ELSEVIER

Journal of Chromatography A, 782 (1997) 289–295

JOURNAL OF CHROMATOGRAPHY A

Short communication

# Separation of quaternary ammonium herbicides by capillary electrophoresis with indirect UV detection

M.T. Galceran*, M.C. Carneiro, M. Diez, L. Puignou

*Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Barcelona, Spain*

Received 2 December 1996; received in revised form 24 April 1997; accepted 25 April 1997

**Abstract**

Suitability of various absorbent carrier electrolytes for the determination of quaternary ammonium ion herbicides ('quats') with indirect UV detection in capillary electrophoresis was investigated. Consideration of the electrophoretic mobility and the molar absorptivity of the chromophore, and the effective separation achieved, suggested that 1-(4-pyridyl)pyridinium chloride hydrochloride (PP) was the best electrolyte for the simultaneous detection of quats. The influence of pH, chromophore concentration, organic modifier, detection wavelength and ionic strength was investigated. The optimal conditions for separation were: 10 m*M* PP (pH 2.5), 10% methanol as carrier electrolyte, applied potential +20 kV, hydrodynamic injection 5 s and UV detection at 205 nm. Figures of merit such as detection limits and run-to-run and day-to-day reproducibility were established. The detection limits for the non-absorbent herbicides were 0.8 $\mu g\ ml^{-1}$. The method was successfully applied to the determination of chlormequat and mepiquat in spiked tap water although the dependence of the detection limits on the sample matrix was observed. © 1997 Elsevier Science B.V.

*Keywords:* Buffer composition; Water analysis; Pesticides; Quaternary ammonium compounds

## 1. Introduction

Quaternary ammonium herbicides ('quats') have been widely used in agriculture for more than ten years. The bipyridyldiylium herbicides, 1,1′-dimethyl-4-4′-bipyridinium (paraquat) and 6,7-dihydrodipyrido[1,2-*a*:2′,1′-*c*]pyrazinediium (diquat) salts are widely used in preharvest non-selective weed control [1]. The pirazolium monocation 1,2-dimethyl-3,5-diphenyl-1H-pyrazolium (difenzoquat) is a more selective herbicide [2,3]. The non-aromatic compounds, (2-chloroethyl)trimethylammonium (chlormequat) and 1,1-dimethylpiperidinium (mepiquat) salts are used as growth plant regulators [4]. Paraquat (PQ) and diquat (DQ) are extremely toxic and are often encountered in cases of accidental and intentional poisonings [5]. Both compounds are pesticides of major economic importance and have been classified as moderately hazardous [6] and have been included in a priority list of herbicides of potential concern in the Mediterranean region [7].

The analysis of quats is made difficult by their aqueous solubility and low volatility. Due to their cationic character, ion-pair high-performance liquid chromatography with direct UV detection is currently used for the simultaneous determination of paraquat, diquat and difenzoquat [8,9]. Capillary electrophoresis (CE), an analytical technique specially suitable for the analysis of ions [10,11], has also been proposed for the determination of the UV-absorbing

*Corresponding author.

0021-9673/97/$17.00 
*PII* S0021-9673(97)00507-4

quats [12,13]. For the determination of the non-absorbing compounds, ion chromatography or CE with conductimetric and potentiometric detection have been reported [4,14]. Fast atom bombardment mass spectrometry (FAB-MS) [15], liquid chromatography–mass spectrometry (LC–MS) [16,17] and recently, CE–MS [18,19] have also been proposed for the characterization of this group of herbicides. Nevertheless, indirect UV detection might be a relative simple and economic solution to the detection of non-absorbing analytes and it has been used to examine similar quaternary ammonium compounds [20,21].

Investigations into the application of indirect UV detection in CE published by Nielen [22] showed that several conditions have to be fulfilled. First, for optimal sensitivity the carrier electrolyte has to generate a high UV-absorption background at a wavelength of minimal absorption of the sample ions. Second, matching running electrolyte mobility with solute mobility is important in order to minimize peak-shape distortions. In this paper the suitability of an absorbing carrier electrolyte for the determination of the non-absorbing quats, chlormequat and mepiquat, and also, the simultaneous detection of the absorbing and non-absorbing compounds, was evaluated. The effect of the chromophore concentration, pH, the addition of an organic solvent and the ionic strength were studied, and the advantages and shortcomings of the procedure are discussed here.

