

# Determination of Free and Total Furfural Compounds in Infant Milk Formulas by High-Performance Liquid Chromatography

Soledad Albalá-Hurtado, M. Teresa Veciana-Nogués, María Izquierdo-Pulido, and M. Carmen Vidal-Carou\*

Unitat de Nutrició i Bromatologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain

HPLC methods are described for the determination of free and total furfurals in liquid and powdered infant milk formulas. (Hydroxymethyl)furfural, furfural, furyl methyl ketone, and methylfurfural were determined simultaneously in one run. Identical HPLC conditions were used to measure free and total furfural compounds, although there were slight differences in sample preparation. The chromatographic technique involved the separation of the furfural compounds in a C<sub>18</sub> column using an isocratic mobile phase (water–acetonitrile) and a variable-wavelength absorbance detector. Linearity, precision, recovery, and sensitivity were always satisfactory. Detection limits ranged from 0.01 to 0.05 µg/mL, and determination limits ranged from 0.02 to 0.06 µg/mL of liquid infant milk or 15% (P/V) reconstituted powdered infant milk. In addition, we report the levels of furfural compounds in 12 commercial infant milk formulas (powdered and liquid) produced in Spain. (Hydroxymethyl)furfural and furfural were higher in infant milk formulas than in powdered cow milks. Levels of total furfural compounds were the same in powdered and liquid infant milk formulas. However, levels of free furfurals were higher in powdered than in liquid infant milks.

**Keywords:** *Furfurals; infant milk; (hydroxymethyl)furfural; Maillard reaction*

## INTRODUCTION

The browning reaction (Maillard reaction) is one of the main alterations observed during heat processing or storage of foodstuffs rich in reducing sugars, particularly at improper temperatures. The reactions between sugars and amino acids have been studied extensively since 1953, when Hodge proposed the first coherent scheme describing the Maillard reaction, up until the most recent symposiums where chemical, physiological, and technological aspects of the Maillard reactions have been discussed (Boekel and Berg, 1993; O'Brien and Labuza, 1994).

The Maillard reaction occurs slowly in dry food systems at low temperatures and is relatively slow in high-moisture foods (Labuza and Saltmarch, 1981). However, in systems showing intermediate moisture content and at temperatures above 35 °C, the Maillard reaction can be the major reaction, causing changes in color (melanoidines), flavor (aldehydes and ketones), functional properties, and nutritional value (blocking or destruction of lysine). The organoleptical changes caused by the Maillard reaction are undesirable in dairy products, except in some confectionery products, and should be minimized by regular processing controls (Caric et al., 1984). Controlling the reduction in the nutritional value of products which have undergone the Maillard reaction (due to decreases in the availability of lysine bonded to lactose) is of particular interest in infant milk formulas because these products may be the only food which the babies receive.

Numerous furfural compounds are formed in processed food (e.g. sterilized or UHT milk, dry foods) during thermal processing or storage at inappropriate temperatures. Therefore, these compounds are useful

indicators for assessing the extent of the Maillard reaction (Guerra-Hernández et al., 1992; Lo Coco et al., 1994). (Hydroxymethyl)furfural (HMF) is the main furfural compound and has been correlated with browning reactions in different foods (Boekel and Berg, 1993; Morales-Navas et al., 1994). The relationships between flavor changes and contents of furfural (F) (Dinsmore and Nagy, 1974; Mijares et al., 1986), furyl methyl ketone (FMC), and methylfurfural (MF) (Nemr et al., 1988; Zhang and Ho, 1990) have also been reported.

HMF and F have been widely used as indicators of the Maillard reaction in numerous foodstuffs, such as juices (Porreta and Sandei, 1991; Lo Coco et al., 1994), honey (Jeuring and Kupperts, 1980; Viñas et al., 1992), spirits (Lo Coco et al., 1992; Villalón et al., 1992), baby cereals (Guerra-Hernández et al., 1992), and breakfast cereals (García-Vilanova et al., 1993). The browning reaction is one of the main alterations observed in heat-treated milk due to the high levels of proteins and lactose (Boekel and Rehman, 1987; Fernandes and Lellan, 1992; Morales-Navas et al., 1994).

