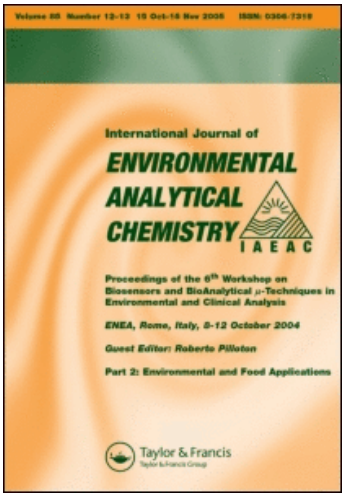

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The Use of Post-Column Dynamic Ion-Pair Extraction for the Hplc Detection of Anionic Additives in Foods

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THE USE OF POST-COLUMN DYNAMIC ION-PAIR EXTRACTION FOR THE HPLC DETECTION OF ANIONIC ADDITIVES IN FOODS

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Applications of post-column ion-pair extraction with absorbance detection to the determination of anionic food additives are discussed. The compounds studied include sodium dioctylsulfosuccinate, artificial sweeteners and free fatty acids. Dyes such as methylene blue and crystal violet provide different extraction and detector wavelength selectivities when used as counter ions. Mobile phase composition, dye concentration, phase ratios and extraction system design influence the sensitivity of the system. Excellent selectivity is shown through application of the approach to a variety of food samples including drink powders, carbonated beverages and juices. Free fatty acids can be determined directly without derivatization at low parts per million levels in samples such as orange juice, margarine and butter using a mobile phase of up to 99% acetonitrile. Absolute detection limits for the additives studied are in the low nanogram range.

KEY WORDS: Post-column ion-pair extraction, anionic food additives, HPLC.

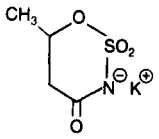
INTRODUCTION

Post-column dynamic ion-pair extraction using fluorescent counter ions has been shown to be very useful for the selective detection of both acidic[1,2] and basic[3–5] analytes after HPLC separation. The principle involves mixing the mobile phase from the column with a solution containing an appropriate fluorescent counter ion. The ionic analyte in the mobile phase forms a neutral ion-pair with the counter ion. The stream is then dynamically mixed with an immiscible organic solvent such as chloroform which extracts the neutral ion pair while leaving excess counter ion in the aqueous phase. The two phases are then separated by means of gravity and wetting in a specially designed separator. The organic phase passes through the detector for measurement. Detection of the analyte is based on the fluorescence of the counter ion part of the ion-pair. This offers an attractive means of detecting non-fluorescent analytes without recourse to chemical derivatization. Advantages of the technique have been recently reviewed.[6]

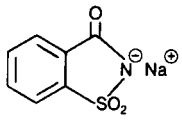
This presentation discusses the extension of the approach to UV-visible absorbing ion-pairs with applications to three different types of anionic food additives with comparisons employing two different basic dyes as counter ions.

SWEETENERS

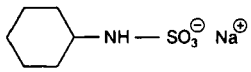
Acesulfam—K



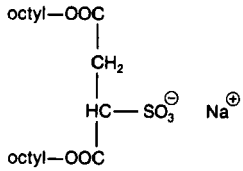
Saccharin



Cyclamate



DSS (wetting agent)



FATTY ACIDS

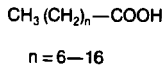


Figure 1 Structures of the compounds studied using post-column ion-pair extraction.

EXPERIMENTAL

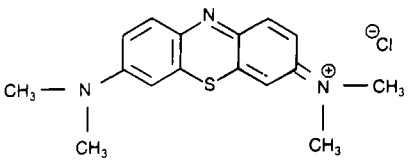
Reagents

All solvents were distilled in glass grade. Water was deionized (Milli-Q^R, Millipore, USA). Methylene blue (Aldrich, USA), crystal violet (Aldrich), sodium cyclamate (Sigma, USA), saccharin (Sigma), acesulfam (Hoescht), DSS (sodium dioctysulfosuccinate, Eastman-Kodak, USA) and C_8–C_{18} fatty acids (Sigma) were analytical grade and used as received. All other reagents were analytical grade. Figure 1 shows the structures of the additives studied while Figure 2 shows those of the two dyes.

