

Analysis of Browning Reaction Products in Foods, Using High Performance Liquid Chromatography

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Introduction

In food systems, perceived flavor is based not only on individual ingredients that make up the product, but also on interactions between these components. Browning is an important reaction in food systems, and affects both the flavor and the color of the product. One type of non-enzymatic browning reaction that occurs in products such as baked goods and soy sauce is the Maillard reaction. When reducing sugars such as glucose are heated in the presence of a free amino acid or a free amino group in a protein chain, a variety of products, including 5-hydroxymethyl-2-furaldehyde (HMF), result. To monitor the progress of a Maillard browning reaction, HPLC with a UV-visible detector can be used to quantitate products such as HMF.¹ In systems containing a number of reaction products, LC with a mass selective detector (LC/MS) would provide more specific information about the reaction products. The application of LC and LC/MS to the study of HMF will be presented.

The Maillard Reaction

The Maillard reaction is one of four primary non-enzymatic browning reactions. This reaction requires the presence of an amino-bearing compound, a reducing sugar, and water. Basic amino acids, such as lysine, are especially susceptible. When the reducing sugar is a hexose, 5-hydroxymethyl-2-furaldehyde, or HMF, is one of the intermediate reaction products, especially at lower pH. The structure of HMF is shown below, and a simplified Maillard reaction scheme is shown in Figure 1.² The formula weight of HMF is 126.1, and the UV_{\max} is 283nm. On further reaction, a number of high molecular weight polymeric materials are formed, resulting in color and flavor changes. These changes may or may not be desirable; some of the negative effects include off-flavors or off-colors, and the potential loss of essential amino acids such as lysine.

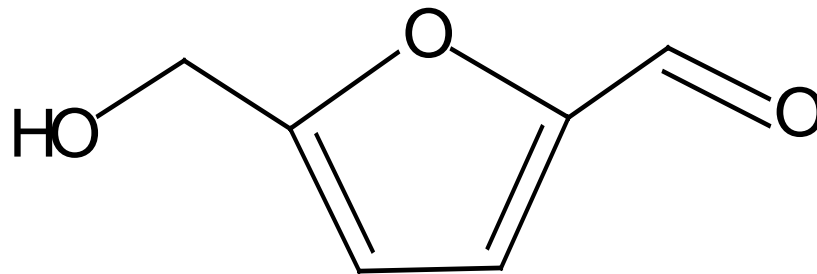
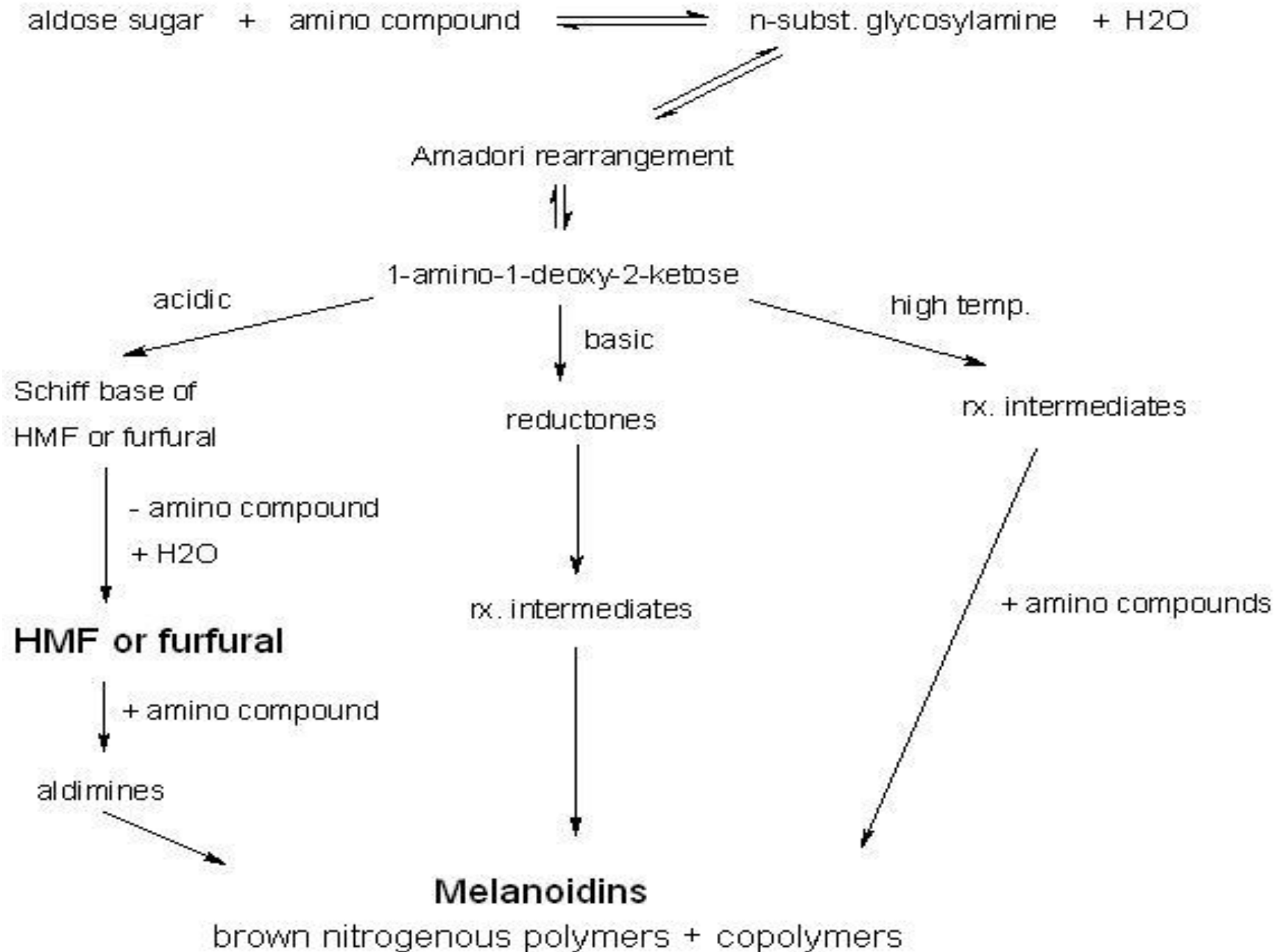


