High-Performance Liquid Chromatographic Determination of Furfural and Hydroxymethylfurfural in Apple Juices and Concentrates

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Key Words

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Apple juice

Summary

A rapid and sensitive method for determining 2-furaldehyde (FUR) and 5-hydroxymethyl-2-furaldehyde (HMF) in apple juices and juice concentrates has been developed. The method for FUR and HMF involves the solid-liquid extraction of the juice by using a C-18 cartridge prior to reversed-phase separation with detection at 280 nm. The mobile phase was acetonitrile-water (8/92, v/v) at a flow rate of 1.0 ml/min. Recoveries from apple juices and juice concentrates spiked at different levels ranged from 94.1 to 104.0 (FUR) and 94.5 to 100.5 (HMF). The quantification limit for both, FUR and HMF, was 5 ppb.

Introduction

Both 2-furaldehyde (FUR) and 5-hydroxymethyl-2-furaldehyde (HMF) are recognized indicators of quality deterioration of fruit juices during the heating process i.e. concentration, pasteurization or storage. HMF has been correlated with color change in fruit juices [1] while furfural is widely accepted as an indicator of flavor changes [2, 3].

Many different analytical techniques have been investigated for the determination of FUR and HMF. Among the different techniques available, colorimetric and chromatographic procedures are the ones most commonly used. Colorimetric methods were used for both FUR and HMF quantification in fruit juices [1–3], spirits [4], honey [5], caramel [6], etc. However, these methods have some disadvantages such as the instability of the color complex formed, the time required and the use of hazardous chemicals. Chromatographic techniques include thin layer chromatography [7], gas chromatography [8], and more recently, high-performance liquid chromatography. HPLC has been used in the quantitative determination of FUR and/or HMF in fruit juices and concentrates [9–12] and other products [13, 6]. Generally, the presence of interfering peaks complicates the HPLC separation of FUR and HMF in fruit juice concentrates especially at low concentrations. For this reason the majority of authors recommend sample preparations such as distillation [10], extraction [14] or clarification [11] before HPLC determination.

This paper is specifically concerned with the application of HPLC methods in the control and the determination of FUR and HMF formed in pasteurized apple juice and juice concentrates. It describes a rapid procedure for the extraction and quantification of FUR and HMF using a C-18 column and UV detection. The HPLC method has been evaluated by complete triplicate analysis and by spiking samples.

Experimental

The chromatographic system consisted of a Waters 510 pump; a Wisp Model 712 automatic injector; a Waters Model 990 diode array detector; and a Digital 380 data station.

Separations were carried out on a 250 × 4.6 mm i.d. column packed with 3 µm Spherisorb ODS-2. The mobile phase was acetonitrile/water (8 : 92) at 1 ml/min, degased with helium prior to use. The analysis was carried out by injecting 25 µl of the sample or standard into the column. Final UV detection was carried out at 280 nm.

Standard solutions of FUR (Fluka Chemie) and HMF (Sigma Chemical) were prepared by dissolving their analytical grade reagents in water with 10 % of methanol.

Sample Preparation

Between 1 and 3 ml of either apple juice pasteurized at 72 °C for 30 min or apple juice concentrate, was pipetted into a syringe and passed through the conditioned C-18 cartridge (1 ml of methanol followed by 2 ml of water).

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After washing the cartridge with 0.5 ml of hexane, the furfurals were eluted with 4 ml of acetonitrile/water (20 : 80). Under these conditions, the furfural recovery up to 5 ppm was greater than 94 %. However, if furfural concentrations were greater than 5 ppm, the amount retained by the cartridge decreased, principally for HMF, and therefore a sample dilution was necessary.

Results and Discussion

For some samples such as spirits, honey or caramel [4–6], FUR and/or HMF determinations by HPLC can be carried out by direct sample injection. However, according to different authors [10, 11, 14], other types of samples such as citrus juices, need tedious sample pretreatments in order to avoid the interferences caused by other compounds present in the sample which elute with retention times close to FUR and HMF. Normally, chromatographic separations were carried out by employing C-18 columns and a mixture of acetonitrile-water or methanol-water as the mobile phase. Recently, Li et al. [12] have reported a new method for furfural separation in citrus juice without sample pretreatment based on its elution by a mixture of tetrahydrofuran-water (0.3 : 99.7). With the aim of developing a rapid and sensitive HPLC method for monitoring the effects of thermal treatments and storage on the apple juice and concentrate quality, we have investigated the possibility of a reversed-phase separation and determination of furfurals. This was tested by the direct injection of apple juice and concentrate samples, using a Spherisorb ODS-2 stationary phase and two mobile phases: acetonitrile-water and tetrahydrofuran-water. As can be seen in Figure 1 neither of the mobile phases was adequate for the furfural separation. Variations between 5–15 % and 0.3–1 % in the acetonitrile and tetrahydrofuran contents respectively yield similar chromatographic results. When major percentages of organic modifier (acetonitrile or tetrahydrofuran) were used, HMF resolution from some substances belonging to the elution front was impaired. By decreasing the percentages the elution times were excessively increased. Consequently, a sample clean-up prior to injection was necessary. In order to do this, solid-liquid extraction using C-18 cartridges is probably one of the easiest sample clean-up methods. As shown in Figure 2 for the two mobile phases employed, solid-liquid extraction greatly simplifies the separation problem. However, the chromatographic peak shape and the separation are better with the acetonitrile-water phase.