Liquid Chromatography

A Beckman gradient HPLC system was used for the separations. For DSS, a Lichrosorb C-18 (10 μm, 10 cm × 4.6 mm i.d.) column (CSC, Montreal) with a mobile phase of acetone – 0.02 M KH_2PO_4 (55+45) was used at a flow rate of 1.5 mL/min. The sweeteners were separated using a μ Bondapak (Waters Assoc., USA) C-18 column (10 μm, 30 cm × 4.6 mm i.d.) with a mobile phase of acetone – 0.02 M KH_2PO_4 (2+98) at a flow rate of 1.3 mL/min. The fatty acids were separated by gradient elution using a Spherisorb ODS-2 (HPLC Technology, UK) column (5 μm, 15 cm × 4.6 mm i.d.) with a moble phase of 79–99% acetonitrile in

METHYLENE BLUE



CRYSTAL VIOLET

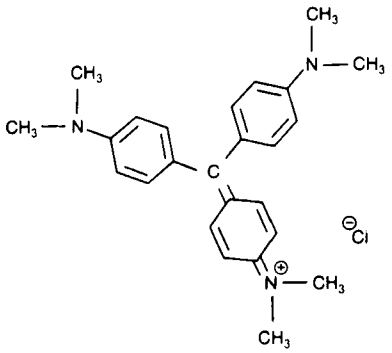


Figure 2 Structures of the dyes employed as counter ions.

water (adjusted to pH 4 with H_3PO_4) over a time of 20 min.[7] The flow rate was 0.8 mL/min.

Post-Column Ion-Pair Extractor

Figure 3 shows the setup for the ion-pair extraction of the artificial sweeteners.[8] Figure 4 shows the apparatus as consturcted for the analysis of DSS[9] and for the fatty acids.[7] For the sweeteners, the dyes were dissolved in $CHCl_3$ (2 mg/L) and pumped at 1.0 ml/min with about 0.3 mL/min passing through the detector. For DSS, the dyes were dissolved in 0.5 M H_3PO_4 (20 mg/L) and pumped at 0.1 mL/min with a $CHCl_3$ flow rate of 1.0 mL/min. For the fatty acids, the dyes were dissolved in 0.02 M KH_2PO_4 (2 mg/L) at a flow of 2 mL/min. The flow rate was 1.0 mL/min. The detectors employed were a Waters M440 UV detector with a 546 nm filter (for DSS and sweeteners) and a Micromeritics Model 788 UV detector set to 651 nm.

Sample Analysis

For the sweeteners and DSS, beverages were prepared as directed on the labels, filtered and injected directly into the HPLC system. The fatty acids were extracted

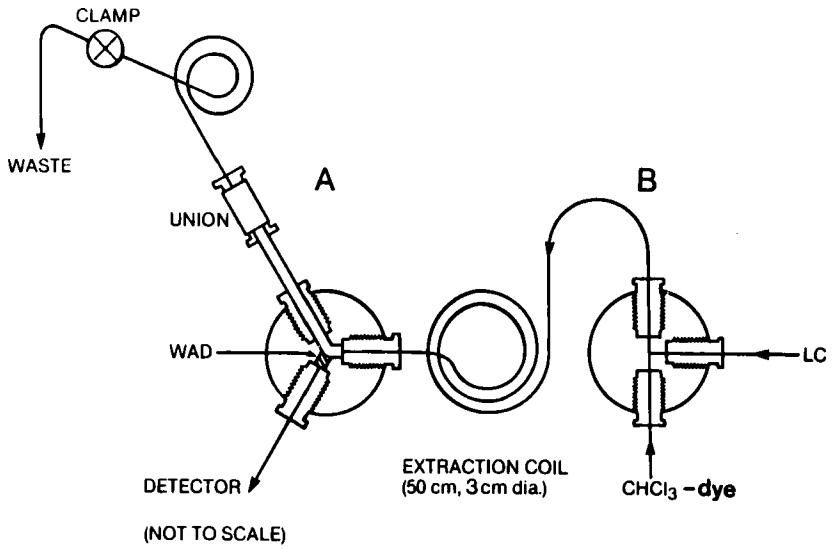


Figure 3 Post-column extraction apparatus as set-up for artificial sweeteners analysis. The LC effluent is mixed with the dye in chloroform at tee "B". The ion pairs are formed and extracted into the organic phase in the extraction coil. The phases are separated in tee "A" where a part of the chloroform flows through the detector.

