Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 984 (2003) 153–158
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Comparison of separation conditions and ionization methods for the liquid chromatography–mass spectrometric determination of sulfonamides

Dal-Ho Kim[a,*], Dai Woon Lee[b]

[a]*Organic Analysis Laboratory, Division of Chemical Metrology and Materials Evaluation, Korea Research Institute of standards and Science, Yusong, Daejon 305-600, Republic of Korea*
[b]*Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea*

Received 24 July 2002; received in revised form 10 September 2002; accepted 28 October 2002

Abstract

The effects of the type and concentration of buffer, composition of the mobile phase and the ionization mode, used for the separation and detection of sulfonamides with LC–MS, were studied. Five typical sulfonamides were selected as target compounds and beef meat was selected as a matrix sample. For the separation of sulfonamides, 0.05 *M* NH_4Ac in 13–15% aqueous acetonitrile, APCI ionization was more effective than ESI with regard to separation efficiency and the detection sensitivity.

Keywords: Ionization methods; Mobile phase composition; Sulfonamides

1. Introduction

Sulfonamides are widely used as veterinary drugs for the treatment of infections and the promotion of growth of livestock and fish [1–6]. Sulfonamide residues in food are an important concern, due to the possibility of risk to human health, such as resistance development and toxicity [2,6]. Many countries, including Korea, have established maximum residue limits (MRLs) of 100 ng/g for most sulfonamides in edible animal tissues and 10 ng/ml in milk [3–5,7].

A number of LC–MS methods have been developed for the analysis of sulfonamide residues in meat [2,4–7], fish [3,8], milk [9–11], egg [12,13], urine [14], and animal manure [15]. Most of the LC–MS methods have been used various separation conditions and ionization methods. The separation and ionization conditions affect the precision, accuracy and sensitivity of sulfonamide analysis. In relation to separation, Ito et al. [6] described optimization of a mobile phase consisting of acetonitrile, methanol, and aqueous formic acid solution. In general, most of the present LC–MS methods were focused on application to different matrices and identification of sulfonamides using MS–MS.

This report focused on the comparisons of separation conditions and ionization methods for sulfonamides in LC–MS. The separation efficiency and the detection sensitivity of LC–MS were examined

*Corresponding author. Fax: +82-42-868-5042.
E-mail address: dhkim@kriss.re.kr (D.-H. Kim).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01773-9

by changing the kind of buffer, the concentration of the buffer, the composition of LC mobile phase, and the ionization method (APCI, ESI).

2. Experimental

2.1. Chemicals

The sulfonamide standards were purchased from Sigma (sulfamethazine, SMZ, S-5632; sulfadimethoxine, SDM, S-7007; sulfathiazole, STZ, S-9876; sulfadiazine, SDZ, S-8626; sulfamethizole, SMTZ, S-6256; Sigma, St. Louis, MO, USA). Sulfapyridine (SP, S-6252, 99%, Sigma), and isotopic enriched sulfamethazine (-phenyl-$^{13}C_6$, atomic purity 90%, CLM-3045, $^{13}C_6$-SMZ; Cambridge Isotope Laboratories, 50 Frontage Road, MA, USA) were used as an internal standard. Acetic acid (HAc, 99.7%; Showa, Tokyo, Japan), ammonium acetate (NH_4AC, 97%; Aldrich, St. Louis, MO, USA), formic acid (FA, 96%; Aldrich), trifluoroacetic acid (TFA, 99+%; Aldrich), sodium phosphate monobasic (NaH_2PO_4, Merck, Darmstadt, Germany) were used as buffers for the HPLC solvent, or for the pH control for samples. Acetonitrile was pesticide grade (Burdick & Jackson, Muskegon, MI, USA). For SPE, LiChrolut EN (200 mg, Merck) was used.

