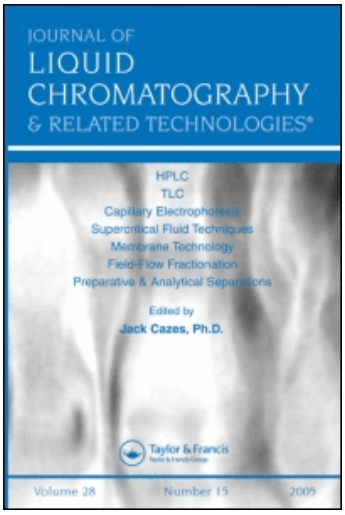

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IDENTIFICATION AND DOSAGE OF 2-FURALDEHYDE AND 5-HYDROXYMETHYL-2-FURALDEHYDE IN BEVERAGES BY REVERSED PHASE CHROMATOGRAPHY WITH A MICROBORE COLUMN

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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

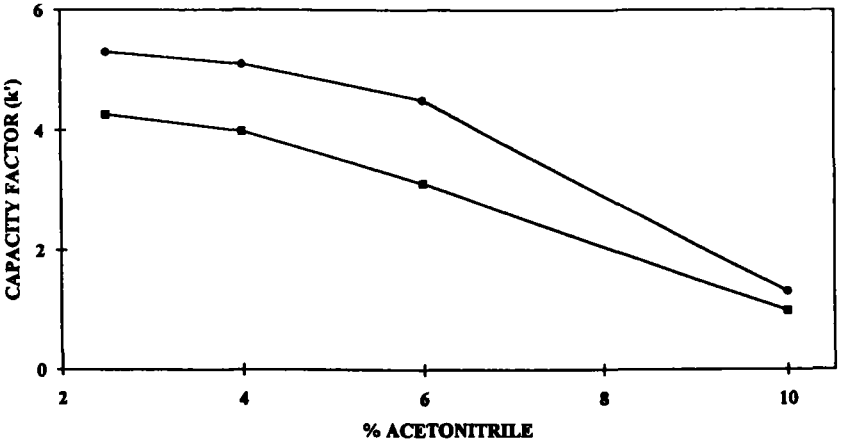

Figure 1. Dependence of the retention factor (k') of HMF (■) and FA (•) on the acetonitrile content in the mobile phase. Chromatographic conditions: column, Supelcosil LC-18 (300 x 1.0 mm I.D.); eluent, acetonitrile-water; flow rate, 60 mL/min; detection by UV absorbance at 280 nm; temperature, 25°C.

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

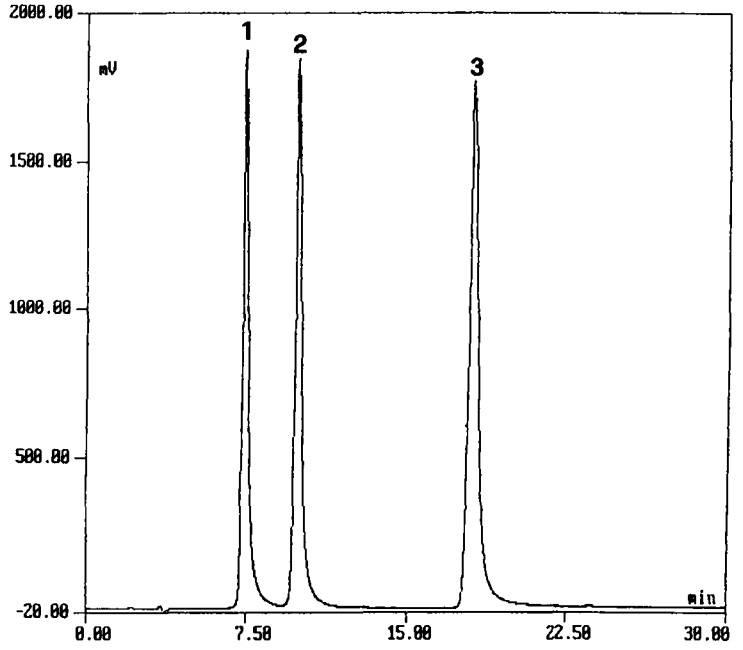

Figure 2. Separation of a standard mixture of HMF (1), FA (2) and MFK (3). Chromatographic conditions: column, Supelcosil LC 18 (300 x 1 mm I.D.); eluent, water-acetonitrile 94:6 (v/v); flow rate, 60 mL/min; detector UV set at 280 nm; temperature, 25°C