Figure 1. Simplified Maillard reaction scheme.



Liquid Chromatographic Analysis of HMF

Liquid chromatography (LC) provides a powerful tool for monitoring reaction products such as HMF in a variety of food and beverage products. Compared to the traditional spectrophotometric methods, LC is a much more specific assay and is less susceptible to interferences. A number of LC procedures have been published,³⁻⁵ most using a reversed phase separation with a C18 or C8 stationary phase and water:methanol or water:acetonitrile as the mobile phase. In order to make the separation compatible with MS detection, water:methanol with 10 mM ammonium formate was used in this work.

The analysis of an HMF standard is shown in Figure 2, with the LC conditions given in Table I. The method is linear over the range of 0.014–213 ppm, as shown in Figure 3. Several types of fruit juice were tested using this method. A grape juice assay is shown in Figure 4. There is at least one compound that interferes with the HMF when using UV detection at 280nm; therefore, some type of sample treatment will be needed.

Figure 2. Analysis of an HMF standard solution by HPLC. Run conditions are given in Table I.

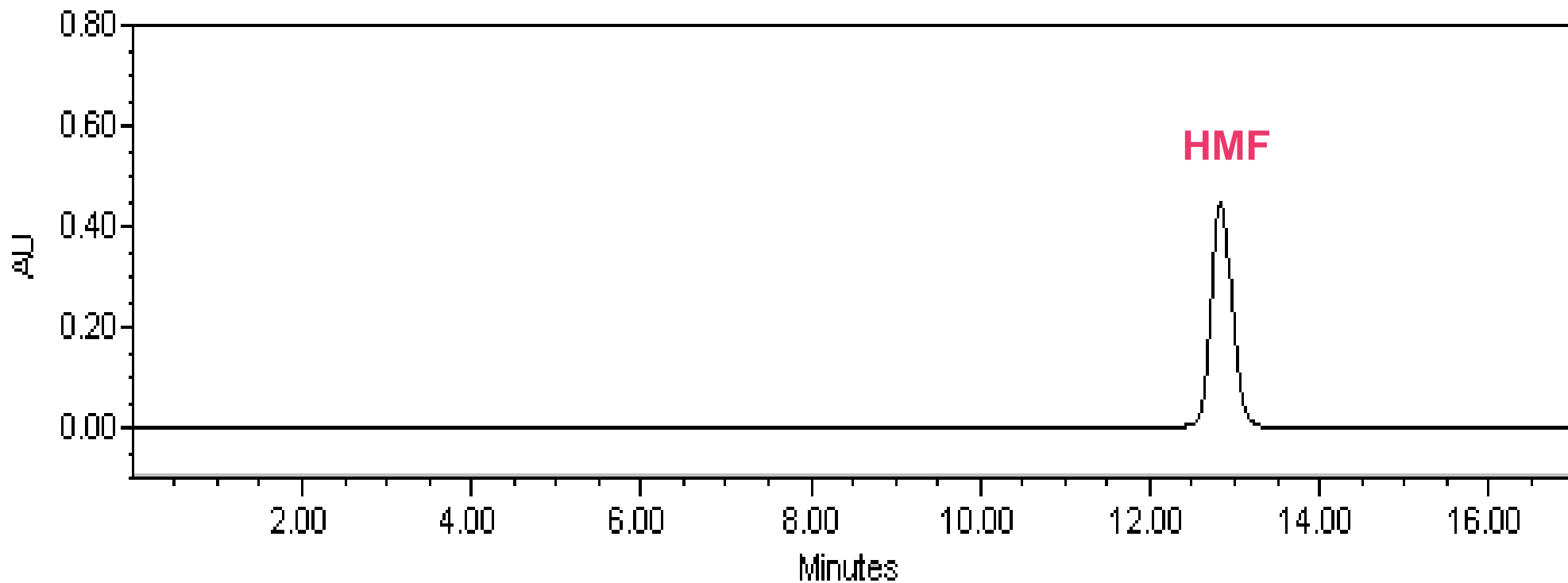


Table I. Chromatographic conditions for HMF analysis by LC.

Column: Ultra C18 (Restek Corp.), 250 mm x 4.6 mm, 5 μ m

Mobile Phase A: 90:10 water:methanol, 10 mM ammonium formate

Mobile Phase B: 10:90 water:methanol, 10 mM ammonium formate

<u>Time (minutes)</u>	<u>% B</u>
0	0
5	0
10	100
20	100
21	0

Flow: 0.5 mL/min.

Temperature: ambient

Detector: UV @ 280 nm

Injection Volume: 10 μ L

Figure 3. Linearity plot for HMF, using LC-UV. Over a concentration range of 14 ppb - 213 ppm, $R^2 = 0.9994$.