## 2. Experimental

### 2.1. Instrumentation

The CE system was an Applied Biosystems (Foster City, CA, USA) Model 270 A with spectrophotometric detection. Electrophoretic data were processed with a Merck–Hitachi Model 2500 integrator. A fused-silica capillary (Supelco, Bellefonte, PA, USA), 72 cm (separation length 50 cm)×50 μm I.D., was used. The temperature was held at 30°C and the applied potential was +20 kV. Indirect UV detection was performed at 257 nm and at 205 nm. Electrokinetic (5 kV, 3 s) and hydrodynamic (by vacuum, 16.9 KPa, 5 s) injection modes were used. The conductivity was measured with a Radiometer conductivity meter CDM83 (Copenhagen, Denmark).

### 2.2. Chemicals

Reagents used for the preparation of buffer solutions were analytical grade; 1-(4-pyridyl)pyridinium chloride hydrochloride (PP) and N,N-methylphenazonium methyl sulphate (MPZ) were obtained from Fluka (Ronronkoma, NY, USA). 1,1′-Di-*n*-heptyl-4,4′-bipyridinium dichloride (HQ) was obtained from Tokio Kasei. Malachite green (MG) was obtained from Merck (Darmstadt, Germany) and *o*-nitroaniline (ONA) was obtained from Carlo Erba (Milan, Italy). Paraquat (PQ) (99%) was purchased from Riedel-de Haën (Seelze, Germany). Diquat (DQ) (97%), difenzoquat (DF) (98%) and mepiquat (MQ) from Chemservice (West Chester, PA, USA). Chlormequat (CQ) (98%) and the internal standard, ethyl viologen (EQ) (99%) from Aldrich (Milwaukee, WI, USA). Fig. 1 shows the chemical structures of the quats investigated in this paper. Water was purified using a Culligan (Barcelona, Spain) system. All solutions were passed through a 0.45-μm nylon filter before use.

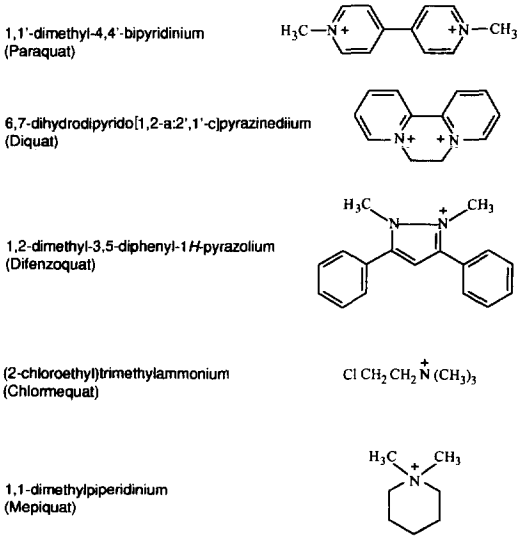


Fig. 1. Chemical structures of the quats.

### 2.3. Procedure

The carrier electrolyte solutions were prepared using 1–15 m*M* PP, MPZ, HQ, MG or ONA as chromophore. Sodium hydroxide (100 m*M*) was used for adjusting the pH of electrolyte solutions to 2.0–6.0. All standard quat solutions were prepared by dilution of 1000 μg ml$^{-1}$ stock solution of each quat. Mixed quat standards were prepared daily at a concentration ranging from 2–50 μg ml$^{-1}$. Before using a new carrier electrolyte solution, the electrophoretic system was conditioned by flushing the capillary with 0.1 *M* sodium hydroxide solution for 20 min, then rinsed with ultrapure water. The capillary was equilibrated with the carrier electrolyte for 120 min before the first run. After each run the capillary was washed with 0.01 *M* sodium hydroxide for 2 min and equilibrated with running buffer for 5 min. Water was used as a marker to measure electroosmotic flow. Apparent mobility ($\mu_{app}$) was related to the electroosmotic mobility ($\mu_{eo}$) of the carrier electrolyte and the electrophoretic mobility ($\mu_{ep}$) of the analyte ($\mu_{app}=\mu_{eo}+\mu_{ep}$). The migration time ($t_m$), which was measured directly from the electropherogram, was related to the apparent mobility by the following: $t_m=l/(\mu_{app}\ E)$; where $l$ was the separation length and $E$, the electric field.

The determination of quats in tap water spiked with 20–40 μg ml$^{-1}$ was performed using the optimal conditions established in this paper. The spiked tap water sample was diluted 1:3 with HPLC water prior to the analysis. The electrophoretic determination was carried out by external calibration using 10 m*M* PP (pH 2.5) with 10% methanol, as electrolyte solution, and hydrodynamic injection (5 s) of the water diluted sample. The applied potential was +20 kV, and detector wavelength was 205 nm.