2.2. Equipment

A chromatograph equipped with a HP 1050 autosampler and pump (Hewlett-Packard, Washington, DC, USA) was used. Phenomenex ODS2 (250× 2.5×5 μm; Phenomenex, CA, USA) was used as a stationary phase. Samples were separated in an isocratic condition. The HPLC conditions were as follows: volume injected: 2 μl; column pressure: 600 p.s.i.; temperature: 25 °C; and flow-rate: 200 μl/min.

A Finnigan LCQ iontrap LC–MS system (Finnigan, San Jose, CA, USA), equipped with an atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) source, was used. The LC–MS system was operated at a high resolution MS scan (Zoom Scan) and a positive-ion mode. In this modes LCQ conducts a high-resolution scan of 10 a.m.u. width, so the protonated positive ions of sulfonamides ($[M+H]^+$; SDZ, m/z=251; STZ, 256; SMTZ, 271; SP, 250; SMZ, 279; $^{13}C_6$-SMZ, 285; SDM, 311) were scanned within a 10-u window. The windows were programmed for the acquisition of different compounds. APCI conditions were: sheath gas flow-rate: 50 (the arbitrary value of LCQ instrument); vaporizer temperature: 450 °C; discharge voltage: 5.5 kV; tube lens offset voltage: 25 V; capillary temperature: 150 °C; and capillary voltage: 10 V. For the ESI experiment: sheath gas flow-rate: 50; spray voltage: 5.5 kV; tube lens offset voltage: 20 V; capillary temperature: 200 °C; and capillary voltage: 20 V.

A high-speed blender (MC1-12–37 ml; Waring, New Hartford, CT, USA) was used for the extraction of sulfonamides in meat.

For the filtration of sample extract or final sample solution, 2.0 μm membrane filter (47 mm, Zefluor; Pall Gelman Lab, MI, USA) and 0.2 μm syringe filter (4 mm, nylon; Whatman, NJ, USA) was used.

2.3. Sample preparation

As a sample matrix, beef meat was purchased from the market and preserved at −20 °C until use. For the preparation of sample, 10 g of pre-ground meat was weighed in a 20-ml bottle, and spiked with five sulfonamides (concentration level of each sulfonamide was 75 ng/g), and internal standards. The sample was transferred into a mini-container of a high-speed blender, and extracted with 20 ml of acetonitrile and 2 g of sodium phosphate for 2 min. The extract was filtered with suction through a 2.0-μm membrane filter. The volume of the filtrate was reduced to 5 ml by reduced pressure rotary evaporation at 40 °C. The Lichrolut EN SPE cartridge was preconditioned with 15 ml of acetonitrile and water. Into the concentrated solution, 100 ml of pure water was added and passed through an SPE cartridge with a flow-rate of 3 ml/min. The SPE cartridges, washed with 2 ml of pure water and enriched sulfonamides were eluted using 20 ml of acetonitrile. Then the solvent was removed to dryness by rotary evaporation. The residue was reconstituted with 250 μl of HPLC eluent and filtered through a 0.2-μm syringe filter to a 300-μl vial insert (part no. 5181-1270; Agilent, Wilmington, DE, USA) for a 2-ml autosampler vial.

3. Results and discussion

The variation of retention time of sulfonamides on ODS2 column by the type of buffer is shown in Fig. 1. When using ammonium acetate as a buffer, most of the compounds show good resolution. In the case of using acetic acid (HAc), formic acid (FA) and trifluoroacetic acid (TFA) as a buffer, the elution time of SDM and SMTZ increased dramatically, on the other hand resolution between compounds decreased. This can be explained as follows. The pK_a values of HAc, FA, and TFA were 4.76, 3.75 and 0.3, respectively. These buffers suppress deprotonation of sulfonamides (pK_a 5.4 (SMTZ) ~7.4 (SMZ)), thus, the interaction between the C_{18} stationary phase and SDM or SMTZ increases relative to other sulfonamides, because these compounds have two or one methyl group, respectively. On the other hand ammonium acetate (pH 6–7) promotes deprotonation, as results the polar interaction between mobile phase is superior to nonpolar interaction between the stationary phase and the methyl group. As the concentrations of HAc or NH_4Ac were rose (HAc, 0.07–0.6 *M*; NH_4Ac, 0.01–0.085 *M*), retention time was decreased, and the improvement in resolution was not significant (Figs. 2 and 3).

