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Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 799–805

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Stereoselective determination of *S*-naproxen in tablets by capillary electrophoresis

M. Fillet *, L. Fotsing, J. Bonnard, J. Crommen

Department of Analytical Pharmaceutical Chemistry, Institute of Pharmacy, University of Liège, CHU B 36, B-4000, Liège 1, Belgium

Received 15 May 1998; received in revised form 25 July 1998; accepted 15 August 1998

Abstract

A capillary electrophoresis (CE) method was developed for the stereoselective determination of the non-steroidal anti-inflammatory drug (NSAID), *S*-naproxen, in tablets. Several β-cyclodextrin derivatives (CDs) were tested as chiral selectors, including sulfobutyl-β-CD (SBCD), carboxymethyl-β-CD (CMCD), dimethyl-β-CD (DMCD) and trimethyl-β-CD (TMCD), in a phosphoric acid/triethanolamine pH 3 buffer. Under these conditions, the analyte was mainly present in an uncharged form and therefore, the use a neutral CD (DMCD or TMCD) alone could not lead to enantiomeric separation. On the contrary, by addition of a charged CD (SBCD or CMCD) to the running buffer, giving the analyte enantiomers an adequate mobility, chiral resolution could be achieved, although the resolution values obtained in this case were not quite satisfactory ($Rs < 1.5$). Dual systems, based on the use of mixtures of charged and neutral CDs, were then investigated. The SBCD/TMCD system was found to be particularly well suited to the enantioseparation of naproxen and after optimisation of the concentrations of both CDs, a resolution value of 5.4 could be obtained. The method was validated for the determination of *R*-naproxen (enantiomeric impurity) in the 0.1–2% range, using the racemic mixture of the analyte. A second validation was performed in the 50–150% range for the quantitation of *S*-naproxen. In both cases, good results with respect to linearity, precision and accuracy were obtained. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; Enantioseparation; Method validation; Naproxen

1. Introduction

The quality control of drugs requires the development of assays to control identity, potency or strength, and purity. For drugs that contain one or more stereogenic centers, the chiral purity may be an important component in the determination of purity and stability. A variety of chiral assay techniques are available to the analyst including nuclear magnetic resonance (NMR), infrared spectroscopy (IR), gas and liquid chromatography (GC and LC), thin-layer chromatography (TLC) and recently, capillary electrophoresis (CE) [1].

CE has proved to possess several advantages over other techniques like LC, e.g. higher effi-

* Corresponding author. Tel.: + 32-4-3664354; fax: + 32-4-3664347; e-mail: marianne.fillet@ulg.ac.be.

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PII: S0731-7085(98)00218-0

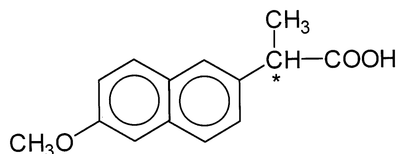

Fig. 1. Chemical structure of naproxen.

ciency, shorter analysis time, faster method development and lower consumption of chiral selector and sample [2–4]. Among several approaches, the addition of cyclodextrins (CDs) as chiral selectors to the background electrolyte (BGE) in CE seems the method of choice for the enantioresolution of drugs. Chiral separation is possible because different degrees in complexation between the two enantiomers and the chiral selector result in mobility differences [4].

Relatively few papers have discussed the validation of chiral CE methods for routine analysis, although such information is becoming more available as CE matures [3–9].

In previous works, complete enantiomeric separation of naproxen, a non-steroidal anti-inflammatory drug (NSAID), was obtained using a neutral CD in buffers having a pH close to the p*K*a of the analyte (pH 5), the electroosmotic flow being suppressed by the addition of hydroxyethylcellulose [10] or a hydrophilic linear polymer [7].

A series of chiral acidic compounds containing carboxy groups, such as NSAIDs, were also enantioseparated by simultaneous addition of anionic and uncharged β-CD derivatives to a phosphoric acid/triethanolamine pH 3 buffer [11,12].

