Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 909–915
IL FARMACO
www.elsevier.com/locate/farmac

Multivariate least squares regression applied to the spectrophotometric analysis of manidipine and its main photoproduct

Gaetano Ragno *, Francesca Aiello, Antonio Garofalo, Giuseppina Ioele, Maria Stefania Sinicropi

Dipartimento di Scienze Farmaceutiche, Università della Calabria, 87036 Arcavacata di Rende (CS), Italy

Received 11 December 2002; accepted 10 February 2003

Abstract

The simultaneous quantitative assay of 1,4-dihydropyridine calcium antagonist manidipine and its main photodegradation by-product has been defined by using a multivariate calibration on UV spectra based on a classical least squares regression. Optimization of the procedure was achieved by means of a calibration model using suitable wavelength ranges singled out from a fractionation scheme thereat defined. Recovery values of 99 and 96% for the drug and the by-product, respectively, were found either in appropriately prepared mixtures and commercial formulations. Quantification limit for the photoproduct concentration was estimated as 1.0% for the reference samples.

Keywords: Classical least squares; Manidipine; Multivariate spectrophotometry; Photodegradation

1. Introduction

Manidipine (MND), 1,4-dihydro-2,6-(dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-[4-(diphenylmethyl)-1-piperazinyl] ethyl methyl ester, is a second-generation calcium antagonist with peculiar and favourable characteristics like the ability in increasing hematic renal flow and giving rise to a remarkable sodium excretion without modifying glomerular filtrate [1–3]. On the other hand, its depressive effect on heart is less marked compared to analogous drugs [4]. Due to its chemical features, MND is sensitive to light, with a behaviour common to all members of 1,4-dihydropyridine class of compounds [5–8]. The main photodegradation product is represented by the aromatic pyridine derivative, generally termed as dehydro-MND (d-MND), devoid of any pharmacological activity. Accordingly, the control of the drug and its by-product, as such or in pharmaceutical formulations, constitutes a noteworthy analytical problem.

MND in human plasma was reported to have been quantitatively determined by chromatographic techniques [9]. No reports have hitherto appeared in the literature concerning with the assay of MND and relative by-products in dosage forms.

Strictly controlled operating conditions should be followed in order to keep light exposure to a minimum, owing to the high drug photosensitivity. Therefore, straightforward analytical methods employing a number of steps as low as possible are preferred.

A method for the simultaneous assay of MND and d-MND is herein described. Quantitative multicomponent analysis using UV–vis spectrophotometry was selected as the most appealing approach to be adopted, because of the wide acceptance recently granted to such a methodology [10–12]. Moreover, one of the main advantage in applying spectroscopic procedures is the possibility of analysing complex mixtures very quickly,

* Corresponding author.
E-mail address: ragno@unical.it (G. Ragno).

0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00149-6

provided that the calibration was already performed. In fact, alternative techniques of wide application, namely HPLC, GC, colorimetric or fluorometric assays, usually result rather slow and demanding during the preparation of samples.

The adopted procedure is based on the application of a multivariate spectrophotometric method utilizing a classical least squares (CLS) algorithm. A system aimed at selecting optimal wavelength subsets is proposed as well, in order to improve the prediction ability.

Validation of the method has been achieved by analysing synthetic mixtures. Good statistical values in terms of accuracy, precision and sensitivity have been so assessed. The whole procedure required only a simple and rapid sample preparation with a negligible light exposure when applied to pharmaceutics.

The photodegradation study herein reported has been performed according to the 'Guide for the Photostability Testing of New Drug Substances and Products' recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonized Tripartite Guideline) [13].

2. Experimental

A red lamp (60 W) for the room illumination was used during all laboratory procedures, in order to minimize drug photodegradation

2.1. Instruments

All ultraviolet absorption spectra were obtained with a Lambda 40P UV–vis spectrophotometer (Perkin–Elmer). The spectra were recorded at a data density of one point per nanometer between 190 and 450 nm, in 10-mm silica quartz cells. The optimized instrumental parameters were: scan rate, 1 nm s^{-1}; time response, 1 s; spectral bandwidth, 1 nm. UV WINLAB 2.70.01 package software (Perkin–Elmer) was used to elaborate the spectral data.