The composition of powdered or liquid infant milk formulas and the heat treatments (drying and sterilization) involved in their manufacture make these products susceptible to the Maillard reaction. The intensity of changes in dairy products caused by the Maillard reaction are dependent upon the severity of the temperature/time ratio during the processing. Furfural compounds from Maillard reaction can also be formed during storage of dairy products which have undergone thermal treatment. The main factors which determine the intensity of changes by the Maillard reaction during storage are water activity and the duration and temperature of storage (Caric et al., 1984). Therefore, furfural compounds could be indicators of food damage during manufacturing or storage.

In contrast to other foods, no data are available about the typical contents of furfural compounds in infant milk formulas, nor about the factors which can favor their

\* To whom correspondence should be addressed (telephone, 34-3-402 45 13; fax, 34-3-402 18 96; e-mail, veciana@farmacia.far.ub.es).

accumulation during processing. Caric et al. (1984) reported that cow milks enriched with lactose, iron, and vitamin A had higher contents of HMF than nonenriched milk. Moreover, the formation of HMF during storage also increased in enriched milk. Infant milk formulas are usually enriched with lactose, vitamin A, and iron. Therefore, these products may contain high levels of furfural compounds.

The majority of methods used to determine HMF and F in foods are based on the method of Kenney and Bassette (1959), who used a colorimetric reaction with thiobarbituric acid (TBA) to measure either free HMF or total HMF (free HMF plus the potential HMF derived from other browning intermediates) in whole milk powder. After some modifications, this procedure can also be applied to liquid milk samples (Craig et al., 1961). However strict control of time and temperature reaction is required because of the instability of the reaction product, which gives high variability of results (Kahlhofer, 1982; Mijares et al., 1986). Moreover TBA is not specific for HMF (Lo Coco et al., 1992; Diaz and Clotet, 1993) and may also react with other carbonyl compounds produced in the Maillard reaction. For this reason, Fink and Kessler (1986) indicated that it is more appropriate to report HMF values than HMF concentration. To prevent interference problems and to increase the speediness of analysis, high-performance liquid chromatographic procedures (HPLC) have been developed for the determination of HMF in milk (Boekel and Rehman, 1987; Morales-Navas et al., 1994). However, no references are available for the simultaneous determination of HMF, F, FMC, and MF in foods, and no methods have been described for the determination of HMF and other furfurals in infant milk formulas.

The aim of the present study was to develop simple and rapid HPLC methods which would allow the determination of free or total HMF, F, FMC, and MF in a single run in both powdered and liquid infant milk, which has recently been introduced in the European market. Data are provided in support of method reliability in terms of accuracy, reproducibility, and linearity. In addition, a preliminary study was carried out on the levels of furfural compounds in Spanish commercial infant milk formulas, and comparisons were made with levels found in commercial powdered cow milks.

## MATERIALS AND METHODS

**Reagents and Standards.** Acetonitrile was of HPLC grade (SDS, Peypin, France). Other chemicals were of reagent grade. Oxalic acid dihydrate was obtained from Fluka (Buchs, Switzerland) and trichloroacetic acid (TCA) from Panreac (Montplet & Esteban S.A., Barcelona, Spain). Double-distilled water was obtained from the Milli-Q System (Millipore Corp., Bedford, MA).

Standards of 5-(hydroxymethyl)-2-furaldehyde (HMF), 2-furaldehyde (F), 2-furyl methyl ketone (FMC), and 5-methyl-2-furaldehyde (MF) were purchased from Fluka. The stock solution was 500 mg/L of each furfural compound in double-distilled water. Intermediate solutions were 5 mg/L for HMF and 1 mg/L for F, FMC, and MF. Working solutions were 0.05, 0.1, 0.2, 0.5, 1, 1.5, 2.0, and 5 mg/L for HMF and 0.05, 0.1, 0.2, 0.5, 0.8, and 1 mg/L for F, FMC, and MF. The concentration of HMF in the standard solutions was higher than those of the other furfurals because HMF is usually the main furfural compound in dairy products. All solutions were filtered through a 0.45  $\mu\text{m}$  filter (Millipore Corp., Bedford, MA), protected from light, and stored at 4 °C no longer than 15 days.

**Samples.** (a) A powdered and a liquid infant milk formula which were stored for 6 months at 37 °C were used to study

methods reliability. (b) Twelve Spanish commercial infant milk formulas (six powdered and six liquid) and 10 commercial powdered cow milks were assessed to find their furfural levels. All commercial samples were analyzed within their shelf-life.

