Pergamon

0731-7085(95)01339-3

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 4/5, pp. 497–503, 1995

0731-7085/95 $9.50 + 0.00

Quantitative analysis of non-steroidal anti-inflammatory drugs by capillary zone electrophoresis*

I. BECHET, M. FILLET, Ph. HUBERT and J. CROMMEN†

Laboratory of Drug Analysis, Institute of Pharmacy, University of Liège, rue Fusch 5, B-4000 Liège, Belgium

Abstract: The potential utility of capillary zone electrophoresis (CZE) for the separation and quantitative determination of some non-steroidal anti-inflammatory drugs (NSAIDs) was investigated. The influence of different parameters on migration times, peak symmetry, efficiency and resolution was studied; these parameters included the nature and concentration of the anionic and cationic components of the separation buffer. A buffer consisting of 75 mM glycine adjusted to pH 9.1 with triethanolamine was found to provide a very efficient and stable electrophoretic system for the CZE analysis of NSAIDs, giving RSD values of about 0.1 and 0.5% for the within-day reproducibility of migration times and peak areas, respectively at a concentration of 25 μg ml^{-1} (n = 5). Response was linear from 2–100 μg ml^{-1} for both sulindac and tiaprofenic acid, for which the LOQ values were 2.8 and 1.9 μg ml^{-1}, respectively, using UV detection at 280 nm. Accuracy for each drug was 102–103%.

Keywords: *Capillary zone electrophoresis; non-steroidal anti-inflammatory drugs; method development; method validation; quantitative analysis.*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of acidic compounds which cover a number of different chemical types: these include derivatives of arylacetic acid, arylpropionic acid, indolic acid and anthranilic acid, as well as oxicams. These compounds are widely used in therapeutics for their anti-inflammatory and analgesic properties. The common structural feature of NSAIDs is an acidic group, characterized by pK_a values in the range 3–6, so that at pH ≥7 these compounds are mainly present in the anionic form. Consequently, capillary electrophoresis (CE) would appear to be an appropriate technique for the direct analysis of this class of drugs. By use of uncoated fused silica capillaries, the electroosmotic flow (EOF) can be made sufficiently high to sweep anionic compounds such as NSAIDs towards the cathode located after the detector [1–2].

Of the few applications of CE to the analysis of NSAIDs published so far, micellar electrokinetic chromatography (MEKC) has been used in most cases [3–6]. Six NSAIDs were separated in phosphate buffer (20 mM, pH 7.0) containing sodium dodecyl sulphate (SDS; 25 mM) [3]. A series of fifteen NSAIDs was directly analysed by capillary zone electrophoresis (CZE) using phosphate buffer (30 mM, pH 7.0) containing 20% acetonitrile [4]. This paper showed that the migration order of these compounds could be completely changed by use of MEKC, 40 mM SDS being added to a borate buffer (50 mM, pH 9.0) [4]. In both cases, the separation was incomplete but some improvement was obtained in the MEKC system by addition of acetonitrile. Several NSAIDs were quantified in different kinds of pharmaceutical formulations using the MEKC buffer described above, except for piroxicam which was determined by CZE with phosphate buffer (30 mM, pH 8.0) [5]. Another group of fifteen NSAIDs was completely resolved by MEKC using a phosphate buffer (40 mM, pH 8.0), to which 104 mM SDS and 3% methanol had been added [6]. The MEKC separation of anionic compounds, including the NSAID naproxen, was improved by addition of tetraalkylammonium ions to a buffer containing SDS [7]. Naproxen could also be separated from other co-formulated active ingredients in pharmaceutical preparations by MEKC, using different anionic surfactants [8] and bile salts [9].

*Presented at the Fifth International Symposium on Pharmaceutical and Biomedical Analysis, Stockholm, Sweden, September 1994.

†Author to whom correspondence should be addressed.

The aim of the present work was to study the electrophoretic behaviour of NSAIDs in free solution and to develop a fast and efficient method for the separation and determination of some NSAIDs by CZE. The influence of different parameters, such as the nature and concentration of buffer ions, was investigated. After selection of suitable operating conditions, the CZE method developed was validated using two typical NSAIDS (sulindac and tiaprofenic acid) as test substances.

