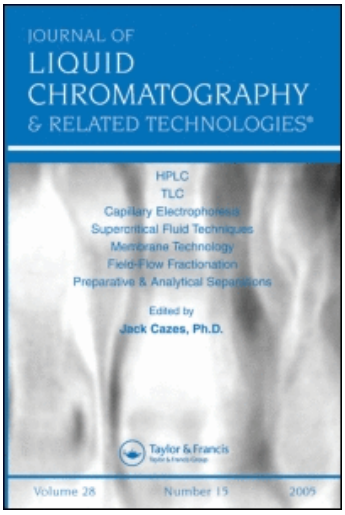

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Multicomponent Analysis of Highly Overlapped Capillary Electrophoretic Peaks Using Multiwave-Length Charge-Coupled Devices Detection

Shaoxiang Xiong[a]; Jianjun Li[a]; Jieke Cheng[a]
[a] Department of Chemistry Wuhan, University Wuhan, People's Republic of China

To cite this Article Xiong, Shaoxiang , Li, Jianjun and Cheng, Jieke(1996) 'Multicomponent Analysis of Highly Overlapped Capillary Electrophoretic Peaks Using Multiwave-Length Charge-Coupled Devices Detection', Journal of Liquid Chromatography & Related Technologies, 19: 13, 2129 — 2141
To link to this Article: DOI: 10.1080/10826079608017146
URL: http://dx.doi.org/10.1080/10826079608017146

J. LIQ. CHROM. & REL. TECHNOL., 19(13), 2129–2141 (1996)

MULTICOMPONENT ANALYSIS OF HIGHLY OVERLAPPED CAPILLARY ELECTROPHORETIC PEAKS USING MULTIWAVELENGTH CHARGE-COUPLED DEVICES DETECTION

Shaoxiang Xiong, Jianjun Li, Jieke Cheng*

Department of Chemistry
Wuhan University
Wuhan 430072, People's Republic of China

ABSTRACT

A chemometric method, Kalman filtering, was applied to deal with three-dimensional electropherograms, obtained by capillary electrophoresis connected to a charge-coupled device multiwavelength fluorescence detector. The capillary electrophoretic peaks of a mixture, which were not effectively separated, were resolved into electrophoretic peaks of individual components to obtain qualitative and quantitative information. The approach has been applied to analyze a mixture of rhodamine fluorescent dyes with excellent results.

INTRODUCTION

Capillary electrophoresis (CE) is an emerging, important technique in analytical chemistry because of its high efficiency, rapid analytical speed, small sample amount, etc.[1-3] In most reports to date, single channel detection is used, and a two-dimensionl electropherogram is produced.

However, multichannel detection, with high speed scanning UV/Vis detectors,[4-8] photodiode array detectors[9-12] and NMR[13,14] is more attractive because it gives a three-dimensional electropherogram with wavelength and retention information from which the separated compounds may be identified.

Charge-coupled devices (CCD) form a new class of multi-channel solid-state detectors which are applied to molecular spectroscopy, atomic spectroscopy, Raman spectroscopy, separation science, image analysis and some analytical chemical aspects.[15-18] It is also used as a sensitive detector for CE. Cheng et al.[19] first used CCD as fluorescence detector for CE and obtained a three-dimensional electropherogram with wavelength information. CCD was used by Chen et al.[20] for the on-line Raman spectroscopic detection, in CE, of a mixture of methyl red and methyl orange.

Sweedler et al.[21] utilized the time-delayed integration mode to detect fluorescein isothiocyanate-labeled amino acids in the 10^{-20} mole range, and quantitatively determined a sub-attomole quantity of bag cell neuropeptides collected from the giant mollusk Aplysia californica.[22] With a linear regression methodology, the multi-wavelength fluorescence electropherogram detected with CCD was used by Karger et al.[23] for the determination of base fragments attached with four kinds of fluorophores and rapid DNA sequencing.

Yueng et al.[24] and Takahashi et al.[25] performed an array capillary electrophoresis for DNA sequencing with CCD.