**HPLC Analysis.** The HPLC system used (Hewlett-Packard, Pennsylvania, PA) consisted of an HP 1050 system controller pump, an HP 1050 Series degassing device, an HP 1100 autosampler with a 20  $\mu\text{L}$  fixed loop injector, and an HP 1050 Series UV detector. Data acquisition was accomplished by a Chemstation system HP 3365-II. The separation was performed on a Tracer Spherisorb ODS 2  $\text{C}_{18}$  column 4.6  $\times$  250 mm, 5  $\mu\text{m}$  particle size (Teknokroma, Barcelona, Spain), with a matching guard cartridge.

**Chromatographic Conditions.** Analysis was carried out isocratically at room temperature using as the mobile phase a mixture of acetonitrile–water (5:95) (v/v) at a flow rate of 1 mL/min. The variable-wavelength detector was set as follows: time = 0 min at 284 nm; time = 10 min at 277 nm; time = 15 min at 274 nm; and time = 23 min at 293 nm. The total run time required was 30 min.

**Sample Preparation.** The sample preparation was based on the Boekel and Rehman (1987) procedure. (1) *Total furfurals.* Mix 15 g of infant milk (liquid or 15% of reconstituted powder) with 5 mL of 0.3 N oxalic acid (prepared fresh daily) in a sealed tube to prevent evaporation. Heat the tube in a water bath at 100 °C for exactly 25 min. After cooling at room temperature, add 3 mL of a 40% (w/v) TCA solution and a magnetic stirring bar. Mix thoroughly for 5 min. over a magnetic stirring plate. Centrifuge 15 min. at 3000 rpm and separate the two phases. Add 10 mL of 4% (w/v) TCA to solid residue obtained, mix thoroughly for 10 min, and repeat centrifugation. Discard solid phase. Combine the two TCA extracts in a 25 mL volumetric flask, and fill to volume with 4% TCA. Filter extracts through a 0.45  $\mu\text{m}$  filter before the LC analysis. (2) *Free furfurals.* The sample preparation is the same as for total furfurals, but the heating at 100 °C for 25 min is omitted.

**Calculation.** The content of furfurals in samples of milk was obtained directly by interpolation from calibration curves. The concentration of each furfural compound (FC) expressed in  $\mu\text{g}/100$  mL of milk was calculated from the following formula:

$$[\text{FC}] = C(V/W)\delta \times 100$$

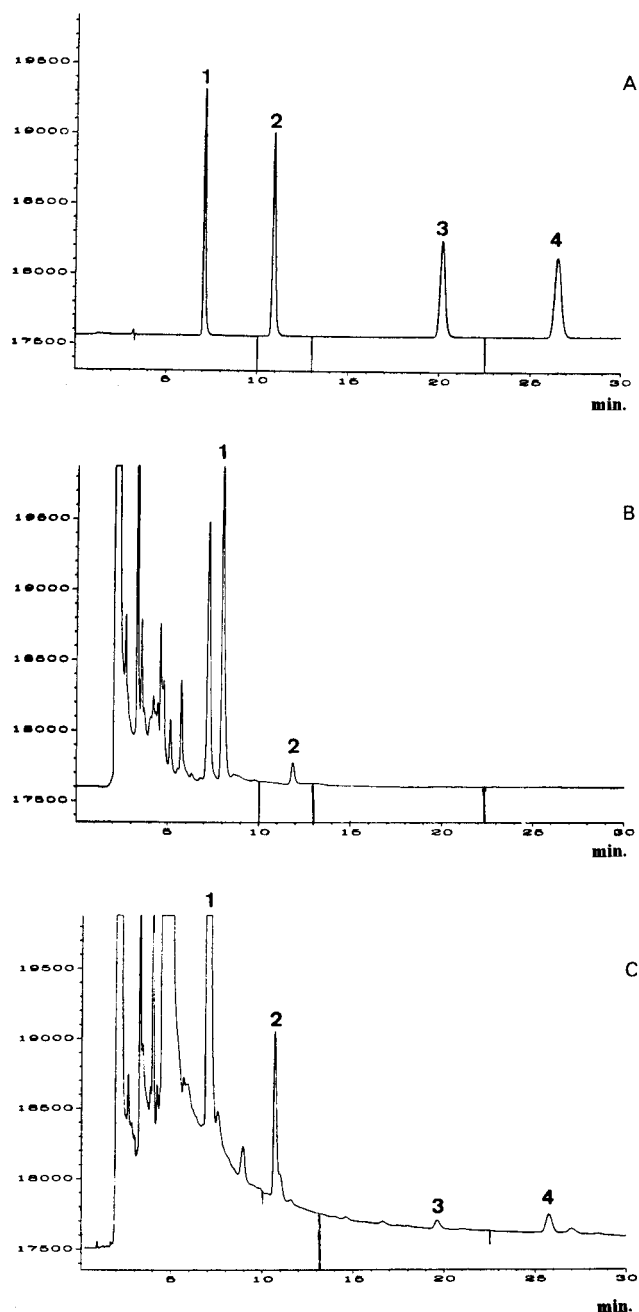
where  $C$  = concentration from the calibration curve ( $\mu\text{g}/\text{mL}$ ),  $V$  = final volume (25 mL),  $W$  = weight of milk (g), and  $\delta$  = density of milk (g/mL).

