable for pharmaceutical samples in aqueous solution in the presence of impurities, while it is simple, rapid and accurate. The results obtained from real samples were compared with those obtained using Ultra Violet spectrophotometry as a reference method. Statistical parameters such as R^2 , REP, RMSEC and RMSECV were evaluated, and number of factors, number of scans and resolution were also optimized. In this method R^2 and RMSEC for proposed procedure have been found to be 0.9983 and 0.1297, respectively.

Keywords: ATR-FTIR, wavelength selection, PLS, naltrexone, determination, spectrometry

1. Introduction

Opiate addiction is a very serious worldwide problem which has now spread to societies [1]. One of the current treatments is to use narcotic antagonists which generally have chemical structures similar to those of opiates and can preferentially occupy the body's opiate receptors and thus block their euphoric effects. This makes opiate intake delight less and removes the addicts' incentive in opiate using [2]. Naltrexone (NTX), cyclopropyl derivative of oxymorphone is a potent, long acting and orally effective, narcotic antagonist (Fig. 1). It is considered as a useful adjunct for the maintenance of abstinence

*Corresponding author: M. Khanmohammadi, Department of Chemistry, Faculty of Science, Imam Khomeini International University, P.O. Box 288, Qazvin, Iran. Tel.: +98 281 3780040; Fax: +98 281 3780040; E-mail: mrkhanmohammadi@gmail.com.

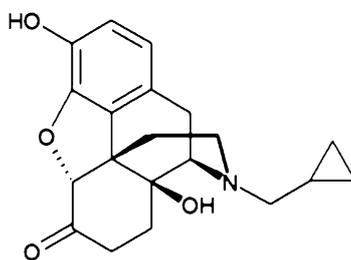


Fig. 1. Chemical scheme of naltrexone.

in the detoxified opioid addicts [3,4] when administered in selected patient groups and in combination with appropriate support mechanisms and psychotherapy. Therefore, it is a promising alternative to methadone, naloxone [5] and to drug-free approaches in the treatment of narcotic addiction. NTX is presented in pharmaceuticals either in liquid or solid form. NTX and its active metabolite 6- β -naltrexol, competitive antagonists at μ - and κ -opioid receptors, and to a lesser extent at δ -opioid receptors. The plasma half-life of naltrexone is about 4 h, for 6- β -naltrexol 13 h. The blockade of opioid receptors is the basis behind its action in the management of opioid dependence it reversibly blocks or attenuates the effects of opioids. Its use in alcohol dependence has been studied and has been shown to be effective. Its mechanism of action in this indication is not fully understood, but as an opioid-receptor antagonist it is likely to be due to the modulation of the dopaminergic mesolimbic pathway which ethanol is believed to activate. Naltrexone is metabolized mainly to 6- β -naltrexol by the liver enzyme dihydrodiol dehydrogenase. Other metabolites include 2-hydroxy-3-methoxy-6- β -naltrexol and 2-hydroxy-3-methoxy-naltrexone. These are then further metabolized by conjugation with glucuronide. Many techniques have been developed for determination of this compound and its major metabolite (6- β -naltrexole) such as thin layer chromatography (TLC) [6], gas chromatography (GC) [7–10], high performance liquid chromatography with electrochemical detection (HPLC-EC) [11], gas chromatography-mass spectrometry (GC-MS) [12] and flow injection analysis (FIA) [13,14]. However, these procedures are very time consuming. An alternative method is therefore required for this type of study. ATR-FTIR spectrometry technique offers some distinct advantages for this purpose. FTIR spectrometry is a fast analytical technique and provides very interesting qualitative and quantitative information [15,16]. Also attenuated total reflectance (ATR) technique makes possible to study aqueous solution in quantitative analysis [17,18]. According to the absence of troubles due to the intercoverage in this technique, sample preparation is much easy and does not require any sample pre-treatment. Partial least squares technique (PLS) has been used to extract the relevant part of information and produce reliable models. Nowadays, this concept is applied by several researchers, according to Martens and Neas algorithm [19,20]. The choice of wavelength would critically affect the future predictive ability of the model. Recently, several selection methods have been developed, e.g. artificial neural network (ANN) [21], Tabu search [22], hybrid linear analysis (HLA) [23], and successive projection algorithm (SPA) [24] in calculation power and being applicable for extremely complex problems. There are some publications dealing with genetic algorithms [25,26].