Another advantage of multi-channel detection is that, with a chemometric method, the three-dimensional data can be processed for the multicomponent analysis of highly overlapped electrophoretic peaks. Based on the differences of in spectra, individual constituents can be accurately identified and quantified from the unresolved electropherogram. In some other cases, this is more important and necessary. For instance, in four color fluorescent labeled DNA sequencing by CE, different length fragments may overlap with each other, owing to the migration speed differences of fluorophores.[26] If one did not exploit the merit of three-dimensional electropherograms from which the positions of different fluorophore-labeled fragments are located, it would be impossible to accurately obtain the DNA sequence.

In liquid chromatography (LC), the chemometric methods, least-squares multiple linear regression,[27,28] Kalman filtering,[29] factor analysis,[30] generalized rank annihilation,[31] multivariate curve resolution,[32-33] etc., have been used for the processing of multichannel data and multicomponent analysis of highly overlapped LC peaks. Similarly, these methods are able to be used for the multicomponent analysis of overlapped capillary electrophoretic peaks. However, there are only a few reports about this.

In this paper, a chemometric method, Kalman filtering,[34] was utilized for the processing of multiwavelength electrophoretic data and multicomponent analysis of highly overlapped electrophoretic peaks. The model compounds, three kinds of rhodamine fluorescent dyes whose spectra are overlapped, were separated by CE with CCD multiwavelength fluorescence detection. In the three-dimensional electropherogram two components' peaks were highly overlapped but, by the use of Kalman filtering, the mixture peak was divided into the peaks of the individual components. The qualitative and quantitative analyses of individual components were further accomplished. It may be concluded that CE with CCD multiwavelength detection is more powerful than with single-channel detection.

EXPERIMENTAL

Separation and Detection System

A CE system with a CCD multi-wavelength fluorescence detector, previously described[35] was used. The excitation beam emitted from a tungsten-bromine source (Shanghai Third Analytical Instrument Factory, China) was focused with a lens (10X magnification) onto the fused-silica capillary (100μm i.d., 370 μm o.d., (Yongnian Optical Fiber Factory, China) at a position located 5.5 cm from the cathodic end of the tube. From the capillary with a length of 51 cm a small section of protective coating was burned off with a gentle flame to form a detection region. The effective length was 45 cm.

The fluorescence emission was collected at right angles to the excitation source, and focused by the two lenses, onto the entrance slit of a polychromator (HR 320, Instruments, SA, Inc., USA), dispersed by a 1200 grooves/mm grating and irradiated on a 512 by 512 element CCD (Model 1530-P, EG & G, Princeton Applied Research, USA). Through an optical fiber, the data of photogenerated charge were transported into a computer for storage, calculation and output of results.

A high-voltage power supply, built in our laboratory, was used to provide a 20 kV potential for electrophoretic separation. The electrical connections were made at both ends of the capillary with platinum wires immersed in 5 mL reservoirs. Samples were introduced by siphoning at a height difference of 10 cm for 5 sec. The CCD chip was cooled with a Peltier device to -40°C, which reduced dark current and background noise. The monitoring of experiments and selection of operation parameters, e.g., exposure time, charge transferring pattern, data collection mode, were controlled by the use of OMA SPEC 4000 software installed in a Gateway 2000 computer.

Reagents

All reagents were of analytical grade; doubly distilled water was used for dilution. Stock solution of rhodamine 590 (R 90), rhodamine 610 (R610), rhodamine 640 (R640), purchased from Exciton Chemical (Dayton, Ohio, USA), were all prepared in 1 x 10^{-4} mol/L. Working solutions were produced by appropriate dilution. The run buffers for all the experiments were 10 mmol/L borates, prepared from sodium borate decahydrate and sodium hydroxide.