**Statistical Analysis.** A nonparametric test was applied since the data were not normally distributed. The Mann–Whitney U–Wilcoxon's test was performed using the Statistical Software Package for Windows 6.0.1 (SPSS Inc., Chicago, IL, 1994).

## RESULTS AND DISCUSSION

HMF, F, FMC, and MF were chosen as analytes, which enables us to understand the extension of heat treatment (Stefano, 1988; Nemr et al., 1988; Zhang and Ho, 1990). HMF and F are the main furfural compounds used to evaluate nonenzymatic browning in foods. However, no previous data are available on the levels of furfural compounds in infant milk formulas. Preliminary assays performed in our laboratory showed that FMC and MF along with HMF and F were present when infant milk was heated to high temperatures (Figure 1). Moreover, when other products such as honey, beer, or brandy were analyzed using the HPLC procedure proposed in this work, we also noticed the presence of F and FMC.

The heating conditions usually employed to determine HMF in foods not only release HMF but also induce its formation (Boekel and Rehman, 1987). The conditions of hydrolysis used in this study prevent the formation



**Figure 1.** Chromatograms of furfural compounds: (A) standard solution of 1  $\mu\text{g/mL}$ , (B) chromatogram of total furfurals of a typical powdered infant milk formula, and (C) commercial powdered infant milk formula heated at 100  $^{\circ}\text{C}$  for 3 h. Peak identities: HMF (1), F (2), FMC (3), and MF (4).

of HMF and remove the need to perform an autocalibration with raw milk (unheated), a procedure used in the most of the methods described previously. We have checked that the method proposed for total furfurals determination yields zero values for furfural compounds in raw milk. Therefore, the content of furfurals in samples depends on the chemical effect of heat treatment during processing and/or changes due to storage conditions.

When spectrophotometric detection is carried out, or when small amounts of furfurals are present, heating during the hydrolysis step is necessary to obtain detectable amounts of furfural compounds. However, if the sensitivity of the analytical technique is increased, as in the HPLC methods, it may be possible to quantify only the furfurals present in samples just as they are

**Table 1. Precision of Method for Determination of Free and Total Furfural Compounds**

compd	liquid infant milk <sup>a</sup>		reconstituted powdered infant milk <sup>a</sup>	
	free furfurals	total furfurals	free furfurals	total furfurals
HMF	47.1 $\pm$ 0.9 <sup>c</sup>	158.9 $\pm$ 1.6	241.5 $\pm$ 1.9	351.4 $\pm$ 3.9
	1.8 <sup>d</sup>	1.6	0.8	1.1
F	8.4 $\pm$ 0.2	22.7 $\pm$ 0.5	7.5 $\pm$ 0.2	19.1 $\pm$ 1.0
	2.5	2.3	2.9	5.4
FMC <sup>b</sup>	5.3 $\pm$ 0.1	5.3 $\pm$ 0.1	5.2 $\pm$ 0.1	5.3 $\pm$ 0.1
	2.6	2.3	2.5	2.2
MF <sup>b</sup>	5.9 $\pm$ 0.1	5.9 $\pm$ 0.1	5.9 $\pm$ 0.1	5.9 $\pm$ 0.1
	2.1	2.1	2.3	2.1

<sup>a</sup> Commercial infant milk formulas stored for 6 months at 37  $^{\circ}\text{C}$ . <sup>b</sup> After addition of 1  $\mu\text{g}$  of both FMC and MF to the samples. <sup>c</sup> Mean  $\pm$  standard deviation in  $\mu\text{g}/100\text{ mL}$ . <sup>d</sup> Relative standard deviation (%).