In this study it has been tried to introduce the ATR-FTIR spectrometry method for quantitative determination of NTX in aqueous solutions, while PLS technique is utilized for wavelength selection.

2. Experimental section

2.1. Apparatus and software

A Bomem (Quebec, Canada) MB-100 FTIR spectrometer equipped with a DTGS (D31B) mid-range detector, a Ge/Sb₂S₃ coated KBr beam splitter and a SiC source, was employed to record the IR spectra using spectratech (Warrington, UK) in compartment contact with sampler horizontal attenuated total reflector with a 45° ZnSe trough plate. PIs-Plus/Iq button to the GRAMS/32 and higher software (Galactic Ind. Co.) was used to process the absorbance data.

2.2. Material

Naltrexone, 17-(cyclopropylmethyl)-4,5 α -epoxy-3,14-dihydroxymorphinan-6-one, was from Iran Pharmaceutical Co. NTX is soluble in distilled water; therefore distilled water may be used as solvent. The NTX real samples were supplied by Iran Polymer and Petrochemical Institute. Other ingredients were lactic acid, glycolic acid and N-methyl pyrrolidone. The lactic acid:glycolic acid:N-methyl pyrrolidone ratio in all of real samples was 1:1:1. In order to optimize and evaluate the prediction capability of the PLS-IR method, aqueous solutions of NTX were prepared in distilled water, containing different amounts of NTX, in the concentration range of 2.5–8.5 (g per 100 ml). Seven standards were prepared for PLS model and 5 other standards of NTX were employed to evaluate this model.

2.3. ATR-FTIR spectrometry procedure and spectral acquisition

A suitable solvent dissolves the analytical samples without reacting and has the least interference at spectral region of the analyte. The ATR-FTIR spectra of distilled water and aqueous solution of NTX with both air and water background are shown in Fig. 2. It is observed in Fig. 2 that some useful signals would appear in the NTX solution spectrum when water is set as the background e.g. in 1700–1750 cm^{-1} region. Obtaining the absorbance spectrum of naltrexone in mid-IR region, the main signals

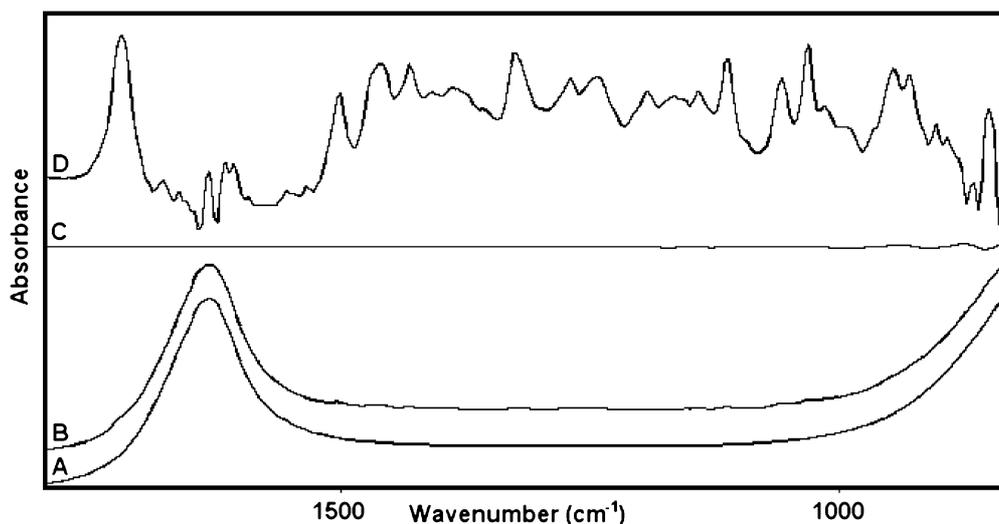


Fig. 2. ATR-FTIR spectra with air background of distilled water (A), NTX aqueous solution (B) and with solvent background of distilled water (C) and NTX (D).

