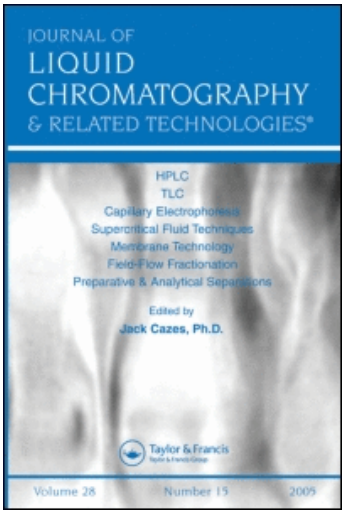


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Alkylated and Sulfonated Diphenyl Oxide Sur-Factant by High Performance Liquid Chromatography

M. Ye[a]; R. Walkup[a]; K. Hill[a]
[a] ManTech Environmental, Research Services Corporation, Ada, Oklahoma

To cite this Article Ye, M. , Walkup, R. and Hill, K.(1996) 'Determination of Alkylated and Sulfonated Diphenyl Oxide Sur-Factant by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 19: 8, 1229 — 1240

To link to this Article: DOI: 10.1080/10826079608006314

URL: http://dx.doi.org/10.1080/10826079608006314

J. LIQ. CHROM. & REL. TECHNOL., 19(8), 1229–1240 (1996)

DETERMINATION OF ALKYLATED AND SULFONATED DIPHENYL OXIDE SURFACTANT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. Ye, R. Walkup, K. Hill

ManTech Environmental
Research Services Corporation
P. O. Box 1198
Ada, Oklahoma 74820

ABSTRACT

Methods for the determination of the anionic surfactant Dowfax 8390 are described. Dowfax is a complex mixture of various alkylated and sulfonated diphenyl oxides. The primary component of Dowfax is monoalkylated disulfonated diphenyl oxide (MADS). This work uses ion pairing chromatography and reverse phase chromatography. Ion pairing chromatography provides a simple and fast method for quantitation of the total concentration of Dowfax surfactant in aqueous solutions. It is shown that suppressed conductivity detection and optical detection at 210 nm have comparable sensitivity. A quantitation limit of 48.4 ppm is achieved with 400 µl injection. The separation of 11 components in Dowfax is achieved using gradient reverse phase chromatography.

INTRODUCTION

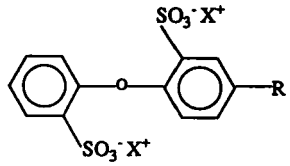
There has been considerable interest in using surfactants to enhance subsurface remediation.[1,2,3] Because surfactants can increase the solubility of hydrocarbon contaminants in groundwater systems, they could potentially greatly reduce the number of pore volumes to be pumped in a cleanup effort.[3] Dowfax, as well as other commercial surfactants, such as T-MAZ ethoxylated sorbitan fatty acid esters,[4,5] Steol CS-330 sodium laureth sulfate[6] and octylphenol polyether alcohols,[7] is a complex mixture of many isomers, homologues and oligomers.

Dowfax 8390 is a commercial blend of alkylated and sulfonated diphenyl oxides. The primary component in Dowfax 8390 is linear C_{16} monoalkylated disulfonated diphenyl oxide (MADS). Approximately 80% of the components are considered to be monoalkylated, with the entire mixture being predominately disulfonated. The other components include monoalkylated monosulfonated diphenyl oxide (MAMS) and dialkylated disulfonated diphenyl oxide (DADS). The dialkylated monosulfonated diphenyl oxide component (DAMS) is typically present at a still smaller concentration. The structural formulas of these components are shown in Figure 1. Multiple isomers exist for each component.

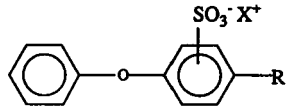
As indicated in previous studies,[6,8] ion pairing chromatography is ideal for the separation of ionic organic compounds. For ionic surfactant CS-330, which consists of a saturated alkyl group, ethoxyl groups and a polar head, $-OSO_3$ Na, we indicated that ion pairing chromatography was the only HPLC separation technique available.[6] However, for Dowfax, since there are two benzene rings in these molecules (Figure 1), reverse phase chromatography, as well as ion pairing chromatography, can be used to separate them.

Reverse phase chromatography with a C_{18} column has been used to separate sorbitan esters previously.[9,10] The limitations using a C_{18} column include the use of a very high concentration of organic solvent (up to 90% isopropanol), long analysis time, and the lack of separation of oligomers.[9,10] As shown in Figure 1, since molecules in Dowfax have two benzene rings and a long hydrocarbon chain which are non-polar, a less hydrophobic packing is needed. A radially compressed C_8 column offers more desirable interactions between the stationary phase, Dowfax molecules and the mobile phase than a C_{18} column. Combined with the use of a mixture of acetonitrile, methanol and sodium phosphate buffer as a mobile phase, and gradient elution, the complex mixture of Dowfax was separated. As demonstrated in the study of T-MAZ oligomers,[5] the use of a C_8 column not only significantly reduced the consumption of organic solvent, but also achieved the separation of the

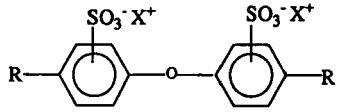
Components in Dowfax 8390



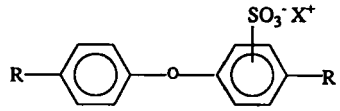
Monoalkylated Disulfonated Diphenyl Oxide (MADS)



Monoalkylated Monosulfonated Diphenyl Oxide (MAMS)



Dialkylated Disulfonated Diphenyl Oxide (DADS)



Dialkylated Monosulfonated Diphenyl Oxide (DAMS)

Figure 1. The molecular formulas of the components in Dowfax 8390.

oligomers. Recently, we have shown that the consumption of organic solvent in the analysis of TMAZ and Triton X-100 can be further reduced using a Waters Delta-Pak C_4 column.[11]

EXPERIMENTAL

Chemicals and Reagents

Dowfax 8390 and MAMS, MADS and DADS were from Dow Chemical Company (Midland, MI, USA). Dowfax is a registered trademark of the Dow Chemical Company. Tetrabutylammonium hydroxide (55% aqueous solution, TBAOH) was from Southwestern Analytical Chemicals, Inc. (Austin, TX, U.S.A.), acetonitrile and methanol from Burdick and Jackson (Baxter Healthcare Corporation, Muskegon, MI, U.S.A.). 18 MΩ water was obtained from a Millipore Milli-Q system (Marlborough, MA, U.S.A.).