The photodegradation studies were performed by means of an irradiation camera Suntest CPS+ (URAI, Milan, Italy), equipped with a Xenon lamp, fixing the wavelength emission between 310 and 800 nm, irradiance power at 350 W m^{-2} and temperature at 25 °C.

2.2. Multivariate analysis software

CLS regression has been performed by use of the software PMULT 1.0 (Perkin–Elmer). The application consists of two steps: a first calibration step in which sample mixtures of known composition were used to extract absorptivities information for all the components; the regression model developed is then used in a second-step to relate spectrum with composition and hence to predict the unknown concentrations of a mixture.

The calibration can be performed using up to 15 reference solutions and 6 wavelength ranges. A linear relationship between absorbance and component concentrations must be ensured. In the calibration step the algorithm furnishes, for each component, three statistic parameters: prediction residual errors sum of squares (PRESS), sensibility (SNB) and selectivity. PRESS represents an index of precision and is calculated by comparison between the known concentrations of the components in the reference samples and the predicted values. SNB represents the contribution of the spectral curves and corresponds to the absorbances of single components at the wavelengths considered. Selectivity is a measurement of the degree of overlapping between the different component spectra.

2.3. Chemicals

MND was obtained from commercial tablets by acetone extraction, followed by two crystallizations from ethanol. d-MND was obtained by direct oxidation of MND, by irradiating a MND 4 mg ml^{-1} hexane suspension in the irradiation camera for 8 h. The pale yellow powder, so obtained, was filtered and washed with pure hexane. The purity and identity of d-MND was confirmed by gas-mass chromatographic analysis, exhibiting a molecular peak m/z 608.

Ethanol and hexane, of analytical reagent grade, were purchased by C. Erba (Italy). PTFE 0.45 μm membrane filters were supplied by Supelco.

Iperten 20 (Master Pharma, Italy) and Vascoman (Takeda, Italy) pharmaceutical specialties were obtained commercially.

2.4. Standard solutions

A calibration set of 15 binary mixtures was prepared in 95° ethanol, with MND concentration within the range 6.00–24.00 μg ml^{-1} and d-MND between 0.25 and 2.00 μg ml^{-1}. For these ranges, each component was demonstrated to respond linearly over the 190–450 nm wavelength range and the contribution of single components in all binary mixtures was shown to be additive.

2.5. Sample solutions

The external validation of the calibration model was achieved over the spectra of a prediction set of synthetic binary mixtures in different ratios, with concentration values within the ranges used for the standard solutions. The assay results of these solutions are listed in Table 3.

For the assay of commercial solid dosage forms, five tablets were weighed and reduced to a fine powder. An amount corresponding to the average of one tablet was accurately weighed, stirred and made up to a volume of 50 ml with ethanol. The suspension so obtained was sonified for 10 min, and then filtered with a PTFE 0.45-μm membrane filter. One milliliter of this filtrate was then diluted to 10 ml with ethanol and analyzed.

2.6. Photodegradation

The exposition to natural light was carried out in the period June–July, constantly exposing the samples from 9 a.m. to 5 p.m. in clear days. The room exposure was performed under artificial light tube. The controlled irradiation was performed in the CPS+Suntest Camera, under a Xenon lamp, fixing a wavelength cut-off to 310 nm.

A 20 μg ml^{-1} MND ethanol solution was placed in a 10-mm quartz cell, perfectly stoppered, and treated

Table 1
Calibration set for binary mixtures of MND and its photodegradation product d-MND (expressed as μg ml^{-1})

Sample	MND	d-MND	MND/d-MND
1	6.01	0.24	25.04
2	6.01	0.48	12.52
3	6.01	0.96	6.26
4	6.01	1.92	3.13
5	12.02	0.24	50.08
6	12.02	0.48	25.04
7	12.02	0.96	12.52
8	12.02	1.92	6.26
9	18.03	0.24	75.13
10	18.03	0.48	37.56
11	18.03	0.96	18.78
12	18.03	1.92	9.39
13	24.04	0.24	100.17
14	24.04	0.48	50.08
15	24.04	0.96	25.04