Investigations into the effect of increasing temperature showed a decrease in analysis time but also a decrease in resolution, due to the elution of other compounds near to HMF and FUR. (Figure 3). Separation is best carried out between 25 °C and 40 °C.

Quantification and Recovery

The quantification of the furfurals was achieved by using the external standard method. Calibration plots were generated by repeated injections of a fixed volume (25 µl) of standard solutions of furfurals of different concentrations, and the resulting plots were stored in the data module. A good correlation of the standards and corresponding peak areas (r = 0.999) over the range 5–20000 ppb was established. A volume of 25 µl of the apple juice or juice concentrate samples (pre-treated according to sample preparation procedure) was then injected and the amount of furfurals was obtained directly from the data module. The data module calibration was checked regularly with standard solutions.

Recovery studies were performed for a commercial pasteurized apple juice and a juice concentrate. Each furfural was spiked at three different concentrations and the results for the pasteurized juice and the juice concentrate are given in Table I.

Typical recoveries ranging from 94–104 % for furfurals at all spiking levels were obtained. These results indicate that

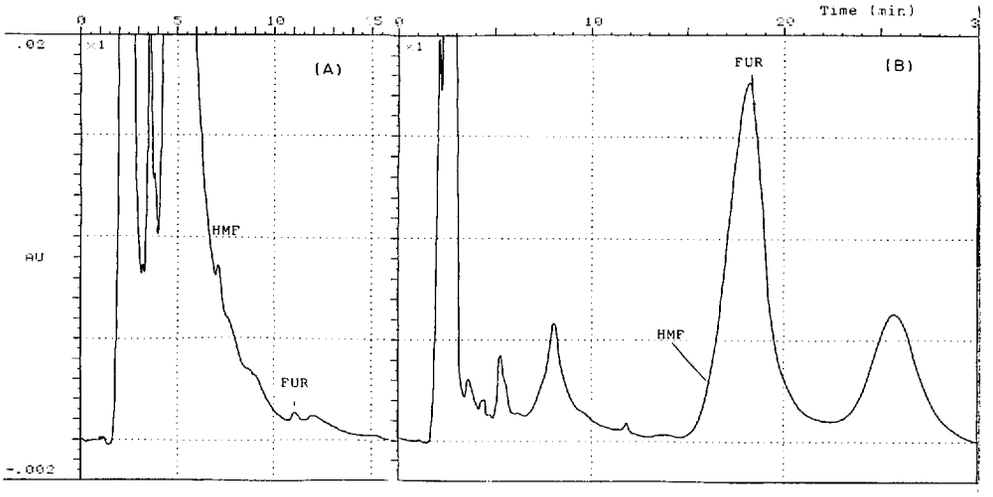

Figure 1

Typical chromatograms of hydroxymethylfurfural and furfural in juice concentrate. Column: Spherisorb ODS-2, 250 × 4.6 mm I.D., 3 µm. Flow rate: 1 ml/min. Temperature: 25 °C. Mobile phase: (A) Water/Acetonitrile (92 : 8). (B) Water/Tetrahydrofuran (99.7 : 0.3).

