

# Simultaneous Determination of 2-Furfuraldehyde and 5-(Hydroxymethyl)-2-furfuraldehyde by Derivative Spectrophotometry

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The reactions of 2-furfuraldehyde (FUR) and 5-(hydroxymethyl)-2-furfuraldehyde (HMF) with 2-thiobarbituric acid (TBA) have been investigated. These compounds react with TBA in an acidic medium, and the reaction is accelerated by heating at moderate temperature. The yellow reaction products show high absorption in the visible region. Chemical variables that affect the reactions have been optimized. The spectral overlapping of the reaction products of FUR and HMF with TBA has been resolved by first-derivative spectrophotometry. The simultaneous determination of FUR and HMF mixtures is accomplished by taking the first-derivative signal at 436 nm for FUR determination and at 414 nm for HMF determination, respectively. The method has been applied to a commercial orange juice and oral rehydration salt formulations.

## INTRODUCTION

The analytical control of furanic aldehydes is necessary in the food industry, because the presence of these compounds is very important in the evaluation of the quality of the processing method and on the organoleptic characteristics of the final product. Nonenzymic browning is considered one of the major causes of quality loss in citrus juices (Hodge, 1953). 5-(Hydroxymethyl)-2-furfuraldehyde (HMF) is the principal decomposition product of the acid-catalyzed hydrolysis of hexoses, and 2-furfuraldehyde (FUR) is the principal product of the hydrolysis of ascorbic acid, by means of the Strecker mechanism (Bonn and Bobleter, 1983).

Both HMF and FUR are useful indicators of temperature abuse in citrus juice. HMF has been correlated with browning in orange juice (Meydav and Berk, 1978), whereas FUR has been shown to increase as flavor deteriorates in commercially processed citrus juice (Dinsmore and Nagy, 1974). Many compounds have been reported to contribute to the heat-abused flavor which characterizes aged citrus juice, and a mathematical equation was developed to represent this relationship for HMF and FUR (Lee and Nagy, 1988). Model systems simulating grapefruit juice were employed to determine the relative reactivity of sucrose, glucose, and fructose in the formation of HMF, at pH 3.5, to investigate the role of this chemical in nonenzymic browning (Lee and Nagy, 1990). These compounds are similarly determined in the quality control of other food preparations. Hence, the content of HMF is determined in sweet wine (Malaga type) to detect the adulteration with unfermented grape juices, and the FUR content is determined in routine analysis of wines (Montilla Gómez et al., 1988). The total content of furanic aldehydes is similarly determined in honey samples and expressed as HMF content, because the high content of glucose and fructose makes HMF the main product formed when the honey is heated or incorrectly stored (Tharasoulou et al., 1986; Jeuring and Koppers, 1980; Salinas et al., 1991). Also, quality control specifications, for oral or injectable solutions containing glucose, normally include a quantitative limit test for HMF and related substances

to control the extent of decomposition (Davidson, 1987; Davidson and Dawodu, 1987, 1988).

Several techniques have been proposed for the analytical determination of these compounds. The spectrophotometric methods proposed have in common that they do not differentiate between HMF and FUR and the need of a previous separation procedure. Hence, Dinsmore and Nagy (1974) described a colorimetric method of determination of 2-furfuraldehyde in citrus juice on the basis of the reaction with aniline and a previous distillation procedure to avoid HMF interference. Alonso et al. (1983) automatized the aniline method, determining HMF in alcoholic beverages by flow injection analysis. Similarly, the described methods based on the Winkler reaction (Winkler, 1955) and the reaction with 2-thiobarbituric acid (TBA) (Meydav and Berk, 1978) allow the determination of the total content of furanic aldehydes.

A method proposed for the determination of both compounds in wines is based on the formation of the corresponding thiosemicarbazone (O'Haver and Green, 1976). A previous distillation step is proposed because of the high overlapping observed in the absorption spectra of both reaction products.

The simultaneous determination of furfural and HMF in mixtures has been proposed by high-performance liquid chromatography (Lee et al., 1986). However, a previous treatment to clarify and filter the samples is necessary.

