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Analysis of linear alkylbenzenesulfonates by capillary zone electrophoresis with large-volume sample stacking

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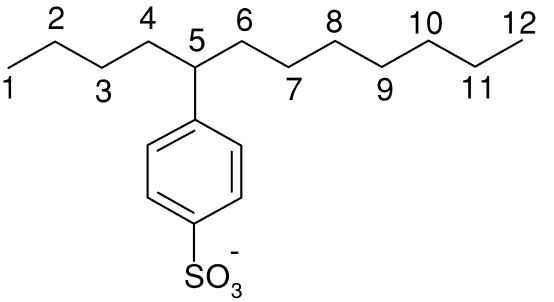
Abstract

A systematic investigation of optimal conditions for determining the homologues of linear alkylbenzenesulfonates (LAS) by capillary zone electrophoresis (CZE) using the large-volume sample stacking technique was presented. The most effective sample stacking and separation conditions was 20 m*M* borate buffer with 30% acetonitrile at pH 9.0, and the sample hydrodynamic injection of up to 90 s at 4 p.s.i. (1 p.s.i.=6892.86 Pa) (around 711 nl). Under such conditions, approximately a 100-fold enrichment factor was achieved based on peak heights. The reproducibility of migration time and quantitative results of stacking CZE can be improved by using internal standards. Quantitation limits of the homologues of LAS were 0.002–0.01 mg/l under these enrichment conditions. The analysis of real samples of laundry and dishwashing detergents was performed. The established high-performance liquid chromatography method was applied to evaluate the stacking CZE method, and compatible results were obtained.

Keywords: Large-volume sample stacking; Sample stacking; Alkylbenzenesulfonates; Linear alkylbenzesulfonates; Surfactants

1. Introduction

Linear alkylbenzenesulfonates (LAS) are the most commonly used anionic surfactants in household laundry and dishwashing detergents. They are used as complex mixtures of C_{10}–C_{13} homologues and of positional isomers where the benzenesulfonate is located at various alkyl carbon positions from the second to the center. An example of a linear dodeylbenzenesulfonate (C_{12}-LAS) isomer is shown in Fig. 1. The detergency depends mainly on the



An example 5-C_{12}-LAS

Fig. 1. Structure of a linear alkylbenzenesulfonate (i.e., C_{12}-LAS isomer).

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structure, the position of the benzenesulfonate group and the alkyl chain length of the hydrophobic part. The homologues and isomeric distributions provide information about the manufacturing process and can be considered as fingerprints of the products, associated with their biodegradability and toxicity. Moreover, varying content of homologues and isomers leads to a wider effectiveness spectrum which contributes to the efficiency of the wash. In order to monitor product control, formulation and application, it is necessary to develop convenient and appropriate analytical techniques for the separation and detection of these surfactants.

The characterization of LAS for product control and formulation has usually been performed by using diverse techniques such as: two-phase titration [1], desulfonation gas chromatography or gas chromatography–mass spectrometry [2–4], capillary electrophoresis [5–7] or high-performance liquid chromatography (HPLC) [8–10]. The HPLC method has been extensively employed in routinely analyzing LAS in various products. However, long analysis times with a relatively high solvent consumption, make this method inefficient. Capillary zone electrophoresis (CZE) has recently become one of the more outstanding separation techniques for analyzing large numbers of charged species [11,12]. The method is preferred to conventional chromatographic techniques in many applications because of its high efficiency, small sample volumes, lower solvent consumption than HPLC, short analysis time and the possibility of rapid development of the method [13,14]. However, in many cases CZE appears to lack sensitivity due to the short optical path associated with the on-column detection. The large-volume sample stacking (LVSS) technique is one of the on-column concentration techniques to improve its detection sensitivity [15–19]. The schematic in Fig. 2 shows the steps of the LVSS technique under reversed polarity conditions. The technique involves hydrodynamic injection of a large volume of sample dissolved in either a lower conductivity buffer matrix or just water. Thereafter, a voltage is applied across the electrodes with ‘‘reversed polarity’’ [outlet positive, therefore reversed the direction of electroosmotic flow, EOF], where the lower conductivity buffer matrix is pumped out from the capillary into the inlet

