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Food Chemistry 64 (1999) 423–427

Food Chemistry

Analytical, Nutritional and Clinical Methods Section

Simultaneous separation and determination of sugars, ascorbic acid and furanic compounds by HPLC—dual detection

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Received 9 January 1998; accepted 25 March 1998

Abstract

An HPLC method was developed for the simultaneous separation and determination of sugars, ascorbic acid, 5-HMF (5-hydroxymethylfurfural), furfural and four other furanic compounds as the possible degradation products of sugars and ascorbic acid on heating and by Maillard reaction. Sucrose, glucose, fructose, ascorbic acid, dehydroascorbic acid, 5-HMF, furfural, DMHF (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone), 2-furoic acid, 2-acetylfuran and furfuryl alcohol were separated on an Aminex HPX-87H column (300×7.8 mm). The mobile phase consisted of acetonitrile and 0.005 mol l^{-1} sulfuric acid aqueous solution (16:84, v/v). Two detectors, a refractive index detector and a photodiode array detector, were used to detect these compounds. Several fruit juice and drink samples were analyzed using this HPLC method. For alcoholic beverages, the content of alcohol was also simultaneously determined by refractive index detector.

1. Introduction

Non-enzymic browning has been considered one of the major causes of quality and colour loss during the processing and storage of food products, e.g. fruit juices. Some furanic compounds, one class of heterocyclic compounds that have been reported in a wide variety of food systems (Yeo and Shibamoto, 1991), were produced from the degradation of sugars and ascorbic acid by heating. 5-HMF (5-hydroxymethylfurfural) and furfural are the principal degradation products of the hydrolysis of hexoses and pentoses, respectively (Espinosa-Mansilla et al., 1992). 5-HMF has been correlated with colour changes in fruit juices while furfural is widely accepted as an indicator of flavour changes (Blanco-Gomis et al., 1991). DMHF (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone), which is an important flavour compound that can be found in various fruits (Krammer et al., 1994; Sanz et al., 1994) and possesses stronger antioxidative activity (Eiserich et al., 1992), is also one of the putative degradation products of sugars (Naim et al., 1993) and can be generated directly from hexoses (Blank and Fay, 1996). 2-Acetylfuran and furfuryl alcohol are also the degradation products of sugars (Lee and Nagy, 1988; Yeo and Shibamoto, 1991). Ascorbic acid is decomposed to form furfural (Rodriguez et al., 1991; Solomon et al., 1995) and other products, which are the same as the intermediates in the degradation of pentoses (Davies and Wedzicha, 1994). 2-Furoic acid is one of the degradation products of an aqueous solution of dehydroascorbic acid, the oxidation form of ascorbic acid (Kimoto et al., 1993; Sawamura et al., 1994).

Although many different analytical techniques have been developed for the determination of 5-HMF and furfural (Bonn, 1985; Blanco-Gomis et al., 1991; Porretta, 1992; Kim and Richardson, 1992; Corradini and Corradini, 1992; Espinosa-Mansilla et al., 1992; Garcia-Villanova et al., 1993; Lo-Coco et al., 1994), no studies concerning the simultaneous determination of sugars, ascorbic acid and furanic compounds have been reported. The aim of the present study is to develop an HPLC method for the simultaneous separation and analysis of these compounds in one chromatographic run.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (HPLC grade), sucrose and glucose were purchased from BDH Laboratory Supplies (Poole, UK).

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0308-8146/98/$—see front matter
PII: S0308-8146(98)00091-0

Fructose and ascorbic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dehydroascorbic acid, 5-HMF, furfural, DMHF, 2-furoic acid, 2-acetylfuran and furfuryl alcohol were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All fruit juice and drink samples were purchased from the local stores.