Derivative spectrophotometry offers the advantage of a higher selectivity than conventional spectrophotometry, and frequently, overlapping spectra can be resolved by this technique. In addition, in many instances, a previous clarification of the samples is not necessary, because the absorption signal due to the turbidity is avoided by the derivative approach, which simplifies the methods. In a previous paper (Espinosa-Mansilla et al., 1992), such a technique has been applied to the simultaneous determination of mixtures of furfural and HMF in honey by using the Winkler method. In this paper, the mixture resolution has been accomplished by using the reaction with 2-thiobarbituric acid.

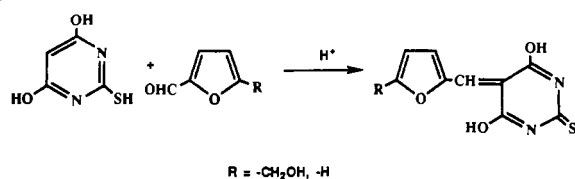
## EXPERIMENTAL PROCEDURES

**Apparatus.** A Beckman DU-50 spectrophotometer connected via RS-232 to an Olivetti PC-286 microcomputer has been used for all absorption measurements. The Beckman Data Leader software, version 3.0, was used for spectra acquisition, storage,

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## Scheme I



and manipulation and analysis of the spectrophotometric data. Differentiation was performed according to the simplified least-squares procedure of Savitzky and Golay (1964). A thermostatic bath, Selecta Unitronic 320 OR, was used for temperature control.

**Reagents.** All experiments were performed with analytical grade chemicals. Doubly distilled and demineralized water was used. 5-(Hydroxymethyl)furfuraldehyde standard solution was a 0.01% (w/v) solution prepared by dissolving 0.01 g of reagent (Sigma) in 100 mL of water. 2-Furfuraldehyde standard solution was a 0.01% (w/v) solution prepared by dissolving 0.01 g of reagent (Sigma) in 100 mL of water. 2-Thiobarbituric acid standard solution was a  $3 \times 10^{-2}$  M solution prepared by dissolving 0.4503 g of reagent (Sigma) in 100 mL of water and sonicating for 20 min.

**Procedures.** In a 25-mL volumetric flask was introduced an aliquot of the sample solution, containing between 12.5 and 100  $\mu$ g of HMF and between 12.5 and 100  $\mu$ g of FUR. Then 8.5 mL of concentrated hydrochloric acid and 7 mL of  $3 \times 10^{-2}$  M TBA solution were added. The samples were heated to 40 °C for 30 min, allowed to cool to 20 °C, and diluted to the mark with water. The absorption spectra were recorded between 300 and 550 nm against a solution containing the same amount of hydrochloric acid. The first-derivative spectra were calculated with a band width of 15 nm. The first-derivative analytical signal was taken as the vertical distance from the first-derivative absorption spectra at  $\lambda = 436$  nm to the base line for FUR and at  $\lambda = 414$  nm for HMF. Calibration graphs are constructed in the same way by using FUR and HMF solutions of known concentrations.

**Applications.** The method has been applied to the determination in orange juice. For that, the appropriate volume of orange juice was introduced in a 25-mL volumetric flask and the recommended procedure was followed except that the same amount of orange juice was used in acid medium, in the absence of TBA, as the background.

The method has been also applied to glucose-containing formulations of oral rehydration salts. For that, the appropriate volume of glucose formulation was introduced in a 25-mL volumetric flask and the recommended procedure was followed.

