Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 71–80
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Evaluation of an International Pharmacopoeia method for the analysis of indinavir sulfate by liquid chromatography

R. Yekkala, H. Lei, J. Hoogmartens, E. Adams *

Laboratorium voor Farmaceutische Chemie en Analyse van Geneesmiddelen, Faculteit Farmaceutische Wetenschappen, Katholieke Universiteit Leuven, O & N 2, Postbus 923, Herestraat 49, B-3000 Leuven, Belgium

Received 24 October 2005; accepted 15 January 2006
Available online 28 February 2006

Abstract

A gradient LC method for the determination of indinavir sulfate (IDV) and its impurities has been recently published in a consultation document of the International Pharmacopoeia, WHO Drug Information. The method uses a base-deactivated reversed-phase C18 column (25 cm × 4.6 mm i.d.), 5 μm kept at a temperature of 40 °C. The mobile phases consist of acetonitrile, phosphate buffer pH 7.5 and water. The flow rate is 1.0 ml/min. UV detection is performed at 220 nm. A system suitability test (SST) is described to govern the quality of the separation. The separation towards IDV components was investigated on 16 C18 columns and correlation was made with the column classification system developed in our laboratory. The method was evaluated using a Hypersil BDS C18 column (25 cm × 4.6 mm i.d.), 5 μm. A central composite design was applied to examine the robustness of the method. The method shows good precision, linearity, sensitivity and robustness. Six commercial samples were examined using this method.

Keywords: Liquid chromatography; Purity testing; Indinavir sulfate; Validation; Column ranking system

1. Introduction

Indinavir sulfate (IDV) is a potent protease inhibitor of the human immunodeficiency virus (HIV) widely used in the treatment against the acquired immune deficiency syndrome (AIDS) and prescribed in combination with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors. IDV is synthesized via the penultimate intermediate by convergent coupling of an optically active mono-substituted epoxide (EPO) and an optically active piperazine-2-carboxamide (CAR), which is further alkylated with picolyl chloride in presence of sulfuric acid to give IDV [1,2] (Fig. 1). Therefore, EPO and CAR can be present as impurities in IDV samples. Indinavir lactone and *cis*-amino-2-indanol can be formed due to amide hydrolysis [2–5]. Several methods have been described for the analysis of IDV, such as potentiometry [2,6,7], thin layer chromatography [2,6], capillary electrophoresis (CE) [3,8–11], liquid chromatography (LC) with electrochemical detection [12], LC with UV detection [2,4,5,13–32] and with mass spectrometric detection [33–49]. Most of these methods were used to monitor IDV in blood. The method described by Aurora Prado et al. [3] allows the separation of IDV from its degradation impurities in capsules, using CE. A few LC methods were published for the assay and purity control of IDV formulations [2,4,5]. LC methods for the purity control of the IDV bulk drug have been published in the Indian Pharmacopoeia (IP) [50], the United States Pharmacopeia (USP) [51], Pharmeuropa [52] and a consultation document of the International Pharmacopoeia (Int. Ph.) [6]. An LC method for IDV capsules has also been published in the United States Pharmacopeial Forum (USPF) [53]. The LC method described by Silva et al. [4] was the basis of the assay methods of the USP and the USPF.

The purpose of this study was to evaluate the LC method described for purity control of IDV in the Int. Ph. monograph, not only for purity control but also for assay. This is useful in case the LC method has to be used to assay dosage forms. Selectivity, limit of detection, limit of quantitation, linearity, repeatability and intermediate precision were examined. Since no brand names are mentioned in the monograph, the suitability on a set of 16 similar columns towards the separation of IDV and

* Corresponding author. Tel.: +3216323443; fax: +3216323448.
E-mail address: erwin.adams@pharm.kuleuven.be (E. Adams).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.01.045

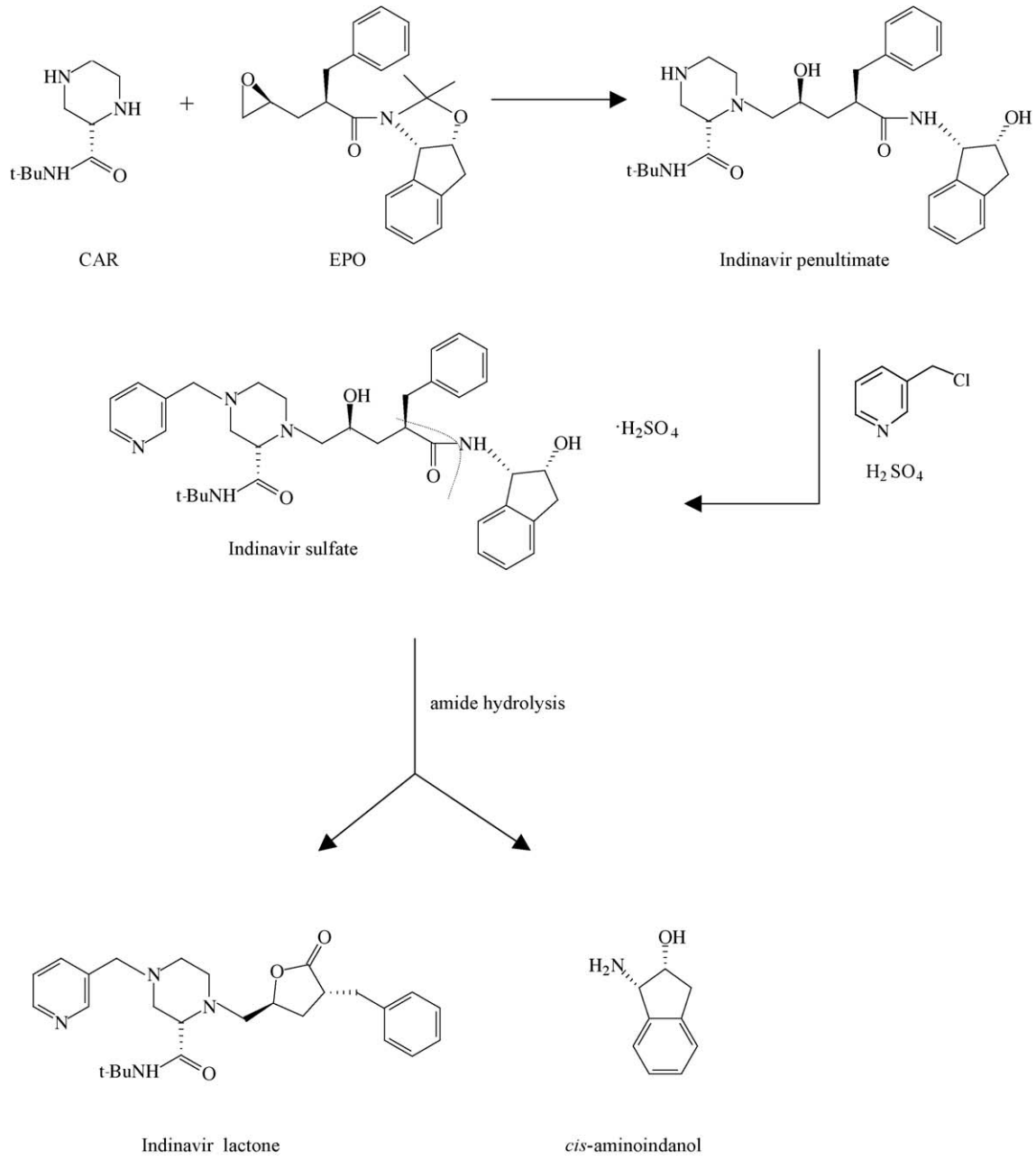

Fig. 1. Synthesis and hydrolysis of IDV.