Materials and Methods

Apparatus

All experiments were performed on a Spectraphoresis 1000 CE instrument (Spectraphysics, San José, CA, USA) 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). An IBM PS/2 Model 90486 was used for instrument control and data handling. Electropherograms were printed on a Laserjet 4 printer (Hewlett–Packard). A capillary column cartridge was obtained from Spectraphysics and fused silica capillaries were provided by Supelco (Bellefonte, PA, USA). The pH of running buffers was adjusted by means of a Delta model 345 pH-meter from Mettler (Halstead, UK).

Chemicals

Glycine was obtained from UCB (Brussels, Belgium). Triethanolamine, Tris-buffer, sodium hydroxide, triethylamine, diethylamine and acetic acid of analytical grade were purchased from Merck (Darmstadt, Germany). Octanoic acid, hexanoic acid and butanoic acid were obtained from Sigma Chemicals (St Louis, MO, USA). Water was of Milli-Q quality (Millipore Corporation, Bedford, MA, USA) and methanol from Janssen Chimica (Geel, Belgium) was of HPLC grade. Sulindac and indomethacin were kindly provided by Merck Sharp & Dohme (Brussels, Belgium). Alclofenac was obtained from Continental Pharma (Brussels, Belgium), tiaprofenic acid from Roussel (Brussels, Belgium) and piroxicam from Pfizer (Brussels, Belgium). Flufenamic acid, flurbiprofen and carprofen were gifts from other sources. All drugs were used and received without further purification.

Electrophoretic method

Electrophoretic separations were carried out with uncoated fused silica capillaries having 50-μm internal diameter and 44 cm length (37 cm to the detector). Before use, the capillary was treated successively with alkaline solutions (1 N NaOH, 0.1 N NaOH), water and running buffer. At the beginning of each working day, the capillary was washed with running buffer for 10 min, while after each injection the capillary was washed with water for 2 min and with buffer for 3 min.

The applied voltage was 15 kV, unless otherwise stated, and UV detection was performed at 280 nm. This wavelength was found to be a good compromise for the sensitive detection of the different NSAIDs tested. Injections were made in hydrodynamic mode for a period of 5 sec, unless otherwise stated. The capillary was thermostatted at 25°C.

The resolution (R_s) and plate number per capillary (N) were calculated according to the standard expressions [10], based on the peak width at half-height.

The asymmetry factor (A_s) was determined using the expression: $A_s = b/a$, where: a is the distance between the perpendicular from the peak maximum to the leading edge of the peak at one-tenth of peak height; and b is the distance between the perpendicular from the peak maximum to the trailing edge of the peak at one-tenth of peak height.

Sample solutions

The standard solutions were prepared by dissolving each NSAID at a concentration of about 5×10^{-5} M (20 μg ml^{-1}) in 7.5 mM glycine adjusted to pH 8.0 with triethanolamine. In this medium, high analyte stability and sample stacking were obtained. The migration order was determined by injecting individual solutions of each NSAID at the same concentration and in the same buffer. Standard solutions of two of the NSAIDs (sulindac and tiaprofenic acid) in the concentration range 2–100 μg ml^{-1} were used for quantitative experiments. They were obtained by dilution with water of a solution of the two NSAIDs at a concentration of 100 μg ml^{-1} each, prepared in the same buffer as specified above.

Results and Discussion

Nature of the anionic component of the buffer

Initially a rather high pH value (9.1) was

selected for the analysis of NSAIDs by CZE. At this pH, the electroosmotic flow has reached a maximum, fairly constant mobility (μ_{eo}) and the NSAIDs are completely ionized, so that their corresponding electrophoretic mobilities (μ_{ep}) were not affected by any slight variations in pH.

