Rapid Determination of Hydroxymethylfurfural in Foods Using Liquid Chromatography-Mass Spectrometry

Application

Food Industry

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Abstract

Hydroxymethylfurfural is a product of food deterioration and is still under investigation for possible toxic effects. It can also be used to monitor food quality. A sensitive and selective LC/MS method for monitoring this compound is presented. The method can quantitatively determine hydroxymethylfurfural in food with a detection limit of 0.005 µg/g. Sample preparation and analytical conditions are given.

Introduction

Hydroxymethylfurfural (HMF) is recognized as an indicator of quality deterioration in a wide range of foods. It is formed as an intermediate in the Maillard reaction and is also formed during acid-catalyzed dehydration of hexoses. Formation of HMF in foods is especially dependent on temperature and pH [1].

In recent years, the presence of HMF in foods has raised toxicological concerns: the compound and its similar derivatives were shown to have cytotoxic, genotoxic, and tumoral effects. However, further studies suggest that HMF does not pose a serious health risk, but the subject is still a matter of debate.

Several HPLC techniques were reported for the determination of HMF in various foods. These techniques use UV detection because of the strong absorption of furfurals at approximately 280 to 285 nm. However, many compounds naturally present or formed in foods during processing may also absorb at this wavelength. Poor chromatographic resolution of these compounds may adversely affect the quantification of HMF during UV detection.

A rapid and reliable liquid chromatography/mass spectrometry (LC/MS) method was developed for the determination of HMF in foods. The method entailed aqueous extraction of HMF, solid-phase extraction (SPE) cleanup and analysis by LC/MS. The separation was performed on a narrow-bore column to shorten the chromatographic run.

Experimental

LC/MS experiments were performed using an Agilent 1100 series HPLC system consisting of a binary pump, an autosampler, and a temperature-controlled column oven, coupled to an Agilent 1100 MS detector equipped with atmospheric pressure chemical ionization (APCI) interface.

Data acquisition was performed in selected ion monitoring (SIM) mode using the interface parameters: drying gas (N_2, 100 psig) flow of 4 L/min, nebulizer pressure of 60 psig, drying gas temperatures of 325 °C, vaporizer temperature of 425 °C, capillary voltage of 4 kV, corona current of 4 µA, fragmentor voltage of 55 eV, and dwell time of 439 ms. Ions monitored for HMF were *m/z* 109 and *m/z* 127. The quantification was performed based on the signal response of the ion having *m/z* of 109.

LC/MS

Flow rate:	0.2 mL/min
Gradient:	ZORBAX Bonus RP, 100 mm × 2.1 mm, 3,5 µm
Mobile phase:	0.01 mM acetic acid in 0.2% aqueous solution of formic acid
Injection:	20 µL out of 1000 µL

MS conditions

Ionization mode:	Positive APCI
Nebulizer pressure:	60 psi
Drying gas flow:	4 L/min
Drying gas temperature:	325 °C
Vaporizer temperature:	425 °C
Skimmer:	20 V
Capillary voltage:	4kV
Fragmentor voltage:	55 eV
Dwell time:	439 ms

The chromatographic separations were performed on a ZORBAX Bonus RP Narrow Bore column (2.1 mm × 100 mm, 3.5 µm) using the isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid at a flow rate of 0.2 mL/min at 40 °C.