its impurities was investigated and a correlation was made with the column classification system developed in our laboratory [54–61].

2. Experimental

2.1. Reagents and reference substances

HPLC-grade acetonitrile (ACN) was purchased from Acros Organics (Geel, Belgium), concentrated sulfuric acid from Fisher Scientific UK Limited (Leicester, UK), phosphoric acid from Riedel-de Haën (Seelze, Germany). Demineralized water was purified in our laboratory by filtering through an ultrapure Milli-Q (Millipore, Milford, MA, USA). Reference standards of indinavir sulfate (IDV) (92.4% on "as is" basis), carboxamide (CAR) and epoxide (EPO) were obtained from the WHO (Geneva, Switzerland). The IDV commercial samples examined were obtained from different companies.

2.2. Preparation of standard solutions

For purity control, IDV solutions were prepared at a concentration of 2.0 mg/ml (100%) and dilutions were made to obtain 2.0 μg/ml (0.1%). For assay, 0.4 mg/ml solutions of IDV reference standard and test solutions were prepared. For the investigation of the separation of IDV on a selected set of 16 reversed-phase C18 columns and the robustness study, a spiked sample was prepared by dissolving 100 mg

Table 1
Gradient program used for assay and purity control of IDV

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	
0–5	93	7	Isocratic
5–25	93–20	7–80	Linear gradient
25–30	20	80	Isocratic
30–35	20–93	80–7	Return to the initial conditions
35–45	93	7	Isocratic

of an IDV commercial sample, 0.5 mg of CAR and 0.2 mg of EPO in 50 ml of mobile phase A. Both commercial IDV and spiked IDV solutions slightly degraded within three days when they were kept at room temperature, but they could be used for several days when they were stored in the refrigerator. However, fresh solutions were prepared for quantification experiments.

2.3. Instrumentation and liquid chromatographic conditions

The LC apparatus (LaChrom, Merck Hitachi, Darmstadt, Germany) (equipment I) consisted of an L-7100 pump, an L-7200 autosampler, an L-7400 UV detector set at a wavelength of 220 nm and a D-7000 interface. EZChrome Elite 4.0 (Merck Hitachi) software was used for data acquisition. The column was kept in a water bath at 40 °C and the temperature was controlled using an EC Julabo thermostat (Seelbach, Germany). A Hypersil BDS C18 column (25 cm × 4.6 mm i.d.), 5 μm (Thermo Hypersil-Keystone, Cheshire, UK) was used. The flow rate was 1.0 ml/min. The injection volume was 20 μl.

For the intermediate precision study, analyses were carried out using a new Hypersil BDS C18 column (25 cm × 4.6 mm i.d.), 5 μm and using a different LC apparatus (LaChrom Elite, Merck Hitachi) (equipment II) consisting of an L-2130 pump, an L-2200 autosampler and an L-2400 UV detector. Other conditions were identical.

2.4. Mobile phase

The mobile phase consisted of ACN—sodium phosphate buffer pH 7.5—water (A; 30:5:65 v/v/v) and (B; 60:5:35 v/v/v). On standing and depending on the room temperature, buffer precipitation was observed in mobile phase B. Therefore, the buffer solution was prepared by dissolving 1.0 g (instead of 1.4 g) of anhydrous disodium hydrogen phosphate in 80 ml of purified water, adjusting the pH to 7.5 by adding phosphoric acid (105 g/l) and dilution to 100.0 ml with purified water. This change in the buffer content did not effect the quality of the separation. The gradient applied is shown in Table 1.

2.5. Selection of a set of 16 C18 columns

The monograph of IDV prescribes in the LC method for related substances a base deactivated reversed-phase C18 column (25 cm × 4.6 mm i.d.), 5 μm. This information is not always sufficient to select a column giving the required quality of separation although the chromatographic conditions given in the monographs may be adjusted to reach the SST. Therefore, it was decided to examine the separation on a set of 16 columns, available in our laboratory and which are at least either base-deactivated or end-capped (the latter were included to check their performance). The columns were chosen based on a column ranking system published in the literature [54–60] and which is also freely accessible on the website of our laboratory

Table 2
The list of C18 columns (25 cm × 4.6 mm i.d.), 5 μm examined and their characteristics provided by the manufacturers

Number	*F*-value	Name of the column	End-capped	Base-deactivated	Pore-size (Å)	Manufacturer/supplier
1	0.000	Hypersil BDS C18	+	+	130	ThermoQuest
2	0.436	ACE C18	+	+	100	Advanced Chrom. Tech./Achrom
3	0.480	Discovery C18	+	−	180	Supelco
4	0.667	Supelcosil LC-18 DB	−	+	120	Supelco
5	2.135	Nucleosil HD	+	−	100	Macherey-Nagel/Filter Service
6	2.303	Validated C18	+	−	100	Perkin-Elmer
7	2.813	Platinum C18	+	+	100	Alltech
8	3.030	Symmetry	+	−	100	Waters
9	3.940	Purospher	+	−	80	Merck
10	4.698	Kromasil EKA	+	−	100	Akzo Noble/SerCoLab
11	4.888	Purospher Star	+	+	80	Merck
12	5.456	Alltima C18	+	+	120	Alltech
13	7.162	Platinum EPS C18	−	+	100	Alltech
14	9.146	LiChrospher	−	+	100	Merck
15	10.477	Apex Basic	+	+	100	Jones Chromatography/Sopachem
16	26.256	Apex ODS II	+	−	100	Jones Chromatography/Sopachem

[61]. The ranking system is based on the determination of four chromatographic parameters. In this system, columns are ranked according to their *F*-values, calculated versus a reference column (in this case, a Hypersil BDS C18 was taken). The chromatographic parameters of the column with the highest *F*-value deviate most from these of the reference column. A list of columns examined in this study with their characteristics provided by the manufacturers and ranked by increasing *F*-values is shown in Table 2.