## 3. Results and discussion

### 3.1. Characteristics of the chromophores

Various chromophores fully ionized at pH 2 were tested. Using *o*-nitroaniline and malachite green no responses were obtained for the quats due to the low apparent mobilities ($\leq 1.7\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$) of both chromophores in relation to the apparent mobilities of the compounds ($5.1\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$). The best results were obtained for PP, which gave higher responses and lower baseline noise. Positive peaks were obtained for the UV absorbing quats, PQ, DQ, DF and negative peaks for the non-absorbing quats, CQ and MQ.

### 3.2. Carrier electrolyte influence

To optimize the separation using PP as chromophore background electrolyte, the effect of its concentration between 1–15 m*M* was studied. An increase in the PP concentration produced a decrease in the electroosmotic flow which, at low concentrations, was in the direction of the cathode ($+0.33\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at 1 m*M* PP) and in the direction of the anode at concentrations higher than 4 m*M* ($-0.90\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at 15 m*M* PP). This reversal of direction could be due to the adsorption of the PP ions onto the silanol groups of the capillary silica surface, which produced a reduction of zeta potential reversing the electroosmotic flow. When increasing chromophore concentration, positive charged solutes appeared to interact rather less with the silanol groups of the capillary surface allowing us to obtain narrower peaks with a slightly higher electrophoretic mobility, thus, a carrier electrolyte containing 10 m*M* of PP was recommended.

On the other hand, an increase in pH brought the electroosmotic flow to more positive values. At low pH, the electroosmotic flow was towards the anode ($-0.59\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at pH 2.0) and, at pH higher than 4.3 a reversion occurred towards the cathode ($+0.25\cdot10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at pH 6.0). This may be related to a decrease in the protonation of the chromophore therefore decreasing its tendency to be adsorbed on the capillary wall, producing a change in both the magnitude and sign of the zeta potential. Losses in resolution between PQ and DQ, and CQ and MQ were observed at high pH values. The loss in resolution was mainly due to a zone broadening related to the adsorption of the cationic herbicides on the silanol groups of the silica. The best results were obtained when adjusting the pH at 2.5.

In addition, the effect of adding methanol to the background electrolyte, 10 m*M* PP at pH 2.5, was studied. For all the compounds, apparent and electrophoretic mobilities decreased when methanol con-

centration increased. The same result was reported for decreased electroosmotic flow, which always appeared in the anodic sense. This behaviour could be related to a decrease in the dielectric constant of the running electrolyte. The addition of 10% methanol improved the separation.

### 3.3. Wavelength effect

Using PP as chromophore, negative responses were obtained for the non-absorbing compounds CQ and MQ and also for the system peak which was generated in the injection plug due to the introduction of discontinuities in the composition of the background electrolyte [23]. Fig. 2 shows the electropherograms obtained at the optimal conditions, 10 m*M* PP (pH 2.5) and 10% methanol, at two different wavelengths, 205 nm and 257 nm. Significant differences can be seen in the electropherograms obtained at both wavelengths. For PQ, EQ and DF the peaks were always positive but for DQ, a positive peak was obtained at 205 nm whereas a negative peak was observed when the wavelength was changed to 257 nm. Negative peaks were due to the variation of the background electrolyte absorbance which depended on molar absorptivities and mobilities of the analytes and the chromophore. For DQ, according to the detection wavelengths a positive or negative peak appeared which could be related to the differences in molar absorptivities between DQ and PP at both wavelengths. At 205 nm the DQ molar absorptivity was higher (DQ:15 952, PP:13 886) and the peak became positive, at 257 nm the molar absorptivity was lower (DQ:2963, PP:17 088) and the peak became negative.