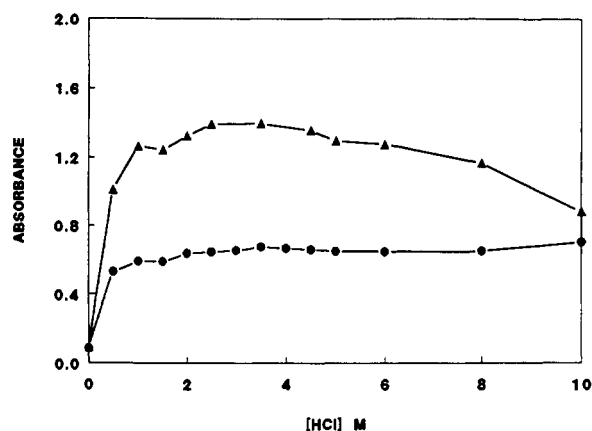
## RESULTS AND DISCUSSION

FUR and HMF react with 2-thiobarbituric acid in strong acid medium, giving rise to the corresponding derivatives according to the equilibrium shown in Scheme I.

The FUR and HMF derivatives are yellow products showing absorption spectra in the visible region with maxima at 414 and 436 nm for the FUR and HMF reaction products, respectively.

**Chemical Variables Optimization.** A study of the influence of hydrochloric acid on the reaction has been undertaken. The results are shown in Figure 1. The reaction with TBA increases as the hydrochloric acid concentration increases. The product of reaction with FUR shows maximum and constant absorption for  $[\text{HCl}] > 3$  M, decreasing slightly for  $[\text{HCl}] > 6$  M. That of HMF shows a constant and maximum absorbance for  $[\text{HCl}] > 3$  M. A  $[\text{HCl}] = 4$  M has been selected as the optimum for a proper development of both reactions.

The influence of TBA concentration on the reaction was investigated. The absorbance of the solutions is maximum and constant for TBA concentrations above  $2 \times 10^{-3}$  M. A  $[\text{TBA}] = 8.4 \times 10^{-3}$  M has been selected as optimum to ensure a sufficient reagent excess for the mixture determination. It has been tested that TBA does



**Figure 1.** Influence of hydrochloric acid concentration on the reaction.  $[\text{TBA}] = 3.6 \times 10^{-3}$  M,  $T^{\circ} = 40$  °C, heating time = 30 min. (▲)  $[\text{FUR}] = 4 \mu\text{g mL}^{-1}$ ,  $\lambda = 414$  nm; (●)  $[\text{HMF}] = 2 \mu\text{g mL}^{-1}$ ,  $\lambda = 436$  nm.

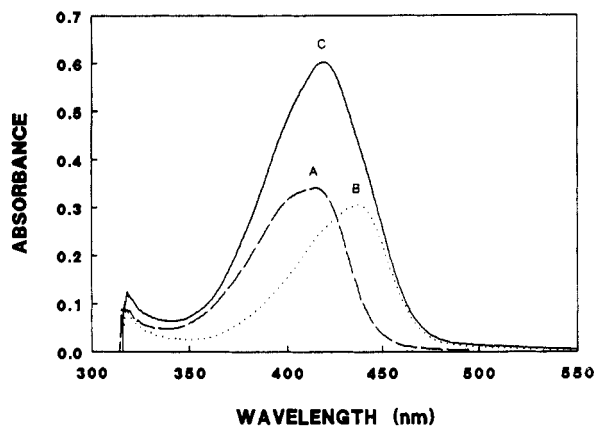
not absorb significantly in the wavelength interval of absorption of FUR and HMF derivatives, and consequently, the determination can be performed using the aqueous acidic medium as the background.

Previous experiments show that the development of the reaction was slow at room temperature. With the aim of accelerating the reaction, the effect of temperature was examined. The temperature was first varied while a constant heating time of 15 min was maintained. The samples were then allowed to cool to 20 °C, and the absorbance was measured. In the case of FUR, there is not a perceptible acceleration of the reaction with temperature, but a decrease of the absorbance is observed for temperatures higher than 50 °C. In the case of HMF, the absorbance slightly increases with temperature up to 40 °C; increasing above 40 °C does not affect the reaction. At a temperature of 40 °C chosen as the optimum, the influence of the heating time was studied. The reaction between FUR and TBA increases as the reaction time increases, reaching a constant level for periods of heating greater than 20 min. The HMF derivative shows first a small decrease in the absorbance when the heating time is increased from 5 to 10 min, and then a constant level is reached. A heating time of 30 min was chosen as optimum for the development of the reaction. In these conditions the solutions are stable for at least 8 h.