In this paper, a simple CE method for the enantiomeric purity control of *S*-naproxen in a formulation has been developed using a dual CD system in a phosphoric acid/triethanolamine pH 3 buffer. The influence of the nature and concentration of CDs (alone and in combination) has been first studied with the aim of obtaining high enantiomeric resolution within short analysis times. The developed method has been then validated by assessing a variety of performance criteria [13–17]. The criteria evaluated include selectivity, linearity, accuracy and precision.

2. Experimental

2.1. Apparatus

All experiments were performed on a Spectraphoresis 1000 CE instrument (SpectraPhysics, San Jose, CA) equipped with an automatic injector, an autosampler, a variable wavelength UV-visible absorbance detector (190–800 nm) and a temperature control system (15–60°C). The pH of running buffers was measured by means of a Delta 345 pH-meter from Mettler (Halstead, UK).

2.2. Chemicals and reagents

Heptakis (2,6-di-O-methyl)-β-cyclodextrin (dimethyl-β-cyclodextrin: DMCD) and heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin (trimethyl-β-cyclodextrin: TMCD) were from Sigma (St Louis, MO). Carboxymethyl-β-cyclodextrin (CMCD) was from Cyclolab (Budapest, Hungary). Sulfobutyl-β-cyclodextrin (SBCD) was kindly provided by Professor Stobaugh (University of Kansas, Laurence, KS).

Table 1
Enantioseparation of naproxen in the presence of one or two CDs[a]

Analyte	Resolution					
	SBCD	SBCD/DMCD	SBCD/TMCD	CMCD	CMCD/DMCD	CMCD/TMCD
Naproxen	1.0	1.5	4.1	<0.7	1.1	2.0

[a] Buffer, 100 mM phosphoric acid/triethanolamine (pH 3) containing SBCD (5 mM) or CMCD (10 mM) and a neutral CD: DMCD or TMCD (10 mM); voltage, −25 kV; temperature, 25°C; wavelength, 210 nm; hydrodynamic injection, 5 s; sample, 20 µg ml^{-1}.

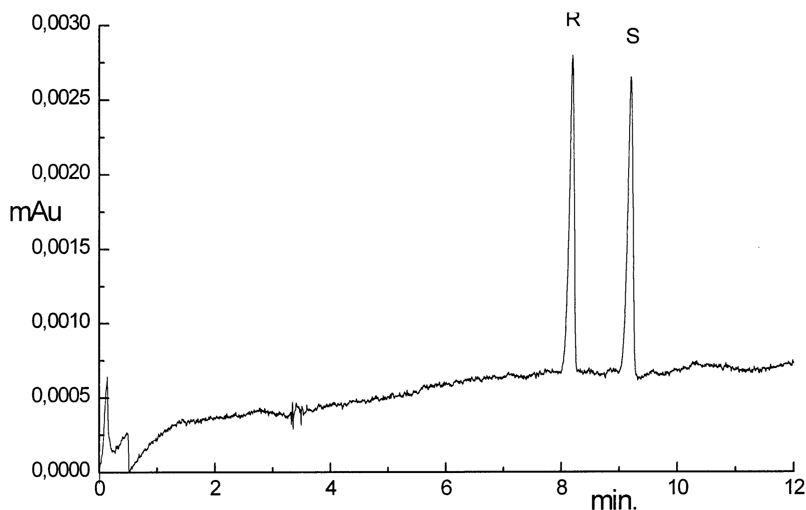

Fig. 2. Enantioseparation of naproxen. Buffer: 100 mM phosphoric acid/triethanolamine (pH 3) containing SBCD (5 mM) and TMCD (20 mM). Hydrodynamic injection: 5 s. Sample: 20 μg ml^{-1} of racemic naproxen in water/methanol (7:3).

Phosphoric acid (85%) and triethanolamine were of analytical reagent grade from Merck (Darmstadt, Germany). Water was of milli-Q quality (Millipore Corporation, Bedford, MA) and methanol was of HPLC grade from Acros (Geel, Belgium). *S*-naproxen were from Sigma and the racemic mixture of naproxen was kindly provided by Professor Blaschke (University of Münster, Germany).

All solutions were filtered through a 0.20 mm pore size filter (Schleicher and Schuell, Keene, NH).