Glycine, octanoic acid, hexanoic acid, butanoic acid and acetic acid were tested as anionic components in the running buffer for the CZE analysis of eight NSAIDs chosen as model compounds. These co-ions were used at the same molar concentration and the pH was adjusted to 9.1 with triethanolamine in all cases. Table 1(a) shows the influence of these co-ions on peak symmetry for three of the NSAIDs. It is well known that in order to avoid peak deformation caused by sample concentration overload (electromigration dispersion), a co-ion with a mobility as close as possible to those of the analytes should be selected [11, 12]. With glycine as co-ion, NSAIDs appear on the electropherogram as symmetrical peaks, whereas peaks with a strong leaning tendency are observed with aliphatic carboxylic acids as co-ions. The latter compounds have higher electrophoretic mobilities than NSAIDs, so pronounced peak deformation is observed with increasing mobility of the co-ion (from octanoic acid to acetic acid). By contrast, at pH 9.1 glycine has a lower electrophoretic mobility, close to those of NSAIDs, and this ensures that the electrophoretic system with this co-ion is less sensitive to peak deformation due to overloading effects. Consequently, glycine buffer provides very high separation efficiency for NSAIDs, with plate numbers per capillary higher than 300 000 (N is about 200 000 with aliphatic carboxylic acids). This favourable effect of glycine on separation efficiency could also be related to the low current (6 μA) generated, in comparison to those obtained with aliphatic carboxylic acids (around 30 μA).

Resolution values obtained for five of the most critical pairs of NSAIDs with the different co-ions tested are given in Table 1(b). Even if the presence of glycine in the separation buffer provides better peak symmetry and higher efficiency, most resolution values are lower with this co-ion than with aliphatic carboxylic acids. A reversal of the migration order of carprofen and piroxicam is also observed with glycine as the co-ion. Glycine gives rise to a two-fold higher electroosmotic flow (45×10^{-5} cm^2 V^{-1} s^{-1}) than do carboxylic acids (ca 22×10^{-5} cm^2 V^{-1} s^{-1}), leading to an important reduction of migration times, but also to a decrease in resolution. Higher electrophoretic mobilities are also obtained for the anionic NSAIDs in the presence of glycine, but the changes in electrophoretic mobilities with the buffer co-ion are smaller than the corresponding differences in electroosmotic mobility. Moreover, the changes in electrophoretic mobilities are generally similar for all analytes, so that they do not have a primary influence on resolution, except for glycine with the pair piroxicam/carprofen, as mentioned above. According to these results, glycine

Table 1
Influence of the anionic component of the running buffer on A_s and R_s

(a) Peak symmetry (A_s)

Analyte	Glycine	Octanoic acid	Hexanoic acid	Butanoic acid	Acetic acid
Indomethacin	1.00	0.78	0.75	0.70	0.68
Piroxicam	1.10	0.88	0.72	0.69	0.68
Flufenamic acid	1.10	0.96	0.86	0.83	0.67

(b) Resolution (R_s)

Analyte pair	Glycine*	Octanoic acid	Hexanoic acid	Butanoic acid	Acetic acid
1–2	3.1	1.9	3.4	3.4	3.4
2–3	0.5	2.4	2.8	2.7	2.2
3–4	0.7	4.1	5.5	5.1	5.1
4–5	0	4.1	5.5	5.1	5.1
5–6	0.7	0	0	0	0

Buffer: anionic component at 75 mM concentration adjusted to pH 9.1 with triethanolamine. Injection: hydrodynamic mode for 4 s. Voltage: 15 kV with glycine; 25 kV with aliphatic carboxylic acids. Analytes: 1, indomethacin; 2, carprofen; 3, piroxicam; 4, flufenamic acid; 5, tiaprofenic acid; 6, flurbiprofen.

Other conditions as described in the text.

* In glycine buffer, the elution order is reversed: 2, piroxicam; 3, carprofen.

provides a very fast and efficient system for the CZE analysis of NSAIDs, whereas hexanoic acid might represent an interesting alternative when a further improvement in resolution is needed, provided low analyte concentrations are injected in order to reduce the peak distortion.

Nature of the cationic component of the buffer

As can be seen from Table 2, the nature of the cationic component of the background electrolyte also seems to exert a significant influence on the CZE separation of the NSAIDs tested, glycine being used as co-ion in all these experiments. Triethanolamine and Tris provide the best peak symmetry (cf Table 2a), while peaks with a strong leaning tendency are obtained with sodium, diethylamine and triethylamine. Although overloading effects are generally considered as being much less dependent on the electrophoretic mobility of the counter-ion, the favourable effect of triethanolamine and Tris on peak symmetry might be related to some extent to their mobilities, which seem to be lower than those of the other cations, as indicated by the lower currents generated in the presence of these two counter-ions (cf Table 2c). The peak deformation observed with sodium, diethylamine and triethylamine might also be due to a slight increase in the mobility of the co-ion, glycine, induced by these counter-ions at the pH used.