Table 2
PRESS and SNB values obtained from application of the calibration step

Wavelength interval	MND			d-MND		
	PRESS	SNB	SNB/PRESS	PRESS	SNB	SNB/PRESS
10 nm intervals						
200–210	5.201	0.120	0.023	4.035	0.153	0.038
210–220	1.420	0.119	0.084	1.230	0.070	0.058
220–230	0.410	0.101	0.246	0.410	0.034	0.084
230–240	0.310	0.061	0.196	0.315	0.052	0.166
240–250	0.632	0.063	0.099	1.351	0.166	0.123
250–260	0.700	0.038	0.054	1.117	0.040	0.036
260–270	1.051	0.016	0.015	1.615	0.015	0.009
270–280	3.699	0.033	0.009	2.589	0.028	0.011
280–290	9.996	0.000	0.000	1.234	0.001	0.001
290–300	3.102	0.003	0.001	4.056	0.008	0.002
300–310	1.511	0.005	0.003	4.250	0.004	0.001
310–320	0.721	0.008	0.011	3.333	0.003	0.001
320–330	0.392	0.015	0.038	1.108	0.001	0.001
330–340	0.310	0.020	0.063	3.964	0.000	0.000
340–350	0.298	0.030	0.102	4.167	0.000	0.000
350–360	0.316	0.049	0.154	6.095	0.000	0.000
360–370	0.341	0.031	0.091	11.245	0.000	0.000
370–380	0.362	0.024	0.065	14.560	0.000	0.000
380–390	0.430	0.010	0.023	43.753	0.000	0.000
390–400	0.689	0.010	0.014	75.147	0.000	0.000
400–410	0.988	0.003	0.003	109.654	0.000	0.000
410–420	2.546	0.000	0.000	122.224	0.000	0.000
Full spectrum						
200–420	0.898	0.318	0.354	0.771	0.196	0.254
Combination of 10 nm intervals with higher SNB/PRESS values						
220–230						
230–240	0.524	0.226	0.431	0.402	0.141	0.351
350–360						
Combination of intervals obtained by mid eight cut-off						
218–243						
224–349	0.256	0.265	1.035	0.371	0.302	0.824
338–370						

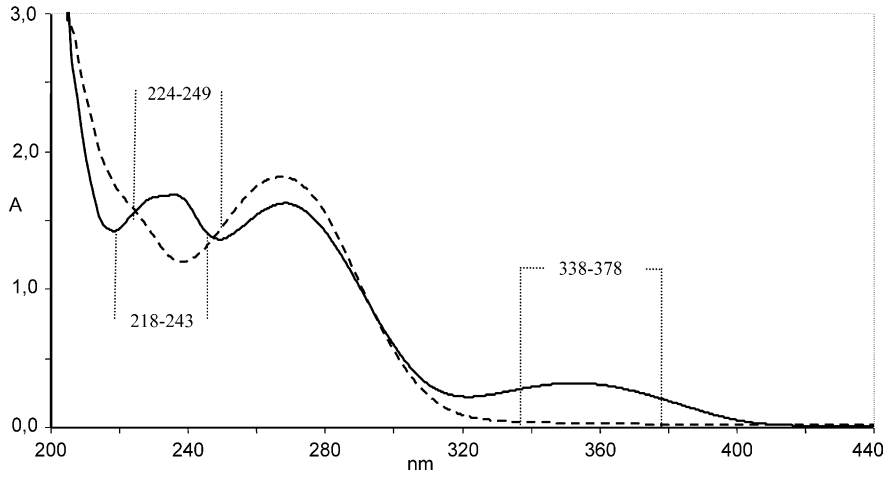

Fig. 1. UV absorption spectra of MND (solid line) and d-MND (broken line).

under the various exposure conditions. MND raw material was distributed in a thin layer, and analyzed at various intervals during exposure. For this aim, 10 mg of powder, accurately weighed, were diluted with ethanol in order to obtaining a concentration of about 20 μg ml^{-1}.

The photostability of the pharmaceutical forms was performed by exposing ten tablets to both natural and artificial lights and analyzed at different times, as reported in 'Sample solutions'. The procedure was replicated on tablets protected by packaging materials.

