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Short Communication

Determination of 5-hydroxymethylfurfural by ion-exclusion chromatography with UV detection

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ABSTRACT

5-Hydroxymethylfurfural (HMF) was determined without interferences in juices, honey, syrup, tomato paste, grape juice concentrate and dehydrated pear by anion-exclusion chromatographic separation and UV detection at 285 nm. The samples were mixed with water, filtered and injected without extensive sample treatment, which is normally required in conventional spectrophotometric or reversed-phase high-performance liquid chromatographic methods. HMF at the 50 ppb level was determined with a signal-to-noise ratio of 6. The recovery of HMF added to honey was 98% at the 10 ppm level and 91% at 30 ppm.

INTRODUCTION

5-Hydroxymethylfurfural (HMF) is the end product of acid-catalyzed hexose dehydration [1] and is often used as an index of deteriorative changes in tomato paste [2], honey [3] and fruit products [4–6]. Its accurate determination in fruit products is therefore important. In honey, HMF is used as an indicator of adulteration with acid-converted invert syrups [7] or of a heating history.

Spectrophotometric methods have been used for many years for HMF [8–11]. The original Winkler method [8] involves toxic *p*-toluidine, and is complicated by uncertainty in the color measurement [10]. The AOAC method for honey relies on the reaction of hydrogensulphite with HMF and has not been applied to other foods or citrus products. Experimental errors are expected if other compounds that absorb at 284 nm and react with hydrogensulphite are present at appreciable concentrations.

Recently, several reversed-phase (RP) high-performance liquid chromatographic (HPLC) methods have been published [12–14] using UV detection at 280–285 nm. Jeuring and Kuppers [12] determined HMF in spirits and honey with minimum sample treatment. Extensive sample treatment [precipitation with potassium hexacyanoferrate(III), Carrez I, and zinc sulphate, Carrez II, centrifugation, and filtration] was needed for citrus juices [13] and tomato products [14]. Lee *et al.* [13] added another step, namely elution with ethyl acetate from a C-18 cartridge following washing with hexane, to remove contaminants in orange juice, and reported a detection limit of 50 ppb[a].

We have been using ion-exclusion chromatography (IEC) with photodiode-array (PDA) detection to monitor changes in food constituents taking place as a result of high-temperature/short-time processing. We noticed that HMF is eluted late from the anion-exclusion column, free from any interference, even if a complex food extract is injected without the precipitation step. Consequently, we

[a] Throughout the article the American billion (10^9) is meant.

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describe in this paper the advantages of the IEC method over the RP-HPLC method, and demonstrate that HMF in complex foods and beverages can be determined by IEC with a high specificity and sensitivity without extensive sample clean-up.

EXPERIMENTAL

Sample treatment

All juice, juice drink, honey, syrup and tomato paste samples were purchased from a local store. Grape juice concentrate was obtained from a manufacturer. Freeze-dried pears are a component of Meal, Ready-to-Eat (MRE), a military ration.

Aseptically processed juice, honey or syrup samples were diluted tenfold with deionized water, filtered through a 0.45-μm nylon 66 membrane filter (Alltech, Deerfield, IL, USA) and injected into either an IEC or a RP-HPLC system without further treatment. Tomato juice, orange juice and grape juice concentrate were diluted ten- or twentyfold with water, centrifuged for 2 min in a Brinkmann (Westbury, NY, USA) Model 5414 Eppendorf centrifuge if necessary, filtered and injected. Freeze-dried pears were homogenized with a twentyfold excess of water using Polytron (Brinkmann) and filtered as above.

Chromatographic analysis

For IEC, an Alltech Model 325 metal-free pump was used to deliver 10 m*M* sulphuric acid eluent at a flow-rate of 0.8 ml/min. A Wescan anion-exclusion HS (sulphonated polystyrene–divinylbenzene) column (100 × 4.6 mm I.D.) and an anion-exclusion guard cartridge (Wescan Instruments, Deerfield, IL, USA) were used. The sample was injected through a 20-μl loop of a Rheodyne injector. The UV detector was either a Waters (Milford, MA, USA) Model 990 PDA detector or a Schoeffel Model SF770 variable-wavelength UV detector. When the PDA detector was used, the UV spectra were obtained in the wavelength range 190–350 nm every 6 s. The three-dimensional (3-D) data were stored in a NEC PowerMate 2 computer and manipulated to obtain a 3-D plot, a contour diagram, a chromatogram at 285 nm or a UV spectrum of a chromatographic peak. The wavelength of the Schoeffel detector was set at 285 nm. The signal obtained at the 0–0.01 absorbance range setting was fed into a Spectra-Physics Model 4270 integrator.

