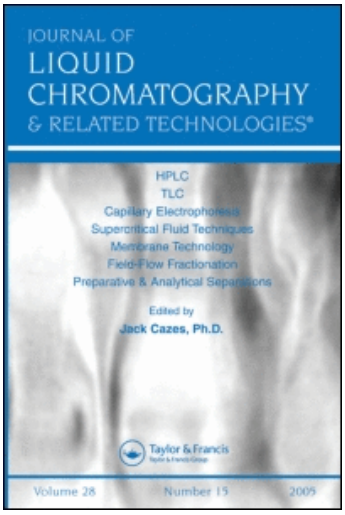

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QUANTITATION OF ALKYL SULFONATES USING UV DETECTOR SENSITIVE "ION-PAIR" REAGENTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Amplification of detector response by means of detector-sensitive ion-pairing reagents demonstrates good sensitivity, linearity, and precision for the quantitation of alkylsulfonate ions by means of ultraviolet absorbance detection at 254 nm.

INTRODUCTION

UV detection of non-UV-absorbing samples was recently shown to be feasible in reversed-phase LC systems by using detector-sensitive "ion-

pairing" reagents in the eluent (1-5). This visualization technique is accomplished without the aid of any reaction type detector or extraction step, and provides novel methods for controlling both retention and detectability which are of value to analytical chemistry methodology from both practical and mechanistic points of view. The explanation of UV visualization in this reversed-phase system is based upon the ion-interaction model (1, 4, 6) which has recently been supported by others (7, 8). This paper identifies the constraints in the use of the UV visualization as a quantitative tool and suggests some guidelines for its successful application.

EXPERIMENTAL

The chromatographic system has been described elsewhere (6) and consisted of a Model 6000A pump (Waters), 4-cm x 4-mm i.d. Porapak C_{18} precolumn (Waters), and a 30-cm x 4-mm i.d. μBondapak C_{18} main column (Waters). The UV detector signal was digitized by a Model ADC-12QZ analog-to-digital converter (Analog Devices) interfaced to a Model 9830A digital computer (Hewlett-Packard) (9). Chromatograms were drawn from the digitized data on a Model 9862A plotter (Hewlett-Packard).

The mobile phase was prepared from HPLC grade methanol and distilled water containing stated amounts of phenethylamine (Fisher) or p-ethyl benzenesulfonate (Rutgers-Nease). The mobile phase was adjusted with hydrochloric acid or perchloric acid (1M) to a nominal stated pH as measured by a glass electrode. Sodium pentane- and hexanesulfonate samples (Eastman Red Label) were usually prepared in the eluent. At 25°C and 2.0 ml/minute flow rate the back pressure of the column was approximately 1.4×10^7 Pa.

RESULTS AND DISCUSSION

Response Sensitivity Of Method

A mobile phase consisting of 6 mM phenethylammonium ion in methanol-water 35:65 was used to visualize non-UV-absorbing straight chain alkyl sulfonates. Figure 1A shows the retention behavior of increasing amounts

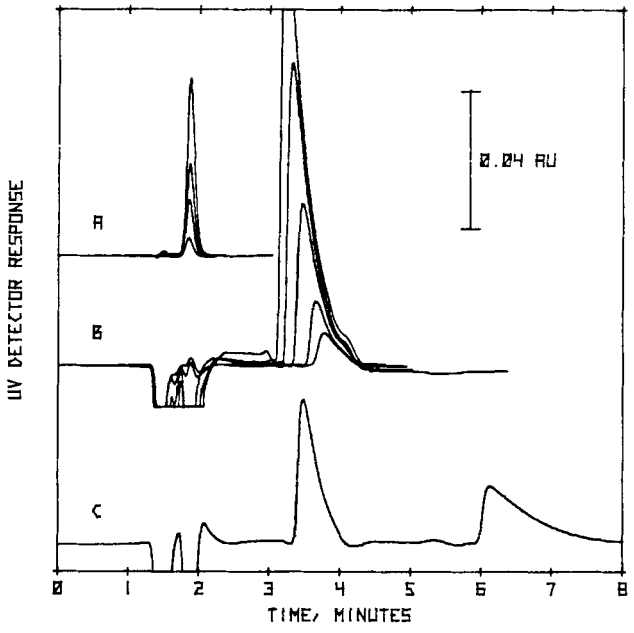

Figure 1. Visualization of alkylsulfonates. UV detector, 254 nm. Mobile phase was methanol-water 35:65, 6 mM phenethylammonium ion, pH = 3 with hydrochloric acid. All samples were made up in the mobile phase. A: Chromatograms obtained for 1, 3, 5, and 10 µL of 10 mM phenethylammonium ion. B: Chromatograms obtained for 1, 2, 5, 10, and 15 µL of 50 mM pentanesulfonate. C: Chromatogram obtained for 10 µL of 25.0 mM each pentanesulfonate (3.5 min) and hexanesulfonate (6.0 min).

