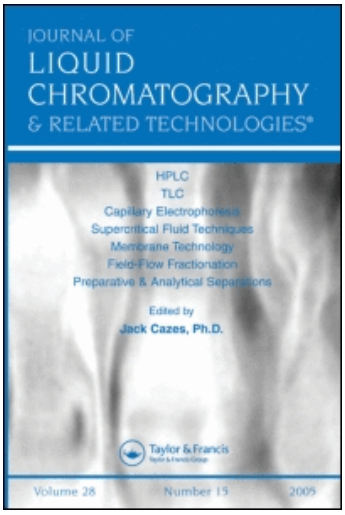

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Aliphatic Amines in Water Using Derivatization with Fluorescein Isothiocyanate and Capillary Electrophoresis/Laser-Induced Fluorescence Detection

W. C. Brumley[a]; V. Kelliher[a]

[a] U.S. Environmental Protection Agency National Exposure Research Laboratory Environmental Sciences Division, Las Vegas, NV

To cite this Article Brumley, W. C. and Kelliher, V.(1997) 'Determination of Aliphatic Amines in Water Using Derivatization with Fluorescein Isothiocyanate and Capillary Electrophoresis/Laser-Induced Fluorescence Detection', Journal of Liquid Chromatography & Related Technologies, 20: 14, 2193 — 2205

To link to this Article: DOI: 10.1080/10826079708006556

URL: http://dx.doi.org/10.1080/10826079708006556

J. LIQ. CHROM. & REL. TECHNOL., 20(14), 2193–2205 (1997)

DETERMINATION OF ALIPHATIC AMINES IN WATER USING DERIVATIZATION WITH FLUORESCEIN ISOTHIOCYANATE AND CAPILLARY ELECTROPHORESIS/LASER-INDUCED FLUORESCENCE DETECTION

W. C. Brumley,* V. Kelliher†

U.S. Environmental Protection Agency
National Exposure Research Laboratory
Environmental Sciences Division
P.O. Box 93478
Las Vegas, NV 89193-3478

ABSTRACT

Detection-oriented derivatization of aliphatic amines and amine functional groups in compounds of environmental interest was studied using fluorescein isothiocyanate (FITC) with separation/determination by capillary electrophoresis/laser-induced fluorescence. Determinative levels down to 10 ppb were studied in deionized and environmental waters. Practical detection limits of 30 to 100 ppb or more were realized, depending on analyte and water source. Criteria were developed to compare results to desired performance in a derivatization method. FITC was judged to be a moderately successful reagent with drawbacks including extensive by-products, lack of specificity to analyte class, requirement to study each potential analyte carefully, and derivatization limits near 30 ppb. Advantages include low cost of reagent, ease in handling, and synthesis of an ionized derivative for free zone electrophoresis.

INTRODUCTION

Various aliphatic amines are of environmental interest due to their toxicity, reactivity, and likely occurrence as a result of their industrial uses.[1] The U.S. EPA, Environmental Sciences Division (CRD-LV), maintains a continuous interest in analytical methods for amines because of their wide occurrence and often problematic analyses. We have recently studied aliphatic amines using indirect UV detection in free zone capillary electrophoresis (CZE).[2] Generally, lower aliphatic amines are considered nonextractable, nonpurgeable volatiles in U.S. EPA methods.[3,4]

Aliphatic amines can present chromatographic problems due to their reactivity and extreme basicity. Derivatization has, therefore, been frequently employed. However, derivatization (e.g., Method 8042[3]) has not achieved as wide adoption in environmental analysis as it has in biological and pharmaceutical analysis. Part of the reason may lie in the tremendous variability of environmental matrices with a real concern for artifact formation from coextractives. Another reason is the desire to determine the analyte directly in the form that occurs in the sample with minimal sample manipulations. For amines, this could encompass primary through quaternary forms.

Gas chromatography (GC) is frequently used to separate amines using a variety of derivatizing agents.[5] Benzenesulfonyl chloride derivatives,[6] imine derivatives derived from pentafluorobenzaldehyde,[7] and isobutyl chloroformate derivatives[8] are representative.