3. Results and discussion

Absorption curves of oxidized by-products of 1,4-dihydropyridines result completely hided by those of the unchanged drugs when registered by customary UV spectra [7,8]. This feature renders the methodology useless in order to satisfactorily quantify little contents of the photoproduct in the presence of larger amounts of the parent drug. Chemometric multivariate calibration methods appeared to be ideal in order to overcome such a drawback. Accordingly, a CLS method has been

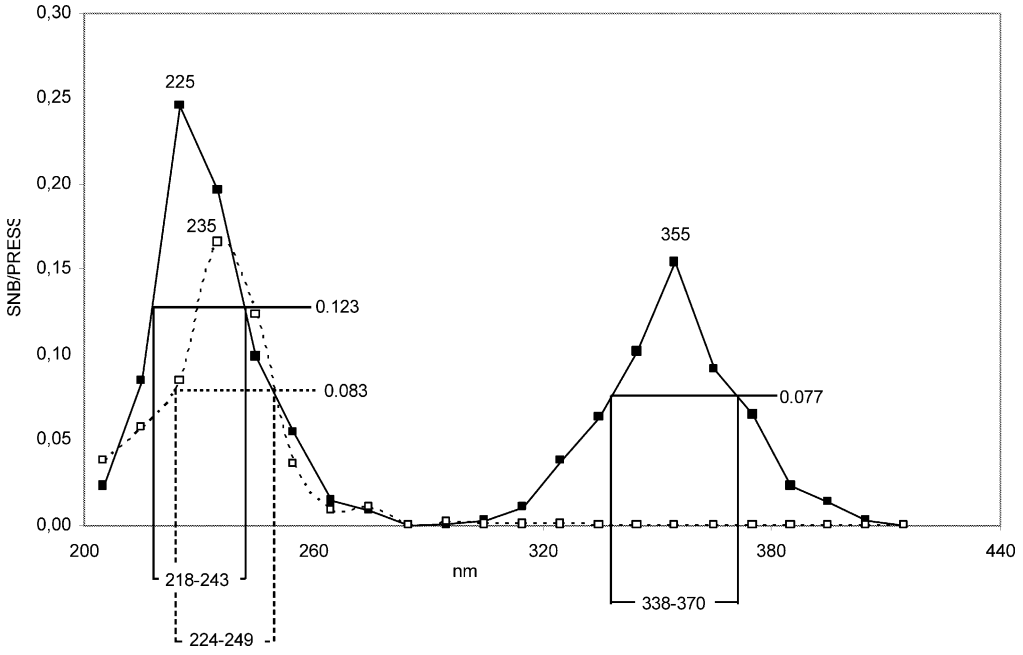

Fig. 2. SNB/PRESS values calculated from calibration on 10 nm wavelength fractions for MND (solid line) and d-MND (broken line).

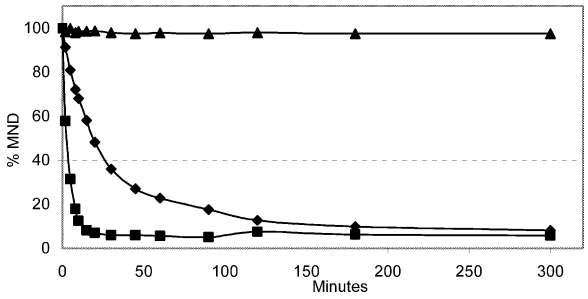

Fig. 3. Photodegradation plots of MND ethanol solutions under laboratory light (▲), sunlight (◆), and Xenon lamp (■).

applied herein for the analysis of dosage forms containing MND and its by-product.

Model calibration was set up using a training set of fifteen binary mixture solutions, selected according to a four level experimental design, and considering the spectral data of the recorded full wavelength range. The composition of these mixtures is reported in Table 1. Nevertheless, the application of such a calibration model to the prediction set gave rise to unsatisfactory results. It is widely accepted that the predictive ability of a model generally might improve by considering only selected wavelength ranges, bearing useful information, and discarding those ones characterized by confusing information and noise [14–16]. The optimization of the multivariate procedure was therefore achieved by a careful selection of the spectral zones to be employed in the calibration model.

Therefore, the spectrum range recorded between 200 and 420 nm was divided into 22 fragments of 10 nm each. Statistical parameters were drawn from the corresponding number of calibration models calculated for each fragment. Since the ratio of SNB vs. PRESS increases proportionally with the model quality, it was taken into consideration as a selection criterion for optimizing the model itself. On the other hand, selectivity was ignored as a not necessary parameter when only two compounds are to be analyzed.

