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Method of Test for Pyridine, Styrene and Eugenyl Methyl Ether in Flavorings

1. Scope

This method is applicable to the determination of pyridine, styrene and eugenyl methyl ether in flavorings.

2. Method

After sample dilution, analytes are determined by gas chromatograph/high resolution mass spectrometer (GC-HRMS).

2.1. Equipment

2.1.1. Gas chromatograph/high resolution mass spectrometer (GC-HRMS): GC-Q Exactive, or an equivalent product.

2.1.1.1. Ion source: electron ionization (EI).

2.1.1.2. Column: DB-5 UI capillary column, 0.25 μm, 0.25 mm i.d. × 30 m, or an equivalent product.

2.1.1.3. Liner: Agilent 5190–2293, Ultra Inert, splitless, single taper, glass wool, 4 mm i.d. × 7.85 cm, 900 μL, or an equivalent product.

2.2. Chemicals

Acetone, GR grade;

Pyridine, styrene and eugenyl methyl ether, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 1 mL and 10 mL.

2.3.2. Centrifuge tube: 15 mL, PP.

2.4. Standard solution preparation

Transfer about 10 mg of each reference standard accurately weighed into a 10-mL volumetric flask, dissolve and dilute to volume with acetone as the standard stock solution. Stored in refrigerator. When to use, mix appropriate volume of each standard stock solution, and dilute with acetone to 0.1~2 μg/mL as the standard solutions.

2.5. Sample solution preparation

Transfer about 0.01 g of the sample accurately weighed into a 1-mL volumetric flask, dissolve and dilute to volume with acetone as the sample solution.

2.6. Identification and quantification

Accurately inject 1 μL of the sample solution and standard solution into GC-HRMS separately and operate according to the following conditions. Identify pyridine, styrene and eugenyl methyl ether based on the retention time and

both the quantitative ion and the qualitative ion with a mass accuracy ≤ 5 ppm. Calculate the amount of pyridine, styrene and eugenyl methyl ether in the sample using the following formula:

The amount of pyridine, styrene or eugenyl methyl ether in the sample (mg/kg) $= \frac{C \times V}{M}$

where,

C: the concentration of pyridine, styrene and eugenyl methyl ether in the sample solution calculated by the standard curve (μg/mL)

V：the final make-up volume of the sample (mL)

M：the weight of the sample (g)

GC-HRMS operating condition (Note):

Column: DB-5 UI capillary column, 0.25 μm, 0.25 mm i.d. × 30 m.

Column temperature: initial temperature: 40℃, 2 min;
temperature gradient rate: 30℃/min;
final temperature: 250℃，5 min.

Injector temperature: 250℃.

Carrier gas: helium, 1.2 mL/min.

Injection volume: 1 μL.

Injection mode: splitless.

Interface temperature: 250℃.

Ion source temperature: 200℃.

Ionization mode: EI, 70 eV.

Detection mode: selected ion monitoring (SIM), detection ions are as follows.

Analyte	Quantitative ion (*m/z*)	Qualitative ion (*m/z*)
Pyridine	79.0417	50.0151
Styrene	104.0621	78.0464
Eugenyl methyl ether	178.0988	147.0804

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. The limits of quantification (LOQs) for pyridine, styrene and eugenyl methyl ether are all 10 mg/kg.

2. Further validation should be performed when interfering compounds are found in the samples.
3. Pyridine, styrene and eugenyl methyl ether may occur naturally in flavorings and specific foods. Though this method can be served as a test of synthetic pyridine, styrene and eugenyl methyl ether in flavorings, it is hard to distinguish from the natural ones. Therefore, the results should be determined comprehensively with the findings of other investigations.