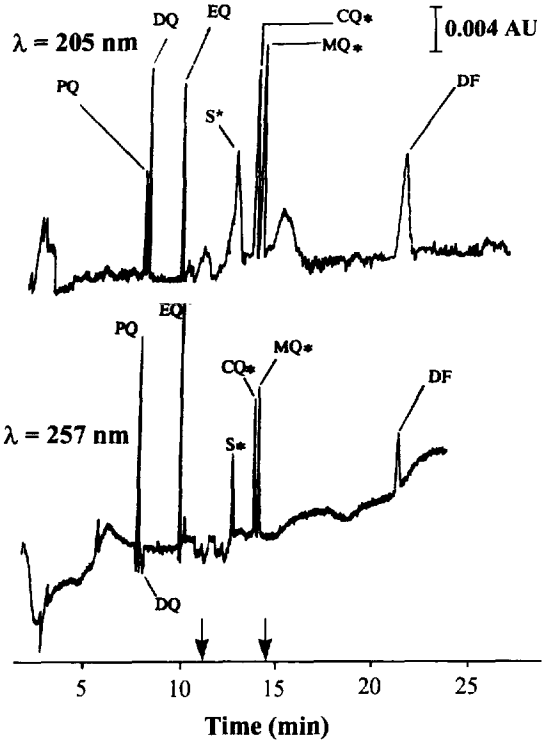


Fig. 2. Electropherograms obtained under optimal conditions, 10 m*M* PP (pH 2.5) 10% MeOH. Applied potential, +20 kV; hydrodynamic injection, 5 s, at different detection wavelength. Standard solution: PQ, 19.6 μg ml$^{-1}$; DQ, 37.3 μg ml$^{-1}$; EQ, 38.0 μg ml$^{-1}$; CQ, 25.2 μg ml$^{-1}$; MQ, 25.5 μg ml$^{-1}$; DF, 27.6 μg ml$^{-1}$. * Negative peak (the arrows indicate a data acquisition interval time during which a negative peak is reversed and plotted as a positive peak).

### 3.4. Sample salinity effect

The effect of sample salinity in the determination of quats by CE and indirect UV detection with hydrodynamic injection was studied. As can be seen from Fig. 3, where the electropherograms obtained for a quat standard solution in HPLC water with increasing amounts of sodium chloride from 0–100 m*M* are given, an important increase in the negative signal of the chromophore with the sample salinity occurred. This can be related to an increase in the conductivity of the injected plug, preventing the migration of the chromophore to the zone. The increase of the conductivity of the sample could also explain the diminished analyte responses, because a decrease in the preconcentration effect in the injected plug occurred. This effect was less important for CQ and MQ. The different behaviour of these compounds, which migrated immediately behind the chromophore might be attributed, according to Beckers [23], to a positive interaction between the chromophore and the peaks.

### 3.5. Application

Quality parameters under optimal conditions using indirect UV detection at 205 nm and both electrokinetic (EK) and hydrodynamic (HD) injection

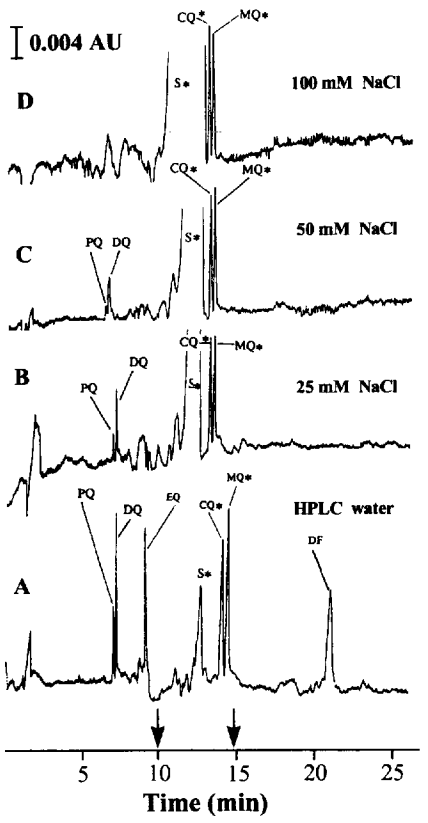


Fig. 3. Electropherograms of a standard solution with increasing amounts of sodium chloride. Electrolyte solution, 10 m*M* PP (pH 2.5) 10% MeOH. Applied potential, +20 kV; $\lambda$=205 nm. Hydrodynamic injection, 5 s. Standard solution as in Fig. 2. * Negative peak (the arrows indicate a data acquisition interval time during which a negative peak is reversed and plotted as a positive peak).

modes are given in Table 1. Detection limits (LODs), based on a signal-to-noise ratio of 3:1, ranging from 0.1–2 $\mu g\ ml^{-1}$ were obtained. The non-absorbing compounds, CQ and MQ, gave low values, 0.8 $\mu g\ ml^{-1}$, using hydrodynamic injection. The LODs for the non-absorbing quats were lower than those (5–8 $\mu g\ ml^{-1}$) reported in the literature for chlormequat [14,20,21]. For the absorbing compounds, direct UV detection provides better detection limits [8], so, the determination of quats, at low concentrations, using indirect UV detection can only be applied to the non-absorbing compounds. Eight replicate determinations of 6 $\mu g\ ml^{-1}$ standard solution of each compound were carried out under optimal conditions to determine run-to-run reproducibilities. Relative standard deviations (R.S.D.) based on the peak area in the range of 4–12% were obtained. In order to test the day-to-day reproducibilities, four replicate determinations of the same standard solution were carried out on four different days. The results for areas ranged from 5–8% for hydrodynamic injection and from 8–17% for electrokinetic injection (Table 1).

