Orbital

The Electronic Journal of Chemistry

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

Orbital 2009, *1* (1), 64-74

Full Paper

High-performance liquid chromatographic method for the determination of nicardipine in pure, pharmaceutical preparations and plasma and its application to pharmacokinetics in humans

Sheikha M. Al-Ghannam, Abeer M. Al-Olyan*

King Faisal University - Girls College of Science, Department of Chemistry, P.O.Box 838, Dammam-31113, Saudi Arabia

Received: 22 October 2008; revised: 25 January 2009; accepted: 29 January 2009. Available online: 07 March 2009.

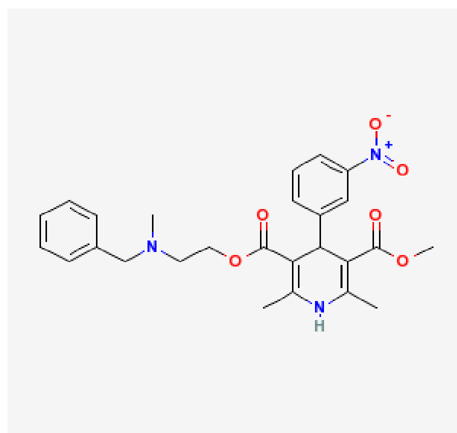
ABSTRACT: *A simple, sensitive and reproducible reversed-phase liquid chromatographic method has been developed and validated for the determination of nicardipine hydrochloride (NC) in pure, pharmaceutical preparations, human plasma and the study of the pharmacokinetics of the drug in human body. Nicardipine in plasma were extracted with hexane-butanol (12:1,v/v) after addition of borate buffer (0.5 M, pH=9.0), and then measured by HPLC-UV using a Waters Symmetry C_{18} column as stationary phase and methanol– triethylamine buffer (0.01M) pH 4 with acetic acid (70:30) as mobile phase. Nicardipine was quantified by ultraviolet absorbance at 353 nm. The method proved to be linear in the pure drug in the ranges of 15-200 ng/mL (r=0.9989) and 5-40 µg/mL (r=0.9995), and for the pharmaceutical preparations and plasma for drug concentrations in the range of 5-40 µg/mL (r =0.9992) and 25-150 ng/mL (r=0.9991), respectively. The lower limit of detection and the lower quantitation limit of NC in plasma were 11.74 and 35.57 ng/mL, respectively. The method is sensitive and reliable for pharmacokinetic studies of nicardipine in humans after the oral administration of immediate-release capsules to healthy subjects.*

Keywords*: HPLC; nicardipine hydrochloride; human plasma.*

*Corresponding author: E-mail: sm_ghannam@yahoo.com

Introduction

Nicardipine hydrochloride, 2-(N-benzyl-N-methylamino)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride, is a calcium antagonist with highly potent vasodilating activity and has been widely used for the treatment of hypertension and cerebrovascular disease[1].



Nicardipine

Although nicardipine is rapidly and completely absorbed from the gastro-intestinal tract after oral administration to humans and laboratory animals, its plasma concentrations are relatively low due to extensive first-pass metabolism in the liver [1, 2]. Thus, a sensitive and specific method for the determination of plasma nicardipine concentrations is required to examine the pharmacokinetics and the relationship between plasma concentrations and pharmacological effects.

Several methods have been reported for the determination of NC in the plasma of human and laboratory animals, including spectrophotometric [3], spectrofluorometric methods [4] gas chromatography GC [5] and voltammetry [6]. Nevertheless, some procedures are rather cumbersome and involve specialized, expensive equipment usually not available in clinical setting, which limits their applications for the analysis of either single patient samples or large numbers of samples resulting from clinical studies.

In recent years, high-performance liquid chromatography HPLC either with ultraviolet [7-11] or electrochemical detection [12, 13] has overcome some of these difficulties and has been extensively used to determine NC in biological samples. This report describes a simple, rapid and sensitive HPLC procedure for the determination of NC in pure, pharmaceutical preparations and plasma by using ultraviolet detection. In this paper, we reported a way of measuring the concentration of NC in pure and pharmaceutical preparations using a HPLC-UV method, with a RP- C_{18} column with low

levels comparing with the other methods. It has been used to determine NC in human plasma samples from a healthy volunteer who had taken NC capsule and provided data on the pharmacokinetics of the drug.