Apparatus

Ion pairing chromatography

Instrumentation was from Waters (Waters Associates, Milford, MA, U.S.A.), which included a Model 996 photodiode array detector, a Model 431 conductivity detector, a Model 600E multisolvent delivery system and a Model 717 autosampler. Separations were accomplished using a Dionex (Dionex Corporation, Sunnyvale, CA, U.S.A.) IonPac NS1 column (4 mm x 250 mm) and a NG1 guard column. A Dionex anion micro membrane suppressor (AMMS-MPIC) and 25 mM sulfuric acid solution were used to suppress the background conductivity of the mobile phase.

The mobile phase was 50% acetonitrile and 3 mM TBAOH in water with a pH value of 11.8. A silica- based reverse phase column cannot be used with this strong basic eluent because it has an operating pH range of 4 to 7.5. Organic polymer packings, such as IonPac NS1 column, have a wide pH range (0 to 14) and they are ideally suited for the separation of molecules in Dowfax. The injection volume was 400 μL at an eluent flow rate of 1.0 mL/min. Data acquisition and processing was accomplished with a Waters Maxima 820 chromatography workstation, which included a system interface module and a NEC PowerMate SX/16 computer.

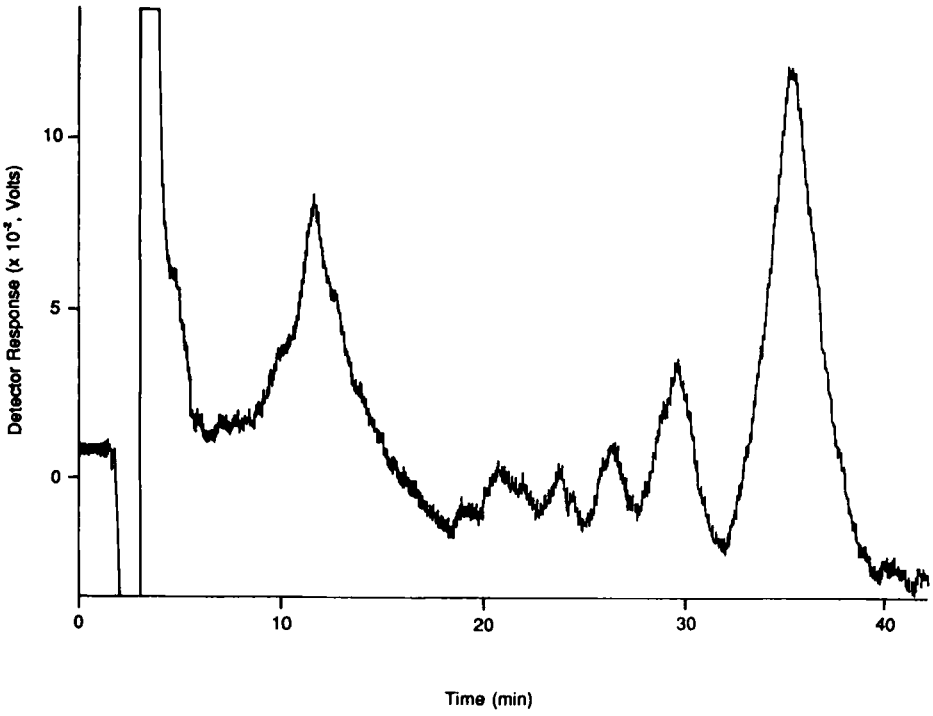


Figure 2. Ion pairing chromatogram of Dowfax. Concentration: 200 ppm.

Reverse phase chromatography

Separations were accomplished using a Waters Nova-Pak C_8 stainless steel column (3.9 x 150 mm). The injection volume was 100 μL with a flow rate of 1.0 mL/min. The linear gradient elution, which used 2 mobile phases: A: (20% acetonitrile, 10% methanol, 0.75 mM NaHCO3 and 2.2 mM Na2CO3) and B: (40% acetonitrile, 25% methanol, 0.75 mM NaHCO3 and 2.2 mM Na2CO3), started at 78% of A and 22% of B, ran to 65% of A and 35% of B at 40 minutes, to 50% of A and 50% of B at 50 minutes and held for 5 minutes, and finally to 100% of B at 60 minutes and held for 20 minutes.

Data acquisition and processing was accomplished with a Waters Millennium chromatography workstation, which used an NEC Image 466es computer. Other instrumentation used was described above.

RESULTS AND DISCUSSION

Ion Pairing Chromatography

The mobile phase, 50% of acetonitrile and 3 mM TBAOH, has a pH value of 11.6 and a conductivity background of 425 μS. With such a high conductivity background, the ratio of signal to noise with a conductivity detector is poor. The micromembrane suppressor removes cations in the column eluent after the oligomer separation is accomplished, reducing the background conductance to 82 μS.

Figure 2 shows the chromatogram of the separation of Dowfax. The concentration of Dowfax in the chromatogram was 200 ppm with 400 μL injection. Several peaks were found in the chromatogram. The peak with retention time at 35 minutes is used in the quantitation because it has the highest response. The integration was from 31.4 to 39.8 minutes. A mixture of benzene, toluene, ethylbenzene, and toluene (BTEX) is contained in the environmental samples, which elute between 5 to 10 minutes and can cause interference with the peaks with short retentions.

Dowfax was analyzed quantitatively with a good degree of precision and accuracy (Table 1). Dowfax standards, 100 and 200 ppm, were analyzed three times, and were used to determine the detection limit. The detection limits were 14.52 ppm, calculated as three times the standard deviation of the mean. From the detection limit of 14.52 ppm, a quantitation limit of 48.42 ppm was estimated, calculated as ten times the standard deviation of the mean. Standards in the concentration range from 50 to 1200 ppm were analyzed four times, and a standard from each concentration was randomly selected to generate calibration curve. As demonstrated by the relative error (Table 2), the calculated concentrations are in a good agreement with the known concentrations.

Since Dowfax molecules consist of UV-absorbing chromophores, two benzene rings, an optical detector can be used. The spectra of four principal peaks (A, B, C and D in Figure 5a) in Dowfax were given in Figure 3, showing strong UV absorption at 200 nm. Figures 4a and 4b show the chromatogram of 50 ppm Dowfax using a suppressed conductivity detector and an optical detector at 210 nm. From the comparison of the noise levels in Figures 4a and 4b, it is clear that the optical detector is as sensitive as the suppressed conductivity detector. With the peak-noise ratio of 6.4 : 1.0 and 6.9 : 1.0 in Figure 4a and 4b, the chromatograms also show that Dowfax at a concentration of 50 ppm can be quantified.