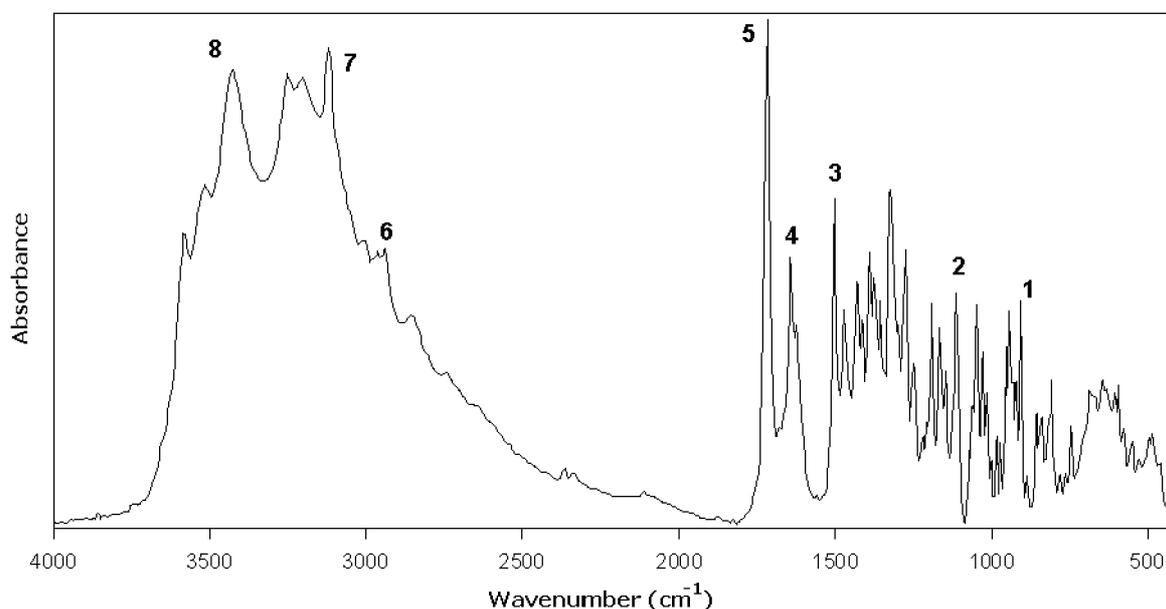


Fig. 3. ATR-FTIR spectrum of NTX in mid-IR spectral region with water background.

were assigned. As shown in Fig. 3, there are several signals due to the complex structure of the analyte. The main assigned bands are:

- (1) C=C banding and substituted aromatic ring,
- (2) ether C–O,
- (3) amine C–N,
- (4) aromatic C=C,
- (5) carbonyl functional group (C=O),
- (6) aliphatic C–H,
- (7) aromatic C–H and cyclopropane ring C–H,
- (8) hydroxyl group.

Investigating the ATR-FTIR spectra of standard samples, an increasing relation was observed between concentration and absorbance intensity of signals, especially in 1000–2000 cm^{-1} spectral region. Aqueous standard solutions of NTX in the concentration range of 2.5–8.5 (g per 100 ml) have intense bands in 1222–1527 cm^{-1} and 1693–1743 cm^{-1} spectral region. According to the main assigned bands, the ATR-FTIR spectra were obtained in the 830–1800 cm^{-1} spectral region, employing cosine apodization. The spectra show that 1222–1527 cm^{-1} and 1693–1743 cm^{-1} spectral regions could be selected for quantitative determination. One of the main advantages of Fourier transformation is the possibility of accumulating a large number of scans, which could provide a better limit of detection for IR measurements. An increase in the number of accumulated scans does not affect the absorbance of the analytes but reduces the noise level of the obtained spectra. Therefore the signal to noise ratio (S/N) is improved [27]. The use of higher resolution achieves more data points, but in a longer time [28]. The use of 10 scans and a nominal resolution of 8 cm^{-1} in the present work seems to be a good compromise in order to obtain a good detection limit for the analyte.