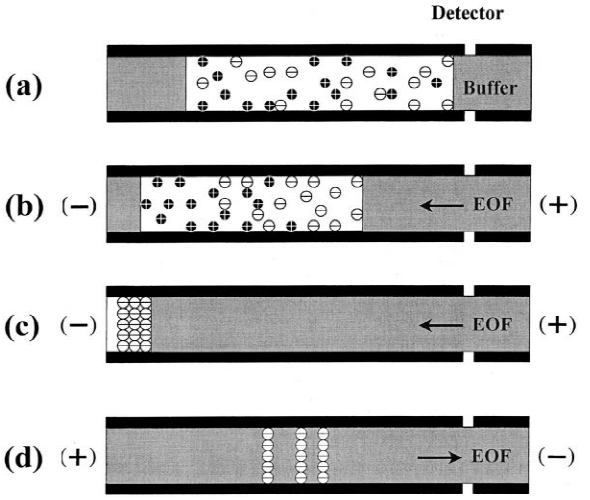

Fig. 2. The schematic of steps of CZE with LVSS under reversed polarity conditions. (a) A large-volume sample (prepared in water) was injected hydrodynamically and a small buffer plug was then injected; (b) the voltage was applied with reversed polarity (reversed EOF direction), the sample-matrix was pushed back into the inlet vial by the EOF; (c) anionic analytes were focused on passing through the concentration boundary; (d) optimal stacking was achieved, the polarity was switched to normal mode and the separation voltage was reapplied for the analytes' separation and detection.

vial and the anionic analytes are focused on passing through the concentration boundary. When the current reaches 95–99% of the original values, the polarity is switched to its ‘‘normal condition’’. The separation voltage is reapplied where the focused analyte zone migrates toward the detector and continues to separate out. This technique has been successfully employed in on-column enrichment of various negatively charged compounds [20–23]. However, CZE with the LVSS technique has not yet been applied to anionic surfactants.

In this paper, we discussed our research into the separation of a LAS mixture by CZE using the LVSS technique. The effect of stacking and separation conditions was considered, and the application to real samples of household detergents was performed. To validate the quantitative CZE method with LVSS, the results were compared to those obtained using the established HPLC method.

2. Experimental

2.1. Chemicals and reagents

Unless stated otherwise, all high purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany) and were used without further purification. Commercial LAS mixture was provided by Taiwan Surfactant Co. (Taiwan) and used as a calibration standard. The composition of the LAS mixture includes four C_{10}–C_{13} alkyl chain homologues and 4–6 benzenesulfonate isomers per homologue. The internal standard, 4-octylbenzenesulfonic acid (C_8-LAS), was purchased from Aldrich. Sodium tetraborate ($Na_2B_4O_7$) separation buffers were prepared at stated concentrations between 5 and 30 m*M* in deionized water and were adjusted to a stated pH between 8 and 10. Stock standard solution (1000 mg/l) of LAS mixture was prepared with methanolic solution (50%, v/v). Working standard solutions were obtained by diluting the stock standard solution with deionized water to appropriate concentrations. The separation buffer was prepared with 30% acetonitrile (ACN) (between 0 and 40%, 30% being optimal, see Section 3) in 20 m*M* $Na_2B_4O_7$ (pH 9.0). Deionized water was further purified with a Minipore water purification device (Millipore, Bedford, MA, USA). The household laundry and dishwashing detergents as powders or liquid form were purchased from local supermarkets. The liquid detergents were diluted with deionized water directly. The appropriate amounts of powder detergents were dissolved and then diluted with deionized water. To prevent capillary blockage, all solutions and samples were filtered through a 0.45-μm membrane filter (Gelman Scientific, Ann Arbor, MI, USA) prior to use.

2.2. Apparatus

All experiments were performed on a P/ACE MDQ system (Beckman-Coulter, Fullerton, CA, USA) equipped with UV–Vis detector. Separations were carried out in untreated fused-silica capillaries (J&W Scientific, Folsom, CA, USA) of 50 μm I.D. and an effective length of 50 cm (total length=60 cm). The UV detector was operated at 200 nm. All electrophoresis runs were performed at a temperature of 25°C. The on-column detection window was made by burning a small section (ca. 3 mm) of the external polymide coating and scraping off the burned residue with methanol. The pH of the solutions was measured by a Mettler-Toledo MP220 pH meter (Schwerzenbach, Switzerland).