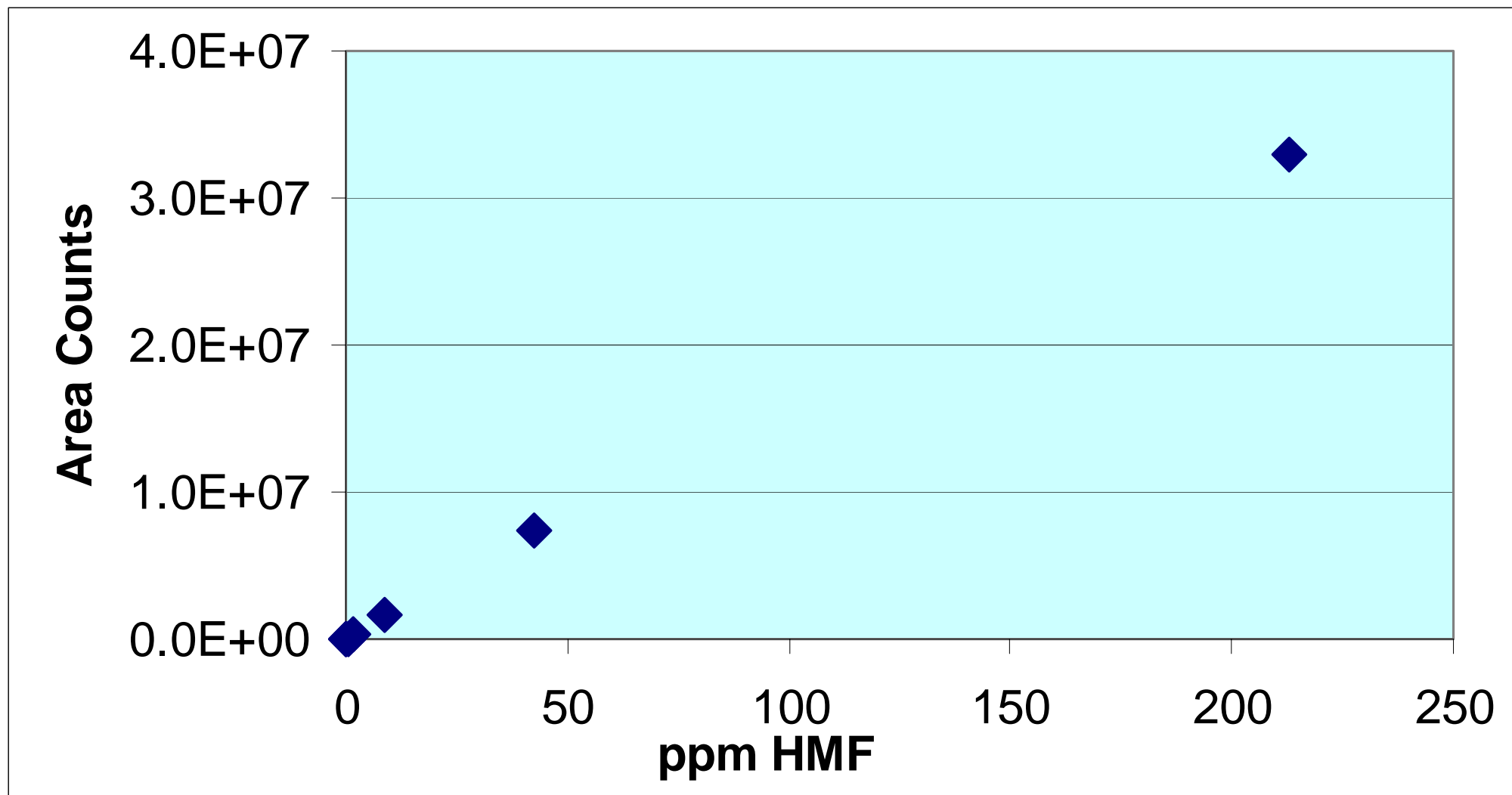