Procedure

The applied voltage and operating parameters of the CCD for fluorescence detection were selected and adjusted. Then the background spectrum was recorded and stored. After the sample was introduced by siphoning, the electrophoresis was initiated by switching on the high-voltage power supply and timer. At an appropriate time, the CCD detection was started and the fluorescence spectra were detected, in succession, using a data gathering mode of automatic background subtraction (Accume-B). Finally, a multi-wavelength fluorescence electropherogram was produced.

The observation vector needed for filtering was composed of spectra chosen from the electropherograms of individual components. The mixture electropherograms were treated with the proposed method, and the qualitative and quantitative information of every constituent were obtained.

Program

Based on the principle of Kalman filtering,[34] a program was compiled with MACRO language provided by the application software OMA SPEC 4000. The observation vectors, made up from standard spectra of the pure compounds, were first input. Then, each spectrum of the mixture electropherogram was processed by the filtering program, and the contributions of each component obtained. If a component is not present in the spectrum, the proportional parameter to its standard spectrum will be less than 10^{-4}. If several components contribute to a spectrum, the proportional parameters of each component to their standard spectra will be obtained, which demonstrates that the electrophoretic peaks are overlapped.

Using the proportional parameters obtained in filtering, the mixture spectra in overlapped electrophoretic peaks are resolved into spectra of individual components. Thus, the overlapping mixture three-dimensional electropherogram is divided into several three-dimensional electropherograms in

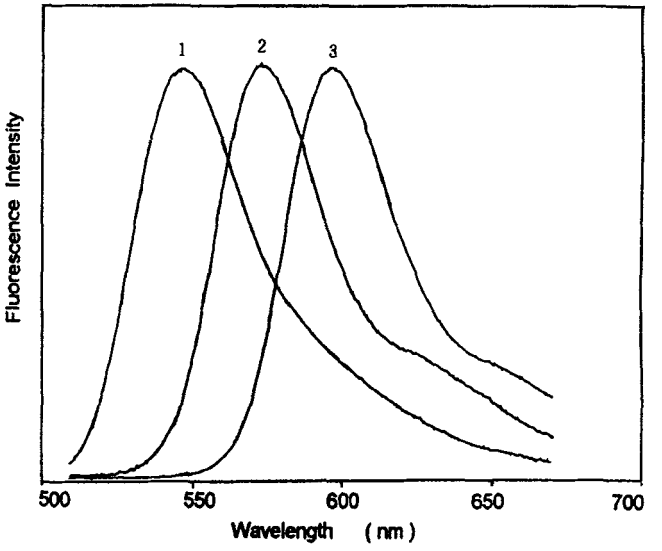

Figure 1. Fluorescence spectra of rhodamine 590 (1), rhodamine 610 (2), rhodamine 640 (3).

Table 1

Quantitative Results of Sample Mixtures

No.	C_{added} x 10^{-4} mol/L R590	R610	R640	C_{found} x 10^{-4} mol/L R590	R610	R640	RSD (%) R590	R610	R640
1	2.50	2.50	2.50	2.61	2.38	2.43	+4.40	-4.60	-2.76
2	2.50	2.50	5.00	2.41	2.39	5.13	-3.60	-4.32	+2.68
3	5.00	5.00	2.50	4.87	5.20	2.40	-2.46	+4.02	-3.80
4	1.25	1.25	2.50	1.32	1.18	2.62	+5.84	-5.20	+4.84

which the electrophoretic characteristics of individual components are shown. Finally, a calculation subprogram described previously[36] was used to obtain the qualitative and quantitative results for components.