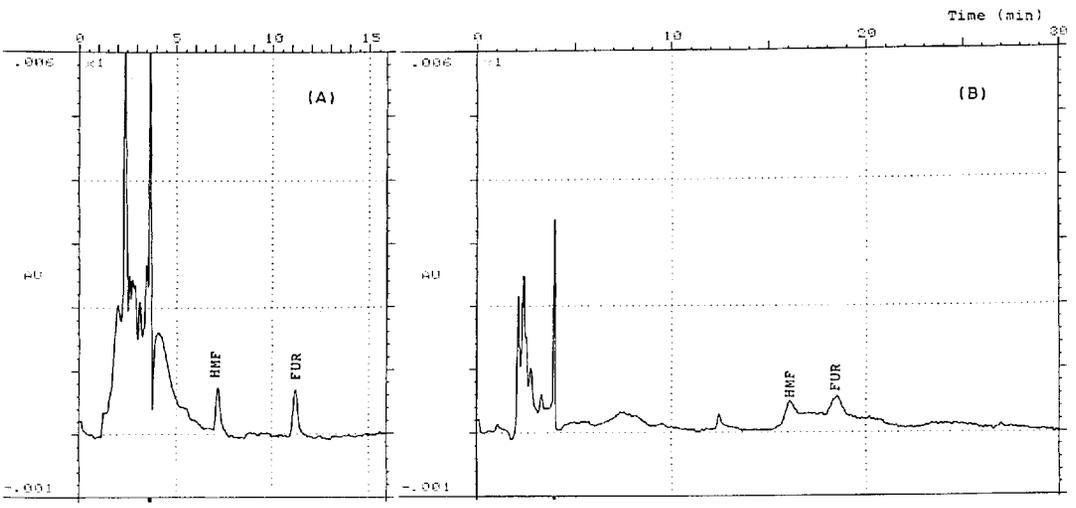

Figure 2

Typical chromatograms of hydrocymethylfurfural and furfural in cleaned-up juice concentrate. Column: Spherisorb ODS-2, 250 × 4.6 mm I.D., 3 µm. Flow rate: 1 ml/min. Temperature: 25 °C. Mobile phase: (A) Water Acetonitrile (92 : 8). (B) Water/Tetrahydrofuran (99.7 : 0.3).

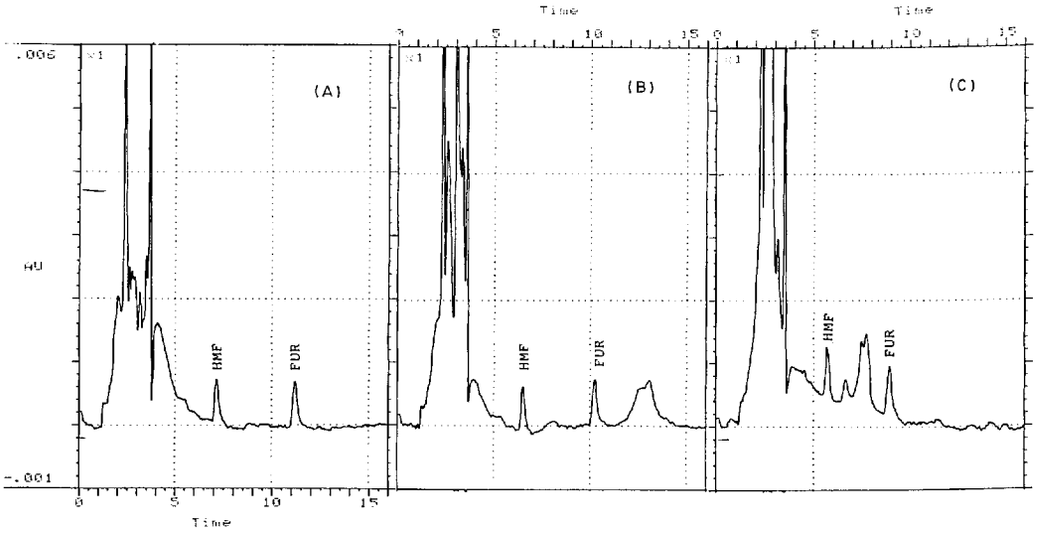

Figure 3

Effect of temperature on the retention of solutes. (A) = 25 °C; (B) = 40 °C; (C) = 60 °C. Other conditions as in Figure 2 (A).

Table I. Recovery studies of furfural (FUR) and hydroxymethylfurfural (HMF) added to pasteurized juice and juice concentrate

Sample	Compound	Amount in sample (ppb)	Amound added (ppb)	Found	% Recovery ± C.V.
Juice Concentrate	FUR	76	50	123	94.1 ± 2.6
			100	172	96.2 ± 1.8
			150	219	95.1 ± 1.8
	HMF	71	50	119	94.7 ± 3.0
			100	168	96.3 ± 2.9
			150	208	97.3 ± 2.3
Pasteurized Juice	FUR	2184	500	2655	94.2 ± 1.2
			1000	3153	96.9 ± 1.4
			1500	3681	99.9 ± 1.5
	HMF	3349	469	3792	94.5 ± 1.9
			938	4240	95.0 ± 1.4
			1407	4763	100.5 ± 1.8

Table II. FUR and HMF contents in juice concentrates (A, B) and in pasteurized apple juices (C, D)

Sample	FUR (ppb)	HMF (ppb)
A	76.0 ± 2.2	71.0 ± 3.2
B	17.9 ± 2.5	118.0 ± 1.5
C	85.0 ± 3.2	3976.0 ± 1.7
D	4368.0 ± 1.1	6698.0 ± 1.8

the method has an adequate degree of accuracy for the analysis of these solutes.

The coefficents of variation for the three replicates of each sample were generally less than 5 %. The limit of quantification for both compounds was 5 ppb.

The results obtained for furfural contents in two different juice concentrates (A, B) and two different pasteurized apple juices (C, D) are summarized in Table II. Samples C and D correspond to pasteurized apple juice analyzed at one month and one year after their manufacture.

Conclusions

HPLC is a rapid and convenient technique for simultaneous analysis of FUR and HMF in apple juice with minimum sample pretreatment. The proposed procedure is simple and sensitive enough for quality control of apple juice during processing and storage and would also contribute to browning mechanism studies.

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