of phenethylammonium ion injected as a sample. Figure 1B shows chromatograms obtained for increasing amounts of pentanesulfonate injected as a sample. The positive peak at about 3.5 minutes is caused by the co-elution of excess UV-absorbing phenethylammonium ion with the non-UV-absorbing pentanesulfonate (10). The two deficiency peaks at approximately 1.5 and 1.8 minutes are a result of the depletion of phenethylammonium ion from the bulk mobile phase. Note that the second deficiency peak occurs at the same retention time as phenethylammonium ion in Figure 1A. Response sensitivity from the injection of phenethylammonium ion (Figure 1A) was 2.62 AU-sec/μmole. Sensitivity to pentanesulfonate (Figure 1B) was 2.08 AU-sec/μmole, suggesting that approximately equal numbers of phenethylammonium and pentanesulfonate ions co-elute. Finally, Figure 1C shows the UV detector responses for the separation of pentanesulfonate and hexanesulfonate using phenethylammonium ion as the UV-absorbing ion-pairing reagent.

Calibration Methods

The chromatograms of Figure 2A are the results of constant volume injections of serial dilutions of 25.0 mM each of pentanesulfonate and hexanesulfonate. The samples were made up in and diluted with the mobile phase. Chromatograms in Figure 2B resulted from different volume injections of the 25.0 mM sample of pentanesulfonate and hexanesulfonate. Both the deficiency peak areas and the positive peak areas are seen to be related to the amount of sample injected and not to the concentration or volume injected. Integration of peak areas showed that for a given injection, positive and negative peak areas were equal within measurement uncertainties indicating that the phenethylammonium ion was depleted from the eluent and eluted later with the pentanesulfonate ion.

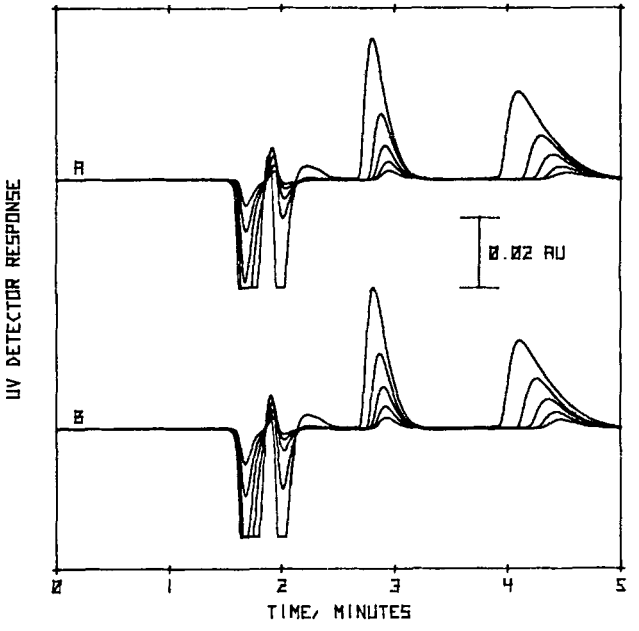

Figure 2. Comparison of constant volume and constant concentration injections of pentanesulfonate (2.8 min) and hexanesulfonate (4.3 min). UV detector, 254 nm. Mobile phase was methanol-water 35:65, 5 mM phenethylammonium ion, pH = 3 with perchloric acid. All samples were made up in the mobile phase. A: 16 µL of 1.6, 3.1, 6.3, 12.5, and 25.0 mM each pentanesulfonate and hexanesulfonate. B: 1, 2, 4, 8, and 16 µL of 25.0 mM each.

Comparison of the peak retention times of Figure 2 (5 mM phenethylammonium ion) and Figure 1 (6 mM phenethylammonium ion) indicates increased retention of the sulfonates with increased concentration of phenethylammonium ion as expected (6, 9).

Table I contains regression parameters (11) for eight sampling situations, showing the sensitivity linearity, and precision obtained in each case. Figure 3 is a representative regression plot of peak area vs.