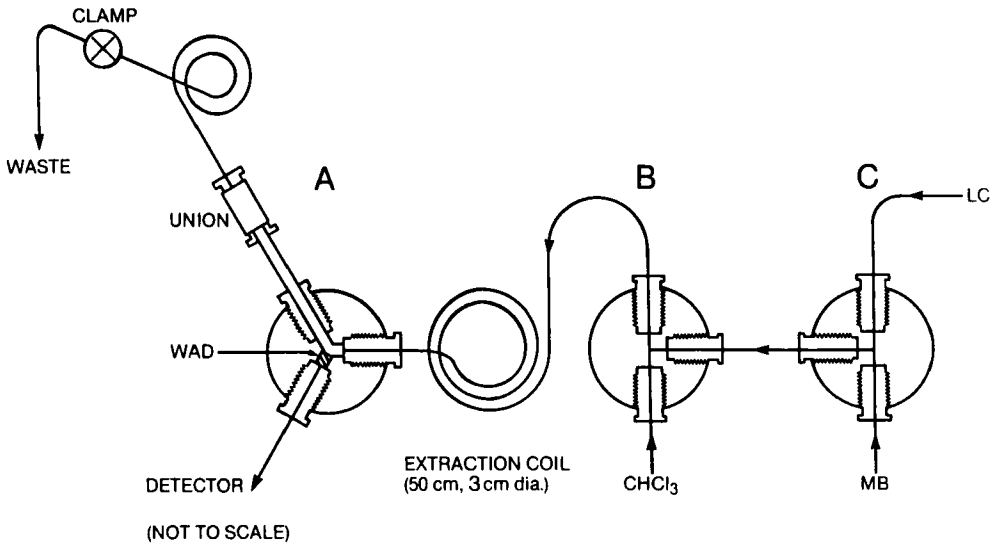


Figure 4 Post-column extraction apparatus as set up for DSS and fatty acid analysis. The LC effluent is mixed with the dye in aqueous solution at tee "C". Chloroform is then added at tee "B". The ion-pairs are extracted in the extraction coil. The phases are separated in tee "A" where part of the chloroform flows through the detector.

from orange juice with dichloromethane, and from butter and margarine with acetonitrile as described elsewhere.[7] The extracts were evaporated to dryness then dissolved in acetonitrile for analysis.

RESULTS AND DISCUSSION

The structures of the compounds studied are shown in Figure 1. The sweeteners, acesulfam-K, saccharin and cyclamate, have an N-SO_2-type moiety. DSS (dioctyl-sulfosuccinate, sodium salt) has a sulfonic acid group, while the fatty acids have in common the carboxyl moiety. The basic dyes evaluated are shown in Figure 2. Methylene blue is more soluble in aqueous solution than crystal violet. The difference in solubilities of the two dyes can lead to some interesting extraction selectivities when applied to the food additives mentioned above.

For the ion-pair extraction of the artificial sweeteners, the counter ion, crystal violet, was dissolved directly in the $CHCl_3$ extraction solvent thus requiring only one post-column pump (Figure 3). DSS and the fatty acids were analysed using the dye, methylene blue dissolved in aqueous solution and mixed with the LC column effluent to form the ion-pairs (Figure 4). The chloroform was intoduced separately to extract them. This arrangement requires an additional pump compared to the system shown in Figure 3. However, in the case of the fatty acids it was necessary to have additional aqueous solution added to the mobile phase (which was predominantly acetonitrile) so that two immiscible phases would result upon addition of the chloroform.

Since the sweeteners are relatively polar in nature, only a small amount of organic modifier (2% acetone or 3% acetonitrile) was required. It was found that under these conditions crystal violet performed much better than methylene blue as counter ion.[8] The detection limits were 20–50 times worse using methylene blue. The difference in the results can be attributed to the differences in chloroform solubility of the resulting ion-pairs with the crystal violet ion-pairs being much more extractible from the aqueous phase than those of methylene blue.

Figure 5 shows the LC separation of a mixture of seven sweeteners and an isomer of aspartame using UV detection at 200 nm. Cyclamate, although present, is not detected. However, using the post-column system it can be readily detected along with acesulfam-K and saccharin as shown in Figure 6. The other sweeteners are not detected with the post-column system. The rise in baseline after 8 min is due to the increase in acetonitrile concentration during the gradient elution part of the run. The increased acetonitrile changes the equilibrium between the organic and aqueous phase resulting in more free crystal violet dye being extracted into the organic phase causing a baseline shift. The use of acetone as a mobile phase modifier would likely reduce this problem considerably as discussed elsewhere.[2,9] Figure 7 compares an isocratic separation obtained using UV absorbance and post-column ion-pair extraction detection in series for cyclamate in a cola beverage. The selectivity of the post-column ion-pair extraction system is clearly seen. Cyclamate is not detected by UV but easily determined with the post-column system. The only sample treatment for this analysis was to dilute and filter the