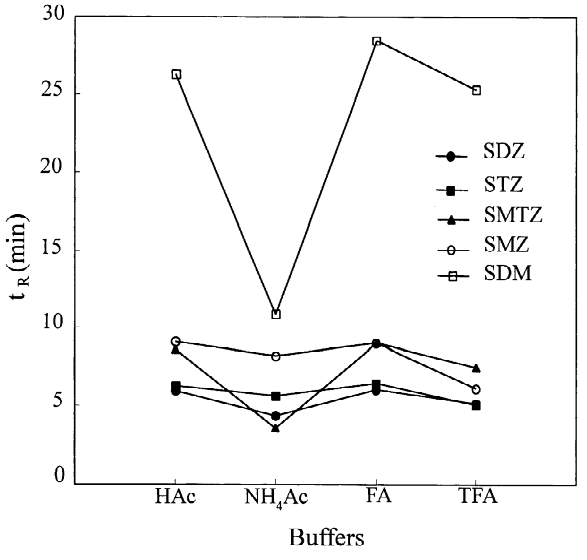

Fig. 1. Variation of retention time of sulfonamides with different buffers (column: ODS2; mobile phase: 20% aqueous acetonitrile, LC–ESI–MS, 5 μg/g standard solution). Additive 1: 0.15 *M* acetic acid; additive 2: 0.05 *M* ammonium acetate; additive 3: 0.03 *M* formic acid; additive 4: 0.01 *M* trifluoroacetic acid.

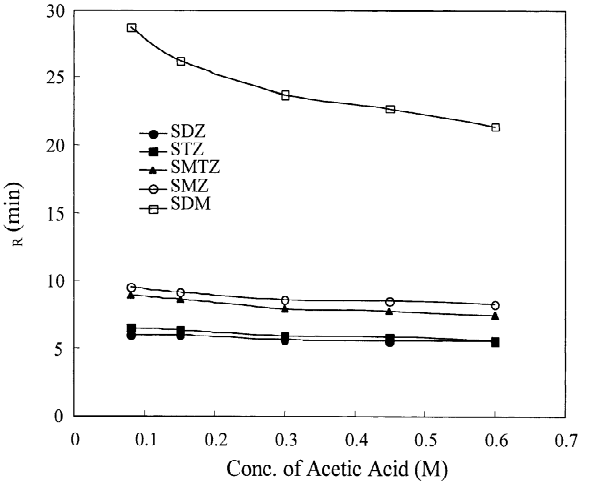

Fig. 2. Variation of retention time of sulfonamides with the concentration of acetic acid in mobile phase (ODS2, 20% aqueous acetonitrile, LC–ESI–MS, 5 μg/g standard solution).

The effect of the ionization method and solvent composition on the separation of LC–MS or response was compared at a constant concentration of NH_4Ac (0.05 *M*) and HAc (0.3 *M*) (Table 1). LC–MS shows an optimum separation by using