Other techniques used to separate amines include supercritical fluid chromatography,[9] thermospray LC/MS,[10] and ion chromatography.[11] Liquid chromatography has often been the separation technique of choice for amines due to the variety of derivatizing agents available to introduce chromophores for UV detection or fluorophores for fluorescence detection.[5] Dansyl chloride derivatives are probably the most familiar[8-11] along with related compounds such as dabsyl and debsyl chlorides.[12] Other derivatives and reagents commonly used include benzoyl chloride[13], m-toluoyl chloride,[14,15] fluorescamine,[16] phenyl isothiocyanate and p-toluenesulfonyl chloride,[17] o-phthaldialdehyde,[18] 3,5-dinitrobenzoyl derivatives,[19] dichlorotriazinyl-fluorescein,[20] acetylacetone,[21] 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-chloride),[22,33] 2,4-dinitrophenyl derivatives,[24] and sodium benzoxazole-2-sulfonate derivatives.[25] More recently, a special reagent, 3-(p-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQ), has been developed for use in capillary electrophoresis.[26] Applications of CE/LIF for analyses of phenoxy acid herbicides have appeared.[27,28] Applications of capillary electrophoresis to

environmental analysis have been growing at a moderate rate and reviews of environmentally related work have been published.[29,30] Applications originally developed for HPLC applications[31] may have a direct counterpart in CE/LIF detection.[32] Recent work involving comprehensive two-dimensional separations has used fluorescein isothiocyanate (FITC)[33] and related compounds with characterization of some of the by-products.[34]

In this work, derivatization of aliphatic amines and amine functions in compounds of environmental interest was studied using FITC with separation/determination by capillary electrophoresis/laser-induced fluorescence. Determinative levels down to 10 ppb were studied in deionized and environmental waters. Criteria were developed to compare results with desired performance in a derivatization method.

EXPERIMENTAL SECTION

Chemicals

All organic compounds were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA) unless otherwise specified. Other chemicals were from standard sources of supply, and all were used as received. Deionized water (ASTM Type II) was used for all aqueous solutions. Solutions were freshly prepared for each experiment. Liquid amines were measured gravimetrically for preparation of standard solutions.

Capillary Electrophoresis

A P/ACE Model 2050 Capillary Electrophoresis System (Beckman Instruments, Fullerton, CA, USA) was used for all capillary electrophoretic experiments. The instrument was fitted with a capillary 57 cm X 75-μm I.D., 50 cm to the detector, with LIF detection using the 488-nm line of the Ar ion laser and a 520DF10 emission filter as well as a notch filter at 488 nm. The temperature of the capillary was 25°C, and electrophoretic runs were about 20 minutes at 20 or 25 kV using a 40 mM phosphate buffer at pH 7.0 with 25% methanol. The capillary was equilibrated with running buffer at the start of each experiment, and washed extensively between runs with 0.1 M sodium hydroxide, water, and running buffer. Migration times, peak widths, and detection limits were estimated directly from the monitor of the data system (software System Gold, Ver. 8.1). Corrected peak areas, as computed by the data system (peak area multiplied by the velocity of the ion [length to the

detector divided by time]), were normalized to the corrected peak area of the internal standard (fluorescein, disodium salt) as a control for the small variations in the nominal volumes of the pressure injections (ca. 5 nL from 1-s injections).

Derivatization and Sample Handling

Derivatization conditions were as follows: 0.1 mL to 1.0 mL of sample (amine in water) ranging from about 10μg to 30 ng (i.e., 30 ppb) was added to a 4 mL vial to which was added 0.1 mL of the derivatizing solution. The derivatizing solution was prepared daily and consisted of 7 to 10 mg of FITC, 300 mg of sodium carbonate (about 300 mM), and diluted to volume in a 10 mL volumetric flask. This solution was kept in the refrigerator when not in use. The reaction mixture was place on a stirrer plate with micro stirrer and capped; the reaction vial was kept in the dark and allowed to react a minimum of 16 hr up to a maximum of 48 hr at room temperature.

The reaction mixture was worked up by transferring the contents of the reaction vial to a 10 mL volumetric flask and diluting to 10 mL with deionized water. A 1-mL aliquot of this solution was added to 1 mL of internal standard, 1 mL of acetonitrile, 1 mL of phosphate buffer, and 1 mL of deionized water. This 5 mL sample was filtered through a 0.2 μm pore filter into the sample vials of the CE instrument.