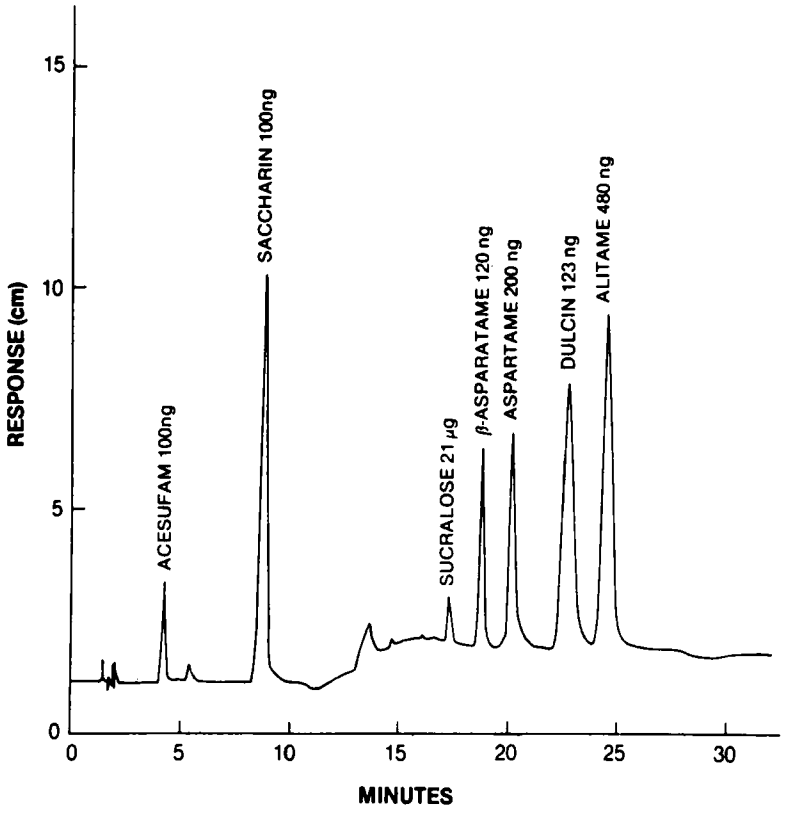


Figure 5 LC separation with UV detection (200 nm) of a mixture of seven artificial sweeteners plus beta-aspartame. Cyclamate is not detected. Gradient elution 3–20% acetonitrile in 0.02 M KH_2PO_4 (pH change from 5.0 to 3.5) from 8–13 min.

drink before injection. The detection limit for cyclamate (2× noise) was about 10 ng per injection. Recovery of cyclamate when added to beverages at 100 μg/mL averaged 105% for three different beverages. The repeatability (coefficient of variation, CV) was 3–6%.

The application of post-column ion-pair extraction to the wetting agent DSS was carried out with different LC conditions and the use of a Technicon proportioning pump for separate mixing of dye solution and chloroform with the mobile phase as shown in Figure 4. DSS is much more lipophilic than the sweeteners and a mobile phase containing 55% acetone was required for a reasonable elution time. Acetonitrile could not be used as the mobile phase modifier under the post-column extraction conditions used. With acetone, a satisfactory phase separation was achieved and the post-column system operated very well. Both methylene blue and crystal violet performed well as counter ions. However, due to the differences in absorbance maxima (657 nm, methylene blue; 588 nm, crystal violet) and partitioning behavior, different concentrations and detector settings were employed. Methylene blue was used for routine determinations.

Figure 8 shows typical results obtained for an orange drink powder spiked with

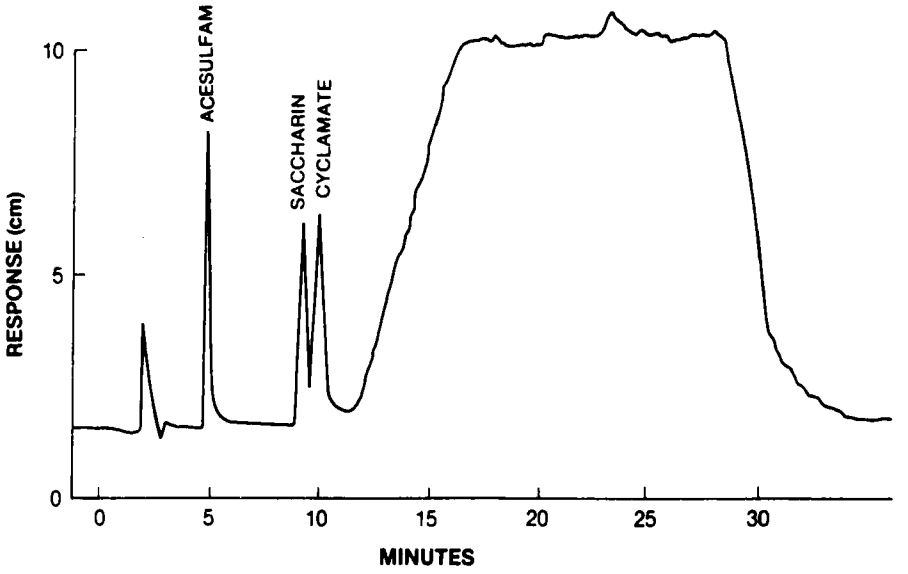


Figure 6 LC separation with post-column ion-pair extraction of a mixture of seven artificial sweeteners. Conditions as describd in Figure 5. Absorbance detection at 546 nm. Only acesulfam-K, saccharin and cyclamate are observed.

