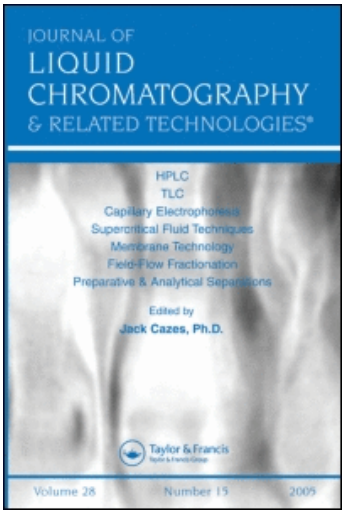

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SINGLE UV-ABSORBING BACKGROUND ELECTROLYTE FOR SIMULTANEOUS DETECTION OF CATIONS AND ANIONS IN CAPILLARY ELECTROPHORESIS

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ABSTRACT

Single UV-absorbing background electrolyte (BGE) has been designed for simultaneous detection of alkali metals and anions. The BGE is composed of a cationic buffer component and an anionic chromophore. Tris, sulfosalicylic acid, trimellitic acid, 1,5-naphthalenedisulfonic acid were tested as the cationic and anionic components in the BGE, respectively. 1,5-naphthalenedisulfonic acid was selected as the anionic chromophore of the BGE for the separation of cations and anions. In Tris/1,5-naphthalenedisulfonic acid BGE, K^+, NH_4^+, Na^+, Li^+, ascorbate, sorbate, benzoate, lactate, acetate, HCO_3^-, phosphate,

formate and fluoride could be detected within 9 minutes with detection limits ranging from 0.1 to 1 ppm under the optimized conditions. This newly developed method allows a larger choice of components to define BGE in simultaneous detection of cations and anions in indirect photometric detection. The applicability of this type of BGE has been demonstrated in the analysis of a real sample. The results obtained were comparable to those obtained by inductively-coupled plasma spectroscopy (ICP) and ion chromatography (IC).

INTRODUCTION

As most small cations and anions lack the necessary intrinsic chromophores for straightforward sensitive detection at any wavelength above 200 nm, indirect UV detection principle is one of the choices that permits the detection of these two classes of ions in capillary electrophoresis (CE). Proper formulation of the background electrolyte (BGE) is the essential step for developing this type of detection method. Although this method has been addressed by many research groups from both theoretical[1-5] and practical[6-10] aspects, the strategies of designing a BGE in CE is still more empirical than rational, especially for simultaneous detection of small cations and anions.

Simultaneous detection and separation of cationic and anionic analytes in CE is useful because more information about the sample compositions and chemical processes can be gained with a single injection. However, the synchronous detection of them is not easy owing to the fact that these two classes of ions tend to migrate in opposite directions under an electric field. The approaches currently developed for this detection method fall into two categories with regard to the operational format. One is that the injection of the sample is performed from both the ends of the capillary and detection of the separated sample zone is accomplished with a single/two-detector locating at the middle/two-end of the capillary.[8,9] The other is that the sample is injected from one end of the capillary and the sample is detected at another end of the capillary.[10,11] In both of the approaches, dual UV-absorbing BGEs were used to probe the cationic and anionic analytes simultaneously. By comparison, the former format is easier for controlling the migration directions of the two classes of analytes but complicated in instrumentation. The latter format is simple in instrumentation but more difficult to ensure that the migration directions of the cationic and anionic analytes would be in the same direction towards the detector.

The method employed to force the cations and anions to migrate in the same direction towards the detector in the latter case is the amplification of the electroosmotic flow (EOF) in bare fused-silica capillaries at higher pHs.[10] It is this high pH condition that limits the choices of appropriate combination of the cationic and anionic chromophores of the BGEs. In addition, two UV-absorbing

components involved in the BGEs make the proper selection of the detection wavelength more critical than that in single UV-absorbing BGEs.[5] These constraints result in the design of BGEs being more sophisticated based on this scheme.