(free furfurals). There is no literature on free furfurals in foods, with the exception of those of Boekel and Rehman (1987) in heated cow milk. The usefulness of free furfurals as indicators of the degree of heating and/or the time and conditions of food storage should be studied.

Scan analysis of standard furfurals indicated that their detection was optimum at 284 nm for HMF, at 277 nm for F, at 274 nm for FMC, and at 293 nm for MF. In general, a compromise wavelength of about 280 nm has been used for the simultaneous detection of furfural compounds, although a slight increase in sensitivity (5–10%) was observed when each analyte was detected at its optimal wavelength absorption. In addition, no disrupting of the baseline was observed in the chromatographic conditions reported (Figure 1). Several kinds of mobile phases previously described for only HMF determination were examined (Boekel and Rehman, 1987; Garcia-Vilanova et al., 1993; Morales-Navas et al., 1994). Acetic/acetate buffer at different concentrations (from 0.04 to 0.08 mol/L) and at different pHs (from 3.6 to 5.0) and methanol–water at different ratios (80:20, 85:15, 90:10) were assayed; however, in all cases, low chromatographic resolution was observed since HMF eluted within the matrix. Assays with water–acetonitrile gave the best results, and the proportion 95:5 water–acetonitrile provided the best resolution (Figure 1). Chromatograms were relatively simple, and furfural compounds were well identified on the basis of retention time by comparison with standard solutions. Relative standard deviations (RSDs) of retention times ranged from 4.0 to 6.5%. Trials were carried out to test the reliability of this method for linearity, precision, recovery, and sensitivity.

**Linearity.** Calibration curves were obtained by using working standard solutions of HMF, F, FMC, and MF from 0.1 to 10  $\mu\text{g/mL}$ , and linearity was verified in this range by analysis of variance of the regression. Coefficients of regression of  $>0.9998$  were obtained for all furfurals ( $p < 0.001$ ). Coefficients of determination ( $r^2$ ) were greater than 99.90% for all standard curves.

**Precision.** Seven determinations were carried out on the two types of samples used for methods validation by using the same reagents and apparatus. FMC and MF (1  $\mu\text{g}$ ) were added to each sample. The RSDs values for HMF, F, FMC, and MF were always satisfactory, according to the Horwitz's formula for intralaboratory studies (Horwitz, 1982). Table 1 shows precision of methods for determination of total and free furfural compounds.

**Table 2. Recovery of Method for Determination of Free and Total Furfural Compounds in Liquid Infant Milk<sup>a</sup>**

compd	initial content, μg/100 mL	content after addition, mg/ 100 mL		Cochran's test <i>G</i> <sub>exp</sub> <sup>d</sup>	recovery, %		
		level I <sup>b</sup>	level II <sup>c</sup>		X ± SD	SDR	
Free Furfurals							
HMF	47.1	66.3 ± 0.8	79.7 ± 0.8	0.608	97.7 ± 1.1	1.1	
F	8.4	14.5 ± 0.3	21.3 ± 0.5	0.724	95.9 ± 2.2	2.3	
FMC	nd <sup>e</sup>	5.3 ± 0.1	10.6 ± 0.2	0.677	76.5 ± 2.0	2.6	
MF	nd	5.6 ± 0.1	11.9 ± 0.2	0.708	86.0 ± 2.3	2.7	
Total Furfurals							
HMF	158.9	222.3 ± 2.8	291.3 ± 2.6	0.549	97.8 ± 0.8	0.8	
F	22.7	28.6 ± 1.1	35.1 ± 1.2	0.542	96.4 ± 3.3	3.3	
FMC	nd	5.2 ± 0.4	10.3 ± 0.4	0.580	75.2 ± 2.0	2.6	
MF	nd	5.9 ± 0.2	11.8 ± 0.3	0.744	85.7 ± 2.2	2.6	