Sample conductivity showed a significant impact on the results obtained with CE and indirect UV detection even though hydrodynamic injection was used. To show the applicability of the method to the routine analysis of real samples, spiked tap water was analyzed. Fig. 4 shows the electropherograms obtained for a tap water sample spiked with the quats. As can be seen, the quats in the water samples (Fig. 4A) were not detected, nevertheless a dilution 1 to 3 of the sample, which produced a decrease in the

Table 1
Quality parameters

| Quat | LOD ($\mu g\ ml^{-1}$) | | | Run-to-run reproducibility R.S.D. (%), $n$=8: | | Day-to-day reproducibility R.S.D. (%), $n$=4: | |
|---|---|---|---|---|---|---|---|
| | HPLC water | | Tap water | HPLC water | | HPLC water | |
| | EK | HD | HD | EK | HD | EK | HD |
| PQ | 0.5 | 2.0 | 5.0 | 10 | 4 | 12 | 6 |
| DQ | 0.1 | 1.5 | 5.1 | 8 | 4 | 12 | 5 |
| CQ | 0.1 | 0.8 | 2.8 | 6 | 5 | 8 | 6 |
| MQ | 0.1 | 0.8 | 2.5 | 8 | 6 | 15 | 8 |
| DF | 1.0 | 1.0 | 6.9 | 12 | 6 | 17 | 7 |

Experimental conditions: 10 m*M* PP (pH 2.5) 10% methanol; +20 kV; $\lambda$=205 nm, hydrodynamic injection: 5 s, electrokinetic injection: 3 s.

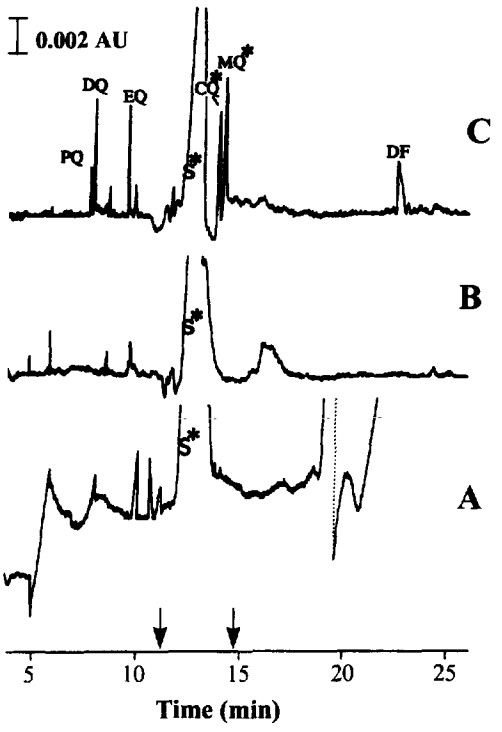


Fig. 4. Electropherograms of tap water. (A) Spiked tap water without dilution: PQ, 19.2 μg ml$^{-1}$; DQ, 37.4 μg ml$^{-1}$; EQ, 38.0 μg ml$^{-1}$; CQ, 25.2 μg ml$^{-1}$; MQ, 25.4 μg ml$^{-1}$ and DF, 27.2 μg ml$^{-1}$. (B) Tap water diluted 1:3. (C) Spiked tap water diluted 1:3. Electrolyte solution: 10 m*M* PP (pH 2.5) 10% MeOH. Applied potential, +20 kV; $\lambda$=205 nm. Hydrodynamic injection, 5 s. * Negative peak (the arrows indicate a data acquisition interval time during which a negative peak is reversed and plotted as a positive peak).

conductivity to about 200 μS cm$^{-1}$, allowed the detection of all the compounds (Fig. 4C). To study the matrix effect on the LODs, tap water was spiked at different concentration levels, and the LODs, based on a single-to-noise ratio of 3:1, were determined (Table 1). The increase in LODs can be partially related to the required dilution of the sample. Nevertheless, the values obtained for the non-absorbing compounds were lower than the values reported in the literature. Only CE–MS showed lower detection limits using electrokinetic injection, although poor reproducibility and linearity were obtained [18]. The procedure established in this paper has been applied in the determination of CQ and MQ in tap water spiked with 25 μg ml$^{-1}$ of each compound giving R.S.D. values ranging from 5–9%. To improve detection limits and prevent matrix effects a clean-up step would be necessary. Nevertheless, the results obtained in this paper show that the method can be used for the analysis of these compounds in environmental water samples.