The phosphate buffer was 40 mM phosphate, pH 7.0 and the internal fluorescein standard was 1 X 10^{-6} M which then resulted in about a 2 X 10^{-7} M solution in the prepared sample. The running buffer for CE was prepared using the 40 mM phosphate buffer but was diluted with methanol to achieve a 25 % level (v/v) in methanol resulting in a phosphate concentration of about 30 mM.

RESULTS AND DISCUSSION

Derivatization Issues

In real environmental samples the contamination level will not be known in advance. Therefore, a concentration in the mM range was chosen for the reagent in order to maintain reagent excess from the 10 ppb level up to the 10 ppm level. A constant level of reagent will also ensure that pseudo first-order rate kinetics will be followed with a half-life independent of initial concentration.[35] The desired detection level in environmental waters was set at

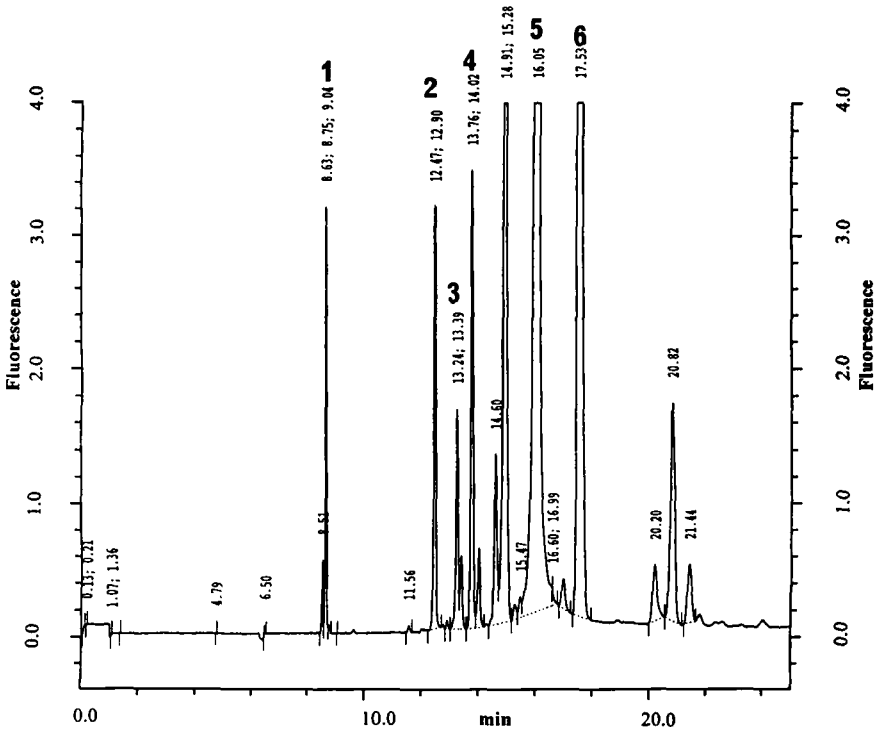

Figure 1. Derivatization of aliphatic amines at 100 ppb in deionized water. Identification of peaks as fluorescein isothiocyanate derivatives: **1**, 1,5-pentanediamine; **2**, 2-[2-aminoethyl]pyridine; **3**, diethylamine; **4**, propylamine; **5**, FITC; **6**, fluorescein internal standard.

10 ppb or lower, if possible. The criteria for the derivatization include: specific for the class of compounds; few or limited side reactions/by-products; separation of products and by-products; low detection limits of desired products (10^{-9} M or below); inexpensive reagent; simple and reproducible reaction conditions; and manageable derivatization of matrix components with separation from target products.