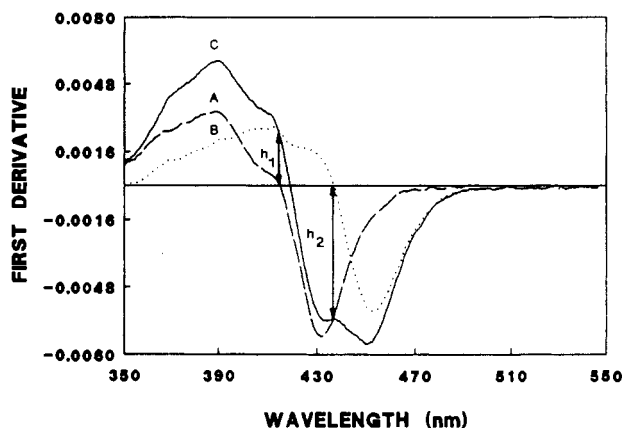
**Spectral Characteristics.** In the optimum conditions selected, the FUR derivative shows an absorption maximum at 414 nm and the HMF derivative at 436 nm. The spectral bands are broad and highly overlapped, which precludes the possibility of simultaneous determination of these compounds by conventional spectrophotometry. In Figure 2 the absorption spectra of FUR and HMF in the presence of TBA and of a mixture of both compounds are shown.

**First-Derivative Spectra.** Figure 3 shows the first derivative of the absorption spectra of the products formed by FUR and HMF with TBA and of a mixture of both. It is observed that, because of the closeness of the two overlapping spectra, they are not sufficiently well resolved to generate two distinct peaks in the first-derivative spectra of the mixture of both compounds.

The technique used to choose suitable wavelengths at which to take measurements, which are proportional to the FUR and HMF concentrations, for the preparation of the calibration graphs was the "zero-crossing measurement" technique. This technique involves the measurement of the absolute value of the total derivative value at an abscissa value (wavelength) corresponding to the zero-



**Figure 2.** Absorption spectra of (A) FUR ( $0.5 \mu\text{g mL}^{-1}$ ), (B) HMF ( $0.5 \mu\text{g mL}^{-1}$ ), and (C) a mixture of both.  $[\text{HCl}] = 4 \text{ M}$ ,  $[\text{TBA}] = 8.4 \times 10^{-3} \text{ M}$ ,  $T^{\text{a}} = 40 \text{ }^{\circ}\text{C}$ , heating time = 30 min.

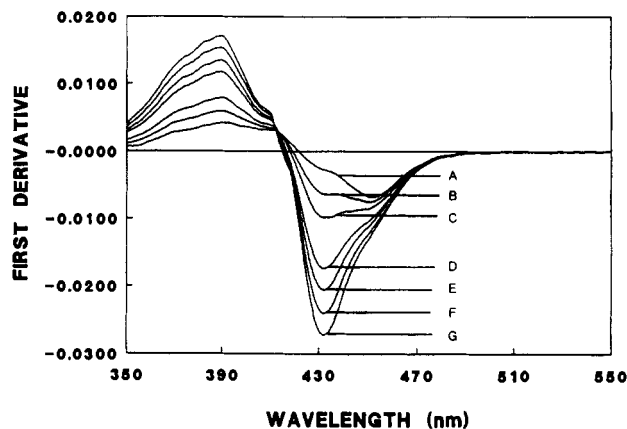


**Figure 3.** First-derivative spectra of the products of reaction of TBA with (A) FUR, (B) HMF, and (C) a mixture of both. Experimental conditions were the same as in Figure 2.