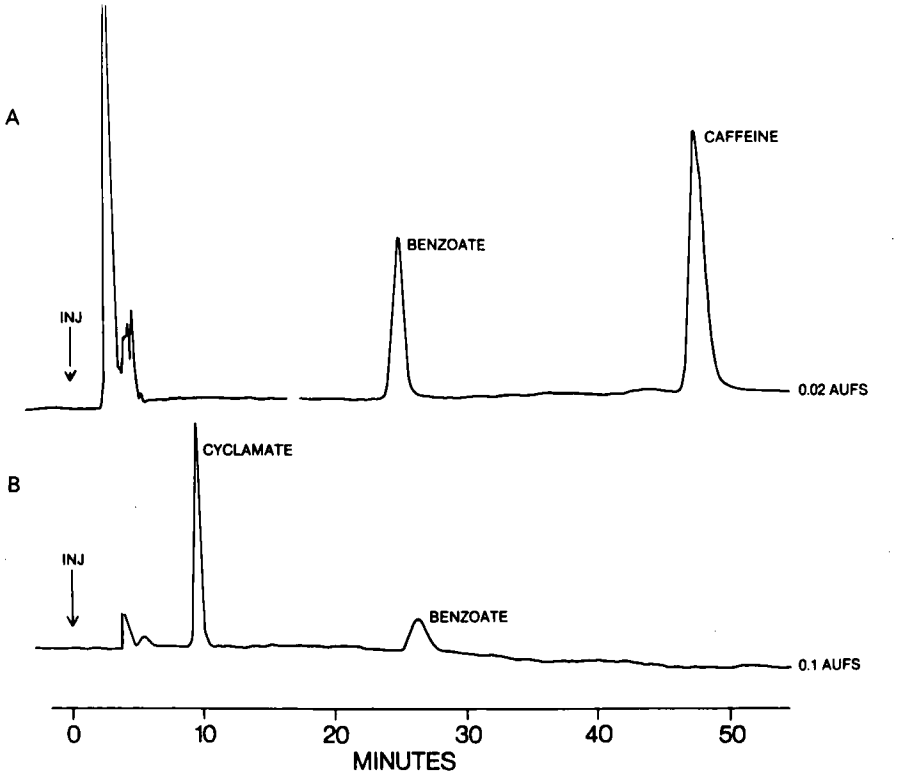


Figure 7 Comparison of (A) UV absorption (200 nm) and (B) post-column ion-pair extraction, for the detection of cyclamate in a diet cola beverage. Mobile phase, 3% acetonitrile in 0.02 M KH_2PO_4. Detector wavelength, 546 nm, for post-column system.

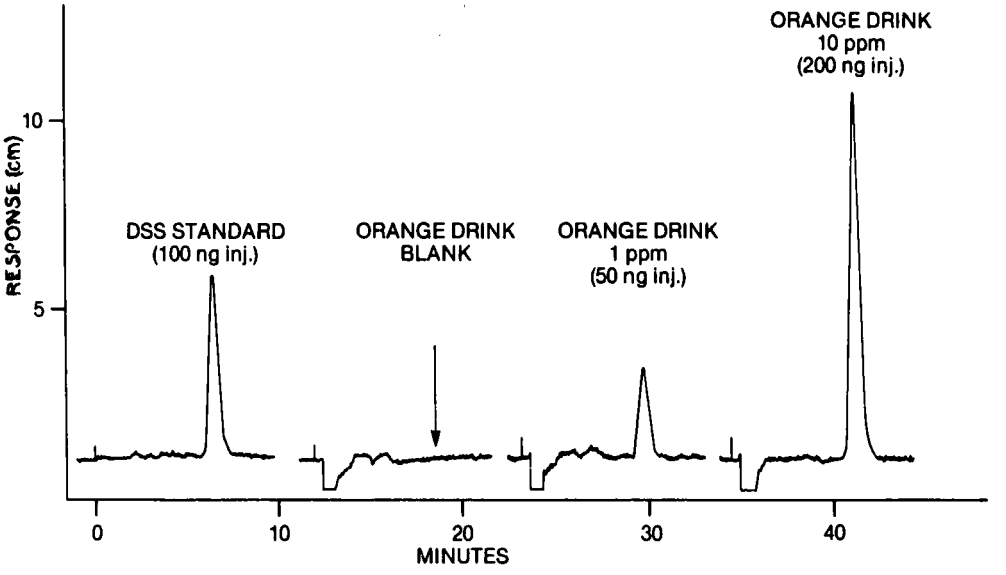


Figure 8 Chromatograms of orange drink samples spiked at 1.0 and 10.0 μg/g DSS. Mobile phase, 55% acetone in 0.02 M KH_2PO_4. Detector wavelength, 546 nm.