3. Results and discussion

3.1. Partial least squares model

Multivariate calibrations are useful in spectral analysis since the overlapping of NTX signals with water signals (see Fig. 2) are calculated and the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and the predictive ability. With the aim of improving the analysis for NTX, a multivariate PLS method was applied for absorption spectra. However, combination of these two spectral ranges $1222\text{--}1527\text{ cm}^{-1}$ and $1693\text{--}1743\text{ cm}^{-1}$ was evaluated by performing PLS calibration method. A set of 7 aqueous standard samples (samples 1–7 in Table 1) were applied as the calibration samples. Also, in order to ensure that the calibration model would provide accurate predictions, 5 other aqueous standard samples were proposed as independent test set (samples 8–12 in Table 2). The concentration of NTX in these standard samples was varied in range of 2.5–8.5 (g per 100 ml). Total absorption spectra were obtained in both $1222\text{--}1527$ and $1693\text{--}1743\text{ cm}^{-1}$ spectral region, following the same procedure as detailed. PLS is often regarded as the major regression technique for multivariate data [29]. PLS has shown an unparalleled application success, both in chemometrics and other fields. Among other features, the PLS approach gives superior interpretation possibilities. In spectroscopy we usually expect linear additivity, and this is especially important for chemical instrumental data. PLS is always

Table 1

Actual and predicted concentrations of NTX (g per 100 ml) in the calibration (1–7) and independent test set (8–12) samples

Sample	Actual concentration	Predicted PLS	Concentration PLS–PLS
1	2.5710	2.5559	2.4635
2	3.5730	3.6534	3.6003
3	4.5720	4.5633	4.6228
4	5.5700	5.2086	5.3722
5	6.5710	6.5056	6.6361
6	7.5720	7.8108	7.7532
7	8.5730	8.5995	8.4736
8	3.0440	2.7468	2.9772
9	4.0440	3.9025	4.0155
10	5.0460	4.8282	5.0242
11	6.0440	6.2599	5.7878
12	7.0440	7.4597	6.6265

Table 2

Statistic parameters obtained from quantitative analysis of NTX aqueous solutions in both calibration models

	Method	
	PLS	PLS–PLS
RMSEC	0.1823	0.1297
RMSECV	0.3330	0.1959
REP	3.2718	2.3283
R^2	0.9967	0.9983

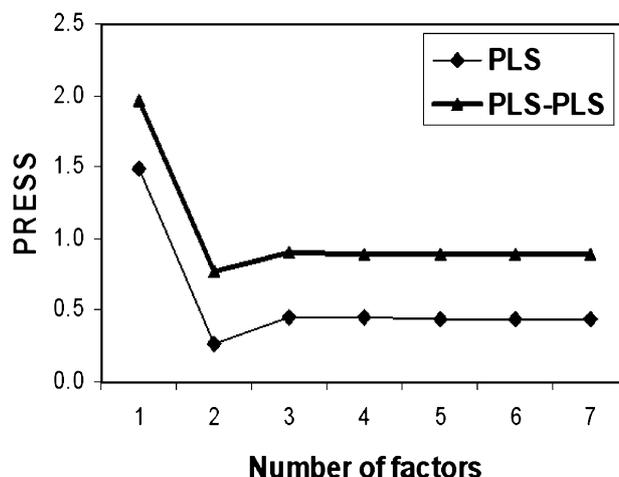


Fig. 4. Diagram of PRESS according to number of factors for both PLS and PLS–PLS models.