Method

Sample Preparation

Finely ground sample (1 g) was weighed into a 10-mL glass centrifuge tube with cap. Carrez I and II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 mL of water, respectively. A total of 100 µL Carrez I and 100 µL Carrez II solutions were added to the sample and the volume completed to 10 mL with 0.2 mM acetic acid. HMF was extracted by mixing the tube for 3 min using a vortex mixer. It was then centrifuged for 10 min at 5,000 rpm at 0 °C. The clear supernatant was further cleaned up by using Oasis HLB SPE cartridge. Prior to use, the SPE cartridge was conditioned by passing 1 mL of methanol and equilibrated by passing 1 mL of water at a flow rate of approximately two drops per second using a plastic 2-mL syringe. The excess water was removed from the cartridge by passing 2 mL of air. One milliliter of aqueous extract was eluted through the preconditioned cartridge at a flow rate of approximately one drop per second using a plastic syringe and the eluate was discarded. The cartridge was washed by passing 0.5 mL of water. Then the cartridge was dried under a gentle stream of nitrogen. HMF was eluted from the cartridge by passing 0.5 mL of diethyl ether at a flow rate of approximately one drop per second using a plastic 2-mL syringe. The eluate was collected in a conical bottom glass test tube placed in a water bath at 40 °C (Zymark Turbo Vap® LV Evaporator) and evaporated to dryness under nitrogen at 3 psig. The remaining residue was immediately redissolved in 1 mL of water by mixing in a vortex mixer for 1 min. Twenty microliters of this test solution was injected onto the HPLC system.

Results and Discussion

Positive APCI-MS analysis of HMF showed both the precursor [M+1] ion and the compound-specific ion [$C_6H_5O_2$] due to loss of water from the protonated molecule. See Figure 1. These characteristic

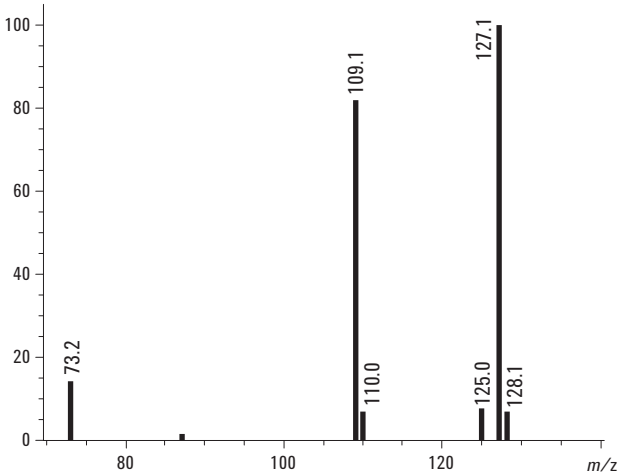

Figure 1. Mass spectrum for HMF obtained with positive APCI.

ions having *m/z* of 127 and 109 were used to monitor HMF in SIM mode. The ratio of these ions (response of ion 127/response of ion 109 = 1.12) was used to confirm the purity of HMF peak. The signal response was linear over a concentration range of 0.05 to 2.0 µg/mL for both ions with correlation coefficients of higher than 0.99. On the basis of a signal-to-noise ratio of 3, the limit of detection (LOD) was determined to be 0.005 µg/mL and 0.006 µg/mL for ions having *m/z* 127 and *m/z* 109, respectively. LC/MS with APCI was found to be a powerful tool that allowed us to determine HMF sensitively and precisely.

The chromatographic separation of HMF was performed on a ZORBAX Bonus RP narrow-bore column. The solution of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid was used as the mobile phase at a flow rate of 0.2 mL/min to increase the ionization yield during MS detection with an adequate separation of HMF in the column from interfering matrix co-extractives. Under these conditions, HMF eluted at 5.087 min with good retention time reproducibility (5.09 ±0.04 min, n = 10). See Figure 2. The capacity factor (k') was determined to be 2.33 for HMF based on the hold-up time of 1.55 min.

Usual approach for the extraction of free furfurals from solid food matrices entails extraction with water followed by clarification using Carrez I and II reagents. Direct LC/MS analysis of aqueous extract showed the presence of interfering compounds. Oasis HLB cartridge packed with a macroporous copolymer of the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone was, there-