To avoid the problem aforementioned, a single UV-absorbing BGE is explored for simultaneous separation and detection of the cations and anions in a single detector instrumental system. The main focus of the present work is on the strategies of formulating the BGEs with respect to simultaneous detection of the cations and anions and their applications in real sample analysis. Results obtained using the present method are compared with those observed by employing other techniques.

EXPERIMENTAL

Chemicals and Solutions

Chemicals used in this work were of analytical grade or better. 1,5-naphthalenedisulfonic acid (97%) and sulfosalicylic acid (A.C.S. reagent) were purchased from Aldrich (Aldrich, Milwaukee, WI, USA). Trimellitic acid (>99%) was obtained from TCI (Tokyo Chemical Industry, Tokyo, Japan). Tris (Electrophoresis purity reagent) was obtained from Bio-Rad Laboratories (Bio-Rad Laboratories, Richmond, CA, USA). All solutions were prepared with Millipore water from a Milli-Q system (Millipore, Bedford, WI, USA). The stock solutions of standard analytes were prepared to ca. 1000 mg/L by dissolving known amounts of each reagent in Millipore water. The sample mixture at ppm levels was prepared daily by stepwise dilution of the standard stock solutions.

Apparatus

CE was performed with a commercial instrument (PRINCE Technologies, The Netherlands), equipped with a DAX data station and connected with a Lambda 1010 spectrophotometer (Bischoff, Leonberg, Germany). Data were collected 10 times per second. A 60 cm x 50 μm I.D uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), with the detection window placed 45 cm from the injection end, was used. Hydrostatic injection was realized in 0.10 min under a pressure of 50 mbar.

The inductively-coupled plasma spectroscopy (ICP) system used in this study was an IRIS Plasma Spectrometer (Thermo Jarell Ash Corporation, Franklin, MA, USA).

Determination of phosphate by ion chromatograpgy (IC) was carried out on a Waters IC-PAK A HR column connected to a Waters 510 HPLC pump and a Waters 431 conductivity detector (Milford, MA, USA). All experiments were carried out at ambient temperature (22-25° C). A pH meter (Model 962 pH/Ion Meter, Metrohm, Switzerland) with ± 0.01 pH resolution was used to measure the pH of the electrolyte solutions throughout the experiment.

Capillary Treatment

Each day, before sample injection, the capillary was rinsed with 1 M NaOH for half an hour, followed by 0.1 M NaOH for 15 min, then Millipore water for 5 min, and lastly the electrolyte solution for another half an hour. Before each sample was injected, the capillary was flushed with the running buffer for 5 min and a voltage of 30 kV was applied to it for 3 min. The total running time for each determination was about 9 min. The capillary was flushed with 0.1M NaOH for 15 min at the end of experiments.

Procedures

All running solutions were prepared daily by pipetting a certain volume of the UV chromophore into a volumetric flask (25 mL) and diluting it with Millipore water to the graduated marker. The pH was adjusted to a specific pH value on the pH meter. Then the electrolyte was transferred to another 25 mL volumetric flask. Prior to use, the running electrolyte solution was filtered through a 0.45 μm membrane filter (Phenomenex, Torrence, CA, USA) and transferred to the two electrolyte reservoirs for separation.

Sample Treatment

The soft drink (Gatorade, The Gatorade Company, Chicago, USA) sample was purchased from the local supermarket. The sample was diluted with Millipore water. After being passed through a 0.45 μm membrane filter (Phenomenex, Torrence, CA, USA), the sample solution was ready for CE and ICP analysis. The dilution ratio for the sample was 1:25.