2.3. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused silica capillaries, 50 μm internal diameter and 44 cm length (37 cm to the detector), provided by Supelco (Bellefonte, PA). The capillary was pretreated successively with alkaline solutions (NaOH 1 M, NaOH 0.1 M), water and running buffer. At the beginning of each working day, the capillary was rinsed with running buffer for 10 min. Between each injection, the capillary was rinsed with buffer for 3 min (~ 6 volumes of the capillary). The applied voltage was − 25 kV (detector at the anode end of the capillary). UV detection was performed at 210 nm and injections were made in hydrodynamic mode for a period of 5 s during the optimisation step (corresponding to 13.3 nl) and for the *S*-naproxen determination. For the enantiomeric purity determination, the injection time was 12 s. The capillary was thermostatted at 25°C. For the electrophoretic experiments, a buffer made of 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine was used.

The resolution (*Rs*) and plate number (*N*) were calculated according to the standard expressions based on the peak width at half height [18].

2.4. Sample solutions

For the optimisation step, the standard solution was prepared by dissolving the racemic mixture of naproxen at a concentration of $\sim 5 \times 10^{-5}$ M (20 μg ml^{-1}) in a mixture of water and methanol (7:3).

The average weight of one tablet was 935.42 mg and as declared, contained 500 mg of *S*-naproxen and several excipients, in particular citric acid, cellulose and methacrylate ammonium copolymer.

For the determination of *R*-naproxen (enantiomeric impurity), standard solutions were prepared by dissolving the racemic mixture of naproxen at five concentrations (1.4, 7, 14, 21, 28

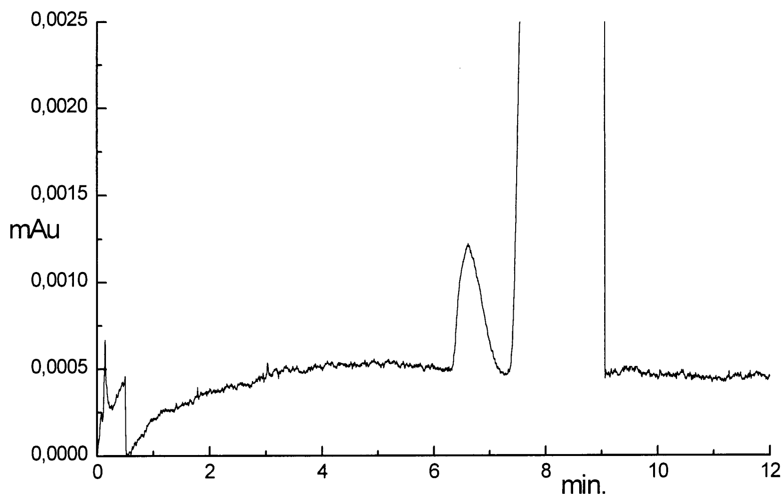

Fig. 3. Enantiomeric purity testing of *S*-naproxen. Buffer: 100 mM phosphoric acid/triethanolamine (pH 3) containing SBCD (5 mM) and TMCD (20 mM). Hydrodynamic injection: 12 s. Sample: 700 μg ml^{-1} of *S*-naproxen in water/methanol (7:3).

μg ml^{-1}) corresponding to five calibration levels ($n = 5$) in a mixture of water and methanol (7:3).

The same handling procedure was then applied to five synthetic mixtures of the dosage form excipients to which the corresponding amount of naproxen was added (spiked placebos) in order to search for possible interferences from excipients over the calibration range tested.

For *S*-naproxen determination, standard and spiked placebo solutions were prepared by dissolving the *S*-naproxen reference standard at five concentrations (40, 60, 80, 100 and 120 μg ml^{-1}) in a mixture of water and methanol (7:3).

3. Results and discussion

3.1. Optimisation of naproxen enantioseparation

All enantiomeric separations reported were performed at 25°C using buffers made of 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine and containing one or two β-cyclodextrin derivatives. In previous papers, we demonstrated the suitability of this kind of BGE for the enantiomeric separation of basic, acidic and even neutral drugs [6,11,12,19].

The pH 3 buffer was here tested for the enantioseparation of the non-steroidal anti-inflammatory drug (NSAID) naproxen. The chemical structure of this drug is given in Fig. 1.