Separation Conditions

In cases where the derivative is ionic, free zone CE provides a convenient separation of analytes. In our work, free zone CE was carried out at pH 7.0

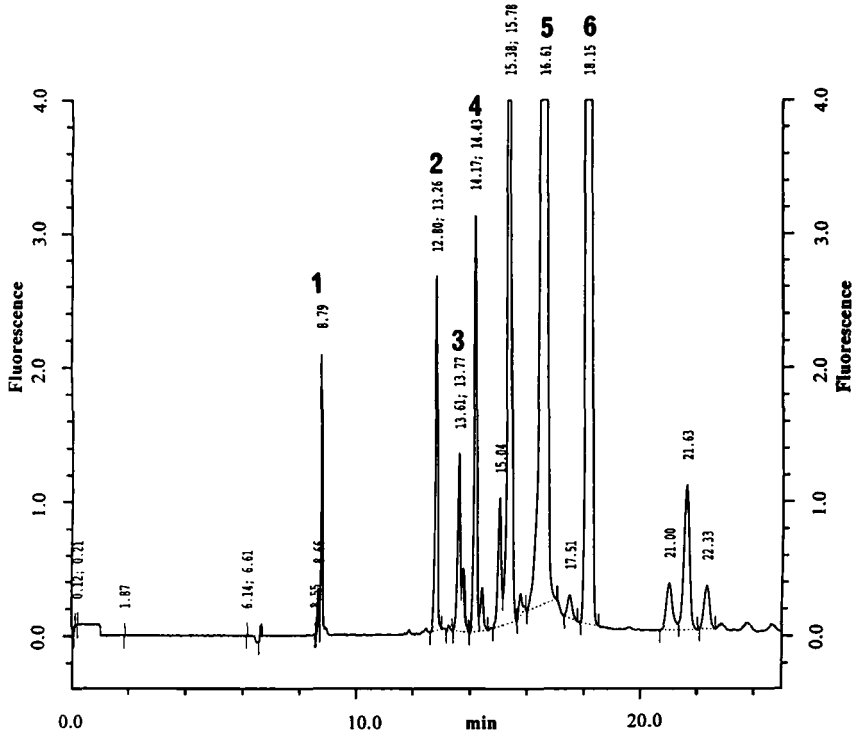

Figure 2. Derivatization of aliphatic amines in spiked ground water at 100 ppb. Identifications as in Fig. 1.

with phosphate buffer and 25 % methanol additive as an electroosmotic (EO) flow suppressor. Micellar electrokinetic chromatography (MEKC) has been used to separate fluorescein-based derivatives of herbicides[28] and amines.[29] In our work, fluorescein disodium salt was a convenient internal standard and migrated after FITC as well as the bulk of the by-products and the desired products. There were, however, later eluting peaks as well.

Detection Levels Achieved in DI Water and Environmental Waters

Standards were derivatized at 10, 30, 100, and 300 ppb in aqueous matrices. Standards representing 10 μg and 1 μg were also subjected to derivatization, representing the amount of material available at 10 and 1 levels. Figure 1 presents results of derivatization at 100 ppb in deionized water