DSS at the equivalent of 1 and 10 μg/g (ppm) in the prepared drink. As can be seen, there are no interferences from the sample matrix. The only sample treatment required was to dissolve a portion of the drink mix in LC mobile phase, filter and inject. Detection limit (2 × noise) was about 10 ng DSS per injection. Repeatability of the method was about 2% (CV) when samples were spiked at 10 μg/mL. Recoveries were generally greater than 90%.

The detemination of fatty acids using the post-column ion-pairing system posed certain problems. Firstly, the mobile phases normally employed for reversed phase LC of fatty acids contain very high percentages of methanol, tetrahydrofuran, or acetonitrile,[10–12] often close to 100% in composition. This makes organic extraction of ion-pairs difficult. This was overcome by employing an extraction system similar to that used for DSS as shown in Figure 4. The major difference was the greatly increased flow of aqueous counter ion (methylene blue) which was necessary in order to mix enough water with the mobile phase so that a good phase separation was achieved with the chloroform. In order to separate the fatty acids of interest, an acetonitrile gradient was used from 79–99%. Acetone was evaluated as a mobile phase since it is much more suitable for the post-column ion-pair extraction. However, poor resolution of the acids was achieved.

While crystal violet was the preferred dye for the artificial sweeteners, it was not useful for detection of the fatty acids under the chosen conditions due to the high concentration of the free dye in the chloroform phase. This led to a high background reducing the sensitivity of the detection. Methylene blue, although not nearly as effective as crystal violet for detection of the sweeteners, proved to be much better for the fatty acids. This was primarily due to its lower solubility in the chloroform phase leading to a lower background absorption. Figure 9 shows the separation of a mixture of free fatty acids. The detection limits (2 × noise) for these

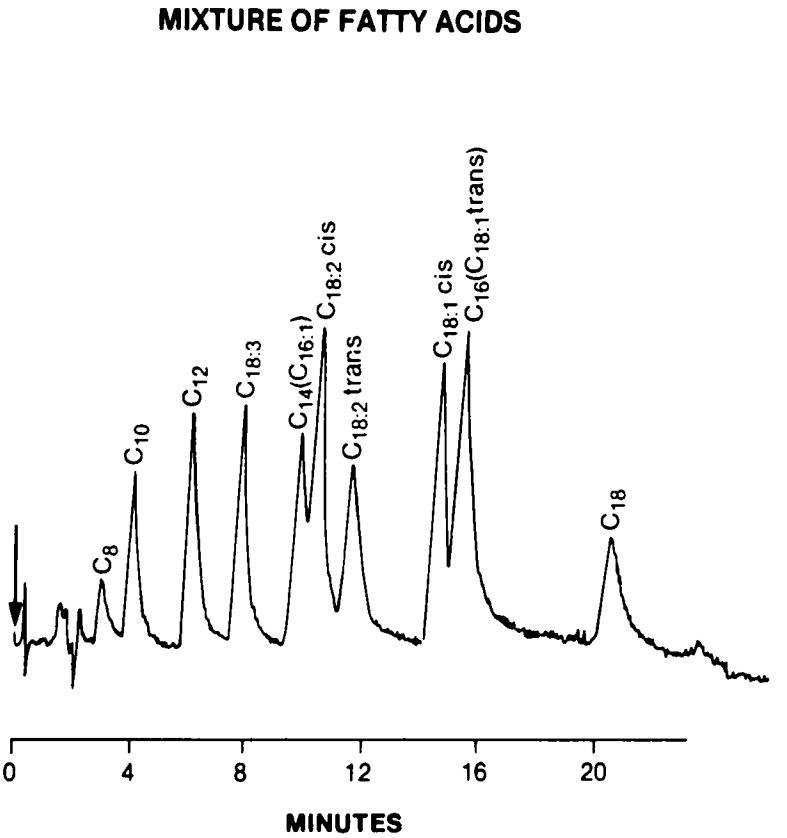


Figure 9 Chromatogram of fatty acids, 200–300 ng each injected. Mobile phase, 79–99% acetonitrile gradient, pH 4.0. Detector wavelength, 651 nm, 0.64 absorbance units full scale.