RESULTS AND DISCUSSION

Composition of the BGE

In the design of BGEs for indirect photometric detection, two aspects should be taken into consideration. One is the buffer capacity of the BGE, the

other is the detection sensitivity permitted by the BGEs. BGEs containing one UV-absorbing chromophore are conventionally used for the detection of one class of analytes, i.e., either cationic or anionic analytes, depending on the signs of the changes of the chromophore and the sample ions. The chromophore in this BGE generally functions as the co-ion of the analytes for this detection scheme. If this type of BGE is employed for the simultaneous detection of cations and anions, the chromophore would function as both the co-ion and the counterion, respectively, towards the different classes of the analytes. As a result, the detection mechanism for this detection scheme is different from that for the individual detection of the ions, where the normal displacement of the chromophore in the BGE by the analytes is responsible for the detection of the ions. In the case of chromophore being a counterion to the analytes, the sample zone absorption is different for different ions and it can be expressed as[5]

$$\Delta A = A^{s}_{obs} - A^{B}_{obs} = C^{s}_{sample} \cdot d \cdot \left(z_{sample} - z_{co-ion} \cdot TR\right) \cdot \frac{\varepsilon_{counterion}}{z_{counterion}} \qquad (1)$$

where A_{obs}, C, d, z, ε represent the observed absorbance of the separated sample zone at the detector, concentration, inner diameter of the capillary, charge number (absolute values) of the ionic species in the BGE (B) and the sample zones (S), and molar absorptivity of the chromophore. TR is the transfer ratios of the analytes which is defined as[5]

$$TR = \frac{z_{sample}}{z_{co-ion}} \cdot \frac{\frac{\mu_{counterion}}{\mu_{sample}} + 1}{\frac{\mu_{counterion}}{\mu_{co-ion}} + 1} \qquad (2)$$

where μ is the effective mobility of the ionic species in question and the other symbols have the meanings as above. The peak patterns observed[5] for different ions could be classified into the following three situations, i.e.,

$\Delta A = A^{s}_{obs} - A^{B}_{obs} < 0$ (normal displacement, negative peak)

$\Delta A = A^{s}_{obs} - A^{B}_{obs} = 0$ (no peak)

$\Delta A = A^{s}_{obs} - A^{B}_{obs} > 0$ (absorbance increase, positive peak)

Through simulation[5] of eq. 1, it was found that analytes with their mobilities less than that of the co-ion in the BGE would be detected as negative peaks, while analytes with their mobilities larger than that of the co-ion would be observed as positive peaks. No peak could be observed if the mobilities of the analytes are equal to that of the co-ion in the BGE. The theoretical

Table 1

Electrophoretic and Spectroscopic Properties of Some Anionic Compounds of BGEs Used in the Present Work

Component of BGE	pKa[12]	Molar Absorptivity[a] ($Mol^{-1}.Cm^{-1}.L$)	Effective Mobility ($10^5 cm^2.V^{-1}.s^{-1}$)	Ref
Cationic				
Tris	8.08	-----	29.5	15
Anionic				
Sulfosalicylic acid	2.49 (pK_1) 12.00 (pK_2)	$4.4x10^4$ (210 nm)	-84	10
Trimellitic acid	2.52 (pK_1) 3.84 (pK_2) 5.20 (pK_3)	$2.4x10^4$ (210 nm)	-92	10
1,5-Naphthalene-disulfonic acid	N/A[b]	$8.9x10^4$ (224 nm)	-74	10

[a] The molar absorptivities of the compounds were obtained by plotting absorbance against concentration with correlation coefficients larger than 0.99.
[b] Data is not available.

prediction of this detection scheme was first put forward by Beckers[2] and Collet et al.[3] But it was only recently demonstrated by Lu et al.[4] and Xiong et al.[5] for the simultaneous detection of the cations and anions. Because of the complicated electropherogram (some cations detected as either peaks or dips) and mechanism involved in this detection scheme, this method has not been applied in any real sample analysis.

Theoretically, either cationic or anionic chromophores could be used as one of the components of the BGE, and they are generally organic amines and acids. Therefore, two types of BGEs for this detection scheme could be formulated. One is composed of the cationic chromophore and a pH adjusting acid. The other is composed of the anionic chromophore and a buffering base.