## 4. Conclusions

The use of PP acting as both carrier electrolyte and detection probe with CE led to an efficient separation of quats, permitting the indirect UV detection of the non-absorbing herbicides chlormequat and mepiquat. Appropriate transfer ratios together with molar absorptivities of the ions involved at 205 nm, allowed a suitable response for the simultaneous detection of the absorbing and non-absorbing quats. The detection limits in HPLC water solution for CQ and MQ, using hydrodynamic injection, were 0.8 μg ml$^{-1}$. However, when tentative analysis in spiked tap water was performed, the effect of the sample matrix produced an unavoidable increase in the detection limits because a dilution 1:3 of the sample was required. In spite of the low sensitivity of the method, this procedure was appropriate for the simple, low cost determination of the non-absorbing quats. Clean-up procedures appropriate for CE analysis are currently being developed to improve the sensitivity of the method for the analysis of complex matrices such as river water samples.

## Acknowledgments

This work was partially financed by the Comissionat per Universitats i Recerca de la Generalitat de Catalunya (contract number 1994SGR01028).

## References

[1] The Agrochemical Handbook, Royal Society of Chemistry, 3rd ed., Cambridge, 1992.
[2] C. Barry, R.K. Pike, J. Assoc. Off. Anal. Chem. 63 (1980) 647.
[3] I. Ahmad, Anal. Lett. 15(A1) (1982) 27.

[4] A. Fegert, U. Schepers, B. Schwarz, Fresenius' J. Anal. Chem. 339 (1991) 441.
[5] G.R. Sagar, Hum. Toxicol. 6 (1987) 7.
[6] G. Ekström, M. Akerblom, Rev. Environ. Contam. Toxicol. 114 (1990) 23.
[7] G. Barceló, in D. Barceló (Editor), Environmental Analysis—Techniques, Applications and Quality Assurance, Elsevier, Amsterdam, 1993, Ch. 5, p. 149.
[8] M.C. Carneiro, L. Puignou, M.T. Galceran, J. Chromatogr. A 669 (1994) 217.
[9] M. Ibañez, Y. Picó, J. Mañes, J. Chromatogr. A 728 (1996) 325.
[10] P. Jandik, W.R. Jones, J. Chromatogr. 546 (1991) 431.
[11] H. Salimimoosavi, R.M. Cassidy, Anal. Chem. 67 (1995) 1067.
[12] Y.Y. Wigfield, K.A. McCormack, R. Grant, J. Agric. Food Chem. 41 (1993) 2315.
[13] M.T. Galceran, M.C. Carneiro, L. Puignou, Chromatographia 39 (1994) 581.
[14] J. Koleček, V. Říha, K. Vytřas, Anal. Chim. Acta 273 (1993) 431.
[15] Y. Tondeur, G.W. Sovocool, R.K. Mitchum, V.J. Niederhut, J.R. Donnelly, Bio. Environ. Mass Spectrom. 14 (1987) 733.
[16] D. Barceló, G. Durand, R.J. Vreeken, J. Chromatogr. 647 (1993) 271.
[17] M. Yoshida, T. Watabiki, T. Tokiyasu, N. Ishida, J. Chromatogr. 628 (1993) 235.
[18] E. Moyano, D.E. Games, M.T. Galceran, Rapid Commun. Mass Spectrom. 10 (1996) 1379.
[19] X.B. Song, W.L. Budde, J. Am. Soc. Mass Spectrom. 7 (1996) 981.
[20] J.R. Larson, C.D. Pfeiffer, Anal. Chem. 55 (1983) 393.
[21] D. Vicherley, M.E. Rose, K. Giles, T.M. Hutton, D.A. Rimmer, J. Chromatogr. A 734 (1996) 339.
[22] M.W.F. Nielen, J. Chromatogr. 542 (1991) 173.
[23] J.L. Beckers, J. Chromatogr. A 662 (1994) 153.