compounds were in the range of 20–30 ng per injection depending upon the compound. It was observed that below a carbon chain length of C_{10}, sensitivity dropped dramatically with the loss of each additional carbon atom. Thus C_8 was three times less sensitive than C_{10} while C_6 was not detected under the conditions employed. Figure 10 shows results obtained for the detection of free fatty acids in orange juice, a predominantly aqueous type sample. Less than 1 μg/g of the compounds could be detected using only a methylene chloride extraction as the sample cleanup. Figure 11 shows an application to high fat samples, butter and margarine, using only an acetonitrile extraction of the samples before analysis. As can be seen both chromatograms are relatively clean and show only the free fatty acid patterns. The results obtained here are in good agreement with values reported elsewhere where extraction, cleanup and derivatization were employed for analysis by gas chromatography[13,14] or LC.[12]

CONCLUSION

Post-column ion-pair extraction employing visible absorbing dyes as counter ions

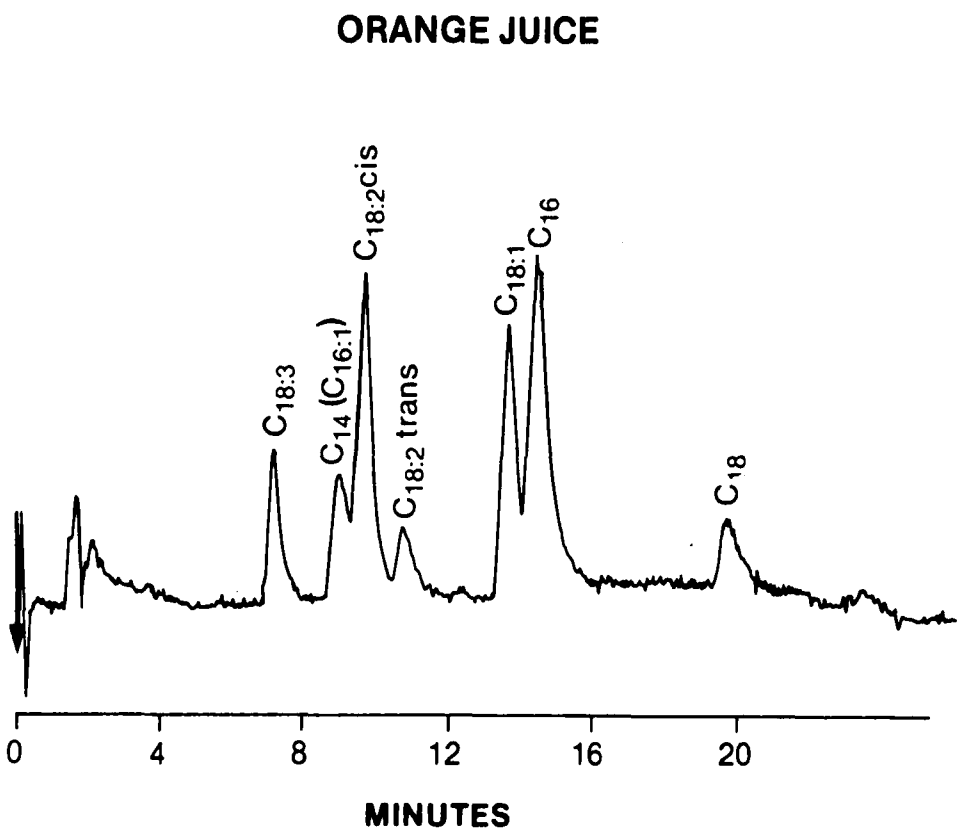


Figure 10 Chromatogram of free fatty acids in orange juice. 0.7–2.3 μg/g of C_{14}–C_{18} fatty acids.

has been shown to be particularly effective in detecting non-absorbing anionic additives in food matrices. In the three applications presented, selectivity was excellent. Detection limits were in the low nanogram range and sample pretreatment was minimal. The technique is ideally suited to routine rapid determinations.