References

1. Zamora, R., Lavado-Tena, C. M. and Hidalgo, F. J. 2020. Oligomerization of reactive carbonyls in the presence of ammonia- producing compounds: A route for the production of pyridines in foods. Food Chem. 304: 125284.
2. Steele, D. H., Thornburg, M. J., Staneley, J. S., Miller, R. R., Brooke, R., Cushman, J. R. and Cruzan, G. 1994. Determination of styrene in selected foods. J. Agric. Food Chem. 42: 1661-1665.
3. Lopez, P., van Sisseren, M., De Marco, S., Jekel, A., de Nijs, M. and G. J. Mol, H. 2015. A straightforward method to determine flavouring substances in food by GC-MS. Food Chem. 174: 407-416.

Reference chromatogram

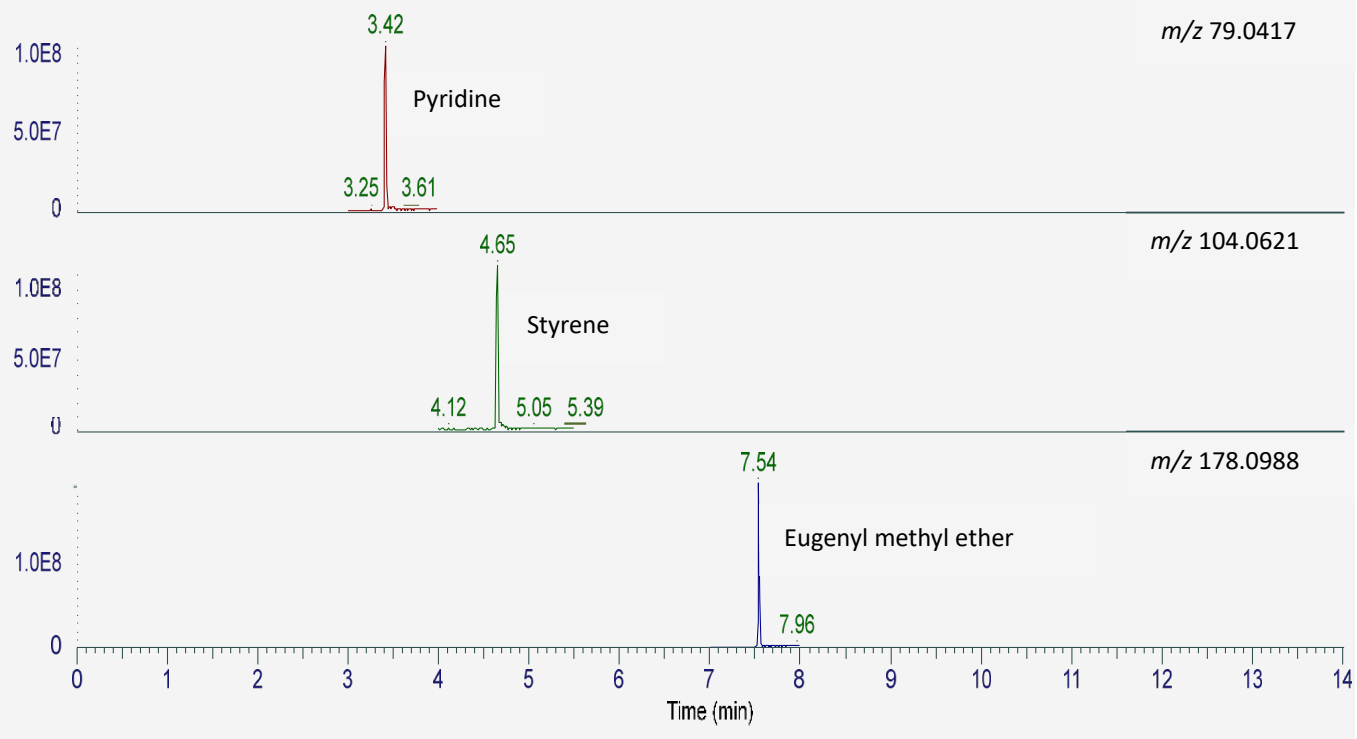

Figure. SIM spectra of pyridine, styrene, and eugenyl methyl ether standards analyzed by GC-HRMS.