Rapid and Sensitive Determination of Acesulfame in Vinegar

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Key Words
HPLC, Acesulfame k, Acclaim Mixed-Mode WAX-1 Column, Artificial Sweetener, Sugar Substitute, Food Additive, Food Safety, Food Analysis

Goal
To develop an efficient high-performance liquid chromatography (HPLC) method for the rapid and sensitive determination of acesulfame in vinegar, in compliance with the Chinese National Standard regulation

Introduction
Acesulfame (structure shown in Figure 1), a synthetic sweetener 200 times sweeter than sucrose, has been extensively used as a sugar substitute in foods and beverages. The Standardization Administration of China allows acesulfame to be added to fermented vinegar products, but limits the added amount to no more than 4 µg/mL.1 Therefore, it is necessary to establish efficient methods to sensitively and rapidly determine acesulfame in fermented vinegar.

The typical method for determination of acesulfame in vinegar uses HPLC, which is also recommended in China GB/T 5009.140-2003.1-3 Table 1 lists details of three methods for determining acesulfame in food and beverages, each using either ammonium sulfate or ammonium acetate as a mobile phase component. Use of ammonium sulfate and ammonium acetate mobile phase buffers with UV detection at ~220 nm may result in less stable baselines than when using other HPLC mobile phases. In addition, the polar compounds present in vinegar samples may interfere with the separation of acesulfame on a C18 stationary phase. To address these issues, the authors investigated using other stationary and mobile phases for this application.

Table 1. Methods for determining acesulfame in food products.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Column</th>
<th>Mobile Phase</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB/T 5009.140-2003</td>
<td>Beverage</td>
<td>C18</td>
<td>0.02 M Ammonium Sulfate/Methanol/Acetonitrile, 10%</td>
<td>UV, 214 nm</td>
</tr>
<tr>
<td>Literature2</td>
<td>Food</td>
<td>C18</td>
<td>0.02 M Ammonium Acetate/Methanol (90:10, v/v)</td>
<td>UV, 230 nm</td>
</tr>
<tr>
<td>Literature3</td>
<td>Liquid Seasoning</td>
<td>C18</td>
<td>0.02 M Ammonium Acetate/Methanol (93:7, v/v)</td>
<td>UV, 230 nm</td>
</tr>
</tbody>
</table>

Figure 1. Structure of acesulfame.
Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system, including:
  - LPG-3400RS Quaternary Pump (P/N 5040.0036)
  - SRD-3400 Integrated Solvent and Degasser Rack (P/N 5035.9245)
  - WPS-3000TRS Well Plate Sampler, Thermostatted (P/N 5840.0020), with 25 µL sample loop (P/N 6820.2415)
  - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - DAD-3000RS Diode Array Detector (P/N 5082.0010), with 2.5 µL flow cell (P/N 6082.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.1 or above
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity, generated from the Thermo Scientific™ Barnstead™ GenPure™ ultrapure water system (P/N 50131948)
- Acetonitrile, HPLC Grade (Fisher Scientific P/N AC610010040)
- Ammonium phosphate monobasic, HPLC Grade, (Fisher Scientific P/N A685-500)
- Sodium hydroxide (NaOH) solution, 50% w/w/Certified (Fisher Scientific P/N SS254-500)
- Acesulfame potassium (Fisher Scientific P/N NC0525062)

Conditions

- Column: Thermo Scientific™ Acclaim™ Mixed-Mode WAX-1, 3 µm, 3 × 150 mm (P/N 070088)
- Mobile Phase: Acetonitrile/100 mM ammonium phosphate (dissolve 13.6 g of ammonium phosphate in 1 L of DI water, adjust pH to 6.8 with 50% NaOH solution), 20:80 (v/v)
- Flow Rate: 0.8 mL/min
- Injection Volume: 10 µL (partial-loop injection mode)
- Temperature: 30 °C
- Detection: UV, 227 nm

Preparation of Standard Solutions

Stock Standard 1
Dissolve 0.01 g of acesulfame potassium standard in 10 mL of DI water. The concentration of Stock Standard 1 will be 1000 µg/mL.

Stock Standard 2
Dilute 400 µL of Stock Standard Solution 1 to 10 mL with DI water. The concentration of Stock Standard 2 will be 40 µg/mL.

Stock Standard 3
Dilute 500 µL of Stock Standard Solution 2 to 10 mL with DI water. The concentration of Stock Standard 3 will be 2 µg/mL.

Standard Solutions for Calibration
For calibration, prepare eight working standard solutions with different concentrations by diluting the proper amount of the stock standard solutions with DI water. The volumes of each solution needed to make the calibration standards are shown in Table 2.