In a similar way as for co-ions, separation efficiency was found to be particularly high (N values per capillary higher than 300 000) with triethanolamine and Tris, i.e. the two counter-ions which give rise to better peak symmetry and lower currents (cf Table 2a and c). Moreover, the high viscosity of triethanolamine might contribute to a reduction of axial diffusion, resulting in an improvement of efficiency [1, 2].

Resolution values given in Table 2(b) can be related to the electroosmotic mobilities obtained with the different counter-ions. The favourable effect of diethylamine, triethylamine and triethanolamine on resolution can be explained by a significant reduction in the electroosmotic flow, as shown in Table 2(c). By contrast, the resolution of the critical pair sulindac-indomethacin is incomplete with sodium and Tris, in accordance with the higher electroosmotic mobilities observed with these two counter-ions (cf Table 2c).

According to the data in Table 2, triethanolamine appears to be particularly well suited as a compound to adjust the buffer pH for the CZE separation of anionic analytes such as NSAIDs. This low mobility compound was selected as the cationic component of the buffer for the CZE analysis of basic drugs [13].

Buffer concentration

The influence of glycine concentration on

Table 2
Influence of the cationic component of the running buffer

(a) Peak symmetry (A_s)

Analyte	Sodium	Diethylamine	Triethylamine	Tris	Triethanolamine
Sulindac	—	0.61	0.45	1.00	0.96
Indomethacin	—	0.71	0.79	0.95	1.00
Piroxicam	0.62	0.72	0.65	1.10	1.10
Tiaprofenic acid	0.52	0.82	0.73	1.20	1.10

(b) Resolution (R_s)

Analyte pair	Sodium	Diethylamine	Triethylamine	Tris	Triethanolamine
1–2	0.7	1.5	1.5	1.3	1.7
2–3	3.2	3.8	3.0	4.5	4.3
3–4	2.8	5.6	8.2	4.9	7.5

(c) Electroosmotic mobility, μ_{eo} ($cm^2\ V^{-1}\ s^{-1} \times 10^{-5}$) and current, I (μA)

	Sodium	Diethylamine	Triethylamine	Tris	Triethanolamine
I (μA)	8.0	7.8	7.8	5.6	6.6
μ_{eo}	+80	+63	+55	+71	+53

Buffer: 75 mM glycine adjusted to pH 9.1; injection: hydrodynamic mode for 2 s. Analytes: 1, sulindac; 2, indomethacin; 3, piroxicam; 4, tiaprofenic acid.
Other conditions as described in text.

migration times and resolution was also investigated. In these experiments, the pH was kept constant by changing triethanolamine concentration in the same molar proportion. A significant increase in migration times and in resolution values was observed when the concentration of glycine (and triethanolamine) was increased. The effect on resolution is shown in Table 3: the increase in resolution with increasing buffer concentration is certainly related to the concomitant reduction of the electroosmotic flow, due to a decrease of the zeta potential across the capillary-solution interface [14, 15]. This favourable effect is particularly useful for the resolution of the critical pair sulindac-indomethacin, which is complete when the glycine concentration reaches 75 mM. Since the three pairs of NSAIDs studied here (cf Table 3) were baseline resolved with a 75 mM glycine solution, adjusted to pH 9.1 with 0.25 M triethanolamine, this buffer composition was selected for the analysis of NSAIDs by CZE.

Method validation

Figure 1 represents an electropherogram obtained under the conditions selected for the separation of the NSAIDs tested. Under these conditions, the five NSAIDs injected are completely separated in less than 6 min and appear on the electropherogram as highly efficient and symmetrical peaks. The baseline disturbance observed on the electropherogram at about 3.5 min corresponds to the electroosmotic break-

Table 3
Influence of glycine concentration on resolution of critical pairs

Glycine concentration (mM)	Sulindac–indomethacin	Indomethacin–piroxicam	Piroxicam–triprofenic acid
10	0	1.3	0.9
25	1.0	2.7	2.6
50	1.1	3.2	4.1
75	1.7	4.0	6.8
100	2.3	4.9	6.8

Buffer: 10–100 mM glycine adjusted to pH 9.1 with triethanolamine.
Other conditions as described in text.