Table 1

Analytical Precision and Detection Limit with the Conductivity Detector

Peak Group	Concentration		Detection Limit (ppm)
	100 ppm	200 ppm	
1	n = 3	n = 3	14.52
	x = 104.3	x = 212.4	
	SD = 4.84	SD = 3.89	
	RSD = 4.6%	RSD = 1.8%	

n: Number of standard solutions analyzed. x: Mean solution concentration (ppm). SD: Standard Deviation. RSD: Percent Relative Standard Deviation (= 100 x (SD / x)). Depection limite was calculated as three times the standard deviation of the mean (3 x SD).

Table 2

Average Peak Area, Relative Standard Deviation, Calculated Concentrations and Relative Error with the Conductivity Detector

Conc'n (ppm)	Ave. Peak Area[a]	(RSD)[b]	Cal. Conc'n	Rel. Error (%)
50	0.7683×10^7	(5.6)	53.4	6.8
100	1.4255×10^7	(4.3)	102.4	2.4
200	2.6132×10^7	(3.2)	184.4	-7.8
400	5.6618×10^7	(4.1)	397.6	-0.6
800	11.7492×10^7	(3.4)	812.5	1.6
1200	17.2030×10^7	(2.9)	1216.9	1.4

[a] The peak areas were averaged from three experimental data.
[b] RSD: Relative Standard Deveiation = 100 x (Standard Deviation/Average Peak Area

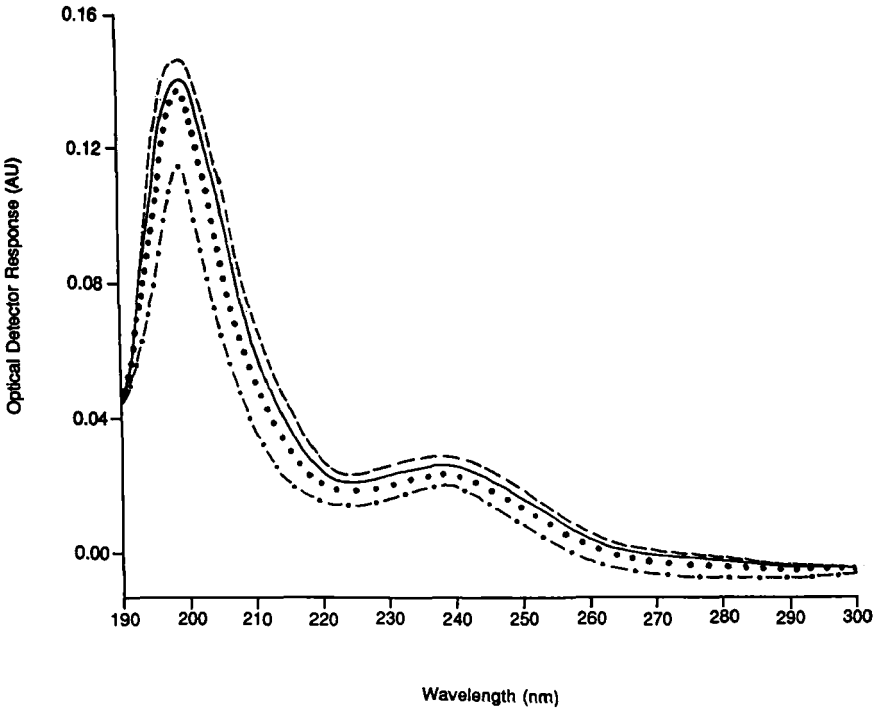


Figure 3. The spectra of 4 principal peaks (A, B, C and D in Figure 5a) in Dowfax. (-----) A, (——) B, (●●●●●) C and (-●-●-) D.

Reverse Phase Chromatography

Gradient elution was used to separate the components in Dowfax. Figure 5a shows the separation of 500 ppm Dowfax. The chromatographic conditions were described in the experimental section. The chromatogram demonstrates the complexity of this industrial chemical with 11 peaks found in the chromatogram. Three components, MADS, MAMS and DADS were analyzed with the identical chromatographic conditions as in Figure 5a. Figure 5b shows the chromatogram of 500 ppm MADS. The close similarity of the chromatograms in Figures 5a and 5b confirms that the primary component in Dowfax is MADS, as suggested by the Dow Chemical Company. The mobile phase used was a mixture of acetonitrile, methanol and aqueous phosphate buffer as described in the experimental section. The use of both acetonitrile and methanol in the mobile phase, rather than using only acetonitrile or methanol, can effectively separate