The SST solution, a commercial IDV sample and an IDV sample spiked with known amounts of CAR and EPO, were used to investigate the influence of the different stationary phases on the separation.

3. Results and discussion

3.1. Optimization of the system suitability test

In a LC method, a system suitability test (SST) solution may be proposed to check the quality of the separation. According to the Int. Ph., a SST solution was prepared by addition of 2.0 ml of sulfuric acid (190 g/l) to 2.0 ml of a 2.0 mg/ml IDV solution and heating in a water bath at 80 °C for 60 min. The resolution between the two major peaks (IDV and the so called system suitability test peak (SSTPK)), with retention times between 15 and 20 min should be not less than 3.5. In order to simplify the Int. Ph. method for the preparation of the SST solution, the same mixture of solutions was heated in boiling water for 10 min. With this modification, similar decomposition and resolution data were obtained. Chromatograms obtained under both SST conditions are shown in Fig. 2.

3.2. Column differentiation based on the SST

Some of the typical chromatograms are shown in Fig. 3. The SST results for all 16 columns are shown in Table 3. According to the Int. Ph. SST requirement, columns 7 and 15 have resolutions below 3.5 and should be considered as "not suitable" for the analysis. When the results are more closely examined, it can be observed that the SST criterion alone does not always give

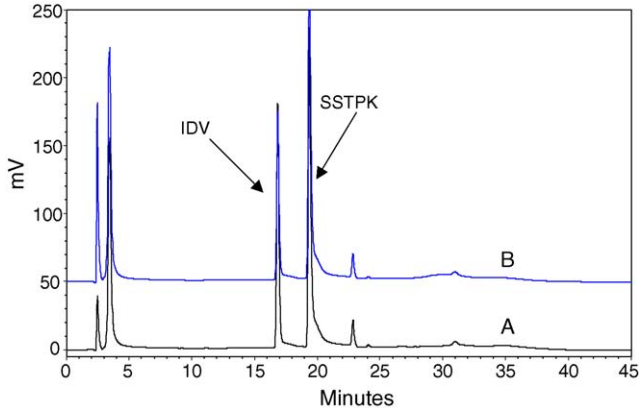

Fig. 2. Typical chromatograms of SST solutions prepared by heating (A) in boiling water for 10 min and (B) at 80 °C for 60 min.

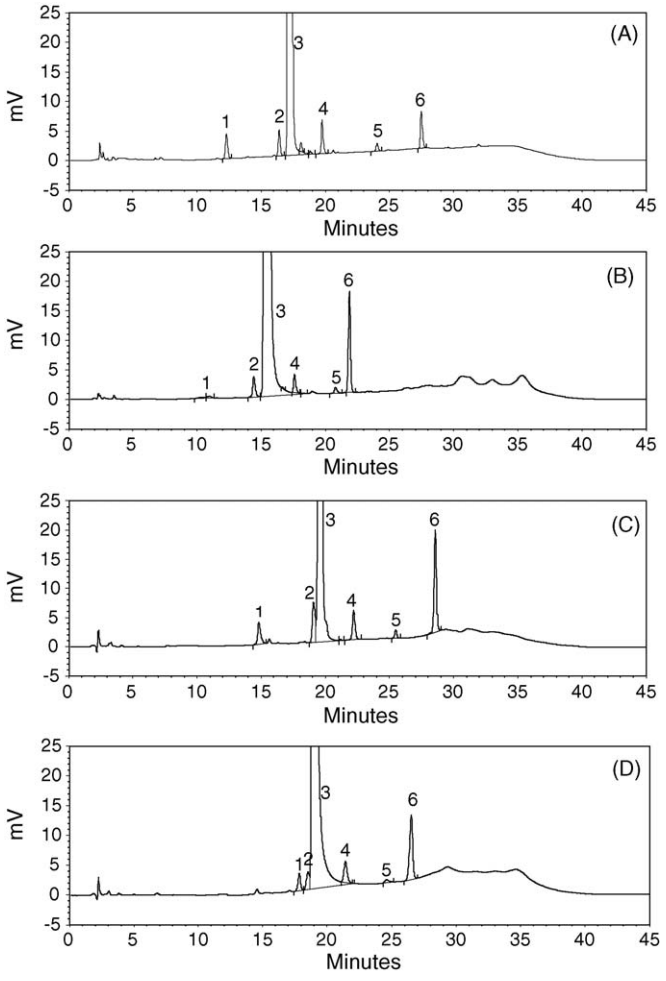

Fig. 3. Chromatograms for purity control obtained on different columns for a spiked IDV sample. (1) carboxamide, (2) unknown impurity 1 (UNK1), (3) IDV, (4) unknown impurity 2 (UNK2), (5) unknown impurity 3 (UNK3) and (6) epoxide (EPO). (A) Hypersil BDS; *F* = 0.000; CRF = 1.00 (column 1), (B) Supelcosil LC-18 DB; *F* = 0.667; CRF = 0.86 (column 4), (C) Validated C 18; *F* = 2.303; CRF = 0.58 (column 6) and (D) Apex ODS II; *F* = 26.256; CRF = 0.23 (column 16).

Table 3
Results of SST and CRF values for the set of C18 columns

Number	Column name	*F*-values	SST	CRF
1	Hypersil BDS C18	0.000	7.721	1.00
2	ACE C18	0.436	5.135	1.00
3	Discovery C18	0.480	6.532	0.94
4	Supelcosil LC-18 DB	0.667	4.150	0.86
5	Nucleosil HD	2.135	5.086	0.00
6	Validated	2.303	5.445	0.58
7	Platinum C18	2.813	1.907	0.00
8	Symmetry	3.030	6.284	0.91
9	Purospher	3.940	6.411	0.82
10	Kromasil EKA	4.698	7.533	1.00
11	Purospher Star	4.888	8.365	0.97
12	Alltima C18	5.456	8.161	0.65
13	Platinum EPS C18	7.162	6.796	0.00
14	LiChrospher	9.146	5.246	0.00
15	Apex Basic	10.477	1.196	0.00
16	Apex ODS II	26.256	4.398	0.23