For simultaneous detection and separation of the cations and anions, higher pHs are favorable to enlarge the EOF and drive the two classes of ions to migrate in the same direction towards the detector. In such cases, only amines with larger pK_a values would be appropriate to act as the chromophore. However, one drawback associated with this type of BGEs is that the deprotonation of the ionized amines at higher pHs, especially when the pH is

Table 2

The Magnitude of the ΔA for Potassium in Each of the BGEs

Anionic Chromopore	TR[a]	$z_{counterion}$	$(z_{sample}-z_{co\text{-}ion}.TR)$ $-\varepsilon_{counterion}/z_{counterion}$ [b]
Sulfosalicylic acid	0.65	-1	15400
Trimellitic acid	0.65	-3	2800
1,5-Naphthalenedi-sulfonic acid	0.67	2[c]	14685

[a] Effective mobility of potassium: 55.3×10^5 $cm^2.V^{-}1.s^{-1}$. All other mobilities used in the calculations are given in Table 1. [b] In the calculation all the charge numbers of the ionic species were used as absolute values without consideration of the sign of them according to the derivation of eq. 1[5]. $z_{sample}=z_{co\text{-}ion}=1$. [c] because the pK_a value of 1,5-naphthalenedisulfonic acid is not available, the charge number of it is assumed as 2.

close to their pK_as, degrades their function as the counterion of the anionic analytes. This results in poor peak shapes for these analytes.[4] In contrary, BGEs containing an anionic chromophore and a buffering counterion have no such problems and they will be explored in the present work.

From eq. 1, we can see that the sample zone absorption in this type of BGE is dictated by the transfer ratios of the sample ions and the molar absorptivity of the chromophore. As a result, a priori knowledge of the electrophoretic behavior of the candidate of the chromophore would be helpful for us to select the most appropriate chromophore of the BGE. For these purposes, the effective mobilities of three organic acids with relatively large molar absorptivities and a cationic buffer (Tris) were determined in a phosphate buffer and are given in Table 1.

Based on the electrophoretic and spectroscopic information of the components of the BGEs, we could not decide which of them was better matched with the anionic analytes of interest. This is because the sample zone absorption is dependent both on the transfer ratios of the analytes and on the molar absorptivity of the chromophore in the given BGE according to eq 1. Given the concentration of the analyte and the capillary dimension, the magnitude and the order of the ΔA for the analytes, e.g., K^+ in each of the BGEs, were worked out and shown in Table 2. From Table 2, we can see that 1,5-naphthalenedisulfonic acid and sulfosalicylic acid are the best choices as the BGEs for anionic chromophore in BGE. As sulfosalicylic acid has previously