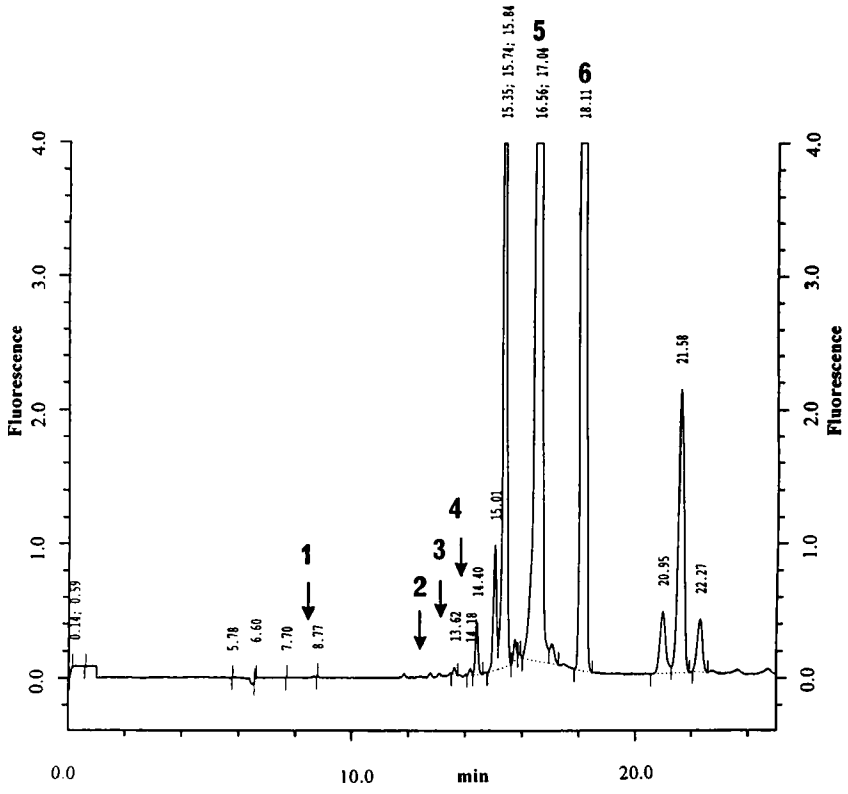

Figure 3. Derivatization of ground water blank. Identifications as in Fig. 1 with indication of where compounds would elute.

for comparison with Fig. 2 and Fig. 3 which represent 100 ppb and blank levels in ground water, respectively. The 100 ppb level appears to be readily achievable in relatively clean water samples, and this is confirmed by the results of ground water spiking. Derivatization at the 30 ppb level in deionized water represents about the lowest level of reliable detection of the amines and is illustrated in Fig. 4.

Results of experiments not reported here suggest that many amine substrates produce more than one product and others react slowly or do not react at all. In view of the known reactivity of isothiocyanates to other

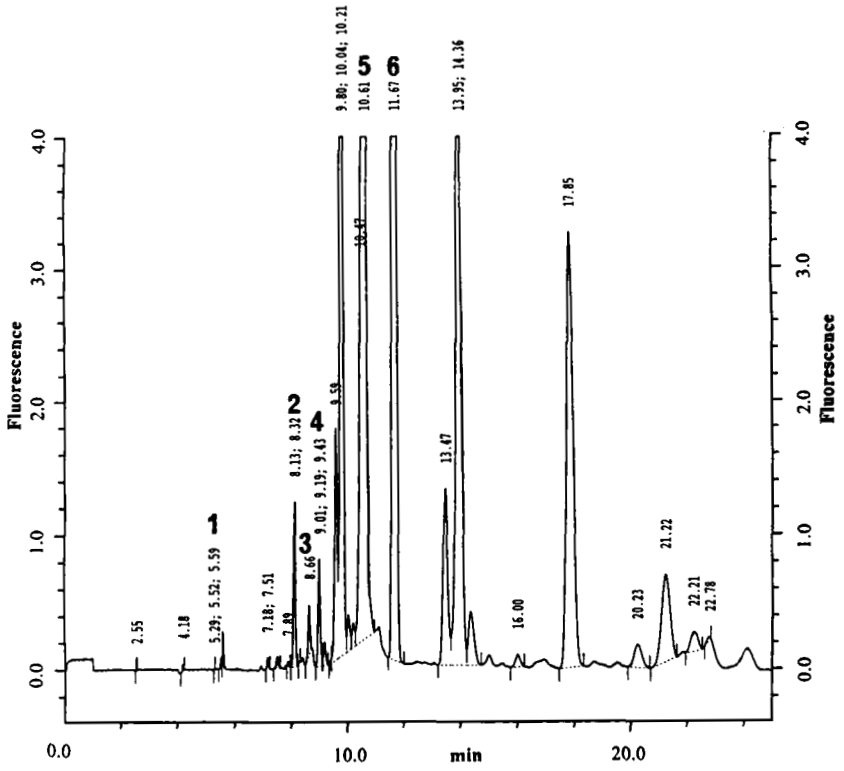

Figure 4. Derivatization of aliphatic amines at 30 ppb in deionized water. Identifications as in Fig. 1.

functional groups such as thiols, care is required in employing such derivatization schemes for real samples. This may explain why much of the literature on CE/LIF with derivatization has been infrequently applied to real samples.