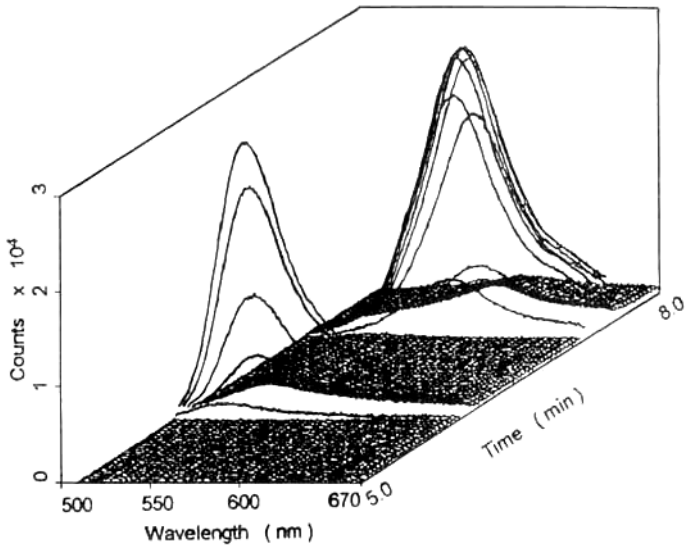

Figure 2. Three-dimensional electropherograms of a mixture. Experimental conditions: Buffer; 0.01 mol/L sodium tetraborate solution, pH 11. Sample: each at 10^{-4} mol/L, introduced by siphoning at a height difference of 10 cm for 5 sec. Applied voltage, 10 kV. The peaks were identified as R590 (1), mixture of R610 and R640 (2).

RESULTS

Fluorescence Spectra

A fluorescence spectrum with a wavelength window of 270 nm is able to be detected by CCD. If the fluorescence spectra from analyses do not overlap with each other, they will be independent peaks in a three-dimensional electropherogram, even though their migration times are equal. This allows their respective qualitative identification and quantification. However, if the fluorescence spectra from analyses are overlapped with each other, and there is little difference in their migration times, the peaks from the analyses overlap in the threedimensional electropherogram. In this case, it is impossible to acquire the qualitative and quantitative information of components unless the mixture peak is mathematically resolved. The fluorescence spectra of R590, R610, R640 are shown in Figure 1. It may be observed that the fluorescence maxima (nm) of RS90, R610 and R640 are 547.5, 577, 601, respectively, and their fluorescence spectra are seriously overlapped. If in electrophoresis several component coelute, mixture spectra are measured.