<sup>a</sup> Commercial liquid infant milk stored for 6 months at 37 °C. <sup>b</sup> Free furfurals: 3 μg for HMF; 1 μg for F, FMC, and MF. Total furfurals: 10 μg for HMF; 1 μg for F, FMC, and MF. <sup>c</sup> Free furfurals: 5 μg for HMF; 2 μg for F, FMC, and MF. Total furfurals: 20 μg for HMF; 2 μg for F, FMC, and MF. <sup>d</sup> Cochran's test: *G*<sub>tab</sub> = 0.8332; DF = 2,7; *p* = 0.05. <sup>e</sup> nd = not detected.

**Table 3. Recovery of Method for Determination of Free and Total Furfural Compounds in Reconstituted Powdered Infant Milk<sup>a</sup>**

compd	initial content, μg/ 100 mL	content after addition, mg/100 mL		Cochran's test <i>G</i> <sub>exp</sub> <sup>d</sup>	recovery, %		
		level I <sup>b</sup>	level II <sup>c</sup>		X ± SD	SDR	
Free Furfurals							
HMF	241.5	337.3 ± 2.6	437.2 ± 2.5	0.617	97.5 ± 0.6	0.7	
F	7.5	13.8 ± 0.6	20.4 ± 0.6	0.546	95.7 ± 3.5	3.6	
FMC	nd <sup>e</sup>	5.3 ± 0.1	10.5 ± 0.1	0.675	76.2 ± 0.8	1.1	
MF	nd	5.9 ± 0.1	11.8 ± 0.2	0.800	85.8 ± 1.7	1.9	
Total Furfurals							
HMF	351.4	510.7 ± 4.7	681.2 ± 7.1	0.691	97.5 ± 0.8	0.8	
F	19.1	24.9 ± 1.3	51.4 ± 1.4	0.561	95.8 ± 3.7	3.8	
FMC	nd	5.2 ± 0.3	10.4 ± 0.3	0.507	74.9 ± 1.9	2.5	
MF	nd	5.9 ± 0.1	11.8 ± 0.2	0.734	85.5 ± 2.4	2.8	

<sup>a</sup> Commercial powdered infant milk stored for 6 months at 37 °C. <sup>b</sup> Free furfurals: 15 μg for HMF; 1 μg for F, FMC, and MF. Total furfurals: 25 μg for HMF; 1 μg for F, FMC, and MF. <sup>c</sup> Free furfurals: 30 μg for HMF; 2 μg for F, FMC, and MF. Total furfurals: 50 μg for HMF; 5 μg for F; 2 μg for FMC and MF. <sup>d</sup> Cochran's test: *G*<sub>tab</sub> = 0.8332; DF = 2,7; *p* = 0.05. <sup>e</sup> nd = not detected.

**Table 4. Methods Recovery and Contents of Free and Total Furfurals in Commercial Infant Milk Formulas and Cow Milk**

compd	liquid infant milk ( <i>n</i> = 6)		powdered infant milk ( <i>n</i> = 6)		cow milk ( <i>n</i> = 10)	
	contents (μg/100 mL)	recovery, %	contents (μg/100 mL)	recovery, %	contents (μg/100 mL)	recovery, %
Free Furfurals						
HMF	15.8 ± 11.8 <sup>a</sup> (0–34.5) <sup>b</sup>	97.8 ± 0.3 <sup>a</sup>	60.3 ± 48.2 (15.5–129.8)	97.9 ± 0.3	5.1 ± 1.9 (2.5–8.7)	97.9 ± 0.2
F	0.6 ± 1.6 (0–2.5)	96.0 ± 0.4	4.8 ± 3.2 (1.7–10.6)	96.0 ± 0.4	nd <sup>c</sup>	96.6 ± 0.8
Total Furfurals						
HMF	124.5 ± 49.4 (38.3–175.2)	97.8 ± 0.2	111.5 ± 58.8 (50.3–189.1)	97.9 ± 0.5	50.7 ± 16.6 (29.8–75.8)	97.9 ± 0.3
F	16.0 ± 5.1 (11.5–24.5)	96.1 ± 0.3	18.4 ± 2.5 (16.2–22.9)	96.0 ± 0.6	7.6 ± 1.8 (4.3–10.1)	96.2 ± 0.6

<sup>a</sup> Values are mean ± standard deviation. <sup>b</sup> Range. <sup>c</sup> nd = not detected.