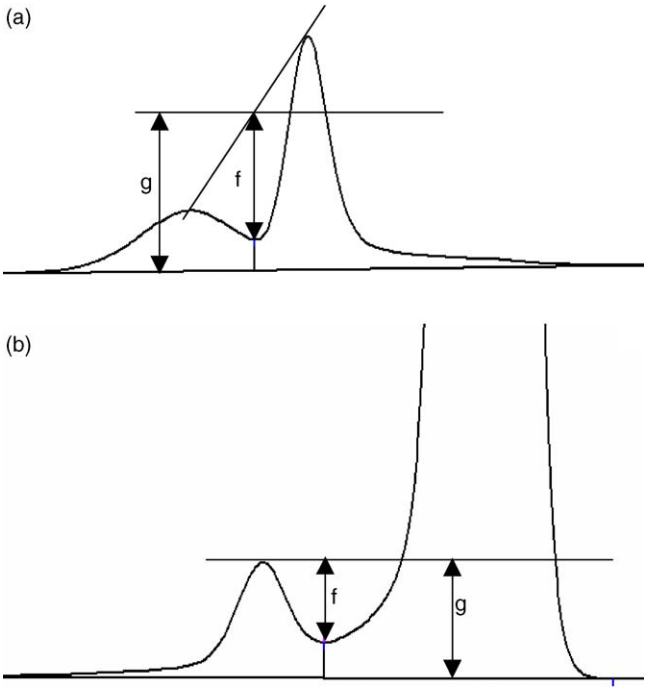

Fig. 4. Illustration of g and f for the calculation of CRF.

the correct/required information, as may be expected. Columns 5 and 14 show co-elution of the UNK1-IDV peak pair, whereas column 13 shows a change in elution order and some of the peaks are co-eluted. Columns 3, 4, 6, 8, 9, 11, 12 and 16, which are suitable according to the SST, do not give overall baseline separation. Although small adjustment of the mobile phase may further improve the separation, it was not adjusted during this study because the aim of the study was to compare the separation of IDV and its impurities on different columns in identical conditions.

3.3. Further column differentiation based on the CRF

The suitability of a column for a separation can also be examined by calculating the chromatographic response function (CRF), a measure of overall selectivity. Of course, this requires the presence of measurable impurity peaks, which is not possible in daily routine analysis, unless reference substances are made available. The CRF is defined as:

$$\mathrm{CRF} = \prod_{i=1}^{n-1} \frac{f_i}{g_i}$$

where n is the total number of peaks, g the interpolated peak height between two peaks (i.e., the distance between the baseline and a line connecting the two peak apexes, at the location of the valley) and f is the depth of the valley, measured from the line connecting two peak apexes [58–60]. See Fig. 4a.

In this IDV analysis, the baseline separation problems are mainly related to the peak pairs of UNK1-IDV or IDV-UNK2. Both UNK1 and UNK2 are relatively small compared with IDV and it is difficult to draw a line connecting the peak apexes. For these peak pairs, the calculation of f and g was slightly adapted as follows: g is the height above the baseline of the smallest peak of the pair and f is the distance between the line parallel to the baseline constructed through the highest point of the small peak and the lowest point of the valley between the two peaks (Fig. 4b). CRF values are always situated between 0 (two or more peaks are co-eluted) and 1 (all peaks are baseline separated). The CRF is a measure of the selectivity and does not take into account the peak shape (while resolution does).

Columns 7 and 15 did not reach the SST requirements. Co-elution of two or more peaks was observed, leading to a CRF of 0. Although columns 5, 13 and 14 were suitable according to SST, they do not separate all peaks. It was observed that baseline separation for all peaks (CRF = 1.00) could only be obtained on three columns (1, 2 and 10).

Columns 4 and 14 show peak broadening for CAR. This can be due to the stationary phase (only base-deactivated), but column 13 (also only base-deactivated) did not show peak broadening.

3.4. Correlation between the column classification and the separation of IDV

In the next step, it was examined whether a correlation could be found between the column classification (taking the Hypersil BDS C18 column as reference) and the separation data for IDV. The quality of the separations was evaluated by the CRF-values. In previous correlation experiments, three ranges of CRF values were examined: $F < 2$, $2 < F < 6$, $F > 6$ [59,60].

It was observed that two out of four columns with $F < 2$ give baseline separation for the analysis of IDV and its impurities (CRF = 1.00). Column 3 (CRF = 0.94) and column 4 (CRF = 0.86) still yield an acceptable separation as can be deduced from Fig. 3. For columns with $F > 2$ the probability to separate IDV from its impurities clearly decreases. When F is between 2 and 6, only one out of eight columns gave CRF = 1.00 and 2 columns were not suitable at all (CRF = 0). For F-values above 6, none out of four columns showed an appropriate selectivity (Table 3).

As it is clearly seen, with the increase of the F-values from 0 to 26, the probability of finding a column with a suitable separation for the analysis of IDV and its impurities has clearly decreased. The column classification system indicates to be a helpful tool for choosing a suitable stationary phase.

3.5. Method validation

3.5.1. Robustness study

In this part, the influence of four (k) chromatographic parameters on the separation was investigated using the Hypersil BDS C18 column. The parameters examined were the amount of ACN, the amount and the pH of the buffer in mobile phase A and the column temperature. Their effects on the resolution of the different pairs of compounds (CAR-UNK1, UNK1-IDV, IDV-UNK2, UNK2-UNK3, UNK3-EPO and IDV-SSTPK) were evaluated by means of an experimental design and multivariate analysis using Modde 5.0 statistical graphic software (Umetrics,