A Waters μBondapak C_{18} column (250 × 4.6 mm I.D.) and an RP guard cartridge were used for RP-HPLC. A Rheodyne injector with a 50-μl loop was used. The Waters Model 510 pump delivered acetonitrile–water (3:97) at a flow-rate of 1.6 ml/min. The Schoeffel detector was used at 285 nm.

A standard solution of HMF (Aldrich, Milwaukee, WI, USA) in deionized water was injected alternately with the samples and the peak heights were compared.

Recovery study

Recovery of 10 and 30 ppm of HMF from honey was studied by both IEC and RP-HPLC. Six 2-g aliquots of honey were weighed into six 50-ml beakers. To two of these beakers 2.0 ml of deionized water were added (control). A 2.0-ml volume of 10 ppm HMF solution was added to two other beakers and 2.0 ml of 30 ppm HMF solution to the two remaining beakers. The contents were mixed with a spatula and left at room temperature for 5 min. Water (16 ml) was added to each beaker and the contents were mixed and filtered. The filtrate from the 30 ppm spiked sample was further diluted twofold before injection. Each filtrate was injected twice. The height of the HMF peak was compared with that of a 2 ppm standard HMF solution. The recovery experiment by the IEC method was repeated with honey a few days later. The recovery of 30 ppm HMF from orange juice was studied by the IEC method on another day.

RESULTS AND DISCUSSION

We first observed elution of HMF from the IEC column, using a PDA detector, while analyzing heat-processed fruit samples. The spectrum of the compound that eluted relatively late from the column (4 min from a high-speed column) showed the characteristic double maxima (230 and 285 nm) of HMF. The compound was collected after the detector and analyzed by gas chromatography–mass spectrometry (GC–MS). The parent molecular weight of 126 and the typical electron impact fragments with *m/z* of 109, 97 and 69 for HMF were observed [15]. When authentic HMF was injected, it was eluted at the same retention time. Subsequently, IEC with fixed-wavelength detection at 285 nm was routinely used.

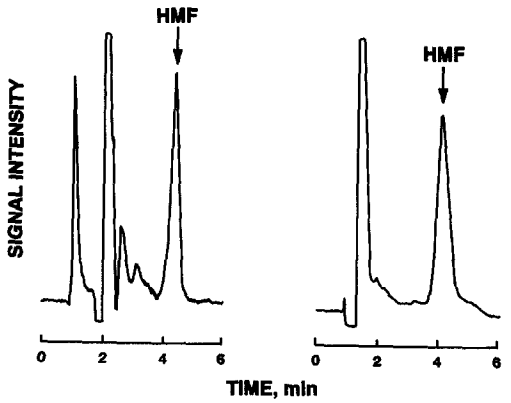

Fig. 1. Chromatograms of *ca.* 2 ppm of HMF in diluted honey (tenfold), obtained by (left) the RP-HPLC and (right) the IEC method. RP-HPLC: Waters μBondapak C_{18} column (250 × 4.6 mm I.D.), acetonitrile–water (3:97) as eluent at a flow-rate of 1.6 ml/min. IEC: Wescan anion-exclusion/HS column (100 × 4.6 mm I.D.), 10 m*M* H_2SO_4 as eluent at a flow-rate of 0.8 ml/min. UV detection at 285 nm.

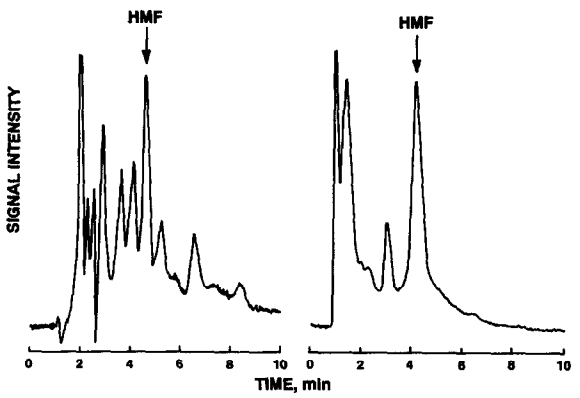

Fig. 2. Chromatograms of 10 ppm of HMF added to tomato juice and diluted tenfold, obtained by (left) the RP-HPLC and (right) the IEC method.

HMF was eluted at *ca.* 4 min after injection in both RP-HPLC (long column, 1.6 ml/min) and IEC (short column, 0.8 ml/min). Fig. 1 shows HMF peaks corresponding to *ca.* 2 ppm in honey, diluted tenfold and injected without Carrez precipitation. Even though the chromatogram given by the IEC method is cleaner, there is also no interference in the RP-HPLC method. On average, 21.5 and 19.4 ppm of HMF were obtained by the RP-HPLC and IEC methods, respectively (Table I, control). The recovery results in Table I show that the recovery of 10 and 30 ppm of HMF added to honey is satisfactory by both methods. The relative standard deviation of the recovery during the day was *ca.* 2% by the IEC method. When 30 ppm of HMF were added to the orange juice, 29.7 ppm were recovered by the IEC method. The recovery of 30 ppm of HMF on three different days, two from honey and one from orange juice, ranged from 91 to 99%.