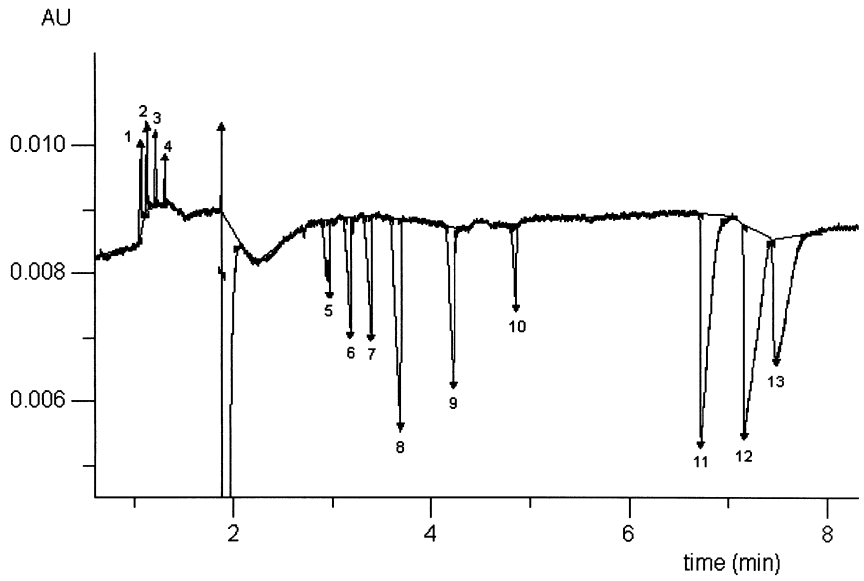

Figure 1. Electropherogram obtained under the optimized condition for a model sample. Peak identification (20 μg/mL each): 1. K^+, 2. NH_4^+, 3. Na^+, 4. Li^+, 5. ascorbate, 6. sorbate, 7. benzoate, 8. lactate, 9. acetate, 10. HCO_3^-, 11. phosphate, 12. formate, 13 fluoride. Separation conditions: 2 mM 1,5-naphthalenedisulfonic acid, 10 mM tris, pH = 8.5, 30 kV, 60 cm x 50 μm fused silica (45 cm to detector), 224 nm, 0.1 min injection time under 50 mbar, ambient temperature.

been used for simultaneous detection,[12] 1,5-naphthalenedisulfonic acid was selected as an alternative for the anionic chromophore in the BGE. Therefore, Tris/1,5-naphthalenedisulfonic acid BGE was employed in the separation of a model sample which contains 4 cations and anions in the following experiments.

Optimisation of the Separation Conditions

The difficulty associated with the simultaneous separation of cations and anions is to drive the anions to migrate to the detector on the cathodic side within a reasonable analysis time. One effective way to force the anions to migrate to the cathodic side is to enlarge the electroosmotic flow (EOF).[10] Generally, high voltage, lower background concentration, and higher pH values could be used to enlarge the EOF.[9,12] Therefore 1,5-naphthalenedisulfonic acid concentration was fixed at 2 mM and the applied voltage was set at 30 kV (normal polarity) to minimize the analysis time. Under these conditions, the only degree of freedom of the electrophoretic system is the solution pH. It was found that all the ions involved in the model sample could migrate in the same

Table 3

Comparison of the Limits of Detection of the Cations and Anions in Single and Dual UV-Absorbing BGEs

Ions	Limit of Detection[a] (μg/mL)	Limit of Detection[b] (μg/mL)
K^+	0.5	2.0
NH_4^+	1.0	5.0
Na^+	0.5	3.0
Li^+	1.0	0.5
Ascorbate	0.5	0.2
Sorbate	0.5	0.2
Benzoate	0.2	0.1
Lactate	0.1	0.09
Acetate	0.2	0.8
HCO^-_3	0.2	----
Phosphate	0.2	----
Formate	0.2	----
Fluoride	0.2	----

[a] Limit of detection is defined as three times the background noise. [b] Limit of detection obtained using dual UV-absorbing background electrolytes:[12] 1,2 dimethylimidazole (4 mM), trimellitic acid (0.9 mM).

direction towards the detector at pH larger than 8. This illustrated that the EOF produced was large enough to offset the intrinsic migration of the anions to the anodic side of the electrophoretic system under the given condition. It was reported[13] that K^+ and NH_4^+could be separated when pH was higher than 8. However, this phenomenon was not observed in the present BGE. By further increasing the solution pH up to 9, K^+ and NH_4^+ could be separated but the peaks corresponding to phosphate, fluoride, and formate overlapped. Therefore, the working pH range in the present BGE was from 8 to 9. By compromising the separation selectivity for the cations and the anions in the model sample, pH 8.5 was finally found to be the most appropriate condition for the separation and detection of all the ions in the model sample. Figure 1 shows the electropherogram of the model sample at the optimal condition. It can be seen that all the cations are detected as positive peaks. This is because the effective mobility of Tris is lower than that of the cationic analytes at the given pH condition as predicted by eq. 1.[3,5] Magnesium and calcium could not be detected in the present BGE. This might be due to the fact that their mobilities are the same as that for the co-ion, Tris, in the BGE at the given condition. As a result, they were not included in the model sample mixture.