2.2. Apparatus

High-performance liquid chromatography (HPLC) was conducted on a Waters liquid chromatograph equipped with two 510 pumps. The samples filtered through 0.45 μm filters were separated using a Bio-Rad Aminex HPX-87H hydrogen form cation exchange resin-based column (300×7.8 mm) packed with sulfonated divinyl benzene–styrene copolymer with a particle size of 9 μm. The column temperature was maintained at 25°C. The mobile phase consisted of acetonitrile and 0.005 mol l^{-1} sulfuric acid aqueous solution. The flow rate was set at 0.5 ml min^{-1}. The samples were injected with a Rheodyne 7725i valve with a 20 μl loop.

2.3. Detection

Two detectors, a Waters 996 photodiode array detector and a Gilson 132 refractive index detector, were used for the simultaneous detection of the sugars, ascorbic acid and the furanic compounds. A tridimensional chromatogram was recorded from 190 to 400 nm for the detection of ascorbic acid and furanic compounds. Peaks were measured at wavelengths of 215, 254 and 280 nm, respectively, to facilitate the detection of these compounds. Chromatographic peaks were identified by comparing retention times and spectra against known standards. All computations were performed using a Waters Millennium 2010 data system.

3. Results and discussion

The Aminex HPX-87H column (Bio-Rad, 300×7.8 mm, 9 μm), an ion-exchange resin column on which the furanic compounds retained strongly (Bonn, 1985), was chosen for the separation of sucrose, glucose, fructose, ascorbic acid, dehydroascorbic acid, 5-HMF, furfural, DMHF, 2-furoic acid, 2-acetylfuran and furfuryl alcohol. In order to reduce the analysis time, Bonn (1985) used the method with an increased flow rate of mobile phase and a higher column temperature (95°C) to elute 5-HMF and furfural, but a high temperature could result in the degradation of these compounds, especially ascorbic acid. Acetonitrile had been chosen as organic modifier to lessen the retention time of the furanic compounds, and this technique has been successfully used for the simultaneous determination of sugars and 5-HMF in glucose injection (Yuan et al., 1995) and drinks (Yuan et al., 1996). Therefore, in the present study, the solvent mixtures (as mobile phase) containing 0.005 mol l^{-1} sulfuric acid aqueous solution and acetonitrile (as organic modifier) were tested for their ability to elute and separate these compounds. The relative proportion of acetonitrile and sulfuric acid aqueous solution in the mobile phase was varied in order to effect separation. The effect of acetonitrile on the retention behaviour of the sugars, ascorbic acid and the furanic compounds was investigated by adding acetonitrile to the mobile phase at concentrations up to 40% (v/v).

While the acetonitrile concentration in the mobile phase changed, the retention times of the sugars did not change significantly. Fig. 1 shows the chromatograms of sucrose, glucose and fructose detected by a refractive index detector when the acetonitrile concentrations were 16% (Fig. 1(a)) and 22% (Fig. 1(b)). In Fig. 1, a negative peak, whose retention time decreased with an increase in acetonitrile concentration, occurs after the elution of fructose. When the acetonitrile concentration was more than 22%, the negative peak would interfered with the determination of sugars.

While increasing the content of acetonitrile in the mobile phase, the retention times of ascorbic acid and the furanic compounds decreased (Fig. 2). For the six furanic compounds, the elution order and selectivity were influenced by the addition of acetonitrile because