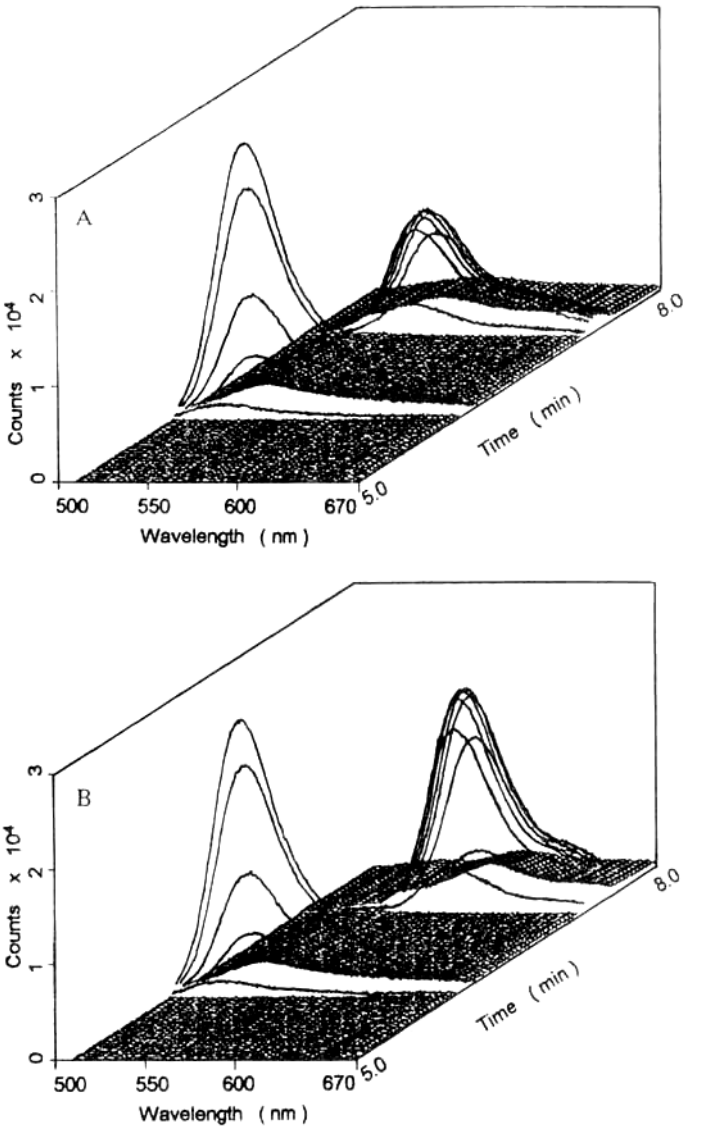

Figure 3. Three-dimensional electropherograms of R590, R610(A) and R590, R640(B) yielded by the proposed method.

Electrophoretic Characteristics of Samples

The experimental results at different pH's and applied voltages show that the migration speed of R590 is the fastest; its retention time is the shortest, hence, it always appears first in the detection window and is baseline separated from the other components. However, the migration speeds of R610 and R640 were slow and their retention times were similar. Therefore, they coelute in the detection window. As a result, two electrophoretic peaks are detected in the three-dimensional electropherogram. The first one is R590, and the last is the mixture peak of R610 and R640 (Figure 2).

This demonstrates that, under the experimental conditions used in this paper, the electrophoretic peaks of R610 and R640 were highly overlapped.

Electropherograms of Individual Components

The three-dimensional electropherograms of individual components, obtained by applying Kalman filtering to data given in Figure 2, are shown in Figure 3. The first peak represents R590, which is same as in Figure 2. The second peak is R610 (Figure 3A) and R640 (Figure 3B). By comparison, one can see that the spectra and sizes of these two peaks are different, which show the respective spectrometric and electrophoretic characteristics of R610 and R640 after the mixture peak was resolved.

The conventional two-dimensional electropherograms, derived from Figure 2 and Figure 3 by applying a calculation subprogram,[36] are shown in Figure 4. It may be observed that the peaks of R610 and R640 are highly overlapped. Without CCD for multi-wavelength detection, followed by a chemometric method, no individual electropherograms and quantitative information would have been obtained.

Results of Quantitative Analyses

The quantitative results of some mixture samples are listed in Table 1. one can see that the proposed method yielded equivalent accuracies for the components R610 and R640, whose peaks are highly overlapped in electropherograms, compared to the component R590 which is completely resolved in the electropherograms.

This demonstrates the performance of Kalman filtering for the multicomponent analysis of highly overlapped multi-wavelength CE peaks detected by CCD.

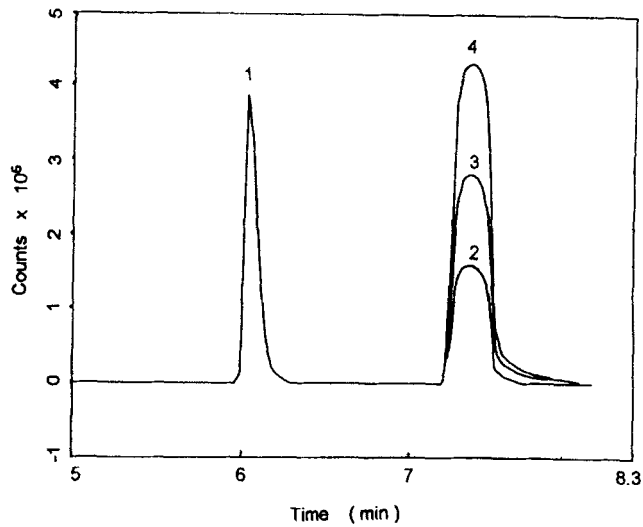

Figure 4. Two-dimensional electropherograms of a sample. The peaks were identified as R590 (1), R610 (2), R640 (3), the mixture of R610 and R640 (4).