TABLE 1

Regression of Peak Areas Vs. Amount Injected[a]

	Slope (AU-Sec/μmoles)		Intercept (AU-Sec)	
	Constant Concentration[b]	Constant Volume[c]	Constant Concentration	Constant Volume
Pentanesulfonate				
alone (n=11)	1.73±0.008[d]	1.73±0.007	0.015±0.002	0.010±0.002
in mixture(n=18)	1.31±0.006	1.33±0.020	0.016±0.001	-0.001±0.005
Hexanesulfonate				
alone (n=10)	1.82±0.020	1.83±0.030	0.008±0.005	0.006±0.012
in mixture(n=18)	1.35±0.080	1.37±0.030	0.011±0.002	-0.010±0.008

[a] Mobile phase was methanol-water 35:65, 5 mM phenethylammonium ion, pH=3 with perchloric acid. All samples were made up in the mobile phase.

[b] 1, 2, 4, 8, and 16 μL injections of 25 mM.

[c] 16 μL injections of 1.6, 3.1, 6.3, 12.5 and 25.0 mM.

[d] Uncertainties expressed as one standard deviation.

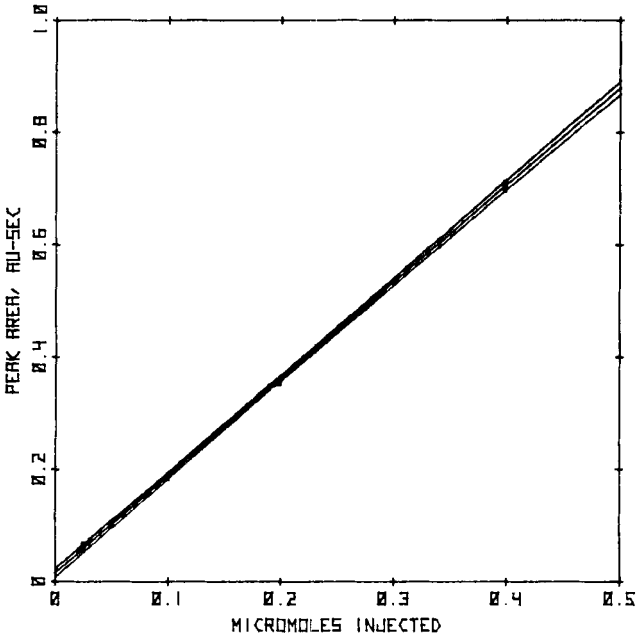

Figure 3. Regression plot of peak area vs. μmoles injected. Data from 16 μL injections of 1.6, 3.1, 6.3, 12.5, and 25.0 m_M_ pentanesulfonate. UV detector, 254 nm. Mobile phase was methanol-water 35:65, 5 m_M_ phenethylammonium ion, pH = 3 with perchloric acid. All samples were made up in the mobile phase. Confidence bands are for the 99.9% level.

μmoles of sample injected (11) for constant concentration injections of pentanesulfonate. At higher detection sensitivity, a relative imprecision (standard deviation/mean) of 3% at 3 nanomoles of pentanesulfonate was obtained. It can be seen from Table I that the slope is not affected by the mode of calibration (constant concentration or constant volume); however, constant volume calibrations appear to give smaller absolute intercept.

It has been reported that in UV visualization, variation of k' with sample size has limited quantitation when using peak heights (5). This data confirms that observation, but suggests that the use of peak area can improve the quantitation. Furthermore, the k' change as sample concentration increases seems to be independant of the charge on the "ion-pairing" reagent. Figure 4 demonstrates the applicability of the visualization

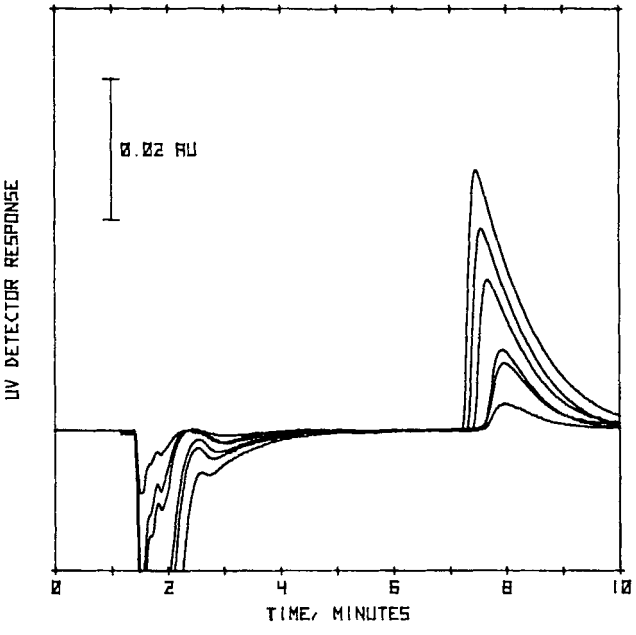

Figure 4. Visualization of octylammonium ion by p-ethylbenzenesulfonate ion. UV detector, 254 nm. All samples were made up in the mobile phase. Mobile phase was methanol-water 50:50, 5 mM p-ethylbenzenesulfonate, pH = 3 with hydrochloric acid. Chromatograms obtained for 2, 5, 6, 10, 15, and 20 µL of octylammonium ion at 25.0 mM. Sensitivity: 4.80 +/- 0.01 AU-Sec/µL octylammonium ion.