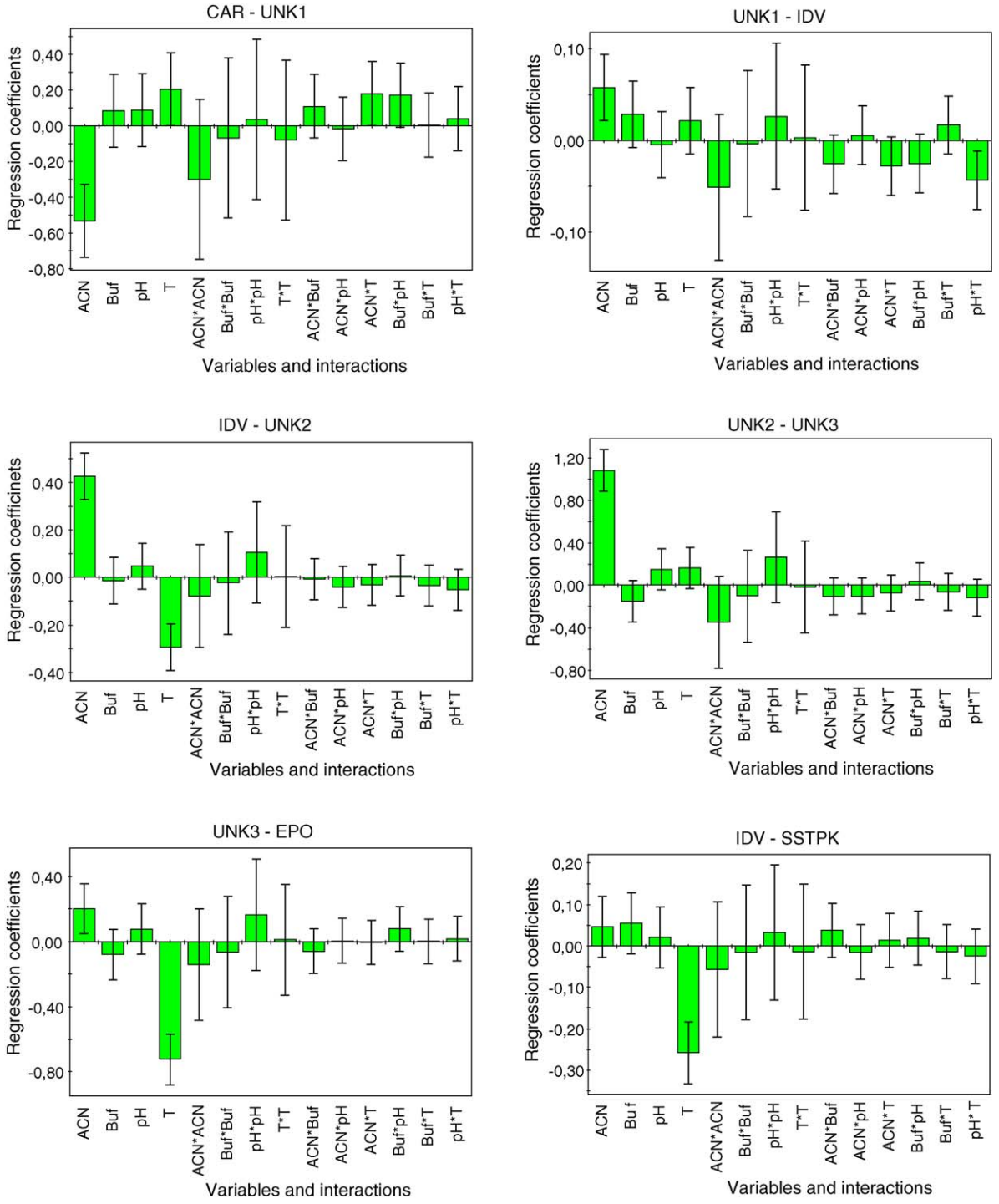

Fig. 5. Regression coefficient plots of the resolution between the peak pairs CAR-UNK1, UNK1-IDV, IDV-UNK2, UNK2-UNK3, UNK3-EPO and IDV-SSTPK, obtained from the robustness study. ACN = acetonitrile; Buf = buffer content; pH = mobile phase pH; T = column temperature.

Umea, Sweden). The chromatographic parameter settings in the experimental design are shown in Table 4.

A central composite face centered (CCF) design was applied. A central composite design consists of points of a two-level full factorial design (2^k), with n replicates of the central point, augmented with $2k$ star points to enable this model to estimate the curvature response. So, $2^k + 2k + n = 27$ experiments were performed, where $k = 4$ is the number of parameters and the central

Table 4
Chromatographic parameter setting applied in the robustness investigation, corresponding to low (−), central (0) and high (+) levels

Parameter	Low value (−)	Central value (0)	High value (+)
Acetonitrile (%)	27	30	33
Buffer (%)	4	5	6
pH	7.2	7.5	7.8
Temperature (°C)	37	40	43

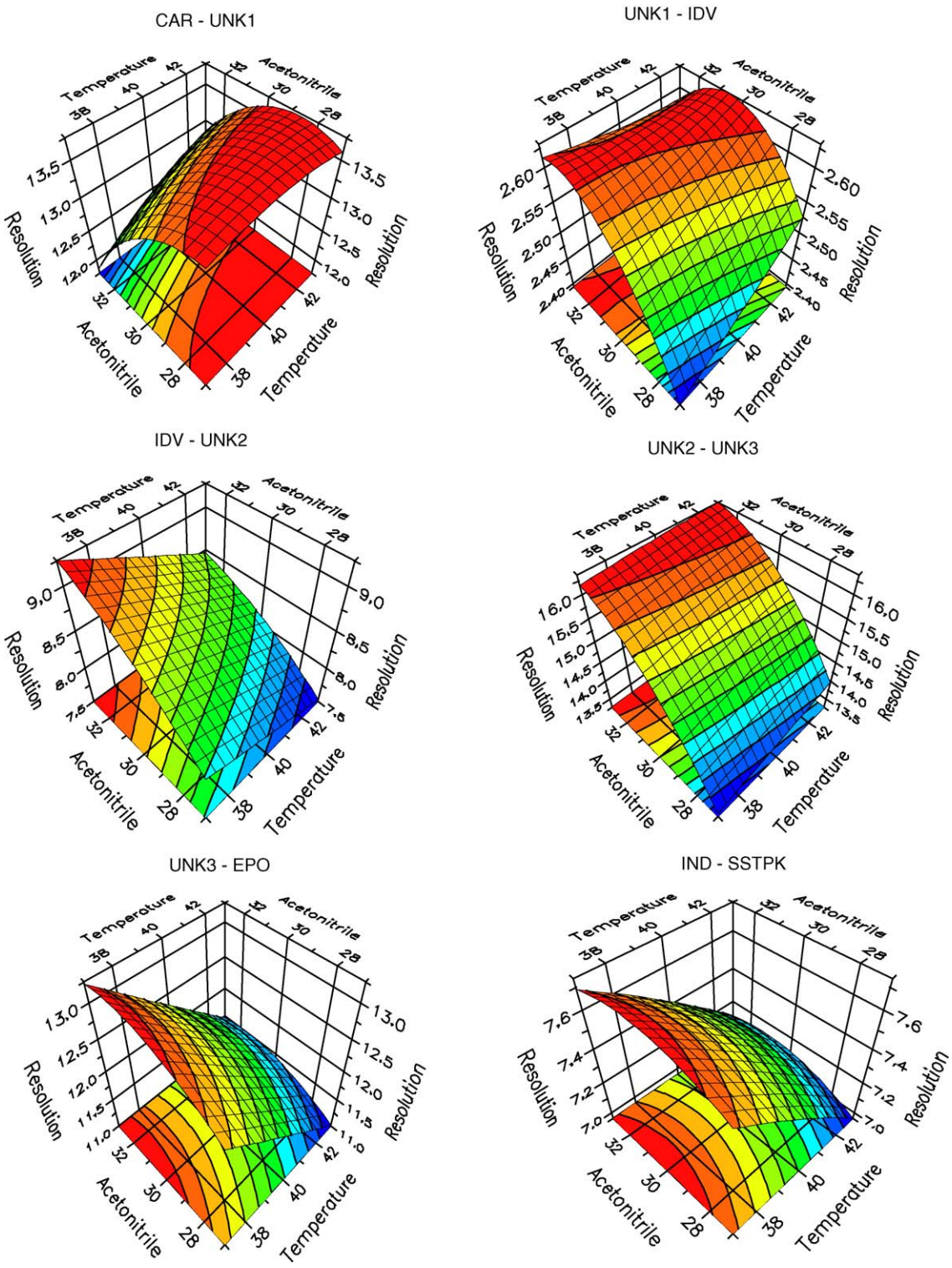

Fig. 6. Response surface plots of the resolution for the pairs CAR-UNK1, UNK1-IDV, IDV-UNK2, UNK2-UNK3, UNK3-EPO and IDV-SSTPK as a function of acetonitrile content in the mobile phase and the column temperature.

point was replicated three times ($n = 3$). The central composite design permits the response surface to be modelled by fitting a second-order polynomial model. The statistical relationship between a response Y and the experimental variables X_i, X_j... is of the following form:

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_i X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \cdots + E$$

where the β's are the regression coefficients and E the overall experimental error. The linear coefficients β_i and β_j describe the quantitative effect of the experimental variables in the model.