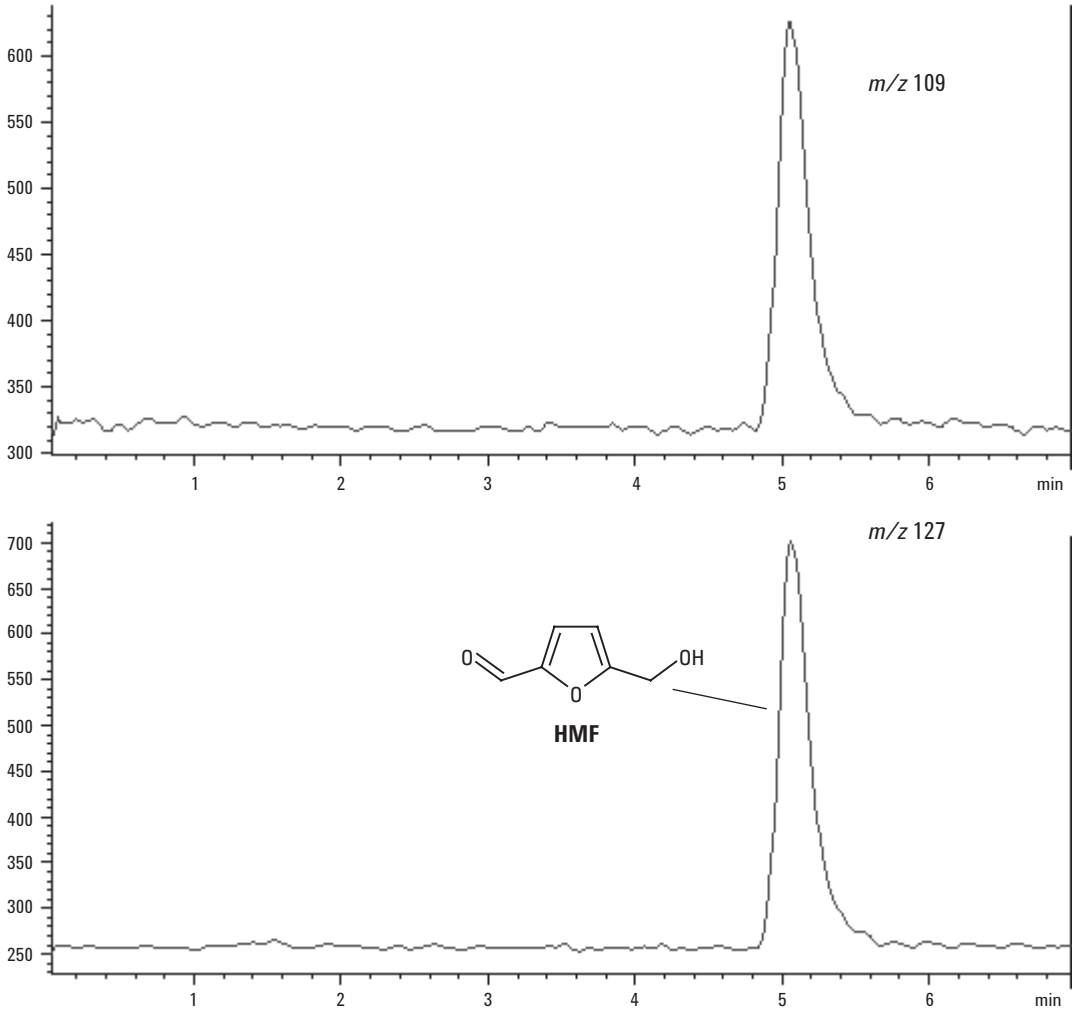

Figure 2. Extracted ion chromatogram (EIC) of an HMF standard (HMF concentration is 100 ng/mL).

fore, used to clean the extract prior to LC analysis. The clear aqueous extract was passed through a preconditioned cartridge. HMF present in the extract strongly interacted with the sorbent material while much of the co-extractives did not. HMF retained in the cartridge was then eluted with diethyl ether. It was determined that 0.5 mL of diethyl ether was sufficient to recover HMF from the cartridge completely.

SPE cleanup brought significant improvement for the detection of HMF using MS in SIM mode. Total ion chromatogram indicated the presence of three major peaks in the sample. HMF peak was identified by comparing both retention time and mass spectral data. The ratio of characteristic ions having *m/z* 127 and *m/z* 109 also confirmed the purity of HMF peak. The compound-specific ion [$C_6H_5O_2$] having *m/z* of 109 was found to be more selective than the parent compound ion. So, the quantification of HMF was performed using the signal response recorded for this ion.