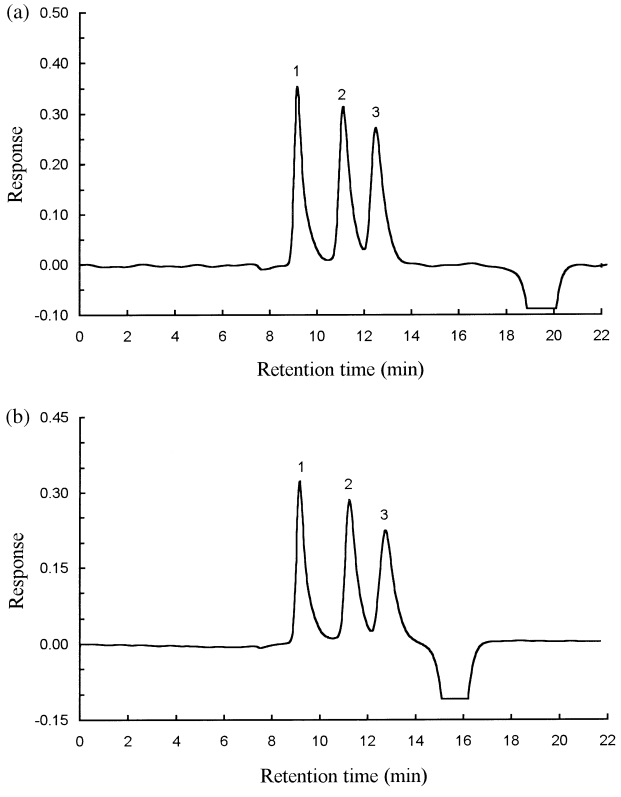

Fig. 1. The chromatograms of sucrose, glucose and fructose detected by refractive index detector when the acetonitrile content was (a) 16% and (b) 22%. Peaks: 1 = sucrose; 2 = glucose; 3 = fructose.

the effects of acetonitrile on the six furanic compounds were different. Fig. 2(a) shows that low acetonitrile concentrations favour the separation of ascorbic acid and dehydroascorbic acid. Fig. 2(b) shows the effect of acetonitrile concentration on the retention times of 5-HMF, DMHF, 2-furoic acid and furfuryl alcohol. Fig. 2(c) shows the effect of the acetonitrile concentration on the retention times of furfural and 2-acetylfuran. The change of acetonitrile concentration in the mobile phase did not significantly affect the retention of the sugars. The best separation for ascorbic acid and the six furanic compounds was therefore obtained with a mobile phase containing 16% (v/v) acetonitrile and 84% (v/v) 0.005 mol l^{-1} sulfuric acid aqueous solution. The temperature of separation was set at 25°C to prevent the degradation of ascorbic acid and the furanic compounds and the hydrolysis of sucrose in the process of separation. Although such a temperature adversely affected the peak shape of the sugars, it was not a problem for the sample analyzed.

A typical chromatogram of the separation of ascorbic acid and the six furanic compounds at this mobile phase composition by photodiode array detection is shown in Fig. 3. The retention times and detection means of the sugars, ascorbic acid and the furanic compounds are summarized in Table 1.

The linearity of the peak–area (A) ratio against the concentration (C) curve for these compounds was investigated and the results are shown in Fig. 4(a) and Fig. 4(b).