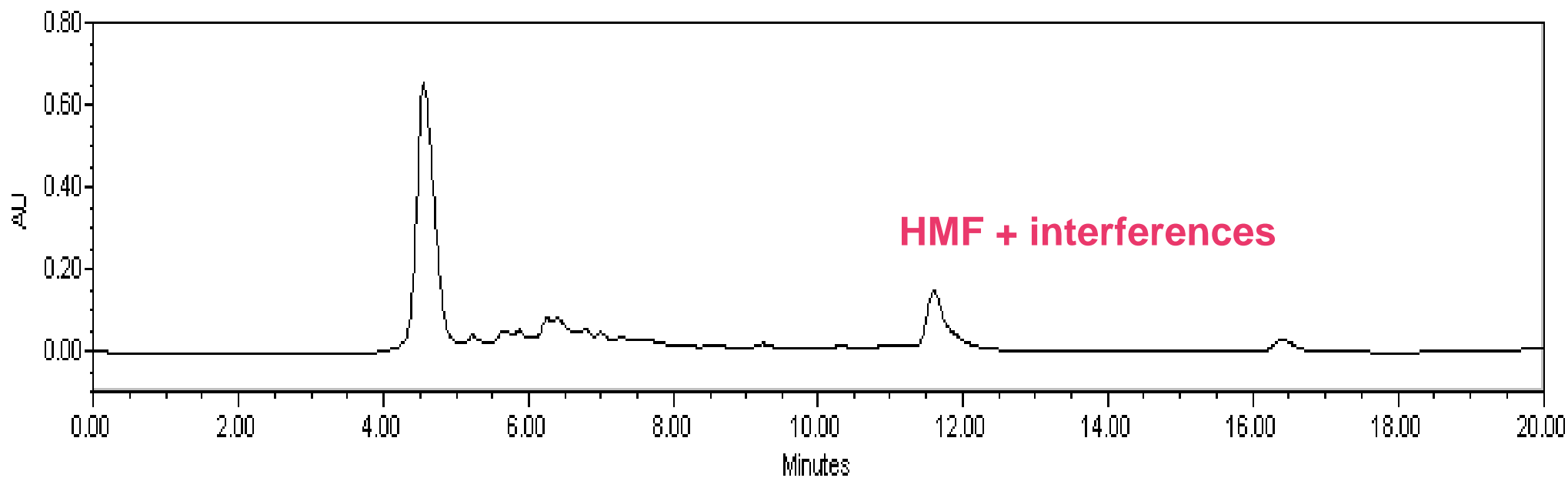