2.3. General electrophoresis and stacking procedures

Before use, the capillary was conditioned with methanol for 10 min at 25°C, followed by 10 min with 1 *M* HCl, 2 min with deionized water and 10 min with 1 *M* NaOH. It was then rinsed with deionized water for 2 min, and followed by 10 min with the running buffer. Between runs, the capillary was washed with 0.1 *M* NaOH for 2 min and deionized water for 2 min before the run.

In the general CZE separation procedure, all samples were hydrodynamically injected into the capillary over 5 s at 0.5 p.s.i. (1 p.s.i.=6892.86 Pa), the volume was approximately 4.9 nl and the applied voltage used was 20 kV. Procedures for LVSS have been described elsewhere [23,24], and were used here with minor modifications. Briefly, the capillary was filled with a separation buffer and large-volume samples of analytes were prepared in deionized water and hydrodynamically injected into the capillary over an injection time of 90 s at 4 p.s.i. (around 711 nl). The small buffer plug (around 90 nl) was then injected after sample injection. At this point, the current decreased due to the lower electrical conductivity of the sample matrix (i.e., water). The ''stacking'' voltage was applied at ''reversed polarity'' (between 10 and 20 kV, 15 kV being optimal, see Section 3) at the sample-inlet end. When the reversed EOF pumped out the sample matrix from the capillary into the inlet vial, the current rose gradually to the initial value. The polarity was then switched to normal mode (20 kV) to allow for the analytes' separation and detection. The stacking period was measured between 1.5 and 2.2 min for optimal stacking efficiency. The stacking and separation steps were done automatically and controlled by

Beckman P/ACE System MDQ Ver. 2.2 software (Beckman-Coulter, Fullerton, CA, USA).

2.4. High-performance liquid chromatography analysis

The procedure used for HPLC analysis was carried out similar to Marcomini et al. [10]. Analyses were performed on a HP-1100 high-performance liquid chromatograph system (Hewlett-Packard, Delaware, USA) connected to an UV–Vis detector operating at 200 nm. A Hypersil-HS-C_{18} column (25×0.46 cm I.D., 0.5 μm packing, ThermoQuest, Runcorn, UK) with a guard column was used at room temperature with an eluent flow-rate of 1 ml/min, with an injection volume of 20 μl. The mobile-phase solvents were ACN (eluent A) and water containing 14 g/l of $NaClO_4$ (eluent B). A 23-min linear gradient from 36% A–64% B to 70% A–30% B was used for analysis. Initial eluent composition was re-established by a 2-min linear gradient, followed by an equilibration time of 5 min. For quantitation, commercial LAS mixtures as standard solutions were prepared by dissolving in methanolic solution (50%, v/v) to construct a five-level internal calibration curve (or average response factor, RF) covering the range 0.3–2.0 mg/ml. The internal standard 4-octylbenzenesulfonic acid (C_8-LAS) of 0.1 mg/ml was added to each standard solution. The precision of the curve, as calculation for each LAS homologue, as indicated by the relative standard deviation (%RSD) of response factors, was 7.1, 3.9, 5.4 and 6.4% for the corresponding C_{10}-, C_{11}-, C_{12}- and C_{13}-LASs. The calibration curves were linear with coefficients of determination of $r^2 \geq 0.99$.

3. Results and discussions

3.1. Evaluation of separation and stacking conditions

3.1.1. Acetonitrile content

Adding organic modifiers to the separation buffer in CZE can affect both the EOF and the electrophoretic mobilities. The decrease in EOF is mainly due to an increase in viscosity [13,14]. Heinig and colleagues stated that ACN is the most effective organic modifier for CZE separation of LAS homologues in wastewater [5]. Without organic modifiers in the buffer, all homologues can not be separated. Fig. 3 displays the results obtained when different quantities of ACN were added to the buffer to separate LAS in a sample dissolved in deionized water. When no ACN was added (Fig. 3a), peaks were poorly separated. As the quantity of ACN was increased, the longer migration time for LAS homologues with better separation was observed. When 30% ACN (v/v) was reached, perfect resolution and better peak shapes were obtained within 15 min. Beyond that, more ACN lead to better separation but increasing the migration time and broad the peak shape. The migration time of the LAS homologue decreases as the alkyl length increases because of the negatively charged molecules, the larger sized ones migrate to the anode (+) slower than the smaller ones due to the electrophoretic mobility of the CZE.