Material and Methods

Apparatus

Experiments were carried out in a Waters 2695 Liquid Chromatograph with autosampler and PDA Waters 2990 Diode array UV/VIS variable wavelength detector (Waters, Milford, USA). The system is twice a year operationally qualified using its software built-in procedures. Chromatographic separations were accomplished using a Waters Symmetry C_{18} 3.9 mm x 150 mm, 5 µm stainless steel column (Waters, Ireland).

Reagents and materials

Nicardipine hydrochloride was kindly supplied by Sigma Chemicals (St. Louis, USA, Batch# 16H0190). Acetonitrile, methanol, ethanol, n-butanol and hexane were of HPLC-grade (Merck, Germany and BDH, UK). All other chemicals and reagents were of the highest analytical grade available.

Mobile phase

The mobile phase consisted of methanol/TEA buffer (0.01M) pH 4 with acetic acid (70:30 v/v). The mobile phase was pumped isocratically at a flow rate of 0.25 mL/min during analysis, at ambient temperature.

Preparation of standards solutions, calibration standards and quality control samples

Nicardipine was prepared by dissolving 100 mg of NC in 100 mL of methanol and kept in a refrigerator. Since NC is light sensitive; almost all experiments were carried out in a darkroom in order to avoid photodecomposition. When all this photo protection was impossible to achieve, samples were protected from light by wrapping the vials with aluminum foil. The extracting solvent consisted of hexane and butanol (12:1 v/v).

Blank plasma was spiked with working solutions of the drugs to achieve the following calibration standard concentrations: 25, 75, 100, 150 and 200 ng/mL. The concentration ranges were selected based on reported analysis of NC [9]. Plasma quality control samples (QCs) which were run in each assay were prepared in the same way, the concentrations of QCs were 25, 75 and 150 ng/mL for NC.

Analytical Procedure

Application of the proposed method to the analysis of pure nicardipine.

Into a series of 10 mL measuring flasks, accurately measured aliquots of standard NC solution in the concentration range of (0.005-40 µg/mL) and completed to volume with the mobile phase. All samples were filtered through 0.45-µm sample filters (RC 25, Sartorius AG, Germany) prior to injection into HPLC system. 20 µL aliquots were injected and eluted with the mobile phase under the reported chromatographic conditions. The calibration curve was constructed by plotting the peak height against the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equation was derived.

Application of the proposed method to the analysis of nicardipine in human plasma.

An aliquot of 1.5 mL of plasma was added to a chemically clean screw-capped glass tube. A 500 µL of borate buffer (0.5 M, pH = 9.0) was added and the solution was mixed well. A 5.0 mL volume of hexane-butanol (12:1 v/v) was poured in the tube as an extracting solvent and vortex-mixed for 1 min and centrifuged for 10 min at 1000 rpm. A 4.0 mL sample of the organic layer was collected and evaporated until dry with nitrogen at room temperature, and then 100 µL of methanol was added to dissolve the residue. After 20 s of vortex mixing, 10 µL of the sample solution was injected into HPLC system under the above chromatographic conditions. The procedure was followed as described under section *Application of the proposed method to the analysis of pure nicardipine*. The nominal content of the drug in plasma was determined using the corresponding regression equation.

Application of the proposed method to the analysis of nicardipine in its capsules.

The contents of ten capsules were emptied as completely as possible, and mixed well. An accurately weighed quantity of the powder equivalent to 5 mg of NC was transferred into small conical flask, extracted with 50 mL of methanol and sonicated for 30 min. The extract was filtered into a 100 mL volumetric flask and completed to volume with methanol. Into a series of 10 mL measuring flasks, accurately measured aliquots of extracted NC solution in the concentration range of (5-40 µg/mL) and completed to volume with the mobile phase. All samples were filtered through 0.45-µm sample filters (RC 25, Sartorius AG, Germany) prior to injection into HPLC system under the above chromatographic conditions. The procedure was followed as described under section *Application of the proposed method to the analysis of pure nicardipine*. The nominal contents of the capsules were calculated using either the calibration graph or the corresponding regression equation.

Validation procedure

The validation parameters were selectivity, extraction recovery, precision and

accuracy. Six batches of blank heparinized human plasma were screened to determine the specificity. The extraction recovery of NC was calculated by comparing the peak area measured for the standard solution considering condensation with that obtained for plasma extracts after the extraction procedure. The precision and accuracy of the assay validation were estimated using the inverse prediction of the concentration of the calibration curve.