Figure 4. Analysis of HMF in grape juice. The juice was analyzed neat, without solid phase clean-up.



Solid Phase Extraction of HMF from Juice Samples

Because of the interferences present in grape juice, sample treatment was necessary. Previous reports of solid phase extraction (SPE) used C18 cartridges with 15-20% acetonitrile as the final extraction solvent.^{6,7} To match the mobile phase in this analysis, methanol was used at concentrations of 20%, 50%, and 100%. Both C18 tubes and carbon molecular sieve (Carboprep™) tubes were tested. Good recoveries were obtained using either 20% or 50% methanol and the C18 phase; however, the lower concentration of methanol resulted in a cleaner final extract. Good recoveries were obtained with the Carboprep™ tubes as well, with 100% methanol.

Extraction Procedure

1. Conditioning
 - a. Apply 3 mL methanol
 - b. Apply 3 mL deionized water
2. Sample Application
 - a. Apply 4 mL sample to moist SPE tube, gravity feed
3. Wash
 - a. Pull remaining sample through tube, using vacuum
 - b. Apply 3 mL water
 - c. Remove excess water from bed under vacuum
4. Elution
 - a. Apply 2 mL elution solvent, gravity feed, dilute to volume

Figure 5. Analysis of HMF in grape juice, following extraction using a C18 SPE tube. HMF was eluted with 20% methanol.