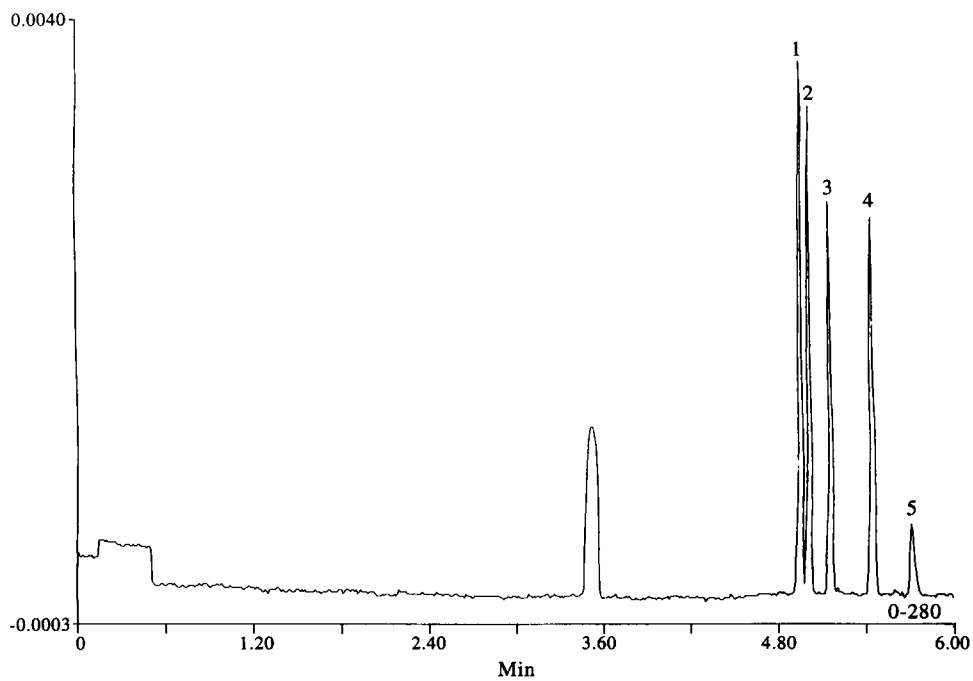

Figure 1
CZE separation of NSAIDs. Buffer: 75 mM glycine adjusted to pH 9.1 with triethanolamine. Detection at 280 nm. Other conditions as described in text. Key: 1, sulindac; 2, indomethacin; 3, piroxicam; 4, tiaprofenic acid; 5, alclofenac.

Table 4
Quantitative figures of merit

Sulindac	Tiaprofenic acid
1. Linearity*	
n = 5; 2–100 μg ml^{-1}	n = 5; 2–100 μg ml^{-1}
y = 672.9x − 105.2	y = 595.7x − 88.2
r^2 = 0.9999	r^2 = 0.9999
2. Precision (RSD%)	
(a) Migration times	
Repeatability (25 μg ml^{-1}; n = 5) 0.10%	Repeatability (25 μg ml^{-1}; n = 5) 0.11%
Reproducibility (25 μg ml^{-1}; n = 3) 1.70%	Reproducibility (25 μg ml^{-1}; n = 3) 1.90%
(b) Peak areas	
Repeatability (25 μg ml^{-1}; n = 5) 0.53%	Repeatability (25 μg ml^{-1}; n = 5) 0.40%
Reproducibility (25 μg ml^{-1}; n = 3) 2.30%	Reproducibility (25 μg ml^{-1}; n = 3) 2.80%
3. Limits of detection and limits of quantification	
LOD = 0.84 μg ml^{-1}; 2.3 × 10^{-6} M	LOD = 0.56 μg ml^{-1}; 2.1 × 10^{-6} M
12.2 pg; 3.4 × 10^{-14} mole	8.1 pg; 3.1 × 10^{-14} mole
LOQ = 2.8 μg ml^{-1}; 7.8 × 10^{-6} M	LOQ = 1.9 μg ml^{-1}; 7.1 × 10^{-6} M
40.5 pg; 1.1 × 10^{-13} mole	26.9 pg; 1.1 × 10^{-13} mole
4. Accuracy (±95% confidence limits)	
(6 μg ml^{-1}; n = 6)	(6 μg ml^{-1}; n = 6)
103.5 ± 3.93%	102.15 ± 3.65%

Conditions as described in text.
*Detection sensitivity at 280 nm: 0.009 AUFS.

through time. However, some groups of NSAIDs (piroxicam-carprofen; tiaprofenic acid-flufenamic acid-flurbiprofen) are not completely resolved in this CZE system.