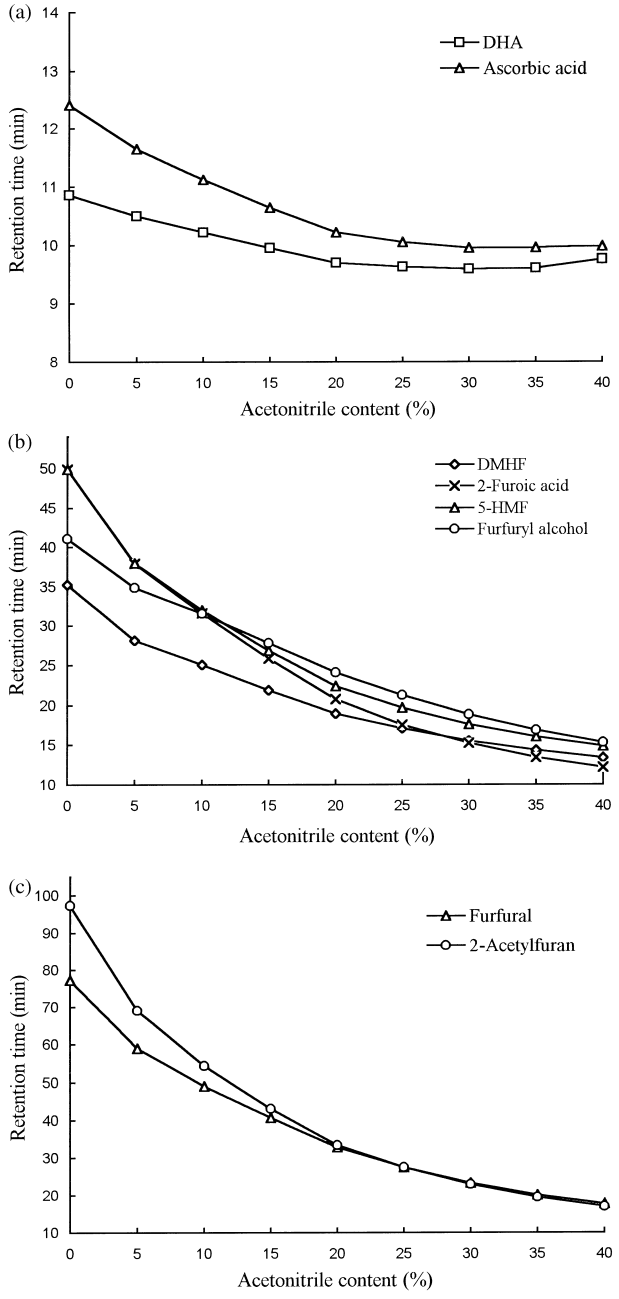

Fig. 2. The effect of the concentration of acetonitrile on the retention times of (a) ascorbic acid and dehydroascorbic acid; (b) 5-HMF, DMHF, 2-furoic acid and furfuryl alcohol; (c) 2-acetylfuran and furfural.

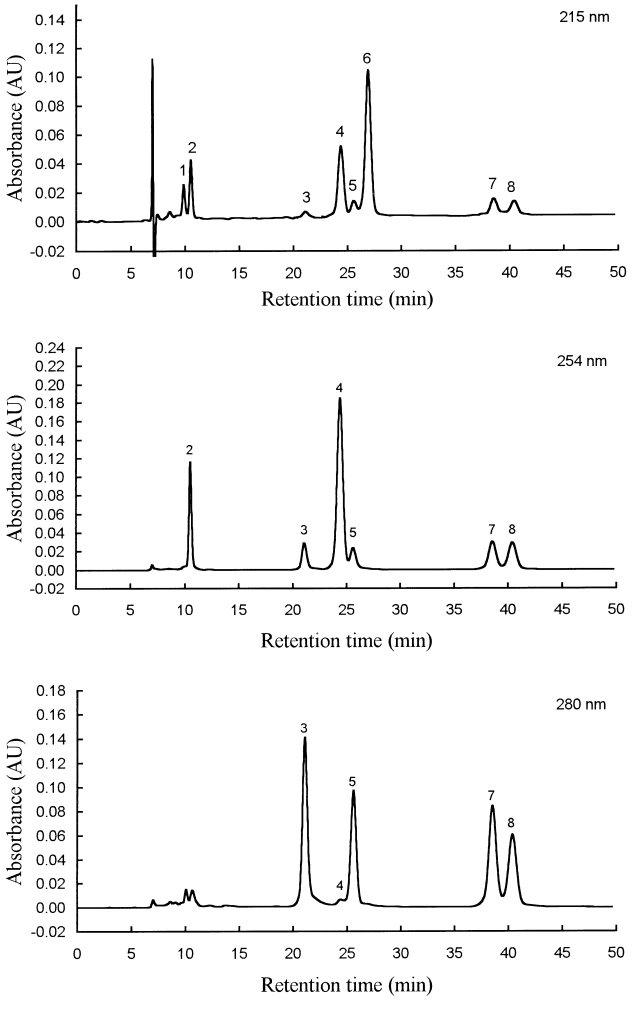

Fig. 3. A typical chromatogram of the separation of ascorbic acid and the six furanic compounds by photodiode array detection. Peaks: 1 = dehydroascorbic acid; 2 = ascorbic acid; 3 = DMHF; 4 = 2-furoic acid; 5 = 5-HMF; 6 = furfuryl alcohol; 7 = furfural; 8 = 2-acetylfuran.