<table>
<thead>
<tr>
<th>Acesulfame Stock Std for Calibration, Conc (µg/mL)</th>
<th>Volume of Acesulfame Stock Std for Calibration (mL)</th>
<th>Volume of DI Water (mL)</th>
<th>Final Volume of Calibration Std (mL)</th>
<th>Final Conc of Calibration Std (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock Standard 2, 40</td>
<td>5.0</td>
<td>5.0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>7.5</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>8.75</td>
<td></td>
<td>5.0</td>
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<tr>
<td></td>
<td>0.625</td>
<td>9.375</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Stock Standard 3, 2</td>
<td>5.0</td>
<td>5.0</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>7.5</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>9.75</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>9.875</td>
<td></td>
<td>0.025</td>
</tr>
</tbody>
</table>

Table 2: Preparation of calibration standards.
Preparation of Samples

Two vinegar samples were purchased from a supermarket in Chengdu, People’s Republic of China.

Dilute 1 mL of the vinegar sample to 100 mL with DI water in a 100 mL volumetric flask. Pass the sample through a 0.45 µm syringe filter prior to injection.

Dilute 5 mL of the 10 µg/mL calibration standard and 1 mL of the vinegar sample with DI water in a 100 mL volumetric flask. The spiked concentration of acesulfame in the vinegar sample will be 0.5 µg/mL. Pass the sample through a 0.45 µm syringe filter prior to injection.

Results and Discussion

Optimization of Chromatographic Conditions

Three columns with different stationary phases were evaluated for the separation of acesulfame: the Acclaim 120 C18, Acclaim PolarAdvantage II (PA2), and the Acclaim Mixed-Mode WAX-1 columns. Under the recommended chromatographic conditions shown in References 1–3, the acesulfame peak tailed with weak retention (close to the void volume) on the Acclaim PA2 column. When using the Acclaim 120 C18 column, the elution of some compounds from a vinegar sample required a long time (nearly 20 min). The Acclaim Mixed-Mode WAX-1 column—which has been applied to the determination of additives including acesulfame in beverages—showed good separation of acesulfame from other compounds found in vinegar samples and was, therefore, used in this application.4

Here, ammonium acetate/ammonium phosphate mobile phase buffers and methonal/acetonitrile mobile phase solvents were evaluated using an Acclaim Mixed-Mode WAX-1 column. Compared to ammonium acetate and methanol, ammonium phosphate/acetonitrile yielded better peak symmetry, a smoother baseline, and a faster separation of acesulfame. Therefore, an acetonitrile/ammonium phosphate mobile phase was used in this work.

In addition, investigations of the concentration and pH value of the mobile phase buffer showed that a 0.1 M buffer concentration at pH 6.8 yielded a reproducible separation of acesulfame in vinegar samples. Figure 2 shows a chromatogram of a 0.025 µg/mL acesulfame standard under the optimized chromatographic conditions.

Reproducibility, Linearity, and Detection Limit

Short-term method reproducibility was estimated by making seven consecutive injections of a vinegar sample spiked with acesulfame to a concentration of 0.5 µg/mL. The retention time RSD was 0.1 and the peak area RSD was 0.4, demonstrating good short-term precision for this HPLC method.

Calibration linearity for UV detection of acesulfame was investigated by making three consecutive 10 µL injections of a standard prepared at eight different concentrations (i.e., 24 total injections). Linearity was observed from 0.025 to 20 µg/mL when plotting the concentration vs peak area (calibration curve shown in Figure 3). The linear regression equation was $A = 0.4874c$ (forced to the origin), where $A$ represents peak area and $c$ represents analyte concentration. The coefficient of determination was 1.000. This calibration curve was used to quantify acesulfame in vinegar samples.

Five replicate injections of an acesulfame standard with a concentration of 0.025 µg/mL were used for estimating the method detection limit (MDL) using a signal-to-noise ratio = 3. The measured MDL of acesulfame was 0.008 µg/mL. The detection limit of acesulfame in a vinegar sample is 0.8 µg/mL (100-fold dilution of the sample), which is much lower than the 4 µg/mL that is specified in GB/T 5009.140-2003.1
Analysis of Vinegar Samples

Acesulfame was not found in either vinegar sample. Figure 4 shows chromatograms of a vinegar sample and the same sample spiked with acesulfame. To judge method accuracy, three injections were made of the two vinegar samples spiked with a 0.5 µg/mL acesulfame standard. The average recoveries were 90 and 96%, respectively, demonstrating good method accuracy.

Conclusion

This work describes an efficient HPLC method using a mixed-mode column with UV detection for a rapid and sensitive determination of acesulfame in vinegar. This approach offers the advantages of simple sample preparation, good method reproducibility, and a wide linear calibration range.

References


3. Dong, M. Simultaneous Determination of Acesulfame, Benzoic Acid, Sorbic Acid and Saccharin Sodium in Compound Liquid Seasoner by Reversed Phase High Performance Liquid Chromatography. Commodities and Quality (Shang pin yu zhi liang) 2013, 3, 244, 252.


Figure 4. A vinegar sample (a) and the same sample spiked with 0.5 µg/mL of acesulfame standard (b).