Variations in Migration Times

Under free zone electrophoresis, variations in migration times (MTs) of target ions are principally due to variation in the EO flow. Corrections to this variation are possible based on the MT of an internal standard, and this is an

Table 1

Observed and Corrected MT's for the Compounds of Figures 1-4 with Percentage Differences Between Observed/Calculated MT's

Cpd.	Fig. 1(std) MT (min)	Fig. 2 MT/corr MT	Fig. 3 MT/corr MT	New std MT	Fig. 4 MT/corr MT	% error MT Fig. 1,2/ std, Fig.4
1	8.63	8.79/8.64	----/8.53	5.62	5.59/5.60	-0.1/0.3
2	12.47	12.80/12.49	----/12.51	8.17	8.13/8.15	-0.1/0.2
3	13.24	13.61/13.26	----/13.27	8.70	8.66/8.69	-0.1/0.1
4	13.76	14.17/13.79	----/13.81	9.06	9.01/9.04	-0.2/0.2
6	17.53	18.15/17.53	18.11/17.53	11.72	11.67/11.72	0/0

excellent reason for using an internal standard. Other reasons include the quality control nature of a response that indicates that all went well with injection, separation, and detection phases. These reasons are in addition to the quantitative use of internal standards.

In a previous paper,[37] we reported a tool for correcting MTs of analytes in subsequent runs to reference MTs of a standard run. The approach is based on the reciprocal relationship between MT and apparent mobility. Apparent mobility is the sum of the intrinsic mobility of the analyte ion and the mobility of the EO flow. Using the MT of an internal standard provides a means to correct for EO flow variations from run to run. Variations due to other factors such as wall interactions, temperature effects, and effects due to coextractives are not accounted for by this procedure.

Table 1 shows observed and corrected/predicted values of MT for four derivatives and the internal standard. The first two columns yield correlations of 0.1 % to 0.2 % for runs performed on the same day (reference last column, first number). Day to day variations may be greater than within the day and effects from using new capillaries predicate a new standard run for a new reference set of data. The last two columns indicate that, a different capillary operating on a different day, the same level of agreement is possible for runs on a single day (reference last column, 2nd number). It has been our experience that MT's can agree within 1% and often in the range from 0.1% to 0.3% as a qualitative tool of identification of peaks (i.e., corresponds to agreement within 0.6 sec to 1.8 sec for a MT of 10 min). This reproducibility is closely comparable to that obtained for retention times with HPLC and capillary GC.

Advantages/Disadvantages of FITC

Under our conditions, FITC was found to be unsuitable at 10 ppb levels in DI water because of the relatively high background levels of by-products. Levels of 30 to 100 ppb in DI water and ground water appeared to be feasible. The limiting features of FITC included large by-product peaks induced by the analytes but not present in pure reagent blanks with analyte. Real samples induced production of even more background peaks. Although the derivatives themselves are detectable at levels of 10^{-9} M, background produced by the reagent with analyte and matrix prevented achievement of going below the 30 ppb level (about 10^{-7} M) in actual samples.

CONCLUSIONS

Unforeseen problems arising from more complex matrices must be assumed. Therefore, more extensive testing of derivatization approaches with potential matrices is certainly necessary. The distinct likelihood of unknown components in environmental matrices further complicates any scheme based on derivatization approaches. One possible approach to the problem of specificity is to employ multidimensional separations to the derivatives[33,34] or CE/MS.[35] This, of course, adds complexity to the analysis and may be reserved for positives in order to remove false positive findings.

NOTICE

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded and performed the research described here. This work has been subjected to the Agency's peer review and has been approved as an EPA publication. The U.S. Government has the right to retain a non-exclusive, royalty-free license in and to any copyright covering this article. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

[†] Enrollee in the Senior Environmental Employee Program, assisting the U.S. Environmental Protection Agency under a cooperative agreement with the National Association for Hispanic Elderly.

1. **Kirk-Othmer Encyclopedia of Chemical Technology**, Fourth Ed., J. Wiley & Sons, New York, USA,1991, Vol. 2.

2. W. H. Matchett, W. C. Brumley, J. Liq. Chromatogr., accepted, 1996.

3. **Test Methods for Evaluating Solid Waste (SW-846)**, Vol. 1B, U.S. Environmental Protection Agency, Washington, D.C., USA, 3rd ed., November 1986.

4. **Code of Federal Regulations**, 40 CFR Pt. 136, App. A, Method 624, July 1, 1988.