Clinical application

A healthy subject who gave written informed consent took part in this study. This study was approved by King Fahad Military Medical Complex (Dhahran). After an overnight fast, the subject was given a single 50 mg oral dose on NC. Blood samples (5 mL) were taken before and 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 9.0 and 12.0 h after drug administration. The plasma was separated by centrifugation at 1000 rpm for 10 min, and stored at -20 ^{o}C until analysis.

Pharmacokinetic analysis

The pharmacokinetic analysis was performed using noncompartmental methods. The area under the plasma concentration-versus—time curve (AUC) was calculated using the trapezoidal rule and extrapolated to infinity. The time course of the plasma NC concentration was used to determine the maximum plasma concentration (C_{max}) and the time (T_{max}) to reach C_{max}. The elimination rate constant (k_{el}) was obtained by the linear regression of the terminal phase and the calculated elimination half-life ($t_{½}$) was $0.693/k_{el}$.

Results and Discussion

Optimization of the chromatographic method

Since the pharmacological activity of nicardipine seems to correlate best with its plasma concentration, a method that reliably measure levels of NC is highly desirable. The separation of the analytes, peak shapes and retention times were optimized by modifying the mobile phase constitution and the flow rate until attaining a good resolution in a relatively short run time for each injection (9.26 min).

The effect of the composition of mobile phase on the chromatographic separation was firstly investigated. The results showed that NC retention time was obviously prolonged with increasing the methanol content or decreasing the buffer content. However, the responses of peak height changed only slightly with varying composition of the mobile phase. The presence of methanol was found to be necessary to ensure a good

separation between NC and plasma interfering peaks. Further, the experimental results showed that the addition of triethylamine in the mobile phase increased the selectivity and sensitivity of NC, because it changed the retention time and sharpened the drug peak. To maintain the quality of the separation, it was also necessary to adjust the pH of the mobile phase. As NC is a basic drug, at low values of pH, NC eluted at shorter time because the high degree of protonation of its tertiary amine function hinders the interactions with the lipophilic stationary phase. An optimal pH of 4.0 was chosen for selecting a proper retention time value that produced no interfering peaks near the specific peak of NC and because is at least 1 pH unit different from the NC pKa (pKa = 7.2), which shifts the equilibrium so that 99% of NC will be in one form. Analytes may sometimes appear as broad or tailing peaks when the mobile phase pH is at, or near, their pKa values.

The separation of the analytes, peak shapes and retention times were optimized by modifying the mobile phase constitution and the flow rate until attaining a good resolution in a relatively short run time for each injection.

From these preliminary studies, we decided to use a mixture of methanol–triethylamine buffer (0.01 M) pH 4 with acetic acid (70:30) as mobile phase at a flow rate of 0.25 mL/min.