Further research in evaluating different dyes for application to other additives including cationic substances should prove to be interesting. A recent publication has already investigated the simultaneous determinations of anionic and cationic surfactants in detergents using parallel post-column extraction systems and different dyes.[15]

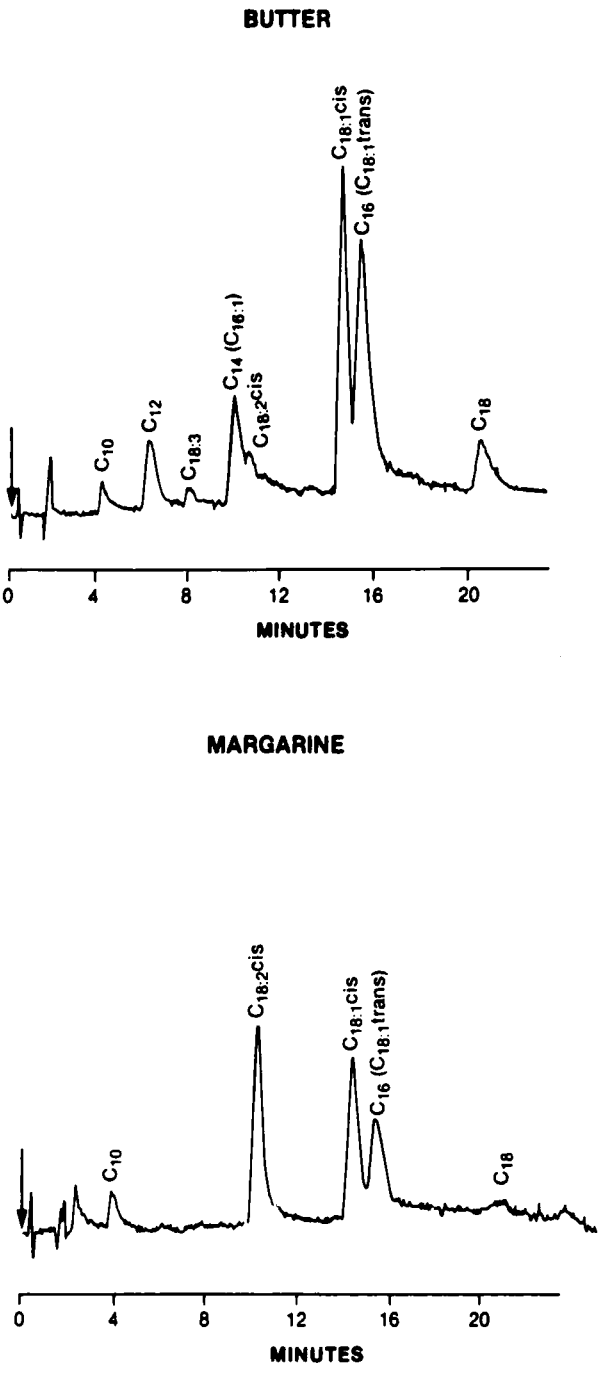


Figure 11 Chromatogram of free fatty acids in butter and margarine. 34–586 μg/g of C_{10}–C_{18} fatty acids.

References

1. C. P. Terweij-Groen, J. C. Kraak, W. M. Niessen, J. F. Lawrence, C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Int. J. Environ, Anal. Chem.* **9,** 45 (1981).
2. F. Smedes, J. C. Kraak, C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr* **247,** 123 (1982).
3. C. Van Buuren, J. F. Lawrence, U. A. Th. Brinkman, I. L. Honigberg and R. W. Frei, *Anal. Chem.* **52,** 700 (1980).
4. J. F. Lawrence, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.* **185,** 473 (1979).
5. J. F. Lawrence, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.* **171,** 73 (1979).
6. J. F. Lawrence, U. A. Th. Brinkman and R. W. Frei, "Ion-Pairing as a Means of Detection in Liquid Chromatography" in *Reaction Detection in Liquid Chromatography,* I. S. Krull, ed., (M. Dekker Inc., New York, 1986).
7. J. F. Lawrence and C. F. Charbonneau, *J. Chromatogr.* **445,** 189 (1988).
8. J. F. Lawrence, *Analyst* **112,** 879 (1987).
9. J. F. Lawrence, *J. Assoc. Off. Anal. Chem.* **70,** 15 (1987).
10. J. W. King, E. C. Adams and B. A. Bidlingmeyer, *J. Liq. Chromatogr.* **5,** 275 (1982).
11. M. I. Aveldano, M. van Rollins and L. A. Horrocks, *J. Lipid Res.* **24,** 83 (1983).
12. A. G. Bailie, T. D. Wilson, R. K. O'Brien, J. M. Beebe and J. D. Stuart, *J. Chromatogr. Sci.* **20,** 466 (1982).
13. J. B. Stack. F. L. Joe, D. G. Cunningham, T. Fazio and J. A. G. Roach, *J. Assoc. Off. Anal. Chem.* **69,** 551 (1986).
14. A. H. Woo and R. C. Lindsay, *J. Dairy Sci.* **63,** 1058 (1980).
15. M. Kanesato, K. Nakamura, O. Nakata and Y. Moridawa, *J. Amer. Oil Chem. Soc.* **64,** 484 (1987).