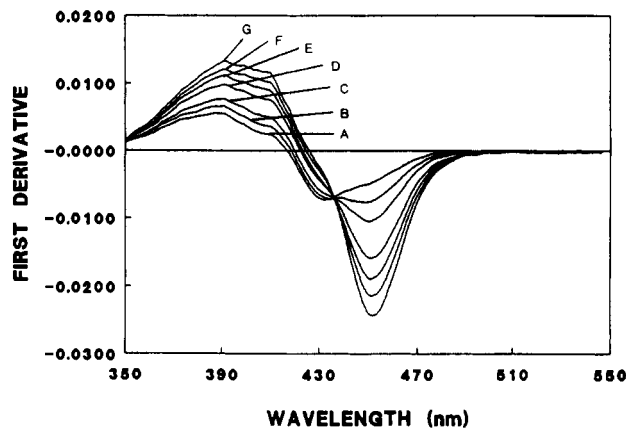
crossing of the spectrum of the interfering component. Hence, the heights  $h_1$  and  $h_2$  in the first-derivative spectrum of the mixture (Figure 3), corresponding to the values taken at the wavelengths 436 and 414 nm, are proportional to FUR and HMF concentrations, respectively.

**Determination of Mixtures of FUR and HMF.** Two calibration graphs were obtained from height ( $h$ ) measurements, for standards containing between 0.5 and 4.0  $\mu\text{g mL}^{-1}$  of FUR, in the absence of HMF and in the presence of 1  $\mu\text{g mL}^{-1}$  of HMF, respectively. The first-derivative spectra obtained in the presence of 1  $\mu\text{g mL}^{-1}$  of HMF are shown in Figure 4. A similar experiment was realized for standards containing between 0.5 and 4.0  $\mu\text{g mL}^{-1}$  of HMF, in the absence and in the presence of 1  $\mu\text{g mL}^{-1}$  of FUR, respectively. The first-derivative spectra obtained in the presence of 1  $\mu\text{g mL}^{-1}$  of FUR are shown in Figure 5.

Calibration graphs were obtained for FUR and HMF by taking the first-derivative signal at  ${}^1D_{436}$  (zero-crossing point for HMF) and at  ${}^1D_{414}$  (zero-crossing point for FUR). In the Table I, the statistical data of the calibration lines obtained are summarized. From the results presented in Table I, it can be deduced that the amplitude of the derivative signal of the mixture, made at the zero-crossing of the derivative spectrum of one of the two components, is a function only of the concentration of the other component, in accordance with the theoretical predictions. Also, the high values of correlation coefficients and the intercepts on the axis (close to zero) indicate the good linearity of all of the calibration graphs of the first-derivative measurements. To test the accuracy and



**Figure 4.** First-derivative spectra calibration of FUR: (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.5, (E) 3.0, (F) 3.5, and (G) 4.0  $\mu\text{g mL}^{-1}$ , in the presence of 1  $\mu\text{g mL}^{-1}$  HMF.



**Figure 5.** First-derivative spectra calibration of HMF: (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.5, (E) 3.0, (F) 3.5, and (G) 4.0  $\mu\text{g mL}^{-1}$ , in the presence of 1  $\mu\text{g mL}^{-1}$  FUR.

**Table I.** Statistical Analysis for Calibration of FUR and HMF by First-Derivative Spectrophotometry

compd	condition	eq <sup>a</sup>	corr coeff
HMF	in the absence of FUR	${}^1D_{414} = 0.0002 + 0.0025C$	0.9997
	in the presence of 1 $\mu\text{g mL}^{-1}$ FUR	${}^1D_{414} = 0.0002 + 0.0022C$	0.9990
furfural	in the absence of HMF	${}^1D_{436} = 0.0002 + 0.0058C$	0.9990
	in the presence of 1 $\mu\text{g mL}^{-1}$ HMF	${}^1D_{436} = 0.0002 + 0.0061C$	0.9997

<sup>a</sup>  $C$  = compound concentration ( $\mu\text{g mL}^{-1}$ ).

precision of the method, 11 successive determinations of a sample solution containing 2  $\mu\text{g mL}^{-1}$  of FUR and HMF were carried out. Relative standard deviations of 0.0149 and 0.0054  $\mu\text{g mL}^{-1}$  and relative errors of 1.56 and 0.60% were obtained for FUR and HMF, respectively (95% confidence). In Table II, the results of the determination of mixtures of both compounds in different ratios are summarized. It can be seen that satisfactory results were obtained with mean recoveries of 99.4 and 110% for FUR and HMF, respectively.