In such a low pH buffer, the acidic naproxen (pKa = 4.2) was mainly present in uncharged form and therefore was migrating close to the electroosmotic flow (low but anodic [6]), the latter making its detection possible at the anodic side of the capillary (reversed polarity mode). Under these conditions, no enantiomeric separation could be expected for this acidic compound from the addition of a neutral cyclodextrin alone (DMCD: dimethyl-β-cyclodextrin or TMCD: trimethyl-β-cyclodextrin). Indeed, even in the presence of enantioselective interactions with this kind of cyclodextrin, no chiral resolution would be observed, due to the lack of significant mobility difference between the free and complex forms of the analyte enantiomers.

On the contrary, the use of a negatively charged β-cyclodextrin derivative (SBCD: sulfobutyl-β-cyclodextrin or CMCD: carboxymethyl-β-cyclodextrin) was found to give rise to the formation of complexes migrating electrophoretically towards the anode and consequently this could lead to the enantiomeric resolution of the drug. As shown in Table 1, naproxen enantiomers

were partly resolved in the presence of 5 mM SBCD ($Rs = 1.0$) and in the presence of 10 mM CMCD ($Rs < 0.7$).

The two anionic cyclodextrin derivatives (SBCD and CMCD) were then tested as chiral additives at different concentrations for the enantioseparation of the acidic drug at pH 3. Resolution values obtained with SBCD and CMCD were fairly constant in the whole concentration range studied (1–10 mM, results not shown here). With both cyclodextrins, a decrease in analyte migration times was observed with increasing selector concentration since the proportion of negatively charged complexes was increased. A 5 mM concentration of SBCD and a 10 mM concentration of CMCD were found to give the analyte enantiomers appropriate migration times (< 10 min) and these concentrations were kept constant in all further experiments.

Table 2
Validation results for *R*-naproxen (enantiomeric impurity)[a]

	R-naproxen
Calibration range (%)	0.1–2
Calibration points	5
Linearity	
Slope	68.16
Intercept	287.20
Coefficient of determination (r^2)	0.9957
LOD–LOQ	
Limit of detection (%)	0.03
Limit of detection (ng ml^{-1})	210
Limit of quantitation (%)	0.10
Limit of quantitation (ng ml^{-1})	700
Accuracy ($k = 3$, $n = 6$)	
Mean recovery ± CI (%) at 0.1%	92.7 ± 8.8
Mean recovery ± CI (%) at 1.0%	100.8 ± 4.3
Mean recovery ± CI (%) at 2.0%	99.8 ± 2.3
Repeatability ($k = 3$, $n = 6$, RSD%)	
0.1%	9.0
1.0%	2.8
2.0%	1.9
Intermediate precision ($k = 3$, $n = 18$, RSD%)	
0.1%	9.6
1.0%	3.3
2.0%	2.0

[a] CI, confidence interval.

Table 3
Validation results for *S*-naproxen (active drug)

	S-naproxen
Calibration range (%)	50–150
Calibration points	5
Linearity	
Slope	1475.4
Intercept	−35.1
Coefficient of determination (r^2)	0.9994
Accuracy ($k = 3$, $n = 6$)	
Mean recovery ± CI (%) at 50%	100.0 ± 2.9
100%	99.4 ± 0.9
150%	99.6 ± 0.9
Repeatability ($k = 3$, $n = 6$, RSD%)	
50%	1.7
100%	1.3
150%	0.7
Intermediate precision ($k = 3$, $n = 18$, RSD%)	
50%	2.0
100%	1.7
150%	1.2

Dual CD systems, using a mixture of a neutral CD (DMCD or TMCD at 10 mM concentration) and a charged CD (SBCD or CMCD, at a concentration of 5 or 10 mM, respectively), were then investigated. For all combinations tested (SBCD/DMCD, SBCD/TMCD, CMCD/DMCD and CMCD/TMCD), resolution values were higher than those achieved with a system containing only SBCD or CMCD (Table 1). This could be explained by a high selectivity for the enantioseparation resulting from the complexation of the drug in uncharged form with the neutral cyclodextrins while interactions with the charged CDs gave only rise to low chiral resolution ($Rs \leq 1.0$).