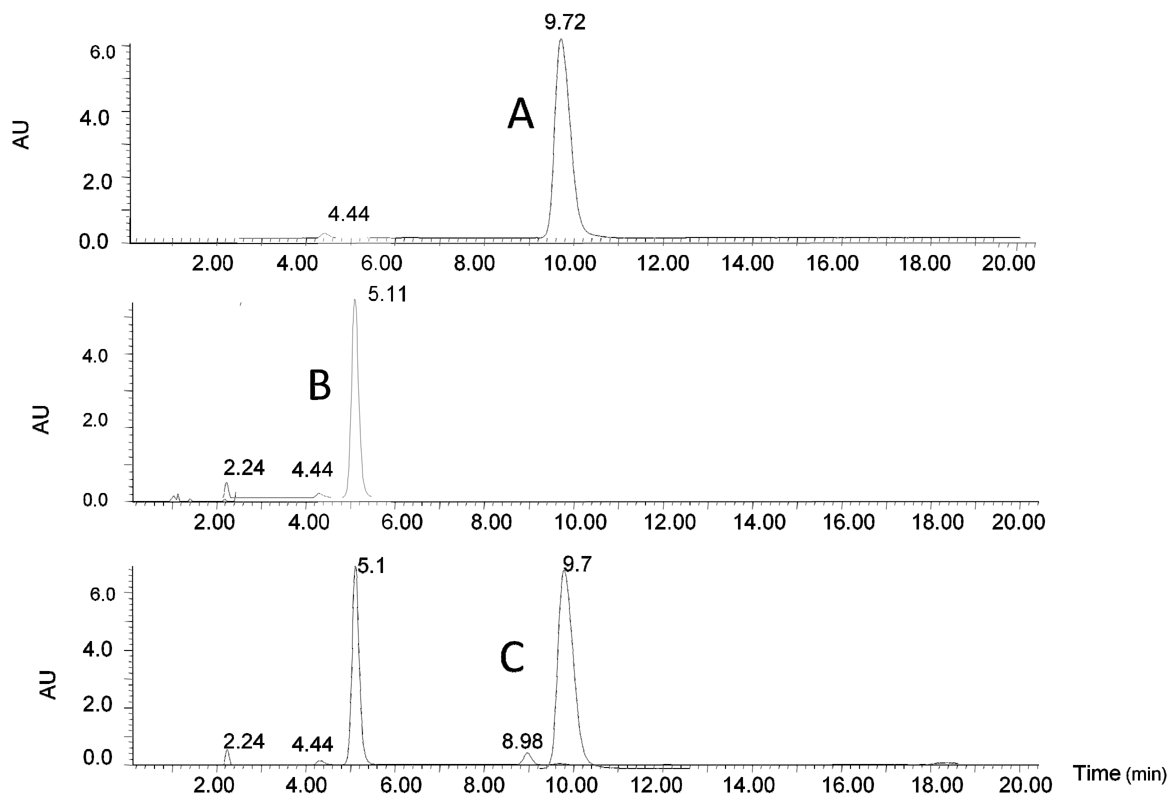


Figure 1. Chromatograms of (A) pure nicardipine (40 µg/mL) t_R = 9.7 min (B) blank plasma (C) after oral administration of nicadipine (210 ng/mL).

Figure 1 shows typical chromatograms for pure nicardipine, blank human plasma and plasma after oral administration of NC. Moreover, Figure 2 shows the chromatogram for NC in its capsules. It can be seen from Figure 1 or 2 that good separation and dectability of NC in human plasma or pharmaceutical preparations were obtained with minimal interference from plasma components or the common excipients present in the capsules. Under the chromatographic conditions described above, the observed mean retention time for NC is approximately 9.0 min. It can be concluded that the proposed method is selective for NC.

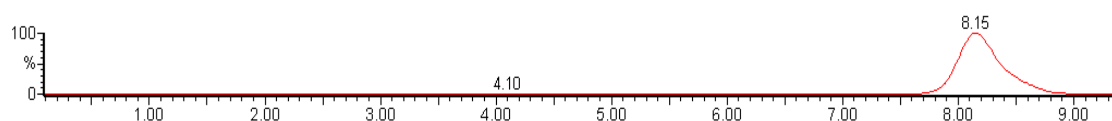


Figure 2. HPLC chromatogram of Nicardipine HCl capsules (25 μg/mL) in methanolic solution with the mobile phase consisting of methanol/TEA buffer (0.01M) 60:40 (v/v) injection volume 10 μL, Flow rate 0.250 mL/min., Detection at 353 nm.

Validation of the method

Assay linearity was evaluated by calibration curves of NC peak-height vs. NC concentration in pure drug ranging from 15-200 ng/mL and 5-40 μg/mL. Using linear regression analysis, an excellent linear relationship between peak-height and NC concentrations was exhibited for concentrations 15-200 ng/mL, y = 0.1636 C +12.505 (r = 0.9989) and for concentrations 5-40 μg/mL, y = 0.6062 C + 0.4046 (r = 0.9995), where *y* refers to the peak height and C corresponds to the concentration of NC. The linear equations for the pharmaceutical preparation and plasma for drug concentrations in the range of 5-40 μg/mL, y = 0.7453 C + 0.5295 (r = 0.9992) and 25-150 ng/mL, y = 0.0713C + 13.699 (r = 0.9991), respectively, which proved excellent linearity in those concentration ranges.

The detection limits (LOD) for the proposed methods were calculated using the following equation [15]:

$$LOD = \frac{3s}{k}$$

Where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits was found to be 11.74 ng/mL for NC in plasma.

The limits of quantitation, LOQ, defined as [15]:

$$LOQ = \frac{10s}{k}$$

According to this equation, the detection limit was 11.47 ng/mL and the limit of quantitation was found to be 35.57 ng/mL for NC in plasma Table 1.