technique in a system of interchanged charges; i.e., a negatively charged "ion-pairing" reagent and a positively charged sample. UV chromatograms for a sample of octylammonium ion were obtained for a mobile phase that contained 5 mM UV-absorbing p-ethylbenzenesulfonate in methanol-water 50:50 at pH 3.

Table I also shows that the slope of the calibration curve for an ionic species is decreased by the presence of other alkyl sulfonates in the sample. Therefore, when using this technique for quantitative analysis, it will be important to investigate the contribution of other ions, both inorganic and organic, present in the sample matrix.

Figure 5 is a plot of the detection sensitivities for pentane- and hexanesulfonate vs. the square root of the conductivity of eluents containing 5 mM phenethylammonium ion and added amounts of potassium perchlorate. The lower ionic strength mobile phases give more sensitive visualization. The slope of the line for hexanesulfonate is about twice that for pentanesulfonate indicating that in this study the less retained compound is less affected by increased ionic strength.

Deficiency Peaks

Figure 6 is a set of chromatograms for constant volume injections of different concentrations of the single sample, hexanesulfonate. As the concentration of hexanesulfonate is increased, both of the negative deficiency peaks increase in area, and the ratio of the first deficiency peak to the area of the second deficiency peak remains constant. Again, in each chromatogram the two deficiency peaks have the same total area as the positive peak. This indicates that the two deficiency peaks are not an effect arising from the number of sample components but instead are characteristic of paired-ion chromatography. It is believed that the first

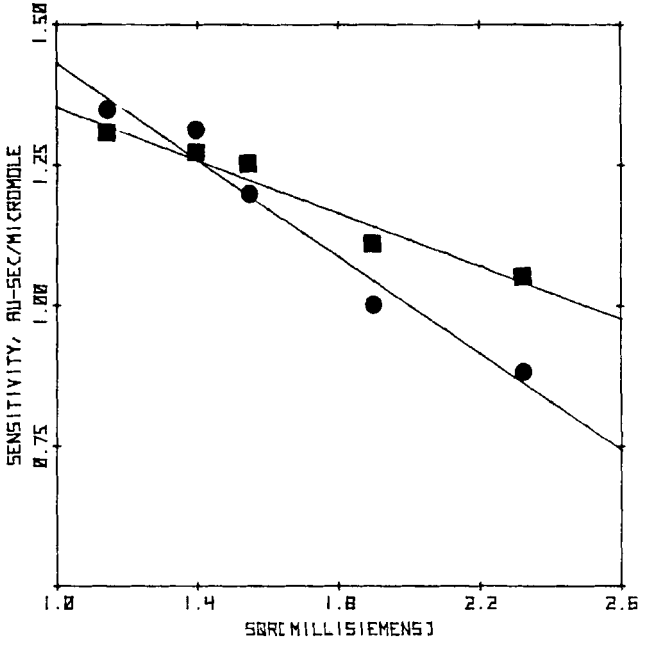

Figure 5. Sensitivity vs. conductivity of eluent. Mobile phase was methanol-water 35:65, 5 mM phenethylammonium ion, pH = 3 with perchlorate. ■ = pentanesulfonate (in mixture), ● = hexanesulfonate (in mixture).

peak is induced by equilibration of the local eluent composition upon injection, and that the second deficiency peak is a vacancy peak for the "ion-pairing" reagent (6, 12, 13).

Mixed Ion-Pair Reagents

In reversed-phase ion-pair LC, mixed reagents have been used to control retention. However, in UV visualization the presence of additional ions can reduce the sensitivity of the method. Therefore, a study was undertaken to demonstrate the inadvisability of using a non-UV-absorbing