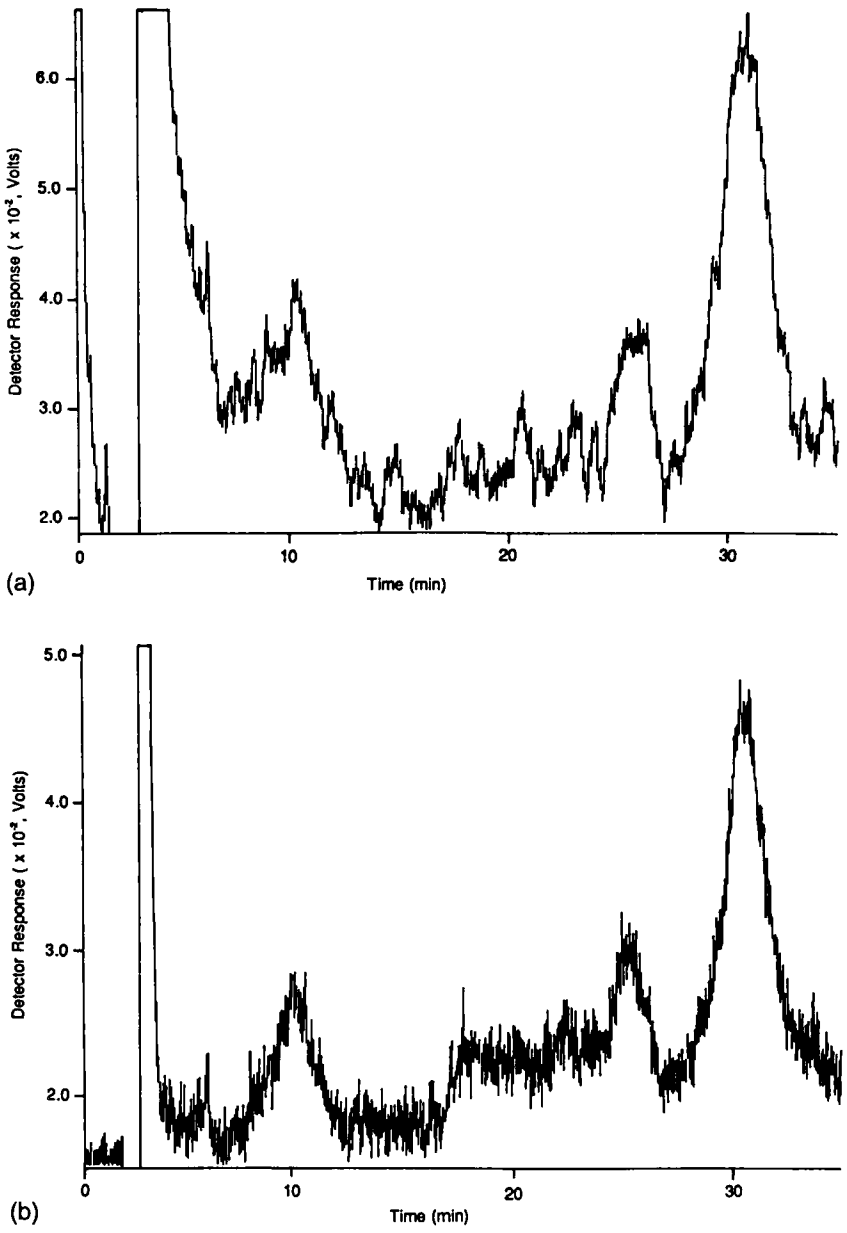


Figure 4. Ion pairing chromatograms of Dowfax. Detection: (a) suppressed conductivity; (b) UV at 210 nm.; Injection volume: 400 μL; Concentration: 50 ppm.

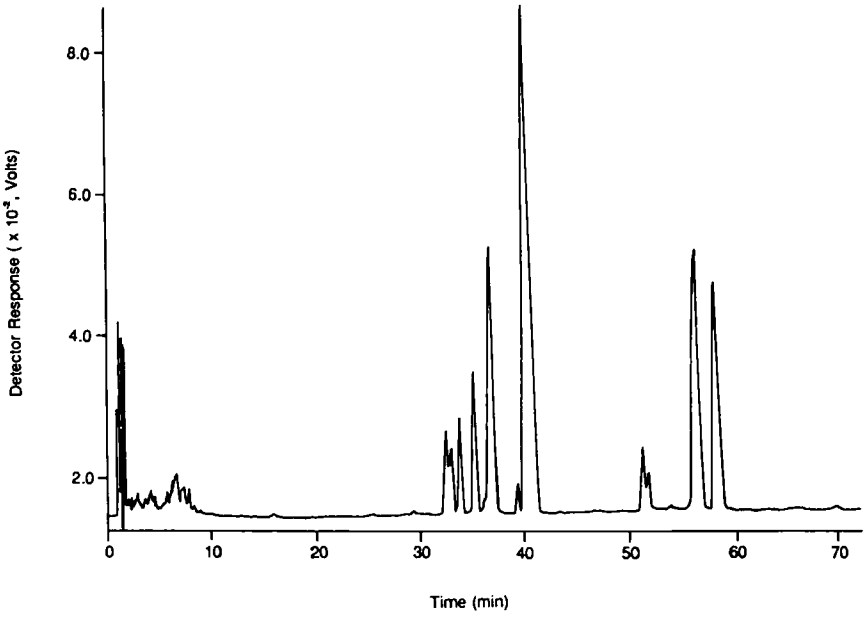


Figure 5. Reverse phase gradient elution chromatograms of Dowfax and MADS. Injection volume: 100 μl; Concentration: 500 ppm; Detection: UV at 239 nm.; (a) Dowfax, (b) MADS.

organic molecules with very subtle hydrophobic differences. Phosphate was added to the mobile phase to vary solute retention and selectivity. In reverse phase chromatography, retention of a polar compound decreases when the addition of salt increases the solubility of the solute in the mobile phase.[12]

Conversely, an increase in retention will occur when the addition of salt decreases the solubility of the solute in the aqueous mobile phase.[12] Dowfax molecules are organic anions. Increase in ionic strength of the eluent can reduce the retention of these molecules and change the separation selectivity.

In a gradient elution, baseline drifting is very significant when the detector is set at low UV wavelength, such as 200 nm. The optical detector was set at 239 nm, which provides a less drifting baseline, though the sensitivity is lower at 239 nm than at 200 nm.

DISCLAIMER

Although the research described in this article has been funded wholly or in part by the U.S. Environmental Protection Agency through Contract #68-C3-0322 to ManTech Environmental Research Service Corporation, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

QA/QC REQUIREMENTS

All QA/QC aspects of this work were performed in accordance with the requirements of the ManTech Environmental Research Services Corporation Quality Assurance Program Plan.

REFERENCES

1. C. C. West, J. H. Harwell, Environ. Sci. & Technol., **26**, 2324 (1992)

2. M. Harper, C. J. Purnell, Environ. Sci. Technol., **24**, 55 (1990).

3. J. D. Rouse, D. A. Sabatini, J. H. Harwell, Environ. Sci. Technol., **27**, 2072 (1993).

4. M. Y. Ye, R. G. Walkup, K. D. Hill, J. Chromatogr., **622**, 323 (1994).

5. M. Y. Ye, R. G. Walkup, K. D. Hill, Chromatographia, **38**, 337 (1994).

6. M. Y. Ye, R. G. Walkup, K. D. Hill, J. Liq. Chromatogr., **17(19)**, 4087 (1994).

7. M. Y. Ye, R. G. Walkup, K. D. Hill, (in preparation,1995).

8. B. A. Bidlingmeyer, F. Vincent Warren, Jr., Anal. Chem., **54**, 2351 (1984).

9. N. Garti, E. Wellner, A. Aserin, S. Sarig, J. Amer. Oil Chem. Soc., **60**, 1151 (1982).

10. Z. Wang, M. Fingas, (in press).

11. M. Y. Ye, R. G. Walkup, K. D. Hill (unpublished results).

12. C. Horvath, W. Melander, J. Chromatogr. Sci., **15**, 393 (1977).

Received July 3, 1995
Accepted July 13, 1995
Manuscript 3910

J. LIQ. CHROM. & REL. TECHNOL., 19(8), 1241–1254 (1996)

IDENTIFICATION AND DOSAGE OF 2-FURALDEHYDE AND 5-HYDROXYMETHYL-2-FURALDEHYDE IN BEVERAGES BY REVERSED PHASE CHROMATOGRAPHY WITH A MICROBORE COLUMN

I. Nicoletti*, C. Corradini, E. Cogliandro, D. Corradini

Istituto di Cromatografia del CNR -
Area della Ricerca di Roma
P.O. Box 10
I-00016 Monterotondo Stazione, Rome, Italy

ABSTRACT

This paper reports the results of a study performed to develop a rapid and straightforward chromatographic method for the identification and dosage of 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (FA), which are recognized indices of deteriorative changes in commercially processed food. The method employs a Supelco microbore reversed phase column (300 x 1.0 mm I.D.), eluted isocratically with a 94:6 (v/v) water/acetonitrile mixture at flow rate of 60 μL/min. Sample is detected at 280 nm in a micro flow cell of 300 nl. Peak purity and identification is assessed by comparing the UV spectra monitored at two points through the chromatographic peak in continuous flow mode in the range from 200 to 400 nm. This method is successfully applied to the identification and

quantitative determination of HMF and FA in alcoholic and non-alcoholic beverages by an internal standard method without any sample pretreatment.