The statistical parameters given by the regression equation were calculated from the calibration graphs, along with the standard deviations of the slope (S_b), the intercept (S_a) on the ordinate and the standard deviation residuals ($S_{y/x}$) and are shown on Table 1.

The accuracy and precision (intra-day precision or repeatability and inter-day or intermediate precision) of this method, evaluated by assaying three spiked plasma samples containing different concentrations of NC, are summarized in Table 2. The differences of the mean value measured from the concentration prepared, expressed in percentages, were only –0.13, -0.22 and –3.88% at 25, 75, 150 ng/mL, which confirmed the accuracy of the method. The range of percentage of relative standard deviation RSD was from 1.9-3.6 % for intra-day analyses and from 2.7-3.7 % for inter-day analyses. The RSD values obtained allow us to conclude that the method has acceptable precision.

Table 1. Optimal and regression characteristics of the proposed method for nicardipine in plasma.

Parameters \ **Concentration**	(25 – 150 ng/mL)
Linear regression equation	A = 0.0713C + 13.699
Correlation coefficient (r)	r = 0.9991
detection limit (ng/mL)	11.74
Quantitation limit (ng/mL)	35.57
Standard deviation of the residual ($S_{y/x}$)	0.1882
Standard deviation of the intercept (S_a)	0.2536
Standard deviation of the slope (S_b)	0.002115

Table 2. Accuracy and precision of the method for the determination of NC in human plasma, expressed as bias (%) and relative standard deviation (RSD), respectively (n=3).

Concentration (ng/mL)	Accuracy Bias (%)	Intra-day precision RSD(%)	Inter-day precision RSD(%)
25	- 0.13	3.6	2.7
75	-0.22	1.9	3.7
150	-3.88	2.7	3.5

To establish the extraction recovery, drug-free plasma was spiked with 25, 75, 150 ng/mL nicardipine. The peak heights of the nicardipine extracted were compared with the peak height of nicardipine at same concentrations in mobile phase injected directly into the HPLC. The extraction recoveries of the low, medium and high concentration were 98.86%, 106.27% and 98.50% for NC.

Results for the determination of NC in pure forms and its capsules were compared with the results obtained by the official method [14] in Table 3. The calculated student's t-test and variance ratio F-test [15] indicate that the proposed method and the official method are equally accurate and precise.

Application to pharmacokinetic studies in human blood

Figure 3 illustrates the profile of plasma concentration versus time for nicardipine in a volunteer. Peak concentration in plasma occurred T_{max} = 1.5 h after ingestion and the peak concentration achieved C_{max} was 210 ng/mL. NC presented a short elimination half-life (2.9 h) with k_{el} (0.26 h^{-1}). These parameters were in accordance with those reported in the literatures [7-11].

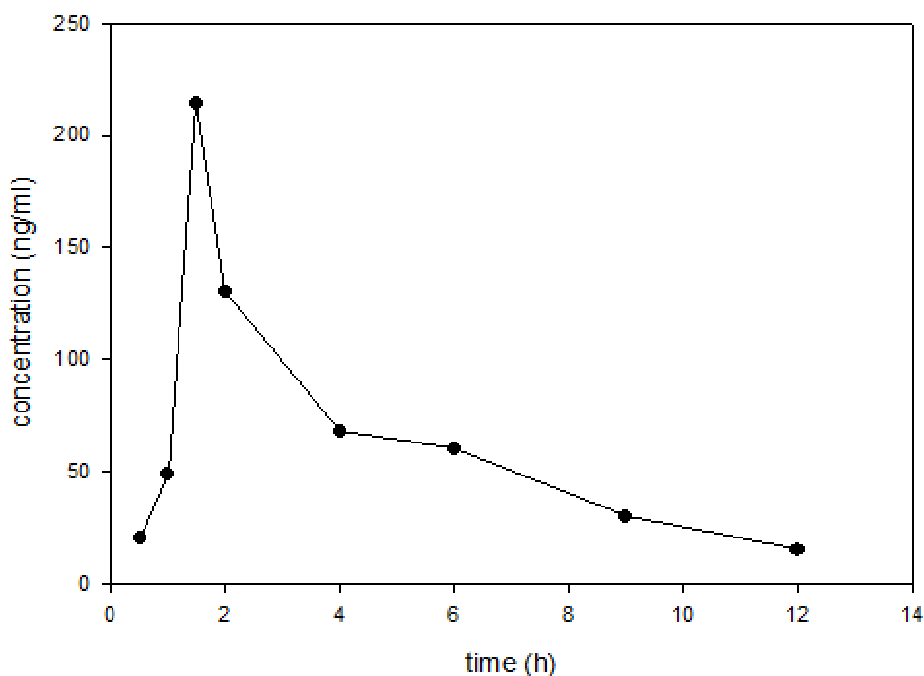


Figure 3. Plasma concentration-time curve after oral administration of 50 mg of nicardipine.