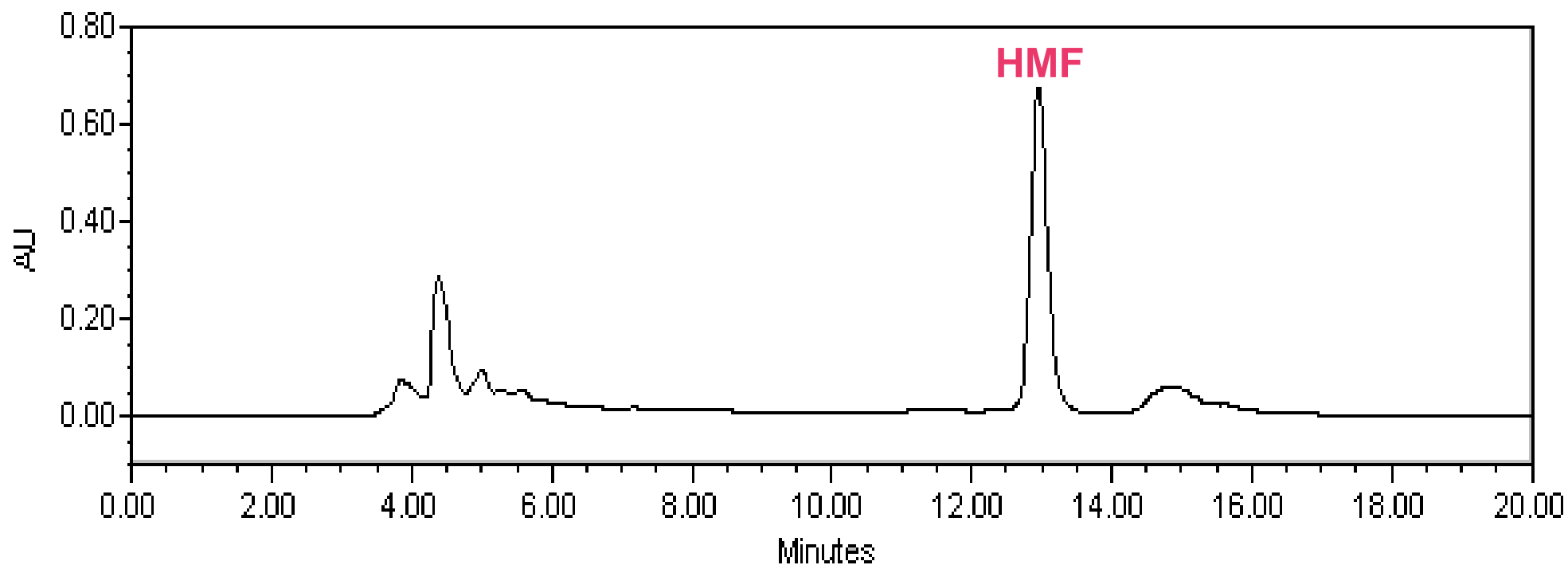


Figure 6. Analysis of HMF in grape juice, following extraction using a C18 SPE