The accuracy of the method was verified by analyzing spiked cereal-based baby foods. The recovery of HMF was determined by analyzing each of the

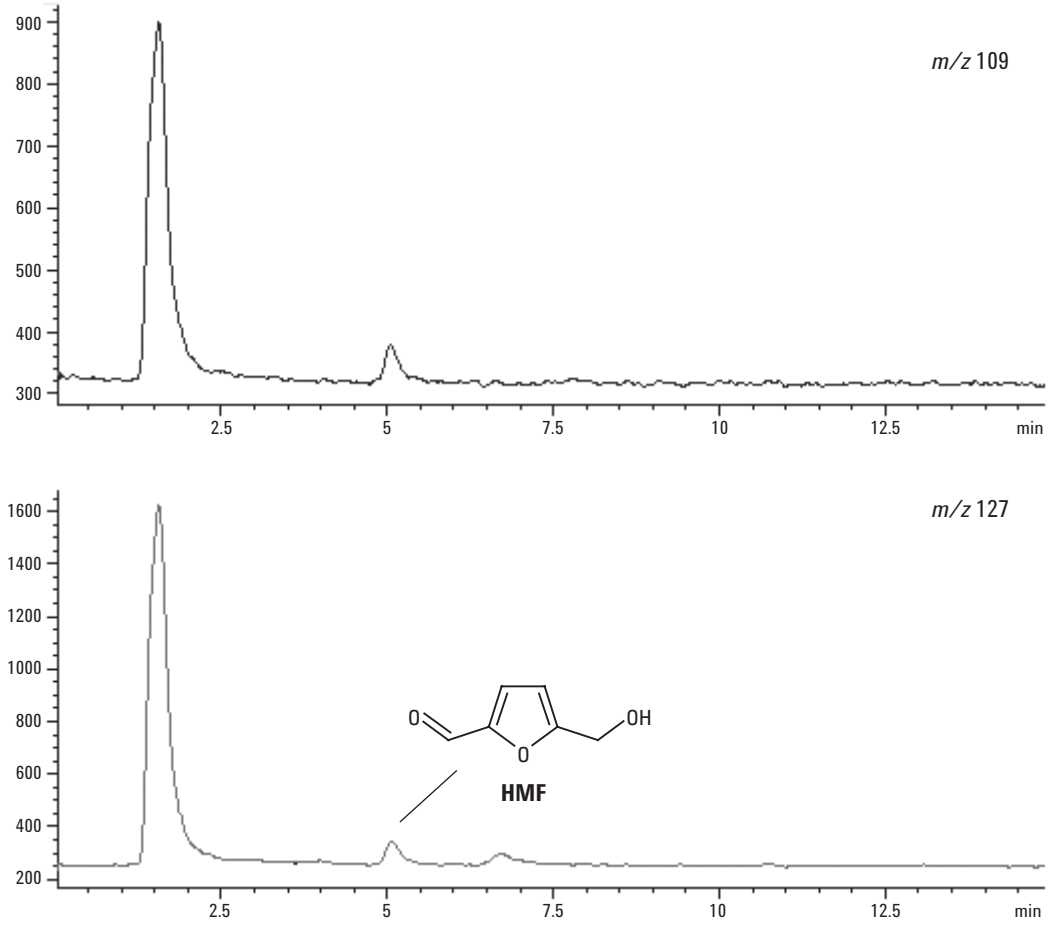

Figure 3. EIC of a fruited yogurt sample (HMF concentration is 0.2 µg/g).