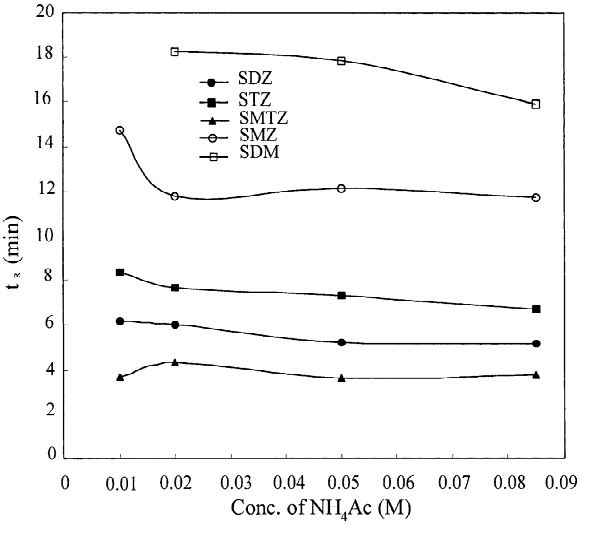

Fig. 3. Variation of retention time of sulfonamides with the concentration of ammonium acetate in mobile phase (ODS2, 15% aqueous acetonitrile, LC–APCI–MS, 5 μg/g standard solution).

Table 1
Effect of experimental conditions on the separation of sulfonamides and on the response of LC–MS

Ionization mode	Acetonitrile (%)[c]	SDZ		STZ		SMTZ		SMZ		SDM	
		t_r (min)	Response[d]	t_r (min)	Response	t_r (min)	Response	t_r (min)	Response	t_r (min)	Response
APCI[a]	10	5.8	730	9.2	650	6.1	380	16.1	910	20.4	610
	15	5.2	920	7.5	800	3.7	510	12.6	1100	18.8	800
	20	4.4	950	5.7	890	3.5	560	8.2	1200	10.9	920
	25	3.8	990	4.3	890	3.2	570	6.2	1200	7.2	920
ESI[a]	15	5.2	26	7.5	40	3.7	20	12.6	270	18.8	190
	20	4.4	16	5.7	29	3.5	11	8.2	200	10.9	150
	25	3.8	15	4.3	30	3.2	12	6.2	190	7.2	150
ESI [b]	10	9.6	490	11.7	720	20.8	510	20.8	1300	–	–
	15	7.0	480	7.9	740	11.8	550	12.4	1400	–	–
	20	5.6	500	5.9	810	7.9	570	8.6	1400	23.8	1400
	25	4.8	590	4.9	960	6.1	700	6.7	1500	13.9	1300
	30	4.4	710	4.4	1200	5.2	760	5.8	1600	9.8	1500

Sample concentration, injection volume and column: 5 μg/g, 5 μl, ODS2, respectively. SDZ, sulfadiazine; STZ, sulfathiazole; SMTZ sulfamethizole; SMZ, sulfamethazine; SDM, sulfadimethoxine

[a] Ammonium acetate was added to the mobile phase in the concentration of 0.05 *M*.

[b] Acetic acid was added to the mobile phase in the concentration of 0.3 *M*.

[c] Aqueous acetonitrile solvent.

[d] Peak area/10000 (n=2).

NH_4Ac instead of HAc. The influence of acetonitrile composition on the retention of sulfonamide was severe. When using HAc as a buffer, the acetonitrile composition of 10–15% could not elute SDM. On the other hand, acetonitrile composition of 20–30% could not separate all compounds (Table 1, Fig. 4B). As shown in Fig. 4C, gradient elution can improve separation when using HAc as a buffer, but the change of solvent composition during elution influences the response of LC–MS.

As the acetonitrile composition in the aqueous solvent rose from 10 to 20 (or 30%), the responses of LC–MS were slightly increased. This can be supposed as a result of the easy desolvation by more volatile acetonitrile. When using NH_4Ac as a buffer, APCI shows a greater response instead of ESI. On the other hand, ESI shows greater response by using HAc instead of NH_4Ac. These results can be explained by the ionization mechanism of the ESI and APCI. In the ESI mode, protonated sulfonamide ions can be easily formed in solution under more acidic conditions. On the other hand, in the APCI mode, H_3O^+ and NH_4^+ ions can be made from solvent, buffer, and nitrogen cations, which are produced by corona discharge to N_2 gas; thus, the acidic H_3O^+ and NH_4^+ ions in the gas phase easily protonate the sulfonamides. As the concentrations of NH_4Ac rose from 0.01 to 0.085 *M*, the response of LC–APCI–MS slightly decreased to 0.05 *M*, then slightly increased to 0.085 *M*, but the improvement of response was not significant.