The cross product coefficient, β_{ij} measures the interaction effect between the variables and the square terms $\beta_{ii}X_i^2$ and $\beta_{jj}X_j^2$ describe non-linear effects on the response [62,63].

The individual and interaction parameter effects on the resolution for pairs CAR-UNK1, UNK1-IDV, IDV-UNK2, UNK2-UNK3, UNK3-EPO and IDV-SSTPK are summarized in Fig. 5. The effects on other peak pairs are not discussed, as the amount present in commercial samples was less than the disregard limit (0.05%). The plots consist of bars, which correspond to the regression coefficients. The magnitude of the variable effects is proportional to the regression coefficients. The bars denoted by variable $i \times$ variable i reflect the regression coefficients for the non-linear effect of that particular variable, where the bars denoted by variable $i \times$ variable j reflect the interaction between the two variables concerned. The 95% confidence limits are expressed by using error lines. A regression coefficient smaller than the error line shows that the variation of the response caused by changing the variable is smaller than the experimental error. Therefore, the effect of variable change is considered insignificant when compared to the response. The coefficients of the terms in the model were estimated by the partial least squares (PLS) method. Statistical analysis of the model gave R^2 values above 0.90 for all resolutions except for the peak pairs CAR-UNK1 ($R^2 = 0.83$), UNK1-IDV ($R^2 = 0.77$) and IDV-SSTPK ($R^2 = 0.85$). These R^2 values correspond to the fractions of variation of the responses that can be explained by the model.

It is observed that the separation under the conditions examined for peak pairs CAR-UNK1, UNK1-IDV, IDV-UNK2 and UNK2-UNK3 is mainly influenced by the amount of ACN present in the mobile phase. ACN has a negative effect on the peak pair CAR-UNK1, while it has a positive effect on the peak pairs UNK1-IDV, IDV-UNK2, UNK2-UNK3 and UNK3-EPO. So, the selectivity increases when the amount of the ACN increases except for CAR-UNK1. For the peak pairs UNK3-EPO and IDV-SSTPK the temperature of the column is the most important factor, while it also has a significant negative influence on the resolution between IDV-UNK2. The effects of the amount of buffer present in the mobile phase and the pH of the buffer are insignificant for all peak pairs. Fig. 6 shows the variation of the resolution for all peak pairs as a function of the amount of ACN present in the mobile phase and the column temperature.

It can be concluded that any changes of the parameter conditions within the examined range will not affect the quality of the separation since the resolution is always above 2.4.

Table 5
Limit of detection (LOD), limit of quantitation (LOQ) and corresponding R.S.D. values for IDV and some of its impurities (2.0 mg/ml = 100%, 20 μl injected)

	IDV	CAR	EPO
LOD			
% (m/m)	0.003	0.008	0.002
Mass on column (ng)	1.2	3.3	0.8
LOQ			
% (m/m)	0.009	0.025	0.006
Mass on column (ng)	3.6	10	2.4
R.S.D. (%, $n = 6$)	1.6	4.6	2.9

Table 6
Linearity data for IDV and some of impurities

Concentrations (μg/ml)	Regression equation y	R^2	$S_{y,x}$	n_c	n_i
IDV					
0.18–2500	$7489\,x + 605115$	0.985	883108	11	3
0.18–500	$11077\,x + 56073$	0.998	98201	7	3
CAR					
0.5–50	$16141\,x - 331$	0.999	489	5	3
EPO					
0.12–50	$99217\,x + 19187$	0.999	31454	5	3

R^2: coefficient of determination; $S_{y,x}$: standard error of estimate; n_c: number of experimental concentrations studied; n_i: number of injections for each concentration; y: peak area; x: concentration injected (μg/ml).

Also, the SST is always above 7.0 for the peak pair IDV-SSTPK.

3.5.2. Quantitative aspects

3.5.2.1. Sensitivity and linearity. The limit of detection (LOD) (corresponding to a signal-to-noise ratio of 3), the limit of quantitation (LOQ) (corresponding to a signal-to-noise ratio of 10) and the corresponding R.S.D. values are summarized in Table 5. The percentages were calculated with respect to the main component nominal value (2.0 mg/ml = 100%, 20 μl injected).

The linearity was checked by separate analyses of IDV, CAR and EPO. The concentrations examined were in the range of LOQ to 125% (0.18–2500 μg/ml) for the main component and in the range of LOQ to 2.5% m/m for the impurities. The percentages used reflect the amount of impurities that can be present in commercial samples. The linearity data obtained for IDV and its impurities are summarized in Table 6. The calibration curves were linear and correlation coefficients (R^2) were good, except for IDV in the range 0.18–2500 μg/ml, probably due to overloading of the detector. For assay, the consultation document of the Int. Ph. prescribes a potentiometric titration. However, a LC method may be used as a replacement on condition that a reference substance with known content is available. IDV was found linear in the range of the LOQ to 25% m/m (0.18–500 μg/ml) of the nominal area (2.0 mg/ml = 100%).

Table 7
Precision data for IDV and some of its impurities

	UNK1	UNK2	UNK3	IDV
Level (%)	0.19	0.16	0.05	99.60
% R.S.D. ($n = 9$)				
Day 1	0.79	1.29	0.91	0.57
Day 2	0.97	1.58	0.84	0.61
Day 3	0.48	2.20	2.92	0.72
% R.S.D. ($n = 27$)				
Days 1–3	1.15	2.28	3.28	0.69
% R.S.D. ($n = 9$)				
Day 4	0.76	0.67	1.85	0.34
% R.S.D. ($n = 18$)				
Days 3–4	0.81	1.59	3.94	0.57

Table 8
Purity control of IDV samples, expressed as IDV (%)

Impurities	Sample number 1	Sample number 2	Sample number 3	Sample number 4	Sample number 5	Sample number 6
Sum of impurities (%)	0.25	0.26	0.40	0.06	0.05	0.40
Total numbers of impurities above disregard limit (0.05%)	3	3	3	1	1	3
Number of impurities above 0.1%	0	1	2	0	0	2

Therefore, a 0.4 mg/ml IDV solution should be used for assay.