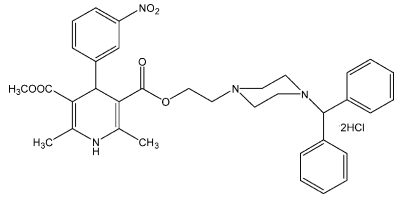

Fig. 4. MND chemical structure

SNB and PRESS ratios are listed in Table 2 and plotted in a graph in Fig. 2. Values relative to the full-range spectrum are reported as well. The highest values resulted to be localized in two separate intervals, centered at 234 and 364 nm, for MND, and a single interval at 225 nm for d-MND. The excellent feature in the 364 nm region can be due to a peculiar absorbance band of MND, not affected by d-MND. The high differentiation between the spectral outlines in the range 220–250 nm led to high values of SNB/PRESS for the two components. On the other hand, the absorbance peaks shown by both components at 269 nm did not give any good information, as they are completely overlapped, so as resulting undistinguishable.

In a first attempt, three intervals at 220–230, 230–240 and 360–370 nm, showing the highest values of SNB/PRESS, have been considered for the calibration model. CLS parameters resulted scantily improved with respect to those deducted by use of the full-range spectrum (see Table 2). Similarly, inaccurate results were estimated when the model was applied to the prediction set.

SNB/PRESS peaks (see Fig. 2) were then cut to various heights in order to single out the most suitable wavelength intervals under every single peak. Subsets so obtained were used to carry out the corresponding number of calibration models and the relative SNB/PRESS values were collected.

The maximum SNB/PRESS option led to best results when wavelength intervals within peaks defined by their mid height were considered. The ranges singled out were the following: 218–243, 224–249 and 338–370 nm (see

Table 3
Determination of MND and photodegradation product d-MND in synthetic mixtures and commercial formulations. Values of laboratory solutions are expressed as μg ml^{-1}

Sample	Nominal		Found					
	MND	d-MND	MND	Recovery	RSD	d-MND	Recovery	RSD
Laboratory solutions (μg ml^{-1})								
1	8.04	1.24	8.24	102.49	2.56	1.20	96.77	3.77
2	12.24	4.16	12.12	99.02	2.20	4.24	101.92	2.84
3	16.32	0.32	15.96	97.79	1.88	0.32	100.00	5.23
4	20.06	0.32	19.96	99.50	1.75	0.30	93.75	4.82
5	24.32	0.24	24.02	98.77	2.42	0.23	95.83	6.20
Pharmaceuticals (mg)								
Iperten 20®	20.00	–	19.56	97.80	1.32	0.24	–	5.30
Vascoman®	20.00	–	19.60	98.00	2.00	0.16	–	7.46

Figs. 1 and 2). Final calibration was then performed by the simultaneous use of all three ranges, since the software allows the use of overlapping wavelengths, emphasizing wavelengths bearing good information. A noteworthy improvement in all the calibration parameters was so achieved and reported in Table 2.

4. Photostability

The proposed method has been applied to study the effects of natural and artificial light on raw material and drug dosage forms, either protected or not by packaging materials.

The graph in Fig. 3 reports the plotting of photochemical decomposition of an ethanolic solution of the drug when exposed to natural and artificial light. It is evident the high photodecomposition rate under natural light and Xenon lamp, with a half degradation amount after just 21 and 3 min, respectively.

The content of d-MND in raw material was found to be approximately 38 and 22% after 5 h, under daylight and Xenon light, respectively. The pharmaceutical forms resulted sufficiently stable, with a decrease of MND title of 10% after 44 h of Xenon lamp exposure for tablets. On the contrary, the pharmaceutics resulted well protected from photodegradation when enwrapped with packaging materials Fig. 4.

5. Validation

The method was validated by means of predictions inferred from the analysis of laboratory binary mixtures containing different amounts of drug and photoproduct. The mean recovery of the method was calculated to lie between 99.51 ± 2.16 and 97.65 ± 4.57 for MND and photoproduct, respectively. A t-test on data obtained from laboratory mixtures was performed in order to verify whether the difference between the true value and the experimental mean was significant. The calculated t in all cases resulted to be lower than a critical value ($t = 2.57$; d.f. 5) at the significance level of 0.05, so that the 'null hypothesis' was confirmed. The results of five replicate determinations on several samples are shown in Table 3.