**Recovery.** Recovery was determined by the standard addition procedure. Standards were added to the liquid sample or to the reconstituted powdered sample. Two addition levels were used for each furfural compound (Tables 2 and 3). Eight determinations were carried out for each addition level. By statistical analysis (Cochran's test), we verified that method accuracy did not depend on furfural content in the sample. Recoveries obtained were always satisfactory and higher than 95% for HMF and F, higher than 85% for MF, and higher than 75% for FMC. The mean recoveries found for each analyte in the two types of samples studied were compared by a Student's *t*-test, and no statistical differences were found between them (*p* > 0.05); hence recovery did not depend on the types of milk sample analyzed. Table 4 provides recovery data for both free and total furfurals in commercial infant milk formulas.

Recoveries were also satisfactory in these samples, always being higher than 95% for all furfurals detected.

**Sensitivity.** To determine the sensitivity of the method, the detection limit (DL) and the determination limit (DtL) were calculated using two criteria. The first was the Long and Winefordner formula (1983), which is based on the repeated analysis of a blank, and is the most common method used for sensitivity estimation in chromatographic procedures. The blank was a raw milk (without heating treatment) which was treated under the same conditions described for milk samples. The second approach was based on the analysis of the low-concentration standard solutions (Castro-Cels et al., 1989). Results in Table 5 are the average of eight determinations. The sensitivity results obtained according to both criteria were very similar for F, FMC, and MF while better results were obtained for HMF

**Table 5. Limits of Detection and Determination<sup>a</sup>**

compd	Long & Winefordner			standard solutions		
	DL, μg/mL	DtL, μg/mL	RSDs, %	DL, μg/mL	DtL, μg/mL	RSDs, %
HMF	0.03	0.04	3.15	0.05	0.06	2.87
F	0.01	0.03	4.80	0.01	0.02	3.65
FMC	0.01	0.02	6.16	0.01	0.03	3.46
MF	0.01	0.02	5.96	0.01	0.03	4.14

<sup>a</sup> DL = detection limit. DtL = determination limit.

when the Long and Winefordner formula was used. In either case, the sensitivity is suitable to determine furfural compounds in milks.

**Content of Furfural Compounds in Infant Milk Formulas.** Only HMF and F were detected in the commercial samples studied. The levels of HMF always were higher than those of F (Table 4). Total HMF and F showed a wide variation between samples, ranging respectively from 38.3 to 189.1 and from 11.5 to 24.5 μg/100 mL of liquid or reconstituted powdered infant milk. No statistical differences were found in the contents of total furfurals between powdered and liquid formulas. Surprisingly, the contents of total HMF and F were much higher ( $p < 0.01$ ) in commercial infant milk formulas than in commercial powdered cow milks, which are products commonly associated with high levels of HMF (Caric et al., 1984; Fink and Kessler, 1986).

With regards to free furfurals, lower values were found in powdered cow milks than in infant milk formulas ( $p < 0.01$ ). However, the levels of free HMF and F were lower in liquid than in powdered formulas. There are no previous data about levels of furfurals in infant milk formulas with the exception of Park and Hong (1991), who reported high contents (264–553 μg/100 mL) of total HMF in Korean infant milk formulas.

The proposed methods to determine total and free furfural compounds in infant milk formulas yield satisfactory results for both liquid and powdered samples. In the literature, many methods have been reported for total HMF determination in foods, but the present study is believed to be the first reported on the simultaneous determination of HMF, F, FMC, and MF and also the first particularly used to analyze furfural compounds in infant milk formulas. The methods proposed are rapid since either no hydrolysis or only a short period of hydrolysis necessary (for measurement of free and total furfurals, respectively). In addition, the proposed methods offer good specificity, precision, and accuracy and can easily be automated.

The levels of furfural compounds were higher in commercial infant milk formulas than in powdered cow milk. The higher levels of vitamin A, iron, and lactose in infant milk formulas compared to cow milks could explain the observed differences in furfural compounds. However, more work should be done to elucidate the influence of raw materials and the processing conditions on the final levels of furfurals in infant milk formulas.

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