INTRODUCTION

5-Hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (FA) have been proposed as general indices of the deterioration of food quality during storage and are also useful as indicators of temperature abuse during process and storage.[1] HMF is one of the best known intermediate products in the Maillard reaction,[2] while FA is the principal product of the hydrolysis of pentoses and is widely accepted as an indicator of flavor changes.[3,4] Many different analytical techniques are available for the identification and dosage and HMF in processed food. Classical methods for the quantitative determination of these components in food are based on colorimetric measurements. These methods have the disadvantage of the instability of the coloured complex formed, the time required, the use of hazardous chemicals, and no one of the methods is specific.[5-8] Chromatographic methods have also been proposed for the determination of FA and/or HMF in different types of food matrices. These techniques include thin layer chromatography,[9] gas chromatography[10,11] and high performance liquid chromatography (HPLC).[1,12-17] The above methods differ in sensitivity and reproducibility, are not free of interferences and some of them require time consuming sample pretreatment or/and the use of relatively large volumes of organic solvents which are toxic and quite expensive.

We are interested in the development of small-scale analytical methods for substances that are involved in the deterioration reaction occurring during food processing and storage. In this respect, we have recently demonstrated that micellar electrokinetic capillary chromatography (MECC) can be successfully employed for the analysis of FA and HMF in fruit juices and honey.[18,19] Reconized advantages of using miniaturized techniques in electrophoresis and liquid chromatography are the increased mass sensitivity, the higher peak efficiency and the smaller sample volume required for analysis.[20,21] Furthermore, the lower volumes of organic solvents, when required, employed by using narrow-bore liquid chromatographic columns reduces health hazard and the cost of solvents and waste elimination

In this paper we report the results of a study aimed at developing an HPLC method for the analysis of FA and HMF in food employing a reversed phase microbore column and multi-wavelength UV detection. In order to select

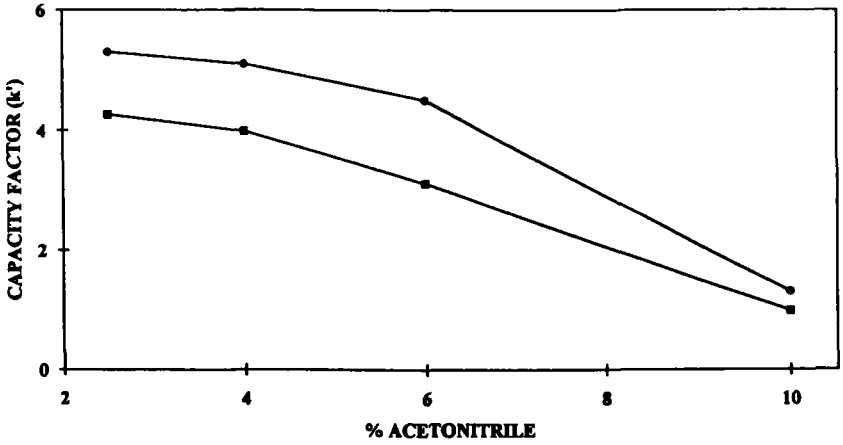


Figure 1. Dependence of the retention factor (k') of HMF (■) and FA (•) on the acetonitrile content in the mobile phase. Chromatographic conditions: column, Supelcosil LC-18 (300 x 1.0 mm I.D.); eluent, acetonitrile-water; flow rate, 60 mL/min; detection by UV absorbance at 280 nm; temperature, 25°C.

the optimum column and chromatographic conditions to perform the rapid and reproducible separations and quantification of the above furaldehydes, the effect of mobile phase composition on the retention time and selectivity was examined using a Supelcosil LC-18 microbore column having 1 mm internal diameter.

The application of this method to the identification and dosage of HMF and FA in some varieties of alcoholic beverages and soft drinks, colored with caramel, from different commercial sources is also described.

EXPERIMENTAL

Instrument and Column

The chromatographic experiments were carried out using an HPLC system equipped with a Model 421A microprocessor controller and a Model 114M single-piston reciprocating pump with the capability of delivering micro flow rates, all from Beckman Instruments, Inc. (Fullerton, CA, USA); a 7520 Rheodyne (Cotati, CA, USA) microsample injector with a 0.5 μL sample rotor and a Model 433 variable-wavelength detector with a standard micro flow cell (300 nl), in conjunction with a Data System 450 software, both from Kontron

Instruments (Milan, Italy). Peak purity and identification was assessed by comparing the UV spectra monitored at two points through the chromatographic peak in continuous flow mode. The reversed phase microbore column employed in the experiments was a Supelcosil LC-18 (300 x 1.0 mm I.D., 5-μm particle size) and was supplied by Supelco (Bellefonte, PA, USA).

Chemicals

HPLC-grade water, acetonitrile and methanol were purchased from Carlo Erba (Milan, Italy). 5-hydroxymethyl-2-furaldehyde (HMF), 2-furaldehyde (FA) and 2-furyl methyl ketone (FMK) were obtained from Aldrich (Milan, Italy). All mobile phases were degassed by sparging with helium before use. All alcoholic beverages and soft drinks were purchased from a local store.

Procedure for Quantitative Analysis

A 2.0 mg/mL stock solution of HMF and FA in methanol-water (10:90 v/v) and 10 mg/mL of FMK in water as the internal standard solution were prepared daily. The stock solution was diluted to produce working standard solutions at five different concentrations within the range 0.1-50.0 μg/mL. An appropriate volume of internal standard solution was added to each solution to give a concentration of 10.0 μg/mL of FMK. Calibration graphs were plotted based on the linear regression analysis of the peak-area ratios, analyzing each working standard solution in quintuplicate.

Alcoholic beverages and soft drinks were diluted with water after the addition of the internal standard solution to give a concentration of 10 μg/mL of FMK. The soft drink samples were initially decarbonated by stirring.