On ESI, for removing ionization suppression phenomenon by residual interference [16,17], the more separation power of LC–MS is needed so the utilization of APCI and NH_4Ac buffer system may have more advantages than ESI with regard to separation and sensitivity.

As shown in Fig. 4, when the compositions of the acetonitrile in eluent were below 20% (Fig. 2A and C), the peaks of some sulfonamides were broad. This can be explained as intracolumn band broadening by the diffusion of the sample band, because the standard mixture of five sulfonamides in 100% acetonitrile was injected to the ODS2 column. This unwanted phenomenon could be resolved by using the same solvent with eluent to make a standard solution and sample solution (Fig. 5).

To demonstrate the separation of sulfonamides in real world sample, sulfonamides and internal standards spiked in meat sample were extracted using

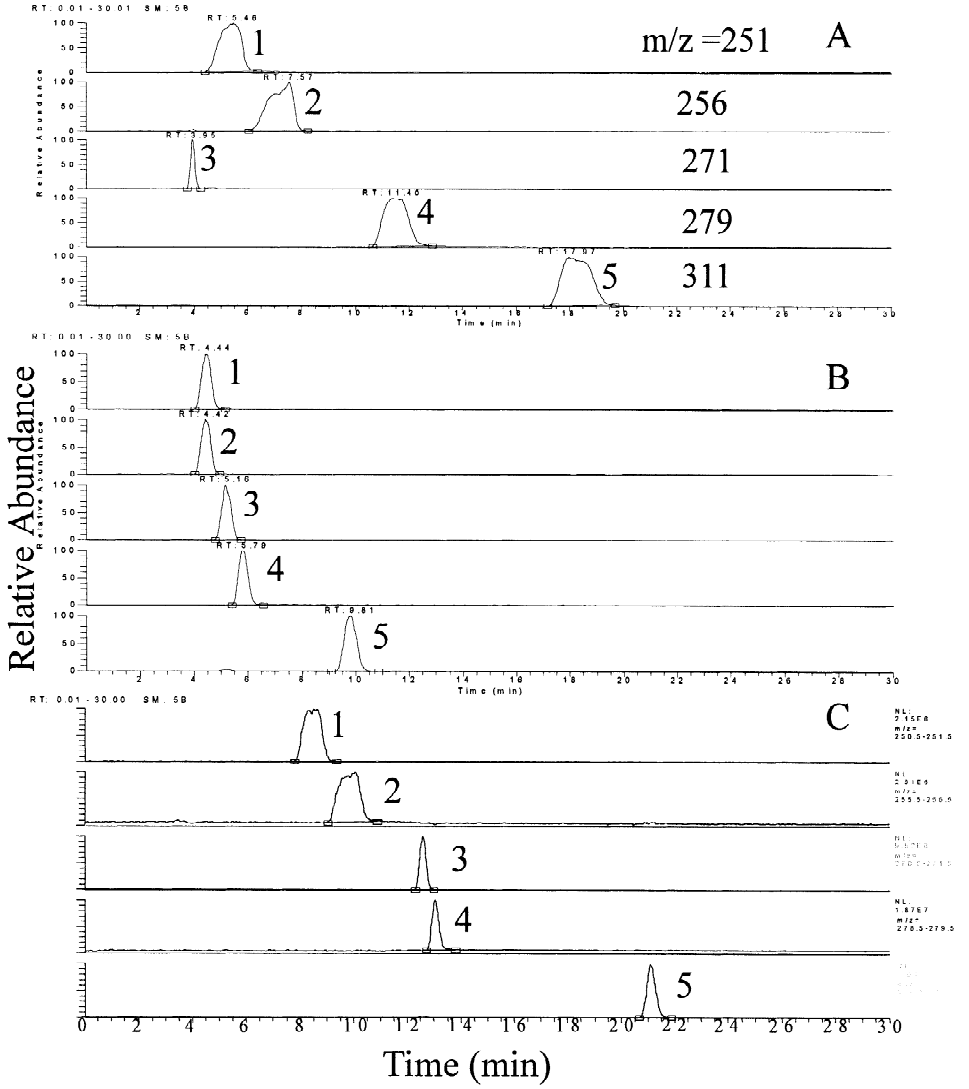

Fig. 4. Extracted ion chromatograms of LC–MS for standard solution (2 μg/g) obtained with three different mobile phases. (A) CH_3CN–water–aqueous 0.1 *M* ammonium acetate (15:35:50, v/v), APCI. (B) CH_3CN–water–aqueous 0.7 *M* acetic acid (30:20:50, v/v), ESI. (C) Gradient from 10:90 to 25:75 (CH_3CN–aqueous 0.7 *M* acetic acid, v/v) for 3 min and isocratic with 25:75, ESI. (1) Sulfadiazine (SDZ), (2) sulfathiazole (STZ), (3) sulfamethizole (SMTZ), (4) sulfamethazine (SMZ), (5) sulfadimethoxine (SDM).

acetonitrile and the extract was cleaned-up and enriched by SPE. The LC–APCI–MS extracted ion chromatogram of the standard solution and the sample solution of meat separated on ODS2 column are shown in Fig. 5. For the separation of some residual interferences, mobile phase composition, slightly changed to acetonitrile–water–0.1 *M* aqueous ammonium acetate (13:37:50, v/v) was used.

4. Conclusions

In this study, separation conditions and ionization methods were explored and compared. When using ODS2 column, 0.05 *M* NH_4Ac in 13–15% aqueous acetonitrile showed best separation. The APCI and NH_4Ac system showed more advantages than ESI and any of the other buffer systems with regard to