The method developed was validated for quantitative performance, using sulindac and triaprofenic acid as test compounds (cf Table 4).

The linearity of the calibration graphs, constructed in the concentration range 2–100 μg ml^{-1}, is demonstrated by the high determination coefficients (r^2 >0.9999, n = 5) observed for the regression lines. Limits of detection (LOD) and limits of quantification (LOQ) calculated from the regression lines are respectively 0.84 and 2.79 μg ml^{-1} for sulindac, and 0.56 and 1.86 μg ml^{-1} for tiaprofenic acid, respectively, with an injection time of 5 s. A further improvement in LOD could be obtained by using longer injection times.

The precision of the method was evaluated by measuring the within-day (repeatability) and between-day variation (reproducibility) of migration times and peak areas for both analytes at a concentration of 25 μg ml^{-1}. Results presented in Table 4 show that the operating conditions selected provide a very stable electrophoretic system, with particularly good repeatability of migration times (RSD around 0.1%) and peak areas (RSD around 0.5%). These results were obtained using neither peak normalization nor internal standardization.

The accuracy was evaluated from a recovery experiment performed on six samples at a concentration of 6 μg ml^{-1}. Mean percentage recoveries are given in Table 4 together with 95% confidence intervals of the mean. The method was found to be accurate for both analytes.

Conclusions

A fast, efficient and reliable method for the CZE separation and determination of NSAIDs has been developed and validated, using a glycine-triethanolamine buffer (pH 9.1). The method has been subsequently applied to the quantitative analysis of some NSAIDs in different pharmaceutical formulations. It should also be applicable to the determination of NSAIDs in biological fluids. Moreover, recent studies made in the authors' laboratory indicate that further improvement in resolution

can be obtained in this CZE system by adding organic modifiers and complexing agents to the buffer.

Acknowledgement — The financial support of the Belgian National Fund for Scientific Research is gratefully acknowledged.

References

[1] J.W. Jorgenson and K.A. Lukacs, *Anal. Chem.* **53**, 1298–1302 (1981).
[2] J.W. Jorgenson and K.A. Lukacs, *J. Chromatogr.* **218**, 209–216 (1981).
[3] R. Weinberger and M. Albin, *J. Liquid Chromatogr.* **14**, 953–972 (1991).
[4] M.G. Donato, E. Van Den Eeckhout, W. Van Den Bossche and P. Sandra, *J. Pharm. Biomed. Anal.* **11**, 197–201 (1993).
[5] M.G. Donato, W. Baeyens, W. Van Den Bossche and P. Sandra, *J. Pharm. Biomed. Anal.* **12**, 21–26 (1994).
[6] C.W. Maboundou, G. Paintaud, M. Bérard and P.R. Bechtel, *J. Chromatogr. B.* **657**, 173–183 (1994).
[7] H. Nishi, N. Tsumagari and S. Terabe, *Anal. Chem.* **61**, 2434–2439 (1989).
[8] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Pharm. Sci.* **79**, 519–523 (1990).
[9] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.* **498**, 313–323 (1990).
[10] *The European Pharmacopoeia*, 2nd edn, Part I, V.6.20.4. Maisonneuve, Sainte-Ruffine, France (1987).
[11] F. Foret, S. Fanali, L. Ossicini and P. Boček, *J. Chromatogr.* **470**, 299–308 (1989).
[12] G.J. Bruin, A.C. van Asten, X. Xu and H. Poppe, *J. Chromatogr.* **608**, 97–107 (1992).
[13] I. Bechet, Ph. Paques, M. Fillet, Ph. Hubert and J. Crommen, *Electrophoresis* **15**, 818–823 (1994).
[14] K.D. Altria and C.F. Simpson, *Chromatographia* **24**, 527–532 (1987).
[15] H.J. Issaq, I.Z. Atamna, G.M. Muschik and G.M. Janini, *Chromatographia* **32**, 155–161 (1991).

[Received for review 22 November 1994; revised manuscript received 8 December 1994]