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

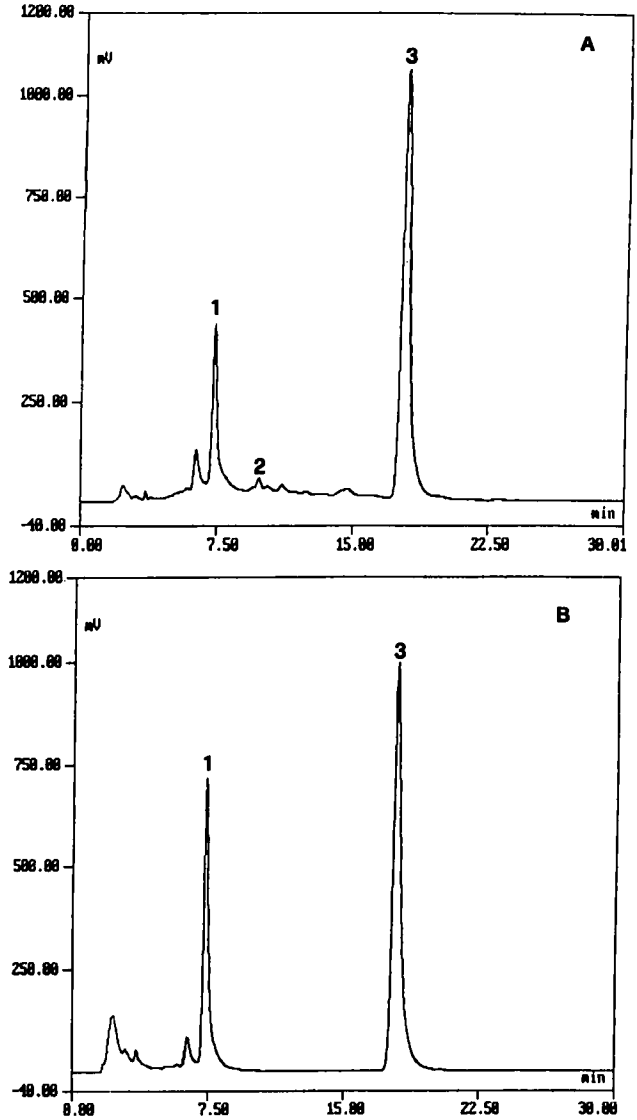

Figure 3. Typical chromatograms of an alcoholic bitter (panel A) and of a soft drink (panel B). Peak identification and chromatographic conditions as in Figure 2.

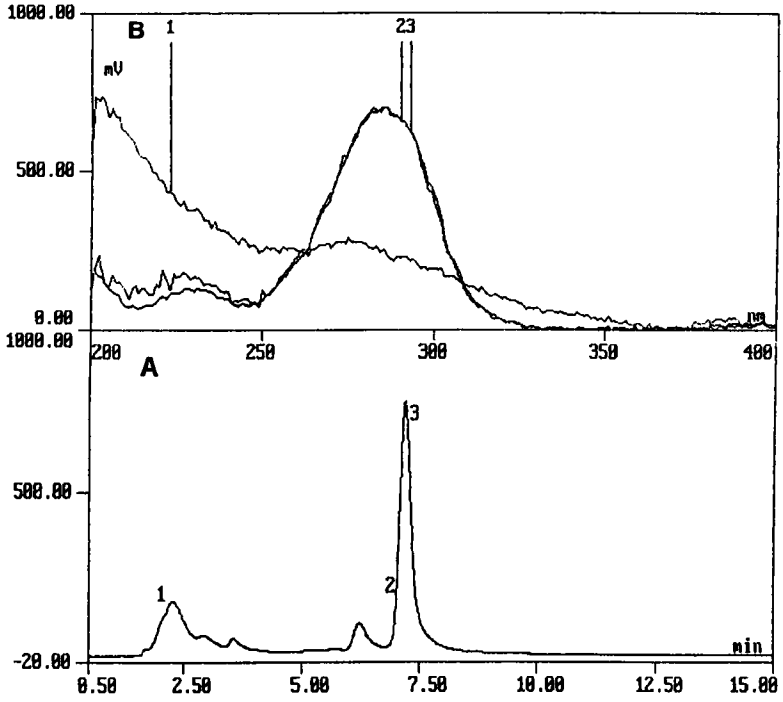

Figure 4. Chromatogram of a sample of a non-alcoholic beverage containing 11.44 mg/mL HMF, the numbers on the chromatogram indicate the points were the UV spectra were monitored (panel A). Chromatographic conditions as in Figure 2. UV spectra from 200 to 400 nm monitored at retention time of 6.99 min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

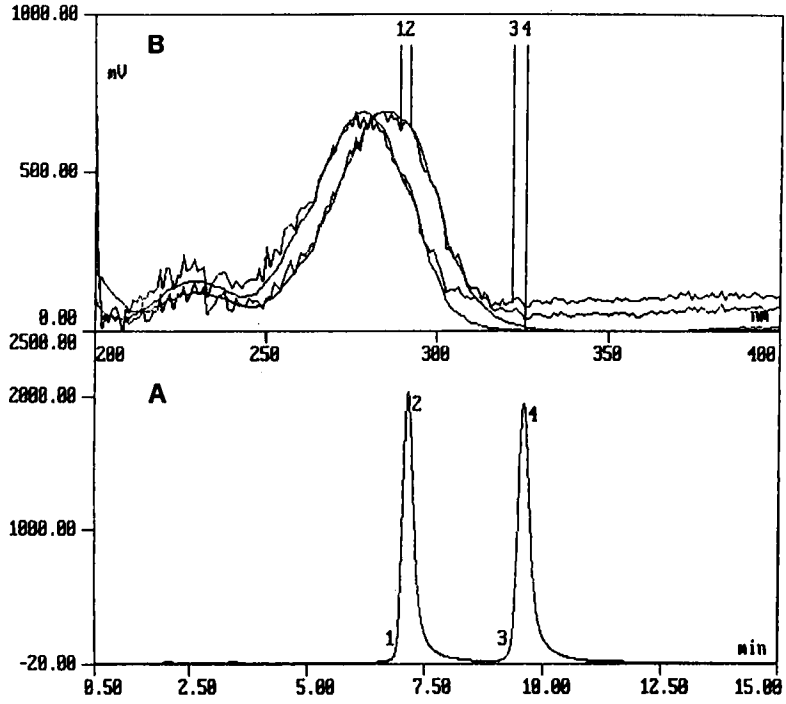

Figure 5. Separation of a standard solution of HMF and FA. Chromatographic conditions as in Figure 2, the numbers on the chromatogram indicate the points were the UV spectra were monitored (panel A). UV spectra from 200 to 400 nm monitored through the peaks at retention time of 6.89 min (spectra 1) and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

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