Limits of Detection

The limits of detection were determined under the condition given in Figure 1 and the signal-to-noise ratio (S/N) was defined as three. The results obtained are presented in Table 3. From this table, we can see that the detection limits for Na^+, K^+, and NH_4^+ are better than those obtained in a dual UV-absorbing BGE.[12] This observation is attributed to the absence of the counteraction effect in UV absorption in the present BGE, which exists in the dual UV-absorbing BGE.[12]

However, the detection limit obtained in the present BGE for the anions is poorer than that in the dual UV-absorbing BGE. The reason for this observation might be that the transfer ratios of the anionic analytes attained in the present BGE are less than those obtained in the dual UV-absorbing BGE given in Table 3. By comparing the effective mobilities of the anionic chromophores used to formulate the BGEs in Table 1 and eq. 2, the explanation is quite reasonable.

Linearity of Calibration and Reproducibility

The usefulness of the method for quantitative analysis was evaluated by examining the dynamic range of the quantitation and reproducibility of peak height and migration time. The concentration ranges of the individual analytes were from at least two times higher than the detection limits upwards to 20 ppm. The correlation coefficients of the calibration lines for each of the analytes ranges from 0.9918 to 0.9999. The reproducibility was calculated for 5 consecutive injections of the model sample with 10 ppm concentrations for each of the analytes. The results were calculated as relative standard deviation (RSD) of peak height and migration time. All results are shown in Table 4.

Application and Comparison

In order to demonstrate the applicability and accuracy of the developed method, a soft drink was chosen as the test sample and the results were compared with those obtained by ICP for cations and IC for phosphate. Figure 2 illustrates a typical electropherogram obtained under the separation conditions as given in Figure 1. Table 5 reports the results obtained by the different techniques.

From Table 5, we can see that the results obtained by the present method are in excellent agreement with those attained by ICP and IC. This shows that the newly developed method is viable for simultaneous detection of cations and anions. This method is more convenient to use than previous methods[10,11] since only one UV-absorbing background is required.

Table 4

Calibration Data and Precision Obtained from Five Consecutive Runs

Ions	Correlation Coefficient	Peak Height[a]	Migration Time[a]
K^+	0.9966	4.46	0.28
NH_4^+	0.9993	2.58	0.26
Na^+	0.9976	2.58	0.26
Li^+	0.9990	5.93	0.36
Ascorbate	0.9947	4.38	0.68
Sorbate	0.9976	3.31	0.71
Benzoate	0.9950	2.85	0.75
Lactate	0.9984	3.06	0.80
Acetate	0.9999	6.79	0.95
HCO_3^-	0.9970	6.18	1.18
Phosphate	0.9960	2.64	1.60
Fluoride	0.9918	4.18	1.87
Formate	0.9934	3.10	1.97

[a] Relative standard deviation (%).

Table 5

Results of Potassium, Sodium and Phosphate in a Soft Drink by CE and Other Methods[a]

Ions	CE[b] (μg/mL)	Other Methods[b] (μg/mL)
Potassium	6.4 (4.3%)	6.3[c] (0.1%)
Sodium	19.9 (1.3%)	20.3[c] (1.4%)
Phosphate	14.4(1.18%)	14.3[d] (2.5%)

[a] Diluted 1:25. [b] Values in parentheses in this table are the relative standard deviations (%) for triplicate measurements. [c] Inductively Coupled Plasma (ICP). [d] Ion Chromatography.