an important tool when there is partial knowledge of the data. PLS can be very robust provided that future samples contain similar features to the original data, but the predictions are essentially statistical. An important feature of PLS is that it takes into account errors in both the concentration estimates and the spectra, so it was used for determination of NTX in real samples. This calibration models was tested by varying the number of PLS factors from 1 to 7 in order to optimize the models. To select the optimum number of factors in PLS algorithm, the cross validation method [30], leaving out one sample at a time, was used. The prediction error sum of squares (PRESS) was calculated each time a new factor was added giving rise to different PLS models. One reasonable choice for the optimum number of factors would be that number which yielded the minimum PRESS. Figure 4 shows calculated PRESS during the number of factors' variation, contributed in model. However, the optimum number of factors was found to be 2 for calibration set. The root mean square error of calibration (RMSEC) was also calculated, using the appropriate expressions [31]. The root mean square error of cross validation (RMSECV), together with relative error of prediction (REP, $n = 2$) and the squared correlation coefficient of regression lines (R^2) for calibration model were also calculated (Table 2).

3.2. Partial least squares as a wavelength selection model

The choice of wavelengths for model building using PLS is critical [32], if the model is to have good future predictive ability (called PLS–PLS in this study). To generate a robust model, wavelengths must be used that are truly 'causal' otherwise they may produce good correlations through mere chance from fortuitous noise trends. To account for this variability, it is expected that a large number of wavelengths will be needed as well as larger than usual number of PLS factors. The selection of causal wavelengths represents a difficult task, particularly with full spectrum multivariate techniques such as PLS. Traditionally, wavelength selection when used with PLS analysis has involved the selection of all wavelengths in a particular region of the spectrum known to contain useful information. Recently a number of selection methods have been proposed where wavelengths are selected through interactive trial and error processes. The most appropriate point to start the wavelength selection process is from a small number of wavelengths known to be useful for predicting the aimed variable in question. To obtain these wavelengths a forward stepwise procedure was used to select useful wavelengths [33]. Leave one out cross

validation was used to determine the best number of factor that is optimum for model in each iteration, then root mean square error of prediction (RMSEP) was calculated and was compared with RMSEP of external validation set, to recognize if the selected variables are appropriate for contributing in model. After finding the best subset instead of full spectrum, the selected model was evaluated by independent test set. REP for independent test set was 4.9132 in PLS–PLS model, while it was found to be 6.0718 in PLS model, which introduces the PLS–PLS as a suitable modification algorithm. Figure 5 shows selected variables using mentioned procedure. The results of calibration (see Table 2) proves the better prediction ability of PLS–PLS model by 34 selected wavelengths.

3.3. Real sample

The UV spectrophotometry method was also applied for determination of NTX in real samples as a reference. Table 3 shows the results obtained on prediction of real samples by FTIR, being compared with UV method. In order to evaluate the precision of the PLS–PLS method, analysis of variance (ANOVA) was applied for obtained data which shows the proposed method to be more precise than the reference method (p -value < 0.05, 95% interval confidence). Note that box plot (Fig. 6) does not show any significant different between results of UV-VIS and PLS–PLS method.