Interaction graphs were plotted to give an indication of the concentration range of furfural that would not interfere with HMF at 414 nm and the concentration range of HMF that would not interfere with FUR at 436 nm. From the results obtained, it is concluded that HMF concentrations of up to about 2.5  $\mu\text{g mL}^{-1}$  do not interfere in the determination of FUR and that FUR concentrations of up to 2.4  $\mu\text{g mL}^{-1}$  do not interfere in the determination of HMF.

**Applications.** The present method has been applied to the simultaneous determination of FUR and HMF in

**Table II. Determination of FUR and HMF in Binary Mixtures**

FUR/HMF ratio	FUR, $\mu\text{g mL}^{-1}$			HMF, $\mu\text{g mL}^{-1}$		
	taken	found	recovery, %	taken	found	recovery, %
2:1	1.00	1.02	102	0.50	0.53	105
5:2	2.50	2.47	99	1.00	1.08	108
2:1	2.00	1.99	99	1.00	1.16	116
3:1	3.00	2.96	99	1.00	1.10	110
1:1	1.00	1.02	102	1.00	1.06	106
1:1	1.00	0.98	98	1.00	1.16	116
2:3	1.00	1.02	102	1.50	1.62	108
1:2	0.50	0.52	104	1.00	1.15	115
3:2	1.50	1.53	102	1.00	1.15	115
1:2	1.00	1.02	102	2.00	2.20	110
2:5	1.00	0.98	98	2.50	2.68	107
1:3	1.00	0.86	86	3.00	3.14	105

**Table III. Results of the Determination of FUR and HMF in Orange Juice**

FUR, $\mu\text{g mL}^{-1}$			HMF, $\mu\text{g mL}^{-1}$		
added	found	recovery, %	added	found	recovery, %
0.00	2.10		0.00	1.45	
2.50	4.55	97	2.50	3.44	82
5.00	7.00	97	5.00	6.52	103
7.50	9.03	92	7.50	9.23	105
10.00	11.76	96	10.00	11.96	105

**Table IV. Results of the Determination of FUR and HMF in Oral Rehydration Salt Preparation**

FUR, $\mu\text{g mL}^{-1}$			HMF, $\mu\text{g mL}^{-1}$		
added	found	recovery, %	added	found	recovery, %
0.00	0.17		0.00	5.43	
6.25	6.30	98	6.25	12.68	110
12.50	12.57	99	12.50	19.47	109
20.83	19.93	95	20.83	25.81	96
25.00	25.03	100	25.00	32.15	105

an orange juice. For that, 5 mL of the orange juice was introduced in a 25-mL volumetric flask and the proposed procedure was followed. To obviate the colored background of the orange juice, the samples were measured against a blank containing the same amount of the juice, in acid medium, in the absence of TBA. In Table III, the results obtained when the standard addition method was applied have been summarized. FUR and HMF concentrations of 2.13 and 1.38  $\mu\text{g mL}^{-1}$  were found, respectively.

The proposed method has been also applied in a pharmaceutical syrup. For that, 2 mL of the glucose formulation was introduced in a 25-mL volumetric flask and the proposed procedure was followed. In this case, the original sample is clear and uncolored. In Table IV, the results obtained when the addition standard method was applied are summarized. FUR and HMF concentrations of 0.14 and 5.80  $\mu\text{g mL}^{-1}$  were found, respectively.

**Conclusions.** The simultaneous determination of FUR and HMF in binary mixtures has been accomplished by combination of spectrophotometry and spectral differentiation. The proposed method is based in the reaction of these furanic aldehydes with 2-thiobarbituric acid. The reaction compares advantageously with the classical method based on Winkler's reaction as the reagents are not toxic and the reaction products are stable. The routine analysis of these compounds in quality control of food and/or pharmaceutical preparations, without previous separation procedures, offers the advantage of speed and simplicity.

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