CONCLUSIONS

It has been demonstrated that single UV-absorbing BGE provides an alternative method for simultaneous separation and detection of cations and anions. Because there is only one chromophore involved in the BGE, this

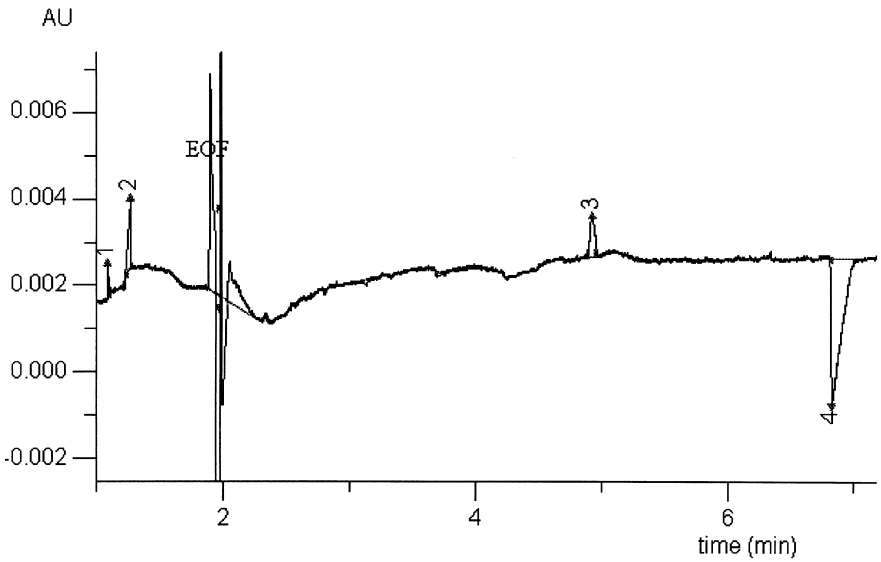

Figure 2. Electropherogram of a soft drink. BGE and separation conditions are the same as in Figure 1. Peak identification: 1. K^+, 2. Na^+, 3. not identified, 4. phosphate.

method permits a large number of chromophores to be used as the components of the BGE. The formulation of the BGE is simple and the detection conditions are easily controlled compared with the dual UV-absorbing BGEs. Although the displacement mechanism involved in this detection scheme is complicated, the complexity of the peak pattern (positive and negative peaks appearing in the same electropherogram) can be reduced by proper selection of the components of the BGE and the detection conditions. The results obtained indicate that the present method has satisfactory detection sensitivity, linear range, and reproducibility and it can be applied in real sample analysis.

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REFERENCES

1. F. Foret, S. Fanali, L. Ossicini, P. Boček, J. Chromatogr. A, **470**, 299 (1989).

2. J. L. Beckers, J. Chromatogr. A, **679**, 153 (1994).

3. J. Collet, P. Gareil, J. Chromatogr. A, **115**, 115 (1995).

4. B. Lu, D. Westerlund, Electrophoresis, **19**, 1683 (1998).

5. X. Xiong, S. F. Y. Li, J. Chromatogr. A, **835**, 169 (1999).

6. A. Weston, P. R. Brown, P. Jandik, W. R. Jones, J. Chromatogr., **593**, 289 (1992).

7. S. C. Beale, Anal. Chem., **70**, 279R (1998).

8. P. Kuban, B. Karlberg, Anal.Chem., **70**, 360 (1998).

9. K. Bächmann, I. Haumann, T. Groh, Fresenius J. Anal. Chem., **343**, 901 (1992).

10. X. Xiong, S. F. Y. Li, Electrophoresis, **19**, 2243 (1998).

11. S. A. Shamsi, N. D. Danielson, Anal. Chem., **67**, 4210 (1995).

12. X. Xiong, S. F. Y. Li, J. Chromatogr. A, **822**, 125 (1998).

13. P. Morin, C. Francois, M. Dreux, Analusis, **22**, 178 (1994).

14. J. A. Dean, ed., **Lange's Handbook of Chemistry**, McGraw-Hill, New York, 1985.

15. T. Hirokawa, T. Gojo, Y. Kiso, J. Chromatogr, **369**, 59 (1986).

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