Table 3. Application of the proposed HPLC method to the determination of NC in pure form and its commercial capsules.

Amount taken (µg/mL)	Pure NC	NC Capsule*	Official method[12]
	% Recovery#	% Recovery#	
1.50×10^{-3}	97.30	-	
7.50×10^{-2}	99.71	-	
1.50×10^{-1}	97.41	-	
2.00×10^{-1}	101.47	-	
5	99.11	101.72	
10	99.86	97.49	
15	99.45	103.87	
20	100.08	97.93	
25	100.50	99.84	
30	100.40	100.31	
35	101.24	98.84	
40	98.73	100.88	
mean	99.61	100.11	100.64% (n=5)
SD	±1.32	±2.26	± 1.08
F	1.49(5.96)	4.38(6.09)	
t	1.22(2.14)	1.49(2.23)	

The figures in parenthesis are the theoretical values of t and F values at 95% Confidence Limit.
*Capsules are product of Pelcard capsules: labeled to contain 50 mg/capsule (GNP, Cairo, Egypt).
The average of three trials.

Conclusion

The present method was sensitive enough to detect low concentrations and the standard curve was linear up to at least 15 ng/mL. The method proved to be, according

to the validation parameters, selective, precise and accurate and also simple, useful and appropriate for studying the pharmacokinetic profile of NC administered to human plasma.

Acknowledgements

This work was supported by Poison Center – Dammam Central Hospital, Dammam, Saudi Arabia and Research and Development Center – Saudi Aramco, Dhahran, Saudi Arabia.

References and Notes

[1] Sorkin, E. M.; Clissold, S. P. *Drugs* **1987**, *33*, 296.
[2] Higuchi, S.; Shiobara,Y. *Xenobiotica* **1980**, *10*, 447.
[3] Escrig-Tena, I.; Alvarez Rodríguez, L.; Esteve-Romero, J.; García-Alvarez-Coque, M. C. *Talanta* **1998**, *47*, 43.
[4] Al-Ghannam, S. M;. Al-Olyan, A. M. *Cent. Eur. J. Chem*. **2008**, *6*, 222.
[5] Rosseel, M. T.; Lefebvre, R. A. *J. Chromatogr. A* **1994**, *668*, 475.
[6] Obendorf, D.; Stubauer, G. *J. Pharm. Biomed. Anal.* **1995**, *13*, 1339.
[7] Uno, T.; Ohkubo, T.; Sugawara, K. *J. Chromatogr. Biomed. Appl.* **1997**, *698*, 181.
[8] Meng, Q. C.; Cheung, A. T.; Guvakov, D.; Weiss, S. J.; Savino, J. S.; Salgo, I. S.; Marshall, B. E. *J. Chromatogr. Biomed. Appl.* **1998**, *718*, 121.
[9] Fernandes, C. M.; Veiga, F. J. B. *Biomed. Chromatogr*. **2003**, *17*, 33.
[10] Eastwood, R. J.; Galustian, C.; Bhamra, R. K.; Holt, D. W. *J. Chromatogr. Biomed. Appl.* **1990**, *530*, 463.
[11] Kobayashi, S. *J. Chromatogr. Biomed. Appl.***1987**, *420*, 439.
[12] López, J. A.; Martínez, V.; Alonso, R. M.; Jiménez, R. M. *J. Chromatogr. A* **2000**, *879*, 105.
[13] Martínez, V.; López, J. A.; Alonso, R. M.; Jiménez, R. M. *J. Chromatogr., A* **1999**, *836*, 189.
[14] The British Pharmacopoeia, HMSO, The Pharmaceutical Press, London, **2004.**
[15] Miller, J. C.; Miller, J. N. Statistics and Chemometrics for Analytical Chemistry, 5th Ed., Prentice Hall, England, **2005**.