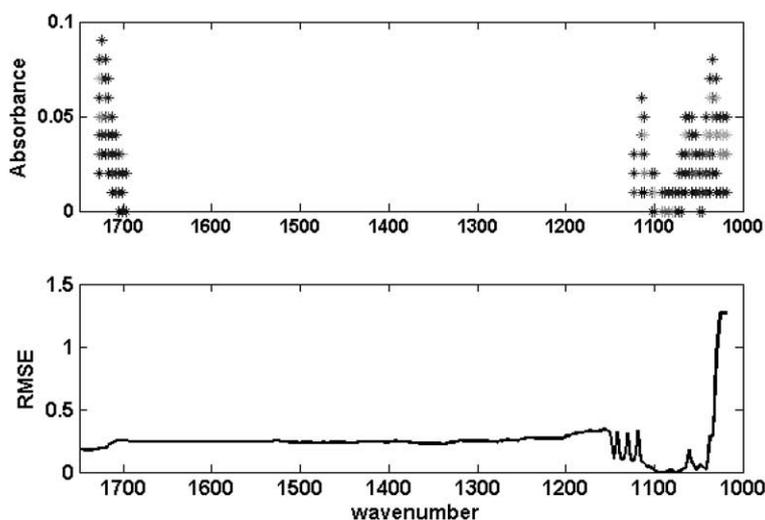


Fig. 5. Selected wavenumbers, utilizing PLS as a wavelength selection method (up) and calculated RMSEP for full spectrum (low).

Table 3

Determination of the NTX concentration (g per 100 ml) in real samples by UV spectrophotometry and ATR-FTIR proposed method

Sample	UV	ATR-FTIR	
		PLS	PLS–PLS
1	4.9890	4.3758	4.6027
2	3.9700	3.7901	3.9545
3	3.5720	3.7454	3.8529
4	3.5290	3.6349	3.7072

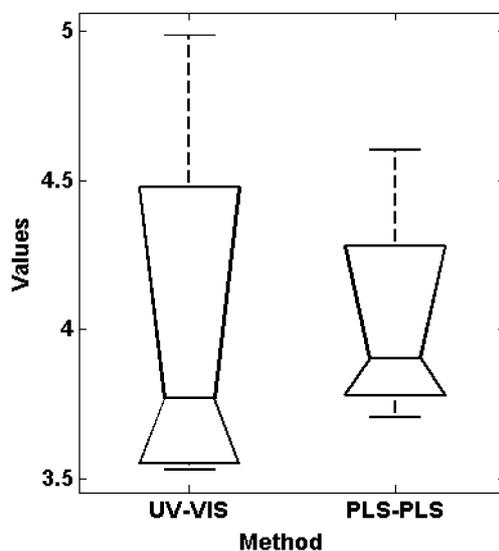


Fig. 6. Box plot of real sample analysis based up on ANOVA test.

4. Conclusion

We have presented a new wavelength selection method for PLS regression. It has been shown that with a sufficient number of ‘causal’ wavelengths, the PLS wavelength selection process selects sufficient additional wavelengths to produce PLS regression models with superior predictive ability over full spectrum based PLS models and over the recently proposed feature selection method. The application of ATR-FTIR technique for determination of NTX is interesting for its simplicity. Applying UV spectrophotometry method for determination of NTX can be useful for analysis of real samples, but it is time-consuming and a pretreatment procedure is also needed. The proposed method (ATR-FTIR) is a rapid and accurate alternative for determination of NTX in real samples without any previous chemical treatment of the sample or a previous separation or extraction step.

References

- [1] A.M. Washton, M.S. Gold and A.C. Pottash, *Adv. Alcohol Subst. Abuse* **4** (1984), 89.
- [2] C.P. O’Brien, R.A. Greenstein and G.E. Woody, Update on naltrexone treatment, in: *The International Challenge of Drug Abuse*, R.C. Peterson, ed., NIDA Research Monograph No. 19, National Institute of Drug Abuse, Rockville, MD, 1978, pp. 315–320.
- [3] F.S. Tennant, R.A. Rawson Jr., A.J. Cohen and A. Mann, *J. Clin. Psychiatr.* **45** (1984), 42–45.
- [4] A.M. Washton, A.C. Pottash and M.S. Gold, *J. Clin. Psychiatr.* **45** (1984), 39–41.
- [5] Y.W. Chien, *J. Parent. Sci. Technol.* **35** (1981), 106–139.
- [6] M.E. Wall, D.R. Brine and M. Perez-Reyes, *Drug Metab. Dispos.* **9** (1981), 369–375.
- [7] K. Verebey, A. De Pace, D. Jukofsky, J.V. Volavka and S.J. Mule, *J. Anal. Toxicol.* **4** (1980), 33–37.
- [8] K. Verebey, M.J. Kogan, A. DePace and S.J. Mule, *J. Chromatogr.* **118** (1976), 331–335.
- [9] K. Verebey, S.J. Mule and D. Jukofsky, *J. Chromatogr.* **111** (1975), 141–148.
- [10] R.H. Reuning, S.B. Ashcraft and B.E. Morrison, in: *Naltrexone Research Monograph*, No. 28, R.E. Willette and G. Barnet, eds, National Institute on Drug Abuse, Rockville, MD, 1980, p. 25.
- [11] P. Zuccaro, I. Altieri, P. Betto, R. Pacifici, G. Ricciarello, L.A. Pini, E. Sternieri and S. Pichini, *J. Chromatogr.* **567** (1991), 485–490.
- [12] K.M. Monti, R.L. Foltz and D.M. Chinn, *J. Anal. Toxicol.* **15** (1991), 136–140.