Among the two neutral cyclodextrins tested, TMCD was found to be the most suited to the enantioseparation of naproxen, especially with SBCD (Table 1, $Rs = 4.1$).

The next step in our investigation was to study the influence on enantioresolution of the concentration of TMCD added to the pH 3 buffer containing 5 mM SBCD. Resolution values were found to increase with TMCD concentration (results not presented here). As shown in Fig. 2, a

excellent separation ($Rs = 5.4$ and migration times < 10 min) was achieved using 20 mM TMCD.

3.2. Validation of the method developed for the determination of *R*-naproxen (enantiomeric impurity)

Under the selected operating conditions (pH 3 buffer made of 100 mM phosphoric acid/triethanolamine containing 5 mM SBCD and 20 mM TMCD), the method was validated for the determination of *R*-naproxen (enantiomeric impurity) using the racemic mixture of the analyte (Fig. 3).

3.2.1. Selectivity

No interference from the formulation excipients could be observed at the migration times of the enantiomers. The migration order (*R*-enantiomer migrating first) was confirmed by spiking experiments.

3.2.2. Linearity

Calibration graphs were constructed at five concentration levels in the range 0.1–2.0% of the nominal concentration of *S*-naproxen in the tablets, and three independent determinations were performed at each concentration ($n = 3$). Linear regression lines were obtained by plotting the normalised peak areas (ratios of peak areas to migration times) versus the analyte concentration using the least squares method.

Firstly, the linearity of the calibration curve for *R*-naproxen in water/methanol (7:3) solution (standard solution) was determined. A straight line passing through the origin and coefficient of determination (r^2) of 0.9969 were obtained. The linearity was also confirmed by an analysis of variance (ANOVA) [15,16]. The same statistical approach was then applied to a synthetic mixture of the excipients of the dosage form to which known quantities of racemic naproxen were added (spiked placebos) corresponding to the different concentration levels in the range tested. The regression parameters are given in Table 2. As earlier, the regression lines were obtained by using the least squares method according to the hypothesis of homoscedasticity [15,16].

3.2.3. Limits of detection and of quantitation

Limits of detection (LOD) and of quantitation (LOQ), corresponding to signal-to-noise ratios of 3 and 10, respectively, were calculated from linear regression analysis performed by plotting the analyte peak height versus the percentage of impurity.

The LOD, the lowest concentration of *R*-naproxen that could be detected, was 0.03%, which corresponds to a concentration of 0.21 μg ml^{-1} and an amount injected of 7.3 pg or 32 fmol. The LOQ, the lowest concentration of *R*-naproxen that could be determined in a purity determination, was 0.10%, which corresponds to a concentration of 0.70 μg ml^{-1} and an amount injected of 24.4 pg or 106 fmol.

3.2.4. Accuracy

Method accuracy was determined by analysing a placebo spiked with racemic naproxen at three concentration levels ($n = 6$) covering the same range as that used for linearity (0.1; 1.0; 2.0%). Mean recoveries with 95% confidence intervals (CI) are given in Table 2. As the theoretical value of 100% was included in the confidence interval, the test procedure could be considered accurate over the range studied.

3.2.5. Precision

Method precision was determined by measuring repeatability and intermediate precision (between-day precision) for *R*-naproxen in spiked placebos ($n = 6$). The study was carried out over 3 days ($k = 3$) at three concentration levels (0.1; 1.0; 2.0%). The RSD values were estimated from repeatability and intermediate precision variances, respectively [16,17].

As can be seen in Table 2, acceptable results with respect to precision were obtained. The RSDs at the LOQ (0.1%) are relatively high but still acceptable for such low levels of impurity.

The *S*-naproxen reference standard was found to contain 0.93% of the R isomer (Fig. 2).

3.3. Validation of the method developed for the determination of *S*-naproxen (active drug)

A second validation was performed in the range 50–150% of the nominal concentration of *S*-

naproxen in the tablets, the five calibration points being injected in triplicate.

The same statistical approach as that described for the *R*-naproxen determination was used. As can be seen in Table 3, the adequate linearity of the calibration graphs is demonstrated by the determination coefficients ($r^2 > 0.999$).