3.5.2.2. Precision. The method was assessed using multiple preparations of a single sample. Three different solutions of the commercial IDV sample each 2.0 mg/ml for purity control and 0.4 mg/ml for assay were analysed in triplicate on a single day. New preparations were made and analyzed on each of four successive days. An intermediate precision study was performed using a new Hypersil BDS C18 column and different LC equipment. Three solutions of the same commercial sample were analysed in triplicate on a single day (day 4). R.S.D. values calculated for IDV, UNK1, UNK2 and UNK3 on triplicate injections in a single day ($n = 9$), three successive days (days 1–3) ($n = 27$) and combining day 3 (equipment I) and day 4 (equipment II) for the intermediate precision are summarized in Table 7.

3.5.2.3. Analysis of commercial samples. The Int. Ph. sets the limit for any individual impurity to be not more than 0.1% and the sum of the impurities to be not more than 0.5% in bulk samples. Six commercial samples of IDV were analyzed for related substances of IDV using the monograph method and results obtained are summarized in Table 8. All samples comply for the sum of impurities whereas samples No. 2, 3 and 6 do not comply for individual impurities. All impurities are expressed as IDV, using a 0.1% dilution (2.0 μg/ml) of the examined sample as the reference.

4. Conclusion

The column classification system indicated to be a helpful tool for choosing a suitable column. It was found that, with the increase of the *F*-values, the probability of finding a column with a suitable separation for the analysis of IDV and its impurities clearly decreased.

The gradient LC method proposed in the Int. Ph. Monograph shows a good separation of IDV from its impurities. This method is robust, precise and linear. However, if it is to be used for assay, more dilute solutions have to be used (0.4 mg/ml instead of 2.0 mg/ml).

Acknowledgements

The authors thank the manufacturers and the suppliers for the gift of columns. The author also thanks the World Health Organization (WHO) for support. E. Adams is a post-doctoral fellow of the Fund for Scientific Research-Flanders (Belgium).

References

[1] B.C. Buckland, S.W. Drew, N.C. Connors, M.M. Chartrain, C. Lee, P.M. Salmon, K. Gbewonyo, W. Zhou, P. Gailliot, R. Singhvi, R.C. Olewinski Jr., W.J. Sun, J. Reddy, J. Zhang, B.A. Jackey, C. Taylor, K.E. Goklen, B. Junker, R.L. Greasham, Metab. Eng. 1 (1999) 63–74.
[2] B.D. Johnson, A. Howard, R. Varasolona, J. McCauley, D.K. Ellison, in: G. Brittain (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 26, Academic Press, San Diego, California, 1996, pp. 319–358.
[3] M.S. Aurora Prado, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, T.J.A. Pinto, M.F.M. Tavares, J. Pharm. Biomed. Anal. 34 (2004) 441–450.
[4] B.C.E. Silva, L.M.M. de Campos, E.A. Nunan, C.D.V. Soares, G.R. Silva, J.A.A. Ribeiro, G.A. Pianetti, Quim. Nova 28 (2005) 50–53.
[5] B. Jancic, M. Medenica, D. Ivanovic, A. Malenovic, Chromatographia 62 (2005) 233–238.
[6] Consultation document of International Pharmacopoeia, WHO Drug Information, 19th vol. Geneva, Switzerland, 2005. pp. 51-55.
[7] B.C.E. Silva, L.M.M. de Campos, G.A. Pianetti, Quim. Nova 28 (2005) 54–56.
[8] W. Gutleben, N.D. Tuan, H. Stoiber, M.P. Dierich, M. Sarcletti, A. Zemann, J. Chromatogr. A 922 (2001) 313–320.
[9] N. Chelyapov, S.A. Jacobs, T.J. Magee, J. Chromatogr. A 853 (1999) 431–437.
[10] W. Gutleben, K. Scherer, N.D. Tuan, H. Stoiber, M.P. Dierich, A. Zemann, J. Chromatogr. A 982 (2002) 153–161.
[11] N.D. Tuan, W. Gutleben, K. Scherer, H. Stoiber, B. Falkensammer, M.P. Dierich, A. Zemann, Electrophoresis 24 (2003) 662–670.
[12] M.R. Fizzano, L. Valvo, M.L. Dupuis, V. Mennella, M. Cianfriglia, J. Pharm. Biomed. Anal. 22 (2000) 307–314.
[13] J.P. Vacca, B.D. Dorsey, W.A. Schleif, R.B. Levin, S.L. Mc Daniel, P.L. Darke, J. Zugay, J.C. Quintero, O.M. Blahy, E. Roth, V.V. Sardana, A.J. Schlabach, P.I. Graham, J.H. Condra, L. Gotlib, M.K. Holloway, J. Lin, I.W. Chen, K. Vastag, D. Ostovic, P.S. Anderson, E.A. Emini, J.R. Huff, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 4096–4100.
[14] K.C. Yeh, P.J. Deutsch, H. Haddix, M. Hesney, V. Hoagland, W.D. Ju, S.J. Justice, B. Osborne, A.T. Sterrett, J.A. Stone, E. Woolf, S. Waldman, Antimicrob. Agents Chemother. 42 (1998) 332–338.
[15] D.M. Burger, M. de Graaff, E.W. Wuis, P.P. Koopmans, Y.A. Hekster, J. Chromatogr. B 703 (1997) 235–241.
[16] G. Aymard, M. Legrand, N. Trichereau, B. Diquet, J. Chromatogr. B 744 (2000) 227–240.
[17] S. Laussine, A. Roux, B. Delhotal-Landes, B. Flouvat, Ann. Biol. Clin. 59 (2001) 169–175.
[18] K. Keil, V.A. Frerichs, R. DiFrancesco, G. Morse, Ther. Drug Monit. 25 (2003) 340–346.
[19] J. Donnerer, M. Kronawetter, A. Kapper, I. Haas, H.H. Kessler, Pharmacology 69 (2003) 197–204.
[20] D.M. Kreuz, A.L. Howard, D. Ip, J. Pharm. Biomed. Anal. 19 (1999) 725–735.
[21] M. Takahashi, M. Yoshida, T. Oki, N. Okumura, T. Suzuki, T. Kaneda, Biol. Pharm. Bull. 28 (2005) 1286–1290.
[22] S.M. Hsieh, H.Y. Yu, S.C. Chang, J. Formos. Med. Assoc. 103 (2004) 191–195.