- [13] J. Martínez Calatayud, S. Sagrado Vivesy and F. Sanmiguel Roche, *Quim. Anal.* **9** (1990), 1–31.
- [14] M.T. Fernández-Abedul and A. Costa-García, *Anal. Chim. Acta* **328** (1996), 67–71.
- [15] F.A. Inon, J.M. Garrigues, S. Garrigues, A. Molina and M. de la Guardia, *Anal. Chim. Acta* **489** (2003), 59–75.
- [16] J.M. Garrigues, M. Akssira, F.J. Rambla, S. Garrigues and M. de la Guardia, *Talanta* **51** (2000), 247–255.
- [17] J. Yang and H.C. Lin, *Analyst* **125** (2000), 1605–1610.
- [18] F.J. Rambla, S. Garrigues, N. Ferrer and M. de la Guardia, *Analyst* **123** (1998), 277–281.
- [19] J. Hernández Méndez, O. Jiménez de Blas and A. Lozano García, *Microchem. J.* **38** (1998), 355–361.
- [20] H. Martens and T. Neas, *Multivariate Calibration*, Wiley, New York, 1991, pp. 116–228.
- [21] D. Svozil, V. Kvasnicka and J. Pospichal, *J. Chemometr. Intell. Lab. Syst.* **39** (1997), 43–62.
- [22] J.A. Hageman, M. Strepal, R. Wehrensand and L.M.C. Buydens, *J. Chemometr.* **17** (2003), 427–437.
- [23] H.C. Goicoechea and A.C. Olivieri, *Analyst* **124** (1999), 725–732.
- [24] M.C.U. Araújo, T.C.B. Saldanha, R.K.H. Galvão, T. Yoneyama, H.C. Chame and V. Visani, *Chemometr. Intell. Lab. Syst.* **57** (2001), 65–73.
- [25] H. Ghasemi, A. Niazi and R. Leardi, *Talanta* **59** (2003), 311–317.
- [26] M. Khanmohammadi, M.A. Karimi, K. Ghasemi, M. Jabbari and A. Bagheri Garmarudi, *Talanta* **72** (2007), 620–625.
- [27] K. Kargosha, M. Khanmohammadi and M. Ghadiri, *Analyst* **126** (2001), 1432–1435.
- [28] B.C. Smith, *Fundamentals of Fourier Transform Infrared Spectroscopy*, CRC Press, Boca Raton, FL, 1996, p. 40.
- [29] K. Kargosha, M. Khanmohammadi and M. Ghadiri, *Anal. Chim. Acta* **437** (2001), 139–143.
- [30] H. Martens and T. Neas, *Multivariate Calibration*, Wiley, New York, 1991, p. 142.
- [31] K. Kargosha, S.H. Ahmadi, A. Ghassempour and M.A. Arshadi, *Analyst* **124** (1999), 367–372.
- [32] S.D. Osborne, R.B. Jordan and R. Kuennemeyer, *Analyst* **122** (1997), 1531–1538.
- [33] Y.F. Maa and J. Heller, *J. Contr. Rel.* **14** (1997), 21–28.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