RESULTS AND DISCUSSION

With the aim of developing a rapid and sensitive HPLC method for the routine analysis of the furanic aldehydes, 2-furaldehyde (FA) and 5-hydroxymethyl-2-furaldehyde (HMF) in alcoholic and non-alcoholic beverages, the systematic investigation of the effect of the mobile phase composition on the

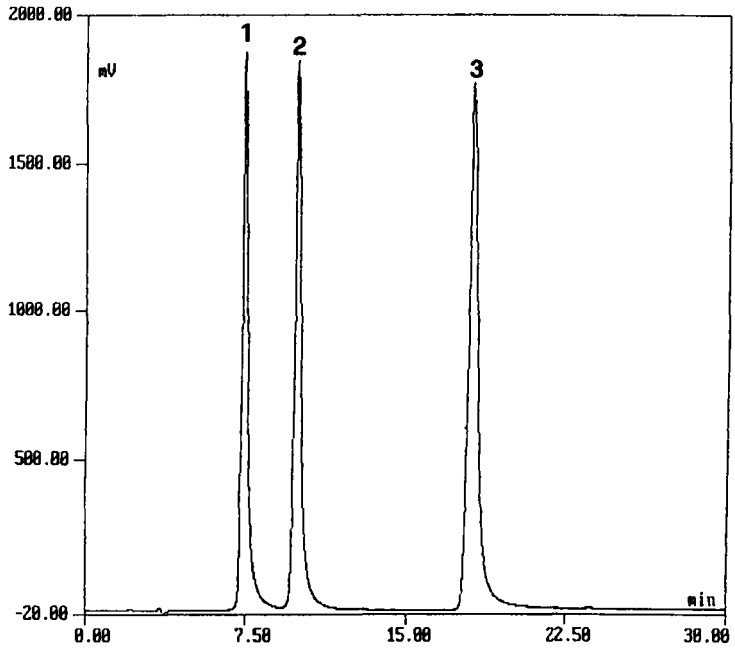


Figure 2. Separation of a standard mixture of HMF (1), FA (2) and MFK (3). Chromatographic conditions: column, Supelcosil LC 18 (300 x 1 mm I.D.); eluent, water-acetonitrile 94:6 (v/v); flow rate, 60 mL/min; detector UV set at 280 nm; temperature, 25°C

chromatographic retention of these furanic compounds in the Supelco LC 18 microbore reversed phase column was performed. The experiments were carried out by eluting under isocratic conditions both real samples and standard solutions with mobile phases containing acetonitrile in water at concentration ranging from 2.5 to 10 % (v/v). Figure 1 shows the retention behavior of the examined furanic aldehydes as a function of the content of acetronitrile in the mobile phase. As expected the retention time of HMF and FA decreased by increasing the concentration of acetonitrile in the mobile phase. Using mobile phases with acetonitrile content higher than 10% (v/v), the resolution of HMF from FA and some substances belonging to the elution front in real samples was impaired. On the other hand, by decreasing the percentage of acetonitrile

Table 1

Retention Time, Standard Deviation (S.A.) and Relative Standard Deviation (R.S.D.) of Multiple Injections of a Standard Solution of 5-Hydroxymethyl-2-furaldehyde (HMF), 2-Furaldehyde (FA) and 2-Furylmethylketone (FMK).Chromatographic Conditions as in Fig. 2.

Compound	Retention Time (min)	Retention Time Mean (min)	S.D. (min)	R.S.D. (%)
HMF	7.39 7.31 7.21 7.24 7.18 7.26 7.16 7.28 7.15	7.25	0.079	1.09
FA	9.60 9.86 9.89 9.69 9.82 9.72 9.61 9.76 9.61	9.71	0.101	1.04
FMK	17.48 17.54 17.76 17.72 17.74 17.48 17.46 17.33 17.38	17.55	0.146	0.83

the elution times were excessively increased. On the basis of these observations, further experiments were carried out with the mobile phase containing 6% (v/v) of acetonitrile which was found to ensure sufficient resolution in a reasonable analysis time.

In order to examine the reproducibility of the retention times, the mean value, the standard deviation (S.D.) and the relative standard deviation (R.S.D.) of the retention times were calculated from the chromatograms obtained by 12 repeated injections of an equimolar solution of FA, HMF and FMK, which was employed as the internal standard in the quantitative analysis. The results are reported in Table 1 and show that the R.S.D.s were batter than 1.09 % for the three compounds.

The dosage of FA and HMF was obtained by an internal standard method. Many commercial compounds structurally related to FA and HMF were evaluated as potential internal standards before selecting 2-furyl methyl ketone (FMK). This furanic compound was selected as the internal standard as it is well resolved from both FA and HMF, is not naturally present in processed

Table 2

Results of the Quantitative Determinations of HMF and FA in Alcoholic and Non Alcholic Beverages

Sample	HMF (mg/L)	R.S.D. (%)	FA (mg/L)	R.S.D. (%)
A1	47.08	2.22	1.21	5.24
A2	147.20	3.47	4.35	5.36
A3	158.87	2.02	11.11	1.65
A4	287.75	1.55	nd	----
A5	432.31	1.81	8.09	3.77
A6	109.20	0.79	nd	----
A7	79.11	1.57	nd	----
A8	291.83	1.54	nd	----
B9	5.40	1.57	nd	----
B10	9.34	0.07	nd	----
B11	343.61	0.12	nd	----
B12	347.18	1.27	1.62	3.04
B13	3.49	5.06	nd	----
C14	187.68	0.01	nd	----
C15	65.25	0.92	nd	----
C16	114.45	1.10	nd	----
C17	3.75	1.32	nd	----

A = bitter (alcoholic beverages made with herbs and/or roobs and colored with caramel); B = aperitif; C = soft drink; nd = not detected; R.S.D .= relative standard deviation. The subscript numbers indicate different commercial sources.

food, does not interfere with the elution pf other species in the examined real samples and is eluted near the peaks of interest. A typical chromatogram of HMF, FA and FMK is shown in Figure 2. It is observed that the peaks of HMF, FA and FMK are well resolved and eluted in less than eighteen minutes. The minimum detectable concentration of HMF and FA was 0.236 ng and 0.116 ng, respectively. The calibration graphs for HMF and FA, obtained by