5. D. R. Knapp, **Handbook of Analytical Derivatization Reactions**, Wiley-Interscience, New York, USA, 1979.

6. H. Kataoka, S. Ohrui, Y. Miyamoto, M. Makita, Biomed. Chromatogr., **6**, 251-254 (1992).

7. M. J. Avery, G. A. Junk, Anal. Chem., **57**, 790-792 (1985).

8. T. Lundh, B. Aakesson, J. Chromatogr., **617**, 191-196 (1993).

9. Z. Wang, H. Xu, C. Fu, Sepu, **8**, 325-327 (1990) (Chinese).

10. M. -L. Henriks-Eckerman, T. Laijoki, J. Chromatogr., **333**, 220-224 (1985).

11. M. C. Gennaro, E. Mentasti, C. Sarzanini, V. Porta, Chromatographia, **25**, 117-124 (1988).

12. J. -K. Lin, S.-S. Wu, J. Chin. Biochem. Soc., **14**, 10-19 (1985).

13. K. D. Duong, H. Kolodziejczyk, D. D. Blanco-Gomis, R. Rosset, Analusis, **19**, 103-106 (1991).

14. K. D. Duong, H. Kolodziejczyk, R. Rosset, *Analusis*, **19**, 154-157 (1991) (French).

15. P. Simon, C. Lemacon, Anal. Chem., **59**, 480-484 (1987).

16. K. Hunter, D. Lindsay, Pestic. Sci., **12**, 319-324 (1981).

17. J. Lehotay, V. Rattay, E. Brandsteterova, D. Oktavec, J. Liq. Chromatogr., **15**, 307-318 (1992).

18. E. Mentasti, C. Sarzanini, O. Abollino, V. Porta, Chromatographia, **31**, 41-49 (1991).

19. A. J. Bourque, I. S. Krull, J. Chromatogr., **537**, 123-152 (1991).

20. R. Siegler, L. A. Sternson, J. Pharm. Biomed. Anal., **6**, 485-492 (1988).

21. Y. Nishikawa, J. Chromatogr., **392**, 349-360 (1987).

22. C. J. Elskamp, G. R. Schultz, Am. Ind. Hyg. Assoc. J., **47**, 41-49 (1986).

23. G. M. Murray, M. J. Sepaniak, J. Liq. Chromatogr., **6**, 931-938 (1983).

24. S. Baba, Y. Watanabe, F. Gejvo, M. Arakawa, Clin. Chim. Acta, **136**, 49-56 (1984) .

25. O. R. Idowu, G. O. Adequyi, J. Liq. Chromatogr., **16**, 2501-2518 (1993).

26. E. A. Arriaga, Y. Zhang, N. J. Dovichi, Anal. Chim. Acta, **299**, 319-326 (1995).

27. Y. Mechref, Z. El Rassi, Anal. Chem., **68**, 1771-1777 (1996).

28. M. Jung, W. C. Brumley, J. Chromatogr. A, **717**, 299-308 (1995).

29. W. C. Brumley, LC.GC, **13**, 556-568 (1995).

30. W. C. Brumley, J. Chromatogr. Sci., **33**, 670-685 (1995).

31. C. J. Miles, H. A. Moye, Anal. Chem., **80**, 220-226 (1988).

32. S. D. Gillman, A. G. Ewing, Anal. Chem., **67**, 58-64 (1995).

33. A. V. Lemmo, J. W. Jorgenson, Anal. Chem., **65**, 1576-1581 (1993).

34. W. D. Cole, R. R. Holloway, C. A. Keely-Templin, D. McManigill, V. K. Smith, T. A. Van d Goor, H. Yin, "Two-Dimensional Separations Using On-Line Liquid Chromatography-Capillary Electrophoresis,", paper presented at the 20th International Symposium on High Performance Liquid Phase Separations and Related Techniques, Jun 16-21, 1996, San Francisco, CA, L2604.

35. K. J. Laidler, **Chemical Kinetics**, McGraw-Hill, New York, 1950, pp.56-57.

36. W. C. Brumley, W. Winnik, "Applications of Capillary Electrophoresis/ Mass Spectrometry to Environmental Analysis", in **Applications of Liquid Chromatography/Mass Spectrometry in Environmental Chemistry**, D. Barceló, ed., Elsevier, Amsterdam, 1996, C12, pp. 481-527.

37. W. C. Brumley, C. M. Brownrigg, J. Chromatogr., **646**, 377-389 (1993).

Received November 20, 1996
Accepted January 31, 1997
Manuscript 4329