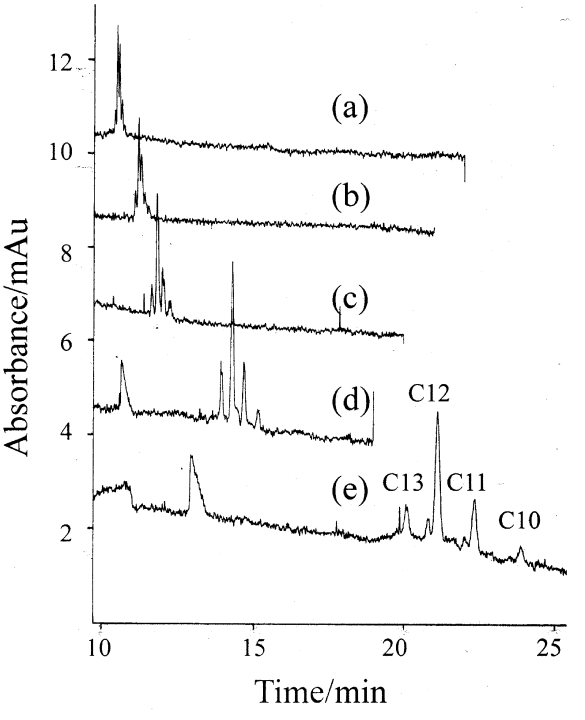

Fig. 3. Separation and peak resolution (*R*, average of all pairs in parentheses) of LAS using acetonitrile in varying proportions (v/v): (a) 0% (*R*=0.5), (b) 10% (*R*=0.6), (c) 20% (*R*=1.3), (d) 30% (*R*=1.8), (e) 40% (*R*=3.4). LAS (20 ppm) in deionized water; separation buffer=20 m*M* $Na_2B_4O_7$ (pH 9.0); voltage=20 kV; temperature=25°C; detection=200 nm.

3.1.2. Buffer concentration and pH

The influence of buffer concentration on the migration time and the separation of LAS homologues was examined in the range 5–30 m*M* of borate buffer at pH 9.0 with 30% ACN. As the concentration of borate was increased, a longer migration time and better peak resolution were obtained. At a concentration of 20 m*M*, perfect resolution and better peak shapes were obtained (results not shown).

Buffer pH is also important in CZE separation because it affects both the charge of the analyte and the strength of EOF. The resolution and peak shape for LAS did not significantly change from pH 8 to 10 (results not shown). A buffer pH of 9.0 was used since no pH adjustment was required at this point.

3.1.3. Stacking periods and voltages

Several investigations demonstrated that the sample concentration effect depends strongly on the ''stacking'' period and the voltage when polarity is reversed [15,16]. Switching back to the normal polarity at the right moment is very important because a back-flush during the ''stacking'' period may cause analytes to be lost. Injecting a small plug of separation buffer after the sample can avoid the possibility of losing analytes. The greatest enhancement of the total LAS peak area was observed when the voltage polarity at 15 kV for 1.8 min was reversed and current was at −10 μA. The stacking effect fell dramatically for longer stacking periods (i.e., 2 min) or higher stacking voltages (i.e., 20 kV).

Fig. 4 shows the electropherogram of the optimial CZE with LVSS technique for on-line concentration of a dilute LAS mixture (0.5 ppm LAS mixture+0.3 ppm of C_8-LAS). With the conditions give in Fig. 4b, detector responses increased with an increasing sample injection time and pressure, corresponding to around 800 nl (711 nl sample+90 nl buffer) injected into the column. To apply the sample stacking technique, the quantitation limits can be reduced to 0.01, 0.004, 0.002 and 0.01 mg/l corresponding to C_{10}-, C_{11}-, C_{12}- and C_{13}-LAS, respectively. Approximately a 100-fold enrichment factor was achieved over the general CZE, from comparison of the LAS peak heights. The advantage of this stacking technique is that the analysis is performed on the sample itself, without the need for pre-treatment or prior manipulation.