tube. HMF was eluted with 50% methanol.

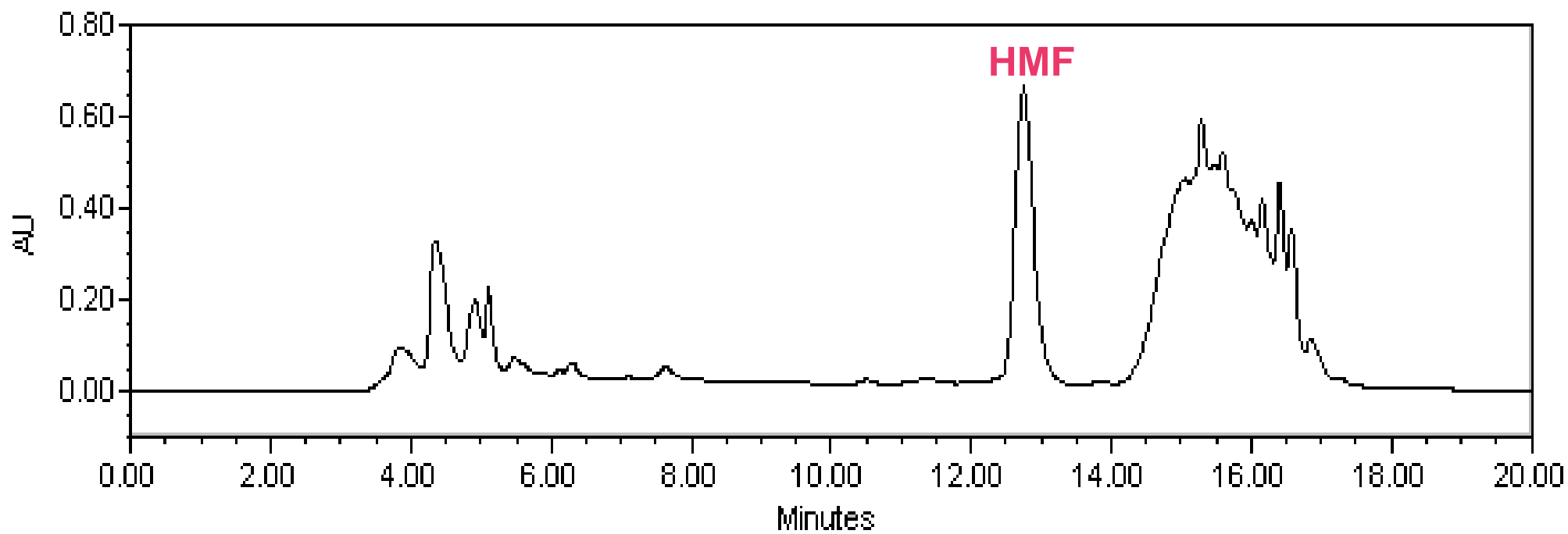
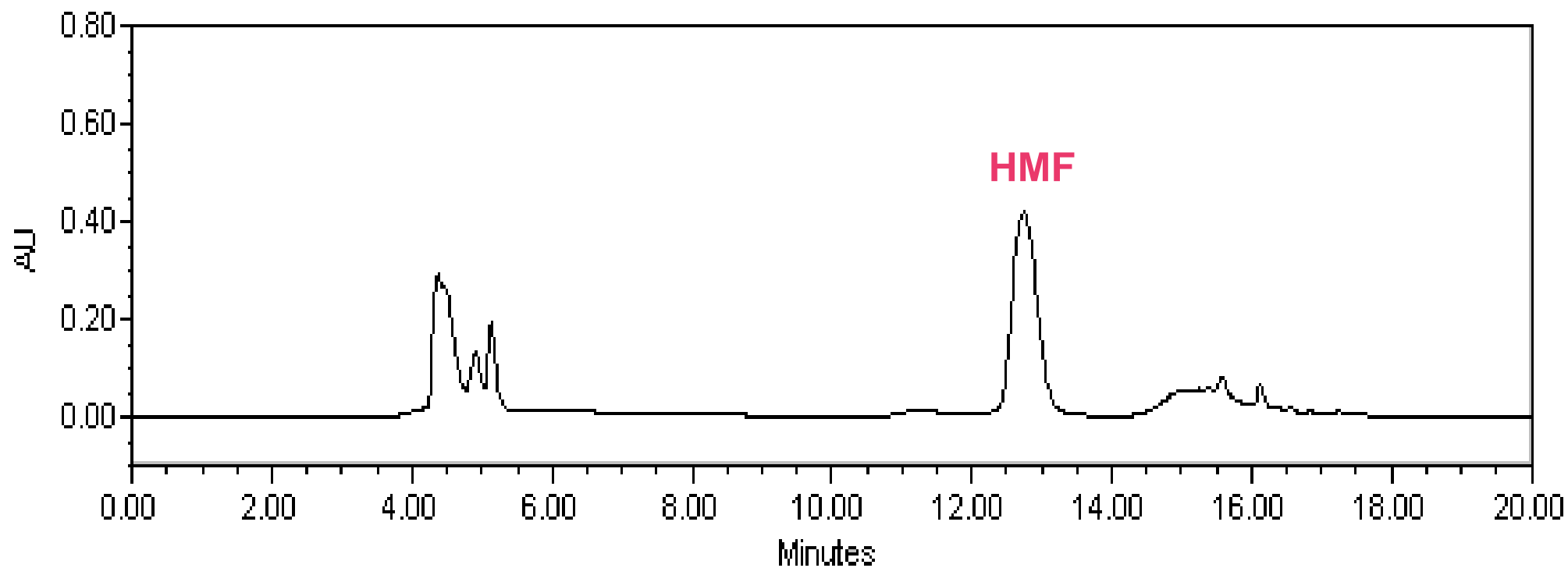