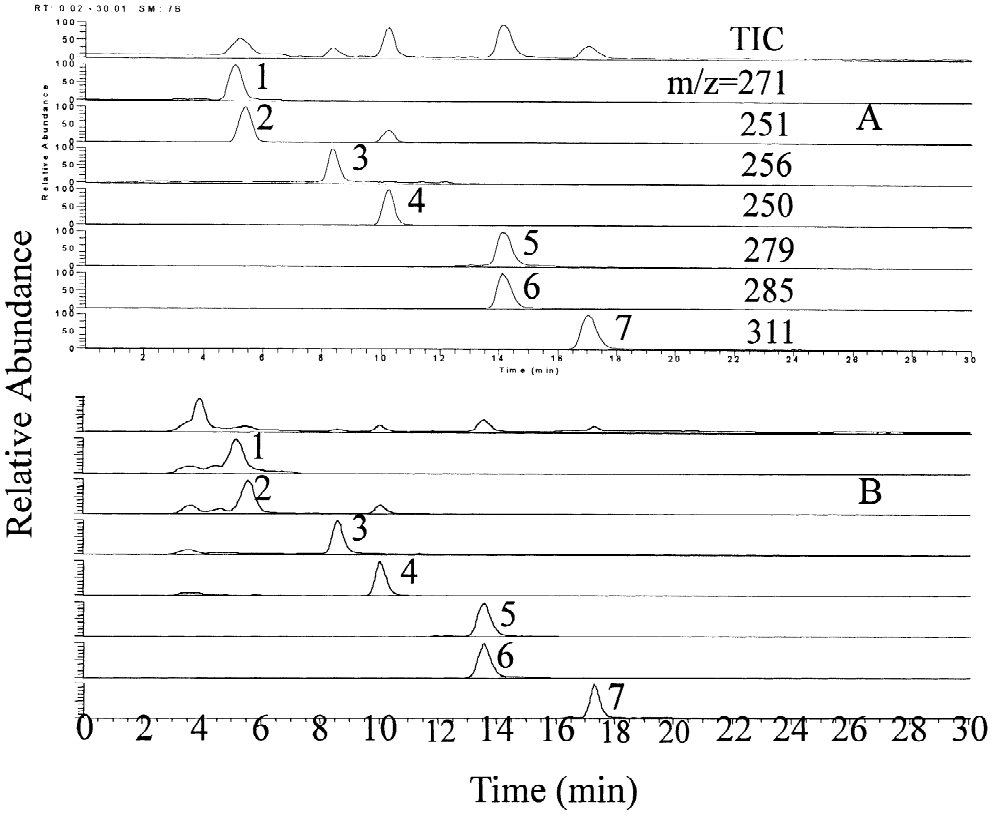

Fig. 5. Extracted ion chromatograms of LC–APCI–MS for standard solution (1 μg/g) and sample solution of meat. (A) Standard solution and (B) meat sample separated using ODS2. (1) Sulfamethizole (SMTZ), (2) sulfadiazine (SDZ), (3) sulfathiazole (STZ), (4) sulfapyridine (SP, ISTD), (5) sulfamethazine (SMZ), (6) $^{13}C_6$-sulfamethazine ($^{13}C_6$-SMZ), (7) sulfadimethoxine (SDM).

separation and detection. The combination of SPE and LC–APCI–MS can provide promising results for the separation and detection of sulfonamides from biological matrices.

References

[1] D.G. Kennedy, R.J.A. McCracken, A. Cannavan, S.A. Hewitt, J. Chromatogr. A 812 (1998) 77.
[2] S. Poter, Analyst 119 (1994) 2753.
[3] T.A. Gehring, L.G. Rushing, M.I. Churchwell, D.R. Doerge, K.M. McErlane, H.C. Thompson, J. Agric. Food Chem. 44 (1996) 3164.
[4] J.O. Boison, L.J.-Y. Keng, J. AOAC Int. 78 (1995) 651.
[5] G. Balizs, L. Benesch-Girke, S. Borner, S.A. Hewitt, J. Chromatogr. B 661 (1994) 75.