The slopes and intercepts of the graphs for standards and spiked placebos, respectively, were found to be not significantly different, with $t_{calc} < 2.05$ ($p = 0.05$) [15,16]. According to the results of this statistical test, it can be concluded that there is no matrix effect and a single-level calibration corresponding to the labelled amount of naproxen could be used in routine analysis.

As can be seen in Table 3, the test procedure could be considered accurate over the range studied. Furthermore, RSD values were estimated at three concentration levels (50, 100, 150%) from repeatability and intermediate precision variances and were in all cases $\leq 2\%$.

3.4. Quantitative analysis of a commercial naproxen tablet

The *S*-naproxen content in tablets was quantified by the CE method described above and was found to be equal to 98.6% of the label claim.

The *R*-naproxen content in a commercial tablet formulation was also quantified by the developed CE method. 0.61% of *R*-naproxen was found in the tablets containing *S*-naproxen.

4. Conclusions

A dual cyclodextrin system using the polyanionic SBCD in combination with the neutral TMCD in a pH 3 phosphoric acid/triethanolamine buffer, was found to be effective for the enantioseparation of naproxen. In these conditions, excellent resolution value was obtained within short migration times (Fig. 2).

The CE method developed was successfully applied to the quantitation of *S*-naproxen and *R*-naproxen (enantiomeric impurity) in a pharmaceutical formulation. Results obtained in method validation confirm that CE can be considered as an interesting and cost effective alternative to liquid chromatography for the quality control of chiral drugs.

Acknowledgements

A research grant from the Belgium National Fund for Scientific Research (FNRS) to one of us (M. Fillet) is gratefully acknowledged. Many thanks are due to Professor Stobaugh (University of Kansas, Lawrence, KS) for providing us with sulfobutyl-β-D and to Professor Blaschke (University of Münster, Germany) for providing us with racemic naproxen.

References

[1] T. J. Wozniak, R. J. Bopp, E. C. Jensen, J. Pharm. Biomed. Anal. 9 (1991) 363–382.
[2] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245–276.
[3] K.D. Altria, D.M. Goodall, M.M. Rogan, Electrophoresis 15 (1994) 824–827.
[4] B. Chankvetadze, Capillary electrophoresis in chiral analysis, Wiley, Chicester, UK, 1997.
[5] K.D. Altria, A.R. Walsh, N.W. Smith, J. Chromatogr. 645 (1993) 193–196.
[6] M. Fillet, I. Bechet, P. Chiap, Ph. Hubert, J. Crommen, J. Chromatogr. A 717 (1995) 203–209.
[7] A. Guttman, N. Cooke, J. Chromatogr. A 685 (1994) 155–159.
[8] R. Porra, M.G. Quaglia, S. Fanali, Chromatographia 41 (1995) 383–388.
[9] L. Liu, L.M. Osborne, M.A. Nussbaum, J. Chromatogr. A 745 (1996) 45–52.
[10] Y.Y. Rawjee, G. Vigh, Anal. Chem. 66 (1994) 619–627.
[11] M. Fillet, I. Bechet, G. Schomburg, Ph. Hubert, J. Crommen, J. High Resol. Chromatogr. 19 (1996) 669–673.
[12] M. Fillet, Ph. Hubert, J. Crommen, Electrophoresis 18 (1997) 1013–1018.
[13] K.D. Altria, J. Chromatogr. 646 (1993) 245–257.
[14] A. Kunkel, M. Degenhardt, B. Schirm, H. Wätzig, J. Chromatogr. A 768 (1997) 17–27.
[15] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: a textbook, Elsevier, Amsterdam, 1988, p. 75.
[16] J. Caporal-Gautier, J.M. Nivet, P. Algranti, M. Cuilloteau, M. Histe, M. Lallier, J.J. N'Guyen-Huu, R. Russoto, S.T.P. Pharma Prat. 2 (1992) 202–226.
[17] C. Hartmann, Analusis 22 (1994) 19–20.
[18] The European Pharmacopoeia, 3rd ed., Part 2.2.2, Council of Europe, Strasbourg, France, 1996.
[19] M. Fillet, L. Fotsing, J. Crommen, J. Chromatogr. A (in press).