DISCUSSION

The experimental results demonstrate that multi-wavelength CE data can be treated by the proposed method. The electropherograms and quantitative results of individual components are obtained, even though their electrophoretic peaks are highly overlapped. Even though the three component system used is simple, the conclusion is general.

In multi-component analysis, two situations may occur. First, although the peak count in the electropherogram increases, all peaks can be sequentially treated and resolved. Second, if the component count in a peak increases, this is not problematic, because Kalman filtering is a rigorous computational method for multi-component analysis; it will not be hard to resolve a mixture peak into the individual component peaks. Nevertheless, owing to the high separation effciency of CE, it is not likely that there are more than two components in a peak.

Other chemometric methods may similarly be used for the treatment of multi-wavelength CE data. That work is in progress. Even though the R610 and R640 are likely to be readily separated from each other by means of other kinds of buffers or by adding selectors, they were selected for the present work as an illustrative example of the CCD, Kalman filtering technique.

In multicomponent analysis by Kalman filtering, the accuracy of the standard spectra which make up the observation vector should be good. If there are errors in standard spectra, the accuracies of analytical results will be seriously affected.[37] In general, spectra at various concentrations of standard solutions are measured, the specific parameters at each wavelength are calculated by a least-squares linear regression and used as the observation vector in multicomponent analysis.

In multi-wavelength spectrometric detection in CE, accurate standard spectra of known concentrations can not be obtained. Therefore, for the resolution of overlapped peaks by Kalman filtering, the strategy applied was to select pure spectra from the electropherograms of individual component, which form the observation vector; this yields the proportional parameter to corresponding component. The procedure is simple and convenient to apply.

In multi-component analysis of overlapped LC peaks,[27-33, 38-40] the assumption is usually made that the peak shapes of each component in single and mixture LC should be identical, i.e., there is a good peak reproducibility under the experimental conditions. Therefore, a procedure is utilized that, at fixed wavelengths, the peak responses at different times are processed. In CE, however, due to the influences of various factors, it is difficult to keep a good reproducibility in peak shape and migration time. Therefore, there is no report about multi-component analysis of overlapped peaks obtained by a single-channel detector.

The procedure used in this paper, i.e., that at fixed migration times, spectra of mixtures are subjected to a multi-component analysis, is able to overcome the disadvantage of poor reproducibility in peak shapes. Under the experimental conditions, the spectra of the common components in the different runs do not vary with small fluctuations in experimental conditions.

Even if the peak shapes change, due to changing of experimental conditions, the proposed method, based on the spectra of individual components, separates the mixture electrophoretic peaks into the electrophoretic peaks of individual components, allowing an accurate qualitative and quantitative analysis.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China for Young Scientists, Foundation of National Education Commission of China and Chengguang Project of Wuhan City.