[6] Y. Ito, H. Oka, Y. Ikai, H. Matsumoto, Y. Miyazaki, H. Nagase, J. Chromatogr. A 898 (2000) 95.
[7] G.K. Kristiansen, R. Brock, G. Bojesen, Anal. Chem. 66 (1994) 3253.
[8] S. Pleasance, P. Blay, M.A. Quilliam, G. O'hara, J. Chromatogr. 558 (1991) 155.
[9] D.A. Volmer, Rapid Commun. Mass Spectrom. 10 (1996) 1615.
[10] D.R. Doerge, S. Bajic, S. Lowes, Rapid Commun. Mass Spectrom. 7 (1993) 1126.
[11] J. Abian, M.I. Chruchwell, W.A. Korfmacher, J. Chromatogr. 629 (1993) 267.
[12] D.N. Heller, M.A. Ngoh, D. Donoghue, L. Podhorniak, H. Righter, M.H. Thomas, J. Chromatogr. B 774 (2002) 39.
[13] J.A. Tarbin, P. Clarke, G. Shearer, J. Chromatogr. B 729 (1999) 127.
[14] G. Bartolucci, G. Pieraccini, F. Villanelli, G. Moneti, A. Triolo, Rapid Commun. Mass Spectrom. 14 (2000) 967.
[15] M.Y. Haller, S.R. Muller, C.S. McArdell, A.C. Alder, M.J.-F. Suter, J. Chromatogr. A 952 (2002) 111.
[16] J. Henion, E. Brewer, G. Rule, Anal. Chem. 70 (19) (1998) 650A.
[17] J.A. van Rhijn, J.J.P. Lasaroms, B.J.A. Berendsen, U.A.Th. Brinkman, J. Chromatogr. A 960 (2002) 121.