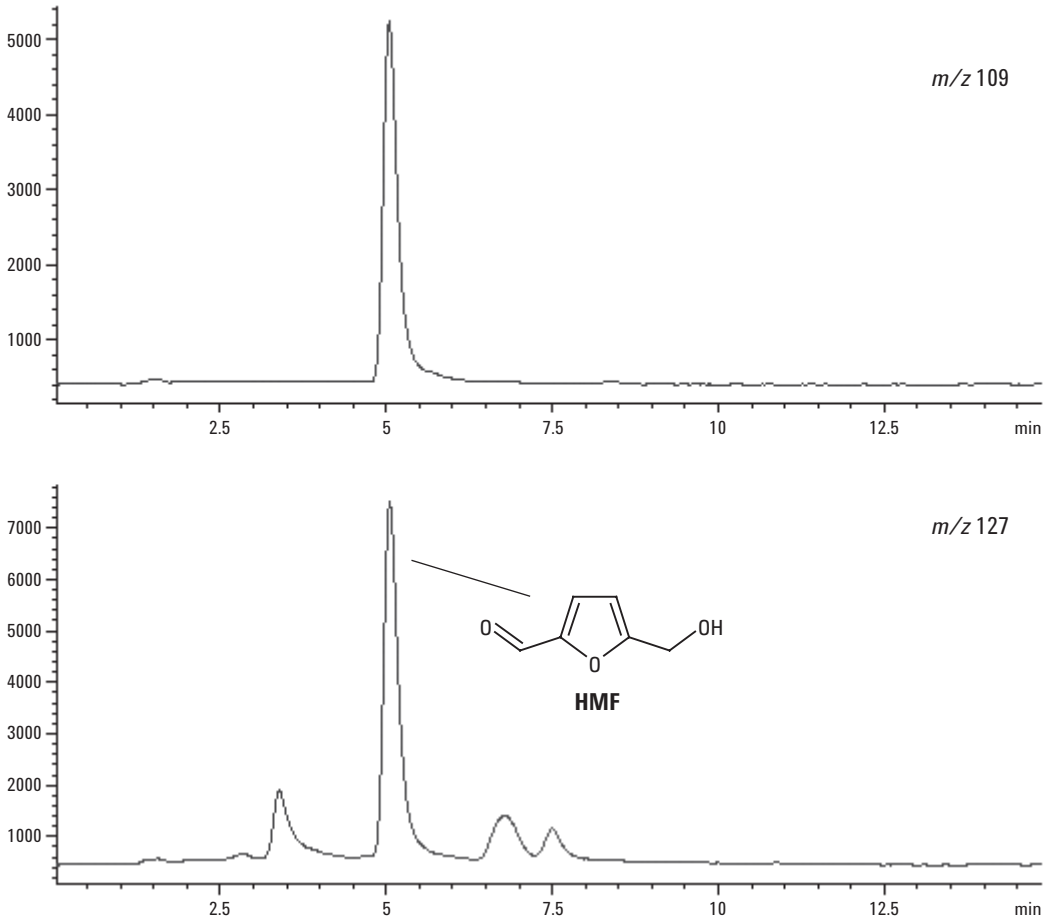

Figure 4. EIC of a crisp bread sample (HMF concentration is 17.5 µg/g).

spiked samples four times for spiking levels ranging from 0.25 to 5.0 µg/g. The mean percentage recoveries exceeded 90% for all levels.

The method is capable of low concentrations, but also high concentrations of HMF in foods precisely and accurately. Figure 3 illustrates the EICs of a fruited yogurt sample having 0.2 µg/g of HMF. It is difficult to measure such a low concentration of HMF using LC coupled to UV detection. Figure 4 illustrates the EICs of a crisp bread sample having 17.5 µg/g of HMF.

Conclusion

The growing attention of the scientific community with regard to the potentially toxic effects of HMF requires new efforts to be made to establish new rapid, reliable, and sensitive methods to determine HMF in real matrices. Previous methods usually dealt with the food items where HMF concentrations are comparatively higher and use extraction procedures that usually do not avoid potential interfering compounds prior to LC analysis. Presence of interferences may be problematic, particularly during the UV detection after LC separation when low concentrations of HMF are being measured in baby foods. The method described in this application combines 1) a rapid separation of HMF from the matrix co-extractives in a narrow-bore column, 2) an efficient cleanup of the extract using SPE, and 3) a selective detection of HMF using MS in a single analytical method.

Reference

1. V. Gökmen, H. Z. Senyuva, Improved method for the determination of hydroxymethylfurfural in baby foods using liquid chromatography-mass spectrometry, *Journal of Agricultural and Food Chemistry*, 2006, 54, 2845–2849.

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Printed in the USA
August 3, 2006
5989-5403EN

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