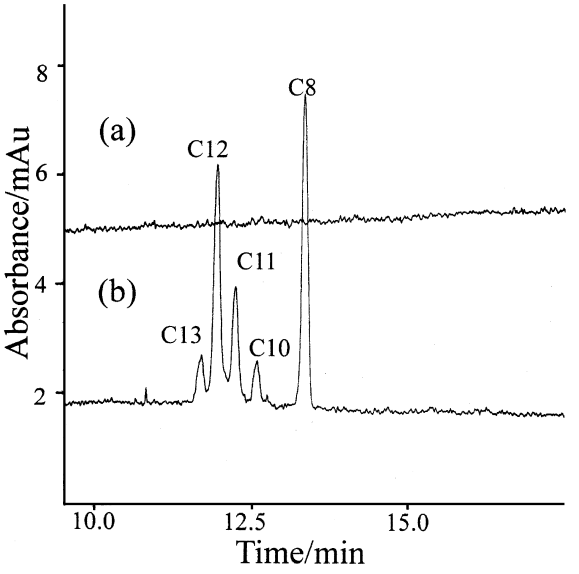

Fig. 4. Comparison the electropherograms of LAS mixture (0.5 ppm in deionized water) by (a) general CZE, hydrodynamic injection of 5 s at 0.5 p.s.i. (around 4.9 nl) and (b) optimized CZE with LVSS, hydrodynamic injection of 90 s at 4 p.s.i. (around 711 nl). Experimental conditions as given in Fig. 3.

3.2. Validation of the stacking procedure

To validate the performance of the sample stacking technique, the reproducibility (in terms of %RSD) and linearity with standard solution mixtures under the optimal conditions described above were investigated. Table 1 summarizes the relative standard deviations (%RSD) of migration time, peak heights, relative migration time and relative peak heights, as well as the linearity of the response in the studies of the LVSS technique. The reproducibility of the technique was tested with eight replicate injections of a LAS standard mixture (0.5 ppm). The %RSD of the migration time and the peak heights were approximately 1.5 and 3.7–6.1%, respectively. However, these values improved when the internal standard was employed. The linearity of the curve for each LAS homologue, as indicated by the %RSD of the response factors, varied from 3.2 to 5.0%. The calibration curves were linear with coefficients of determination $r^2 \geq 0.988$. The %RSD of the relative

Table 1
Reproducibility, linearity of response and response factors using the CZE with LVSS technique

	LAS mixture			
	C_{10}	C_{11}	C_{12}	C_{13}
Reproducibility (N=8, using 0.5 ppm of total LAS)				
Migration time (%RSD)	1.5	1.5	1.6	1.6
Peak height (%RSD)	5.8	3.7	4.1	6.1
Relative migration time (%RSD) (I.S. C_8-LAS, 0.3 ppm)	0.1	0.2	0.1	0.1
Relative peak height (%RSD)	4.9	3.9	2.0	4.5
Linearity of response				
Correlation coefficient (r^2)	0.988	0.997	0.998	0.997
Response factor (%RSD) (I.S. C_8-LAS, 0.3 ppm)	5.0	3.2	3.9	4.7
Relative migration time (%RSD)	0.1	0.2	0.3	0.1

Concentration range: 0.3–5 ppm, six-levels.

migration time was around 0.2% when various LAS concentrations were injected. These results demonstrate that the CZE with LVSS technique provides high reproducibility and excellent linearity.

3.3. Applications

Table 2 demonstrates the versatility of this stacking technique listing the homologues concentrations

Table 2
Results and comparison of LAS determination in household detergents using the CZE with LVSS and HPLC techniques

Detergents	Homologous conc. (mg/g)				$t_{calculated}$ [a]
	C_{10}	C_{11}	C_{12}	C_{13}	
1 (stacking CZE)	4.9	40.0	41.3	12.2	
2 (stacking CZE)	8.2	35.8	52.3	14.6	
3 (stacking CZE)	5.1	40.8	45.5	15.8	
4 (stacking CZE)	8.3	33.2	34.6	10.2	2.95
4 (HPLC)	10.2	36.2	41.3	18.0	
5 (stacking CZE)	19.0	63.0	45.4	23.9	0.21
5 (HPLC)	13.2	56.4	50.0	28.7	
6 (stacking CZE)	6.9	57.9	72.1	12.6	0.045
6 (HPLC)	7.9	56.0	70.6	14.7	
7 (stacking CZE)	1.4	7.2	7.2	3.2	0.51
7 (HPLC)	3.4	5.8	5.7	2.2	
Relative migration time (%RSD for CZE analysis)	0.2	0.3	0.3	0.3	
Estimated of limit of quantitation (mg/l)					
Stacking CZE	0.01	0.004	0.002	0.01	
HPLC	0.2	0.08	0.04	0.2	

[a] From Ref. [25] Eqs. (4)–(10) and (4)–(11). $t_{calculated} = d/s_d\sqrt{n}$; $s_d = \sqrt{\Sigma(d_i - d)^2)/n - 1}$, where d_i is the individual differences between results for each sample, d is the average difference between methods A and B and n is the number of pairs of data (four in this study).