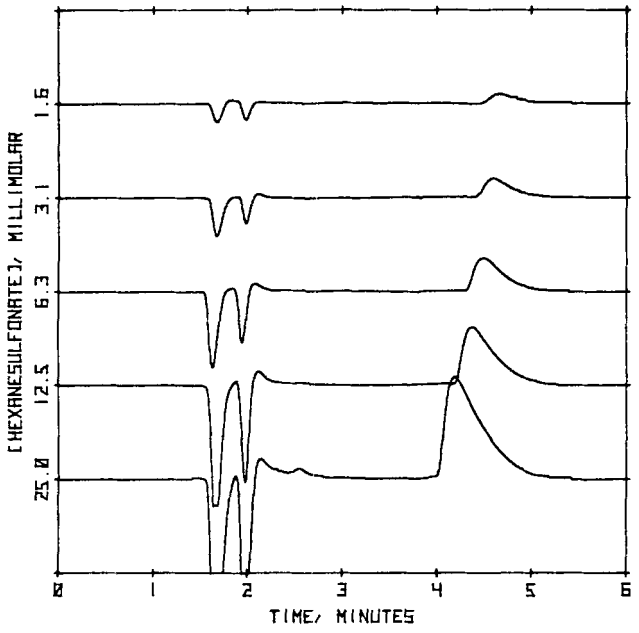

Figure 6. Individual chromatograms for 16 µL injections of 1.6, 3.1, 6.3, 12.5, and 25.0 mM hexanesulfonate. UV detector, 254 nm. All samples were made up in the mobile phase. Mobile phase was methanol-water 35:65 5 mM phenethylammonium ion, pH = 3 with perchloric acid.

ion-pairing reagent to adjust the retention time of the sample and a second UV-absorbing ion-pairing reagent to visualize the sample. Mobile phases composed of methanol-water 35:65 with 5mM octylammonium ion (the retention adjusting reagent) and either 1.0 mM or 2.0 mM phenethylammonium ion (the visualizing reagent) were prepared. The sensitivities in Table 2 confirm that both octylammonium ion and phenethylammonium ion participate in the retention of alkyl sulfonate (4,10). Thus, less of the UV-active phenethylammonium ion co-elutes with the alkyl sulfonate, which results in a decreased sensitivity for the alkyl sulfonate (6).

Table 2

Effects of Mixed "Ion Pairing" Reagents Upon the Visualization Sensitivity

(Octylammonium ion), mM	(Phenethylammonium ion), mM	Pentanesulfonate sensitivity, AU-Sec/μmole
5.0	1.0	0.058±0.010
5.0	2.0	0.121±0.003
0.0	5.0	1.31 ±0.01
0.0	6.1	1.59 ±0.04

CONCLUSION

The use of detector-sensitive ion-pairing reagents for the quantitation of otherwise non-detectable ionic compounds is an alternative to pre- and post-column derivatization techniques. Because only compounds of opposite charge are visualized by a UV-absorbing ion-pairing reagent, this technique enjoys an additional selectivity: the detection of neutral and similarly charged compounds will not be enhanced (2, 6). Although this work has used UV-absorbing ion-pairing reagents to demonstrate the amplification of detector response in reversed phase systems, the technique is applicable to other forms of detection as well, (e.g., electrochemical or fluoresence detection). This visualization technique offers a unique mechanistic probe into the fundamental nature of ionic interactions in liquid chromatography.

REFERENCES

1. B. A. Bidlingmeyer, S. N. Deming, W. P. Price, B. Sachok, and M. Petrusek, Advances In Chromatography, 14th International Symposium, Lausanne, Switzerland, September 24-28, 1979.

2. N. Paris, Anal. Biochem., 100, 250 (1979).

3. B. A. Bidlingmeyer, S. N. Deming, B. Sachok, 13th International Symposium On Chromatography, Cannes, France, July 2, 1980.

4. B. A. Bidlingmeyer, J. Chromatogr. Sci., 18 525 (1980).

5. N. Paris, J. Liquid Chromatogr., 3 (11), 1743 (1980).

6. B. A. Bidlingmeyer, S. N. Deming, S. N. Price, W. P. Sachok, M. Petrusek, J. Chromatogr., 186, 419 (1979).

7. J. K. Knox and R. H. Hartwick, J. Chromatogr., 204, 3 (1981).

8. S. N. Deming and R. C. Kong, Advances In Chromatography, 16th International Symposium, Barcelona, Spain, September 28 - October 1, 1981.

9. S. N. Deming, M. L. H. Turoff, Anal. Chem., 50, 546 (1978).

10. E. Tomlinson, T. M. Jefferies, C. M. Riley, J. Chromatogr., 159, 315 (1978).

11. S. N. Deming, S. L. Morgan, Clin. Chem., 25 (6), (1979).

12. C. N. Reilley, G. P. Hildebrand, J. W. Ashley, Jr., Anal. Chem., 34, 1198, (1962).

13. K. Slais, M. Krejci, J. Chromatogr., 91, 161 (1974).