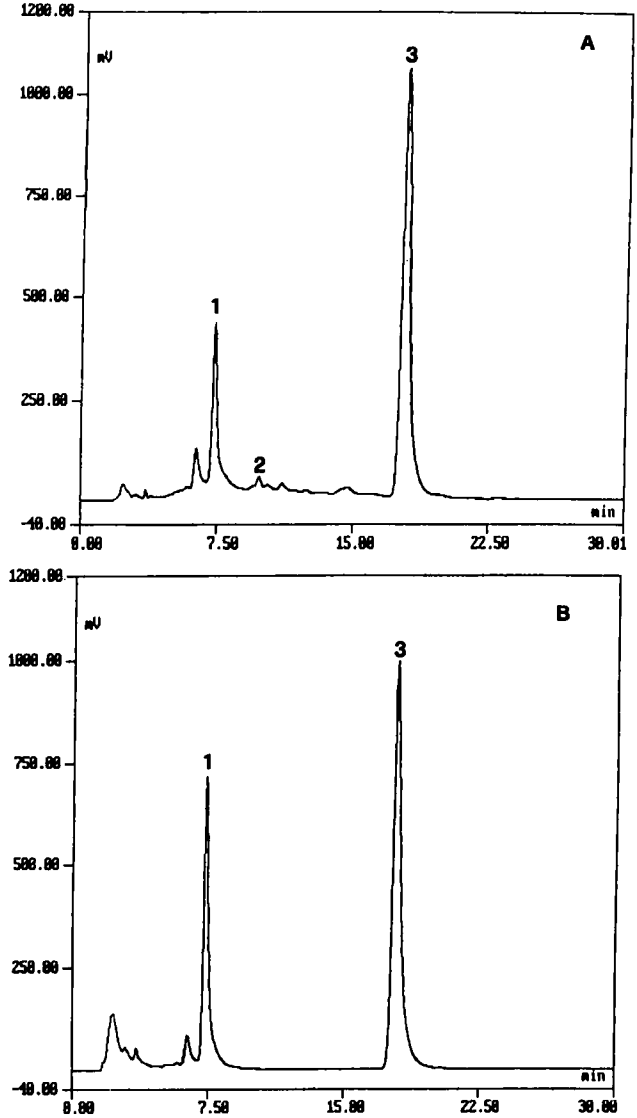


Figure 3. Typical chromatograms of an alcoholic bitter (panel A) and of a soft drink (panel B). Peak identification and chromatographic conditions as in Figure 2.

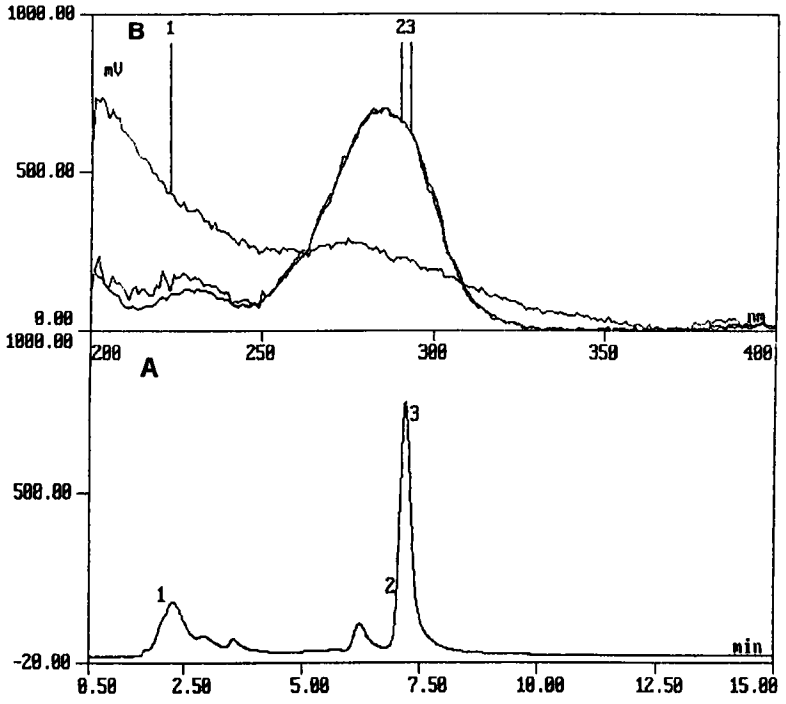


Figure 4. Chromatogram of a sample of a non-alcoholic beverage containing 11.44 mg/mL HMF, the numbers on the chromatogram indicate the points were the UV spectra were monitored (panel A). Chromatographic conditions as in Figure 2. UV spectra from 200 to 400 nm monitored at retention time of 6.99 min (spectra 2) and at retention time of 7.19 min (spectra 3), respectively (panel B).

the peak-area ratio method showed excellent linearity over the concentration range 0.1-50 μg/mL with correlation coefficients r = 0.9998 and 0.9987 respectively, and nearly passed through the origin.

The present method was employed to analyze the HMF and FA content in several varieties of alcoholic bitter beverages and soft drinks coloured with caramel. The beverages were directly injected onto the column without any sample pretreatment, except that they were diluted with water to a concentration range that would ensure no significant loss of resolution due to

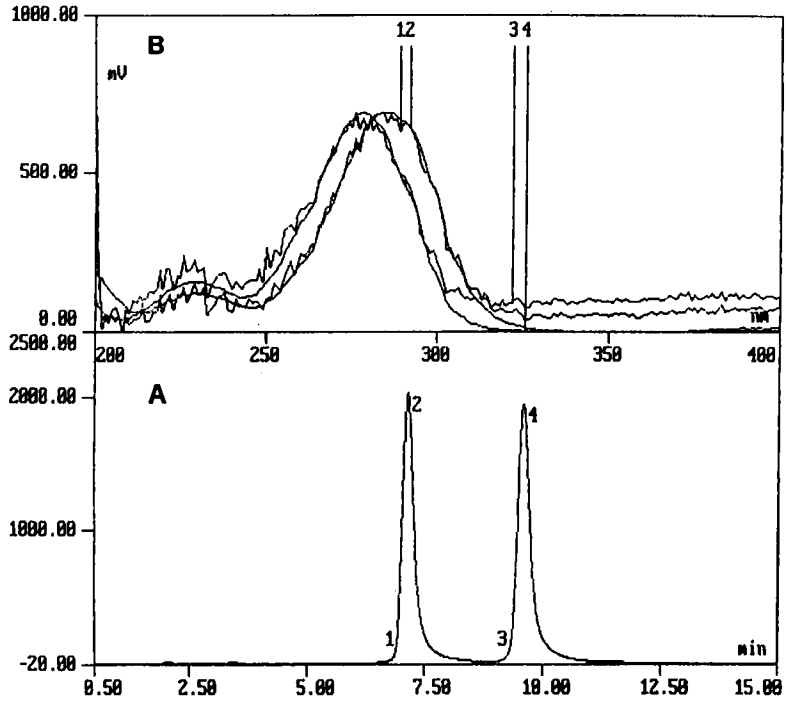


Figure 5. Separation of a standard solution of HMF and FA. Chromatographic conditions as in Figure 2, the numbers on the chromatogram indicate the points were the UV spectra were monitored (panel A). UV spectra from 200 to 400 nm monitored through the peaks at retention time of 6.89 min (spectra 1) and 7.16 min (spectra 2) for HMF and at retention time 9.30 min (spectra 3) and 9.62 min (spectra 4) for FA, respectively (panel B).

overloading of the microbore column. The diluted samples were then filtered through a 0.22 μm single use membrane filter after the addition of the internal standard solution and adjustment of the sample volume to the appropriate value. Results of three replicate determinations are summarized in Table 2 and typical chromatograms are shown in Figure 3.