TABLE I

RECOVERY OF ADDED HMF FROM HONEY BY REVERSED-PHASE AND ION-EXCLUSION CHROMATOGRAPHY

Sample	RP-HPLC[a]		IEC[b]	
	Observed (ppm)	Recovered (ppm)	Observed (ppm)	Recovered (ppm)
Honey, control	21.5 (0.6)	–	19.4 (0.3)	–
Honey, 10 ppm spike	31.2 (0.6)	9.7 (0.3)	28.6 (0.4)	9.2 (0.2)
Honey, 30 ppm spike	50.0 (2.0)	28.5 (1.4)	47.6 (0.9)	28.2 (0.7)

[a] Average of four determinations with standard deviation in parentheses.
[b] Average of eight determinations with standard deviation in parentheses.

The chromatogram for tomato juice obtained by the RP-HPLC method was much more complex, and quantitation of HMF was subject to an uncertainty because of the interfering peaks. Approximately 1 ppm of HMF in the juice was measured by the IEC method. Fig. 2 shows results obtained by both methods for tomate juice spiked with 10 ppm of HMF and diluted tenfold before injection. It appears that further sample treatment, such as Carrez precipitation, is needed for the RP-HPLC analysis [14]. The chromatogram on the right demonstrates that accurate determination of HMF without precipitation is possible by the IEC method. A similar chromatogram was obtained for tomato paste and 102 ppm of HMF was measured.

Lee *et al.* [13] noted that orange juice contains compounds that are eluted with the HMF by the RP-HPLC method. They used Carrez precipitation and elution with ethyl acetate from a C_{18} cartridge,

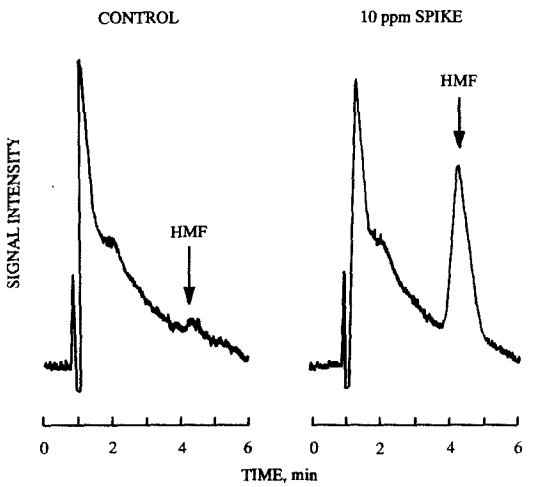

Fig. 3. Chromatograms of orange juice with (right) and without (left) addition of 10 ppm of HMF after twentyfold dilution, obtained by the IEC method.

following a hexane wash. The ethyl acetate had to be dried before chromatography. When orange juice (reconstituted from frozen juice) was diluted twenty-fold with water, filtered and injected, the chromatogram shown on the left in Fig. 3 was obtained by the IEC method. The chromatogram was relatively clean around 4 min, and a small peak corresponding to about 0.05 ppm of HMF was observed (1 ppm in the juice). When the juice was spiked with 10 ppm of HMF and diluted twentyfold, a peak corresponding to 0.55 ppm, shown on the right, was obtained as expected. When the orange juice filtrate was analyzed by the RP-HPLC method using acetonitrile–water (3:97) as eluent, no significant interference was observed.

The method was applied to a variety of aseptically processed juice drinks, and HMF concentrations ranging from 2.0 to 19.4 ppm were obtained. The chromatograms were very clean, and no interference was observed with any samples tested. HMF was also observed without interference in syrup (12 ppm), grape juice concentrate (22 ppm) and dehydrated pears stored at 38°C for 1 year (34 ppm).

At the highest sensitivity setting of the UV detector (0–0.01 absorbance range), a signal-to-noise ratio of 6 was observed when 50 ppb HMF solution was injected. The peak height between 0 and 10 ppm was linear with a correlation coefficient of 0.99. The HMF solution was stable in water for several hours at room temperature. The detector response was also very stable; therefore, automated analysis should be possible.

The IEC separation has been successfully used for the determination of weak acid anions, such as sulphite [16,17], nitrite [18–20] and ascorbic acid [21]. The polymeric resin of the IEC column is also widely used for carbohydrates. This paper demonstrates that a rapid and accurate determination of HMF, which is an important carbohydrate degradation product and a useful quality indicator in food and beverage products, is possible without extensive sample treatment when IEC is used with UV detection.

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