The six furanic compounds, furfural (280 nm: $A = 437335C$; $R^2 = 0.9993$), 5-HMF (280 nm: $A = 344202C$; $R^2 = 0.9995$), 2-acetylfuran (280 nm: $A = 312578C$; $R^2 = 0.9992$), DMHF (280 nm: $A = 50566C$; $R^2 = 0.9998$), 2-furoic acid (254 nm: $A = 308794C$; $R^2 = 0.9993$), furfuryl alcohol (215 nm: $A = 15275C - 12737$; $R^2 = 0.9992$), present linearity between 0.1 and 100 mg l^{-1}. Ascorbic acid (254 nm: $A = 11475C - 3611$; $R^2 = 0.9976$) presents linearity between 1 and 100 mg l^{-1}. Dehydroascorbic acid (230 nm: $A = 1010C - 1007$; $R^2 = 0.9991$) presents linearity between 5 and 100 mg l^{-1}. Sugars present linearity between 2 and 20 mg ml^{-1} and the correlation coefficients (R^2) were 0.9995, 0.9995 and 0.9991, respectively for sucrose, glucose and fructose. The lower detection limits were 0.003 mg l^{-1} for 5-HMF (280 nm), 0.004 mg l^{-1} for furfural (280 nm) and 2-acetylforan (280 nm), 0.005 mg l^{-1} for 2-furoic acid (254 nm), 0.03 mg l^{-1} for DMHF (280 nm), 3 mg l^{-1} for furfuryl alcohol (215 nm), 0.1 mg l^{-1} for ascorbic acid (254 nm) and 1 mg l^{-1} for dehydroascorbic acid (230 nm).

Table 1
Retention times and absorption maxima of ascorbic acid and furanic compounds

No.	Compounds	Retention time (min)	Absorption maxima (nm)	Detection
1	Sucrose	9.1	—	RI
2	Dehydroascorbic acid	9.8	226.5	UV (230 nm)
3	Ascorbic acid	10.5	244.1	UV (254 nm)
4	Glucose	11.1	—	RI
5	Fructose	12.5	—	RI
6	DMHF	21.1	287.7	UV (280 nm)
7	Alcohol	22.0	—	RI
8	2-Furoic acid	24.4	252.3	UV (254 nm)
9	5-HMF	25.6	284.1	UV (280 nm)
10	Furfuryl alcohol	26.9	215.9	UV (215 nm)
11	Furfural	38.5	277.0	UV (280 nm)
12	2-Acetylfuran	40.4	273.5	UV (280 nm)

UV = ultraviolet; RI = refractive index.