[23] N.L. Rezk, R.R. Tidwell, A.D.M. Kashuba, J. Chromatogr. B 805 (2004) 241–247.
[24] P.D. Walson, S. Cox, I. Utkin, N. Gerber, L. Crim, M. Brady, K. Koranyi, Ther. Drug Monit. 25 (2003) 649–656.
[25] J. Faux, N. Venisse, G. Le Moal, A. Dupuis, S. Bouquet, Chromatographia 58 (2003) 421–426.
[26] M.L. Turner, K. Reed-Walker, J.R. King, E.P. Acosta, J. Chromatogr. B 784 (2003) 331–341.
[27] E. Marchei, R. Pacifici, G. Tossini, R. Di Fava, L. Valvo, P. Zuccaro, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 2325–2336.
[28] M. Sarasa-Nacenta, Y. Lopez-Pua, J. Mallolas, J.L. Blanco, J.M. Gatell, X. Carne, J. Chromatogr. B 757 (2001) 325–332.
[29] C. Marzolini, A. Telenti, T. Buclin, J. Biollaz, L.A. Decosterd, J. Chromatogr. B 740 (2000) 43–58.
[30] P. Remmel, S.P. Kawle, D. Weller, C.V. Fletcher, Clin. Chem. 46 (2000) 73–81.
[31] M.L. Foisy, J.P. Sommadossi, J. Chromatogr. B 721 (1999) 239–247.
[32] A.L. Jayewardene, F. Zhu, F.T. Aweeka, J.G. Gambertoglio, J. Chromatogr. B 707 (1998) 203–211.
[33] C. Armbruster, H. Vorbach, F. Steindl, I. El Menyawi, J. Antimicrob. Chemother. 47 (2001) 487–490.
[34] E. Dailly, L. Thomas, M.F. Kergueris, P. Jolliet, M. Bourin, J. Chromatogr. B 758 (2001) 129–135.
[35] A. Volosov, C. Alexander, L. Ting, S.J. Soldin, Clin. Biochem. 35 (2002) 99–103.
[36] V.A. Frerichs, R. DiFrancesco, G.D. Morse, J. Chromatogr. B 787 (2003) 393–403.
[37] H. Pelerin, S. Compain, X. Duval, F. Gimenez, H. Benech, A. Mabondzo, J. Chromatogr. B 819 (2005) 47–57.
[38] S. Colombo, A. Beguin, A. Telenti, J. Biollaz, T. Buclin, B. Rochat, L.A. Decosterd, J. Chromatogr. B 819 (2005) 259–276.
[39] J.M. Poirier, P. Robidou, P. Jaillon, Ther. Drug Monit. 27 (2005) 186–192.
[40] W. Egge-Jacobsen, M. Unger, C.U. Niemann, M. Baluom, S. Hirai, L.Z. Benet, U. Christians, Ther. Drug Monit. 26 (2004) 546–562.
[41] K.M. Rentsch, J. Chromatogr. B 788 (2003) 339–350.
[42] K.M.L. Crommentuyn, H. Rosing, L.G.A.H. Nan-Offeringa, M.J.X. Hillebrand, A.D.R. Huitema, J.H. Beijnen, J. Mass Spectrom. 38 (2003) 157–166.
[43] U.S. Justesen, C. Pedersen, N.A. Klitgaard, J. Chromatogr. B 783 (2003) 491–500.
[44] E. Gangl, I. Utkin, N. Gerber, P. Vouros, J. Chromatogr. A 974 (2002) 91–101.
[45] J. Chi, A.L. Jayewardene, J.A. Stone, T. Motoya, F.T. Aweeka, J. Pharm. Biomed. Anal. 30 (200) 675–684.
[46] P. Villani, M. Feroggio, L. Gianelli, A. Bartoli, M. Montagna, R. Maserati, M.B. Regazzi, Ther. Drug Monit. 23 (2001) 380–388.
[47] A.L. Jayewardene, B. Kearney, J.A. Stone, J.G. Gambertoglio, F.T. Aweeka, J. Pharm. Biomed. Anal. 25 (2001) 309–317.
[48] L.L. Lopez, X. Yu, D.H. Cui, M.R. Davis, Rapid Commun. Mass Spectrom. 12 (1998) 1756–1760.
[49] S.K. Balani, E.J. Woolf, V.L. Hoagland, M.G. Sturgill, P.J. Deutsch, K.C. Yeh, J.H. Lin, Drug Metab. Dispos. 24 (1996) 1389–1394.
[50] Indian Pharmacopoeia, Addendum 2002. The controller of Publications, Delhi, India, pp. 910–912.
[51] United States Pharmacopeia, 28th ed., U.S. Pharmacopeial Convention, Rockville, MD, USA, 2005, pp. 3247–3248.
[52] Pharmeuropa, 17th vol., European Directorate for the Quality of Medicines, Strasbourg, France, 2005, pp. 483–485.
[53] United States Pharmacopeial Pharmacopeial Forum, 26th vol., US Pharmacopeial Convention, Rockville, MD, USA, 2000, pp. 1641–1643.
[54] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, B. Noszál, E. Roets, D.L. Massart, J. Hoogmartens, Pharmaeuropa 14 (2002) 288–297.
[55] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, Zs. Kovács, B. Noszál, E. Roets, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 977 (2002) 39–58.
[56] T. Iványi, Y. Vander Heyden, D. Visky, P. Baten, J. De Beer, I. Lázár, D.L. Massart, E. Roets, J. Hoogmartens, J. Chromatogr. A 954 (2002) 99–114.
[57] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, Zs. Kovács, B. Noszál, P. Dehouck, E. Roets, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 1012 (2003) 11–29.
[58] P. Dehouck, D. Visky, Y. Vander Heyden, E. Adams, Zs. Kovács, B. Noszál, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 1025 (2004) 189–200.
[59] P. Dehouck, D. Visky, G. Van den Bergh, E. Haghedooren, E. Adams, A. Kerner, Y. Vander Heyden, D.L. Massart, Z. Kovacs, B. Noszal, J. Hoogmartens, LC-GC Europe 17 (2004) 592–601.
[60] D. Visky, E. Haghedooren, P. Dehouck, Zs. Kovács, K. Kóczián, B. Noszál, J. Hoogmartens, E. Adams, J. Chromatogr. A, in press.
[61] Column classification, Katholieke Universiteit Leuven, http://pharm.kuleuven.be/pharmchem/columnclassification, 2005.
[62] D.L. Massart, R.G. Brereton, R.E. Dessy, P.K. Hopke, C.H. Spiegelman, W. Wegscheilder, Chemometrics Tutorials, Elsevier, Amsterdam, 1990.
[63] R. Carlson, Design and Optimization in Organic Synthesis, Elsevier, Amsterdam, 1992.