Figure 7. Analysis of HMF in grape juice, following extraction using a Carbobrep™ SPE tube. HMF was eluted with 100% methanol.



LC/MS Analysis of HMF

For specific detection of HMF, a mass spectrometric (MS) detector can be used, in a scanning or selected ion mode (SIM). In addition to monitoring the HMF (M+H)⁺ ion at m/z 127, a wider mass range can be scanned to identify other components in the sample extract. This method is not as sensitive as the UV assay; however, it does provide more information about the composition of the sample, and may allow identification of other reaction products.

Figure 8. Detection of HMF, using a mass spectrometer.

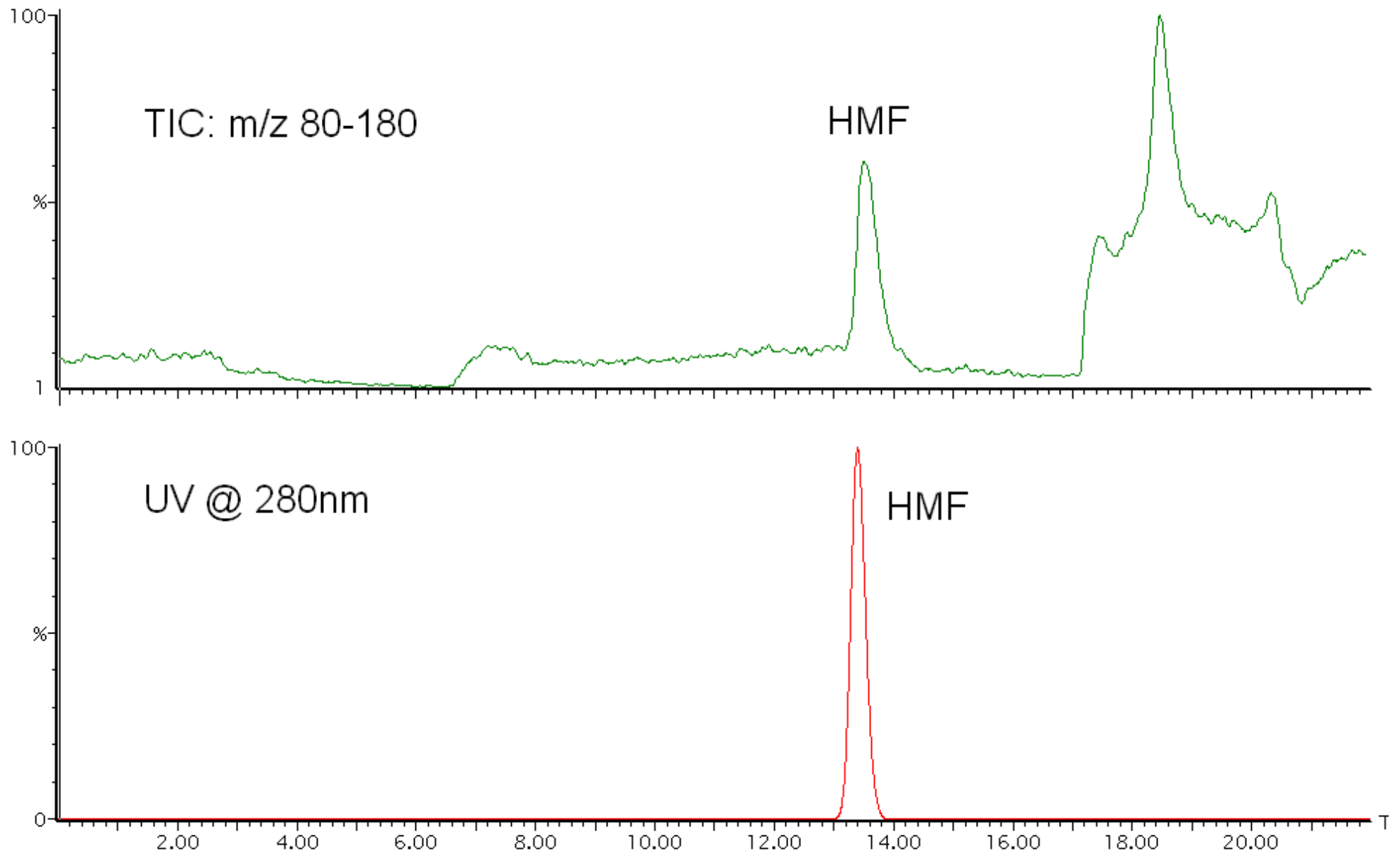


Table II. Experimental conditions for the LC/MS analysis of HMF.

HPLC Conditions

Column:	Ultra C18 (Restek Corp.), 250 mm x 4.6 mm, 5 μ m
Mobile Phase A:	90:10 water:methanol, 10 mM ammonium formate
Mobile Phase B:	10:90 water:methanol, 10 mM ammonium formate
Gradient:	Same as in Table I
Inj. Volume:	10 μ L
Flow Rate:	0.3 mL/min to UV detector, 0.2 mL/min to MSD

MSD Conditions

Detector:	Micromass ZMD
Mode:	ESI+
Capillary V:	3.08V
Extractor V:	13V
Ion Energy:	0.5
Multiplier:	650
Source Temp:	100°C
Desolv. Temp:	200°C
Gas Flow:	500 L/hr.
SIM ion:	m/z = 127
TIC range:	m/z = 80 – 180

Summary

Liquid chromatography provides a sensitive tool for monitoring products from the Maillard browning reaction in foods, such as HMF. Compared to traditional spectrophotometric assays, compounds such as HMF can be selectively determined. Because of the potential for interferences when using UV detection, samples should be prepared by solid phase extraction. Good recoveries of HMF from grape juice were obtained using either C18 SPE tubes or Carbo prep™ tubes. An MS detector can be used to monitor the reaction products. Because the MS provides molecular weight information, it is possible to obtain more detailed information about the reaction.

References

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