The absorption of too low d-MND content shows about the same intensity of the spectral baseline noise, so leading to wrong results. The limit of quantitation for d-MND was experimentally calculated as the lowest concentration showing a mean error lower than 14% (equivalent to almost three times the standard deviation value). Accordingly, series of standard binary solutions with a MND concentration of 20.00 μg ml^{-1} and a d-MND concentration variable between 0.10 and 1.00 μg ml^{-1} were analyzed. The quantitation limit resulted to range anyway from 0.20 to 0.25 μg ml^{-1}, equivalent to an impurity level of almost 1.0%.

Several MND commercial formulations were analogously subjected to analysis by means of the proposed procedure. When applied to pharmaceutics, the method required only a simple and rapid sample preparation which was demonstrated to avoid any detectable photodegradation during the entire manipulation process. The recorded results were in agreement with the content of drug declared on confection labels. The photodegradation product content of assayed pharmaceutical specialities was found anyhow to be lower than 1.2%.

Acknowledgements

This research was supported by grants from M.I.U.R (Italy).

References

[1] Y. Shibouta, T. Kitayodhi, G. Titoh, K. Nishikawa, Calcium channel blocking of manidipine hydrochloride (CV-4093 2HCl) in vitro and in vivo, Jpn J. Pharmacol. 48 (1988) 463–472.
[2] K. Arakawa, Y. Kaneko, O. Iimura, Efficacy and safety of manidipine hydrochloride in patients with essential hypertension: a dose-finding study in monotherapy and combined therapy with thiazide diuretics or β-blocking agents, Jpn Pharmacol. Ther. 17 (1989) 2681–2712.
[3] A. Nagaoka, Pharmacologic characteristics of a new calcium-antagonist, manidipine: beneficial effects on renal circulation and vascular changes, Am. Heart J. 125 (1993) 560–565.
[4] M.I. Hase, M.O. Hase, S. Igawa, Y. Takayama, T. Ootake, F. Sugimura, Reduction of left ventricular mass by manidipine hydrochloride, Am. J. Hypertens. 11 (1998) 81A.
[5] J.A. Squella, A. Zanocco, S. Perna, L.J. Nuñez-Vergara, A polarographic study of the photodegradation of nitrendipine, J. Pharm. Biom. Anal. 8 (1990) 43–47.
[6] W. Shulan, M. Wenshi, W. Xiang, Determination and stability of nimodipine injection by HPLC, Yaowu Fenxi Zazhi 11 (1991) 81–84.
[7] G. Ragno, C. Vetuschi, UV derivative spectrophotometric assay of nicardipine and its photodegradation product, Die Pharmazie 53 (1998) 628–631.
[8] G. Ragno, A. Garofalo, C. Vetuschi, Photodegradation monitoring of Amlodipine by UV derivative spectrophotometry, J. Pharm. Biom. Anal. 27 (2001) 19–24.
[9] T. Ohkubo, T. Uno, K. Sugawara, Liquid chromatographic determination of manidipine in serum with electrochemical detection, J. Chromatogr. 687 (1996) 413–418.
[10] D.M. Haaland, E.V. Thomas, Partial least squares methods for spectral analyses. Relation to other quantitative calibration methods and the extraction of qualitative information, Anal. Chem. 60 (1988) 1193–1202.
[11] I. Duran-Meras, M. de la Pena, A. Espinosa-Mansilla, F. Salinas, Multicomponent determination of flavour enhancers in food preparations by partial least squares and principal component regression modeling of spectrophotometric data, Analyst 118 (1993) 807–813.
[12] K.N. Andrew, P.J. Worsfold, Comparison of multivariate calibration techniques for the quantification of model process streams

using diode-array spectrophotometry, Analyst 119 (1998) 1541–1546.
[13] ICH Harmonized Tripartite Guideline 'Stability Testing: Photostability Testing of New Drug Substance and Product', draft no. 4, 13, 6.10.1996.
[14] H. Martens Harald, Improved selectivity in spectroscopy by multivariate calibration, J. Chemom. 1 (1987) 201–219.
[15] J.C. Da Silva, M.C. Laquipai, Method for rapid screening of chlorophenols using a reduced calibration set of UV spectra and multivariate calibration techniques, Anal. Lett. 31 (1998) 2549–2563.
[16] C. Vetuschi, G. Ragno, Improvement of multivariate UV analysis by selecting wavelengths and instrumental parameters, Anal. Lett. 35 (2002) 559–575.