REFERENCES

1. Q. Liu, J. Cheng, Trace Anal., **7(3/4)**, 1-9 (1991).

2. W. G. Kuhr, C. A. Monning, Anal. Chem., **64**, 389R-407R (1992).

3. C. A. Monning, R. T. Kennedy, Anal. Chem., **66**, 280R-314R (1994).

4. H. Stuppna, H. Sturm, N. Mulinacci, F. Vincieri, Chromatographia, **37**, 579-583 (1993).

5. C. Delgado, F. A. Tomas-Barberan, T. Talou, A. Gaset, Chromatographia, **38**, 71-78 (1993).

6. P. F. Cancalon, C. R. Bryan, J. Chromatogr., **652**, 555-561 (1993).

7. D. E. Hughes, P. Richberg, J. Chromatogr., **635**, 313-318 (1993).

8. J. Harms, G. Schwedt, Fresenius J. Anal. Chem., **350**, 93-100 (1994).

9. S. Kobayashi, T. Ueda, M. Kikumoto, J. Chromatogr., **480**, 179-184 (1990).

10. J. Vindevogel, P. Sandra, L. C. Verhager, J. High Res. Chromatogr., **13**, 295-298 (1990).

11. W. Thormann, P. Meier, C. Marcolli, F. Binder, J. Chromatogr., **545**, 445-460 (1991).

12. W. Beek, R. VanHoek, H. Engelhardt, Electrophoresis, **14**, 540-546 (1993).

13. N. Wu, T. L. Peck, A. G. Webb, R. L. Magin, J. V. Sweedler, Anal. Chem., **66**, 3849-3854 (1994).

14. N. Wu, T. L. Peck, A. G. Webb, R. L. Magin, J. V. Sweedler. J. Am. Chem. Soc., **116**, 7929-7930 (1994).

15. J. V. Sweedler, R. B. Bilhorn, P. M. Epperson, G. R. Sims, M. B. Denton, Anal. Chem., **60**, 283A-291A (1988).

16. P. M. Epperson, J. V. Sweedler, R. B. Bilhorn, G. R. Sims, M. B. Denton, Anal. Chem., **60**, 327A-335A (1988).

17. J. V. Sweedler, CRC in Anal. Chem., **24**, 59-101 (1993).

18. C. W. Earie, M. E. Baker, M. B. Denton, R. S. Pomeroy, Trends in Anal. Chem., **12**, 395-403 (1993).

19. Y. Cheng, R. D. Piccard, T. Vo-Dinh, Appl. Spectros., **44**, 755-765 (1990).

20. C. Chen, M. D. Morris, J. Chromatogr., **540**, 355-363(1991).

21. J. V. Sweedler, J. B. Shear, H. A. Fishman, R. N. Zare, R. H .Scheller, Anal. Chem., **63**, 496-502(1991).

22. J. V. Sweedler, J. B. Shear, H. A. Fishman, R. N. Zare, Proc. SPIE, **1439**, 37-40 (1992).

23. A. E. Karger, J. M. Harris, R. F. Gesteland, Nucleic Acids Res., **19**, 4955-4962 (1991).

24. K. Ueno, E. A. Yueng, Anal. Chem., **66**, 1424-1431(1994).

25. S. Takahashi, K. Murakami, T. Anazawa, H. Kambara, Anal. Chem., **66**, 1021-1026 (1994).

26. L. M. Smith, S. Fung, M. W. Hunkapiller, T. J. Hunkapiller, L. E. Hood, Nucleic Acids Res., **13**, 2399-2412 (1985).

27. A. Cladera, E. Gomez, J. M. Estela, V. Cerda, J. Chromatogr. Sci., **30**, 453-458 (1992).

28. T. W. Ryan, J. Liq. Chromatogr., **16**, 1545-1560 (1993).

29. T. Rotunno, Chromatographia, **34**, 56-62 (1992).

30. Y. Z. Liang, O. M. Kvalheim, H. R. Keller, D. L. Maddart, P. Kredile, F. Erni, Anal. Chem., **64**, 946-953 (1992).

31. E. Sanchez, B. R. Kowalski, Anal. Chem., **58**, 496-499 (1986).

32. I. Scarminio, M. Kubista, Anal. Chem., **65**, 409-414 (1993).

33. R. Tauler, D. Barcelo, Trends in Anal. Chem., **12**, 319-327 (1993).

34. S. D. Brown, Anal. Chim. Acta, **181**, 1-26 (1986).

35. S. Xiong, J. Li, J. Cheng, Sepu, in press.

36. S. Xiong, J. Li, J. Cheng, Chem. J. Chinese Univ., in press.

37. S. Xiong, J. Li, J.Cheng, H. Yuan, Fenxi Kexue Xuebao **11**, 3-7 (1995).

38. T. L. Cecil, R. C. Rutan, Anal. Chem., **62**, 1998-2004 (1990).

39. H. R. Wilk, S. D. Brown, Anal. Chim. Acta, **225**, 37-52 (1989).

40. H. R. Keller, D. L. Massart, Anal. Chim. Acta, **246**, 379-390 (1991).

Received June 19, 1995
Accepted October 5, 1995
Manuscript 3889