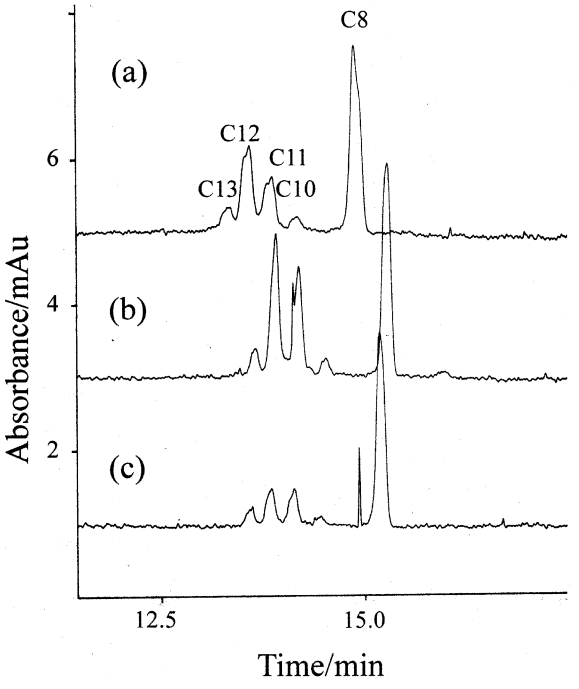

Fig. 5. Electropherograms of CZE with LVSS for separation of LAS homologueus in detergents from different manufactures: (a) detergent 2, (b) detergent 6 and (c) detergent 7.

of LAS mixtures detected in household detergents and the results of HPLC analysis. Fig. 5 shows the typical electropherograms of CZE with LVSS for the separation of LAS homologues in detergents. Each of these separations shows the shoulders and variations in peak widths, which are mostly likely the positional isomers, and it can be seen that the relative amounts of these isomers vary with the manufactures. The %RSD of the relative migration time was around 0.3%. The peak identification and quantitation were performed by relative migration time and response factors, respectively, using C_8-LAS as an internal standard. The variation in the homologues distribution from different manufacturers was observed with the total LAS content ranging from 1.9 to 15.1% (although some of the homologues' distributions were not given by the manufacturers). Table 2 compares the quantitative results obtained from the CZE with LVSS and the HPLC method. Using the student's *t*-test procedure to compare individual differences [25], the two methods for LAS determination were evaluated. In this test, the CZE with LVSS and HPLC methods were used to make single measurements on several samples, such that no measurement was duplicated. The *t*-test was to determine whether the two methods yielded the same results ''within experimental error'', or was one systematically different from the other in certain confidence levels. The test was applied to detergents 4–7. The calculated *t*-values (in Table 2) were less than the table list *t*-value (3.182) [25] at 95% confidence with three degrees of freedom. Therefore, these two methods are not significantly different at the 95% confidence level. However, the CZE with LVSS technique consumed less than 200 μl of solvent for each analysis, whereas HPLC consumed around 50 ml. The sensitivity of the stacking CZE method is better compared to established HPLC methods.

4. Conclusion

The analytical procedure developed herein demonstrates that the CZE with LVSS technique offers a reliable, sensitive and convenient analytical technique for determining LAS in commercial product samples. In separating LAS by stacking CZE, acetonitrile content and buffer concentration are the two most important separation parameters that most affect the migration time and the resolution of LAS homologues. As expected, CZE with LVSS analysis leads to better peak shapes, higher efficiency and sensitivity, and consumes significantly less solvent than is required in HPLC analysis. The reproducibility of the migration time and the quantitative results of CZE with LVSS can be improved by internal standards. The automatic CZE with LVSS technique has the potential to become a more efficient and useful method for LAS analysis than the established HPLC methods.

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