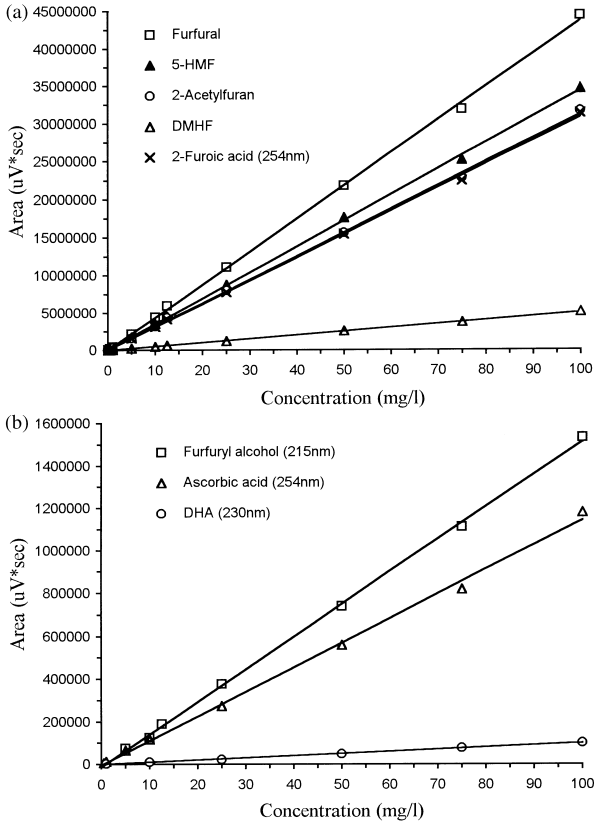

Fig. 4. Calibration curves of (a) furfural (280 nm), 5-HMF (280 nm), 2-acetylfuran (280 nm), DMHF (280 nm) and 2-furoic acid (254 nm); (b) furfuryl alcohol (215 nm), ascorbic acid (254 nm) and dehydroascorbic acid (230 nm).

The repeatability (relative standard deviation) of the proposed method for five replicate injections and the results of the standard addition recovery study for ascorbic acid and the furanic compounds are shown in Table 2.

As an application, the contents of the sugars, ascorbic acid and the furanic compounds in some commercial drinks and fruit juice concentrates with or without added vitamin C were determined. The sample was directly injected without any sample pretreatment

Table 2
Repeatability of determination and recoveries of standard addition in commercial fruit juices and drinks ($n = 5$)

Compounds	Mean value (mg/l)	RSD (%)	Added (mg l^{-1})	Recovery (%)
Dehydroascorbic acid	5.0 *	4.82	5.0	95.4
Ascorbic acid	1090	4.56	5.0	96.1
5-HMF	18.96	1.35	5.0	99.7
Furfural	9.40	2.01	5.0	100.2
DMHF	5.02	1.95	5.0	98.6
2-Furoic acid	10.67	2.82	5.0	99.3
2-Acetylfuran	0.22	2.16	5.0	97.8
Furfuryl alcohol	10.0 *	2.23	5.0	101.3

* for standard solution
RSD = relative standard deviation.

Table 3
Contents (mg l^{-1}) of furanic compounds in fruit juice concentrates and drinks ($n = 3$)

Samples	5-HMF	Furfural	DMHF	2-Furoic acid	2-Acetylfuran
Orange juice	4.16	3.72	4.04	3.39	—
Apple juice	18.96	9.40	5.02	10.67	—
White grape juice	14.20	0.26	0.81	0.92	—
Red grape juice	16.88	0.40	0.78	1.12	—
Pear juice	6.30	7.05	4.10	33.35	—
Apple grape juice	21.94	8.77	2.02	10.68	—
Cola	9.52	0.23	—	0.32	0.22
Beer	2.96	—	2.61	0.37	—
White wine	1.02	—	1.34	0.91	—
Red wine	1.32	—	0.96	1.78	—

Table 4
Contents ($mg\,ml^{-1}$) of ascorbic acid and sugars in fruit juice concentrates and drinks ($n=3$)

Samples	Ascorbic acid	Sucrose	Glucose	Fructose
Orange juice	0.83	12.95	41.45	44.01
Apple juice	1.09	6.48	30.88	69.10
White grape juice	—	—	62.86	67.31
Red grape juice	—	—	64.26	70.00
Pear juice	1.15	9.00	21.21	63.00
Apple grape juice	0.58	5.35	37.88	71.03
Cola	—	—	48.78	50.72
Beer	—	—	—	—
White wine	—	—	8.86	11.38
Red wine	—	—	9.46	6.95

except dilution and filtration. The samples were diluted according to the concentrations of ascorbic acid and sugars in samples. For fruit juice without added vitamin C, the samples were diluted by three to four times. For fruit juice with added vitamin C, the samples were diluted by 8 to 12 times. The results of determinations ($n=3$) are shown in Tables 3 and 4. In all samples analyzed, no furfuryl alcohol was detected. 2-Acetylfuran was detected only in the cola sample. For the wine and beer samples, the content of alcohol could also be determined simultaneously. The retention time of alcohol was 22.0 min under the conditions.

Acknowledgements

This research was supported by the CRCG (the University of Hong Kong Committee on Research and Conference Grants) and RGC (the Hong Kong Research Grants Council).

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