Peak purity is an important consideration in quantitation. The UV variable wavelength detector used here can measure both retention times and absorption spectra in continuous flow mode, and peak components can therefore be identified by comparison of the peak spectra with those of

Table 3

Recovery of HMF and FA from an Alcoholic Bitter

Compound	Amount in the sample (mg/L)	Added (mg/L)	Found (mg/L)	Recovery (%)
HMF	15.887	2.004	17.953	100.35
	15.887	3.006	18.428	97.54
	15.887	5.010	20.339	96.87
FA	0.6650	0.2088	0.8750	101.95
	0.6650	0.4142	1.0366	97.12
	0.6650	0.8352	1.5028	101.20

Table 4

Recovery of HMF from a Soft Drink

Compound	Amount in the sample (mg/L)	Added (mg/L)	Found (mg/L)	Recovery (%)
	11.445	2.004	132.03	98.17
HFM	11.445	3.006	142.55	98.65
	11.445	5.010	164.55	100.23

standards. Figure 4 shows the chromatogram of a soft drink in which HMF was detected at a concentration of 11.44 μg/mL. The spectra of a the component eluting with a retention time of 7.18 minutes was compared with that of a sample of standard HMF eluted under identical conditions (Figure 5). It is observed that the spectra reported above the chromatogram in Figure 4 are almost identical to the spectra displayed in Figure 5, confirming that the peak component eluting with a retention time of 7.18 minutes was HMF.

In order to determine the accuracy of the method, recovery studies were carried out. Known amounts of HMF and FA were added to a variety of commercial alcoholic and non-alcoholic beverages and the resulting spiked samples were subjected to the entire analytical method. Three different amounts

of HMF and FA were added to the samples. All samples were injected three times and an average of the response area ratio was the basis for the found concentrations. The recoveries were calculated based on the difference between the total concentration determined in the spiked samples and the concentration observed in the non-spiked samples. Results with the relative standard deviations for all samples were of the same order as those reported in Table 3 for a commercial alcoholic bitter and in Table 4 for a soft drink. It can be seen that the average recoveries lied between 96.87 and 101.95%, indicating that the method has an adequate degree of accuracy.

CONCLUSIONS

Reversed phase high performance liquid chromatography using the Supelco LC 18 microbore column appears to bea useful and versatile procedure for the rapid and direct determination of HMF and FA in beverages. The developed method is highly reproducible, the quantification is linear over a wide range of concentrations, and the results of the recovery studies show good accuracy. The method is simple and no sample pretreatment is required, except dilution with water to the appropriate concentration range. Furthermore, the low volume of acetonitrile required for the analysis (about 6 μL per run), due to the microbore format of the column, drastically reduces the cost of the analysis, health hazard and the environmental impact of the waste connected to the use of this organic solvent in reversed phase chromatography.

ACKNOWLEDGEMENTS

E.C. was the recipient of a postdoctoral fellowship from CNR (National Research Council).

REFERENCES

1. H. S. Lee, S. Nagy, J. Food Sci., **53**, 168-172 (1988).

2. J. E. Hodge, J. Agric. Food Chem., **1**, 928-943 (1953).

3. H. L. Dinsmore, S. Nagy, J. Food Sci.. **37**, 768-770 (1972).

4. H. L. Dinsmore, S. Nagy, J. Assoc. Off. Anal. Chem., **57**, 332-335 (1974).

5. R. M. Mijares, G. L.Park, D. B.Nelsen, R. C. McIver, J. Food Sci., **51**, 843-844 (1986).

6. Z. F. Li, M. Sawamura, H. Kusunose, Agric. Biol. Chem., **52**, 2231-2234 (1988).

7. D. Tu, S. Xue, C. Meng, A. Espinosa-Mansilla, A. Muñoz De La Peña, F. Salinas López, J. Agric. Food Chem., **40**, 1022-1025 (1992) .

8. A. Espinosa-Mansilla, F. Salinas, J. J. Berzas Nevado, J. Assoc. Off. Anal. Chem., **75**, 678-684 (1992).

9. R. E. Berry, J. H. Tatum, J. Agric. Food. Chem., **13**, 588-590 (1965).

10. J. Shimizu , M. Watanabe, Agric. Biol. Chem., **43**, 1365-1366 (1979).

11. K. MacNamara, J. High Resolut. Chromatogr., Chromatogr. Commun., **7**, 641-643 (1984).

12. D. Blanco Gomis, M. D. Gutiérrez Alvarez, L. Sopeña Naredo, J. J.Mangas Alonso, Chromatographia, **32**, 45-48 (1991).

13. H. J. Kim, M. Richardson, J. Chromatogr., **593**, 153-156 (1992).

14. M. Villalón Mir, J. Quesada Granados, H. López, Gª De La Serrana, M. C. López Martinez , J. Liq. Chromatogr., **15**, 513-524 (1992).

15. F. J. Morales, C. Romero, S. Jimenez-Perez, Chromatographia, **33**, 45-48 (1992).

16. B. García-Villanova, E. Guerra-Hernández, E. Martínez- Gómez, J. Montilla, J. Agric. Food Chem., **41**, 1254-1255 (1993).

17. F. Lo Coco, C. Valentini, V. Novelli, L. Ceccon, J. Liq. Chromatogr., **17**, 603-617 (1994).

18. D. Corradini, C. Corradini, J. Chromatogr., **624**, 503-509 (1992).

19. C. Corradini , D. Corradini, J. Microcol. Sep., **6**, 19-22 (1994).

20. R. A. Wallingford, A. Ewing, "Capillary Electrophoresis" in **Advances in Chromatography**, Vol. 29, J.C. Giddings, E. Grushka, P.R. Brown, Eds, Marcel Dekker, New York, 1989, pp. 2-76.

21. R. P. W. Scott, "Small-Bore Columns in Liquid Chromatography" in **Advances in Chromatography**, Vol. 22, J.C. Giddings, E. Grushka, P. R. Brown, Eds, Marcel Dekker, New York, 1983, pp.247-294.

Received September 23, 1995
Accepted October 10, 1995
Manuscript 3177