Spectrophotometric Flow-Injection Method for Determination of Sorbic Acid in Wines

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ABSTRACT: A simple, rapid, and accurate spectrophotometric flow-injection method was developed for the determination of sorbic acid in wines based on the oxidation of sorbic acid with $K_2Cr_2O_7/H_2SO_4$ at 100°C, followed by reaction of the resulting malonaldehyde with thiobarbituric acid, also at 100°C, to give a red product, the absorbance of which is measured at 532 nm. The method requires no prior distillation of the wine and allows a sampling rate of 40 per hour. The calibration graph was linear from 0 to at least 15 $\mu g \text{mL}^{-1}$ (the linear correlation coefficient was 0.9997), and the detection limit (signal-to-noise ratio, 3) was 0.14 $\mu g \text{mL}^{-1}$. The relative standard deviation of ten replicate determinations was 1.58%. The method was applied successfully to the determination of sorbic acid in red and white wines. © 1999 John Wiley & Sons, Inc. Lab Robotics and Automation 11: 299–303, 1999

INTRODUCTION

The years that followed the end of World War II saw a widespread increase in the consumption of semidry wines, which must be held in sterile bottles to avoid further fermentation. Sorbic acid is a fatty acid with no hazardous effects on human health. Its low cost has allowed its use against bacterial, yeast, and mold proliferation, and as an acidifying agent in nonalcoholic beverages. Since 1959, French winemakers were allowed to use it with no legal obligation to state the presence of it on their labels. The microbial action of sorbic acid focuses on yeasts and molds, and to a lesser extent, on bacteria. Sorbic acid can be added to wine as such or in the form of potassium sorbate. Its use for preservation is avoided because it gives the resulting wine a geranium aftertaste unless some sulfurous acid is also used, bottles are expeditiously sealed, and their contents promptly consumed [1]. The legal low concentration of sorbic acid makes its determination significant to routine wine analyses.

Reported analytical methods for the determination of sorbic acid are mainly photometric and chromatographic (HPLC). The most widely used photometric method for determining sorbic acid relies on its oxidation with a mixture of $K_2Cr_2O_7$ and $H_2SO_4$ in a water bath at 100°C, followed by reaction of the resulting malonaldehyde with thiobarbituric acid, also at 100°C, to give a red product, the absorbance...
of which is measured at 532 nm [2±5]. For this colorimetric method to be effective, the reaction mixture with thiobarbituric acid must be cooled in an ice bath. This method is time-consuming because it entails the prior distillation of the wine for about 45 minutes in order to isolate the sorbic acid from the potentially interfering sample matrix.

Another widely used method involves distilling the wine, acidifying the distillate with HCl, and measuring its extinction at 260 nm [3±5]. This method and that of thiobarbituric acid provide comparable, satisfactory results in the determination of sorbic acid in wines. Both are official choices for this purpose.

The Oficial Diary of the European Community recommends a similar method, involving the distillation of the wine, and the interferent substances are eliminated by evaporation of the distilled product to dry and the addition of calcium hydroxide. The absorbance is measured at 256 nm [6].

A variant of the previous method was developed to avoid distillation of the wine, which, as noted earlier, is time-consuming. The acid is quantified from extinction measurements at 256 nm following removal of interferences on activated carbon [7]; however, the amount of sorbent to be used must be precisely estimated because any excess can also retain some analyte. To avoid distillation, Ziemelis and Sommers developed, another method in which the determination of sorbic acid relies on its extraction into 2,2,4-trimethylpentane and the measurement of the absorbance of the extract at 255 nm. This gives an accurate determination of sorbic acid concentration, and this method is quicker than, but of comparable accuracy to the official procedure [8±10].

After spectrophotometry, the HPLC technique is undoubtedly the second most widely used approach to the determination of sorbic acid in wines. It is highly specific and involves minimal sample preparation. Furthermore, these analyses are fast, from 2 to 3 minutes [11] to about 10 minutes [12±14]. However, no reports on the use of flow-injection (FI) technique for the determination of sorbic acid in wines are found in the literature [15].

In this article, we propose an automatic method for determination of sorbic acid based on the previously mentioned reaction with thiobarbituric acid. The method requires no prior distillation of the wine, which allows a sampling rate of 40 per hour, is more sensitive and more selective, and uses less sample and reagent compared with previously mentioned photometric methods.

**EXPERIMENTAL**

**Reagents**

All reagents used were of the highest purity available and of at least analytical-reagent grade. Doubly deionized water (18 MΩ) was used throughout.

A sorbic acid standard solution (100 µg mL⁻¹) was prepared from sorbic acid (97%, Aldrich, Gil-lingham, Dorset, UK). Working standard solutions were prepared daily by appropriate dilution as required.

A 1:1 mixture of 0.01 N K₂Cr₂O₇ and 0.3 N H₂SO₄ was prepared from potassium dichromate (>99%, Aldrich, Millwaukee, WI, USA) and sulfuric acid (95–97%, Merck, Darmstadt, Germany).

2-Thiobarbituric acid solution (0.5%) was prepared daily from 2-thiobarbituric acid (Aldrich, Gil-lingham, Dorset, UK).

**Instrumentation and Procedure**

A Hewlett-Packard (Avondale, PA, USA) Model 8452A diode-array detector interfaced to a Vectra QS/165 computer that delivered results through an HP Laserjet IIP was used. The flow manifold consisted of a Gilson (Worthington, OH, USA) Minipuls-3 peristaltic pump, a Rheodyne (Cotari, CA, USA) Type 50 six-port rotary valve, and a Hellma (Jamaica, NY, USA) OS 1.000 flow cell. Mixing tubes and reactors were made of Teflon, and pump tubes were made of vinyl.

A schematic diagram of the FI system is shown in Figure 1. It operated as follows: first, the carrier solution (deionized water) is pumped into the system as a blank to establish a baseline. Samples or standard solutions are pumped at 0.7 mL min⁻¹, and 225 µl is injected through the valve into the system and is merged with a stream of a 1:1 mixture of 0.01 N K₂Cr₂O₇ and 0.3 N H₂SO₄ (oxidizing mixture). Both streams are mixed in the reactor R₁ (200 cm × 0.5 mm i.d.) where the oxidation of the sorbic acid is produced. The resulting malonaldehyde is mixed in the reactor R₂ (500 cm × 0.5 mm i.d.) with a stream of thiobarbituric acid solution (0.5%). Both reactors are immersed in a water bath at 100°C. After, the mixture is cooled rapidly when passed through the reactor R₃ (200 cm × 0.5 mm i.d.), which is immersed in an ice bath. The absorbance of the red product formed is measured at 532 nm. The ice bath is very important because it provides a better baseline and improves the reproducibility.

**Determination of Sorbic Acid in Wines**

A 1.0 ml volume of wine was diluted to 25 mL with deionized water in a calibrated flask. This solution...
Figure 1. Schematic diagram of the FI system. P, peristaltic pump; Vi, injection valve; R₁, R₂, and R₃, reactors (200, 500, 200 cm × 0.5 mm i.d., respective).

Figure 2. Calibration graph.

TABLE 1. Effect of Some Interferences on the Determination of 3.0 μg mL⁻¹ of Sorbic Acid

<table>
<thead>
<tr>
<th>Species</th>
<th>Species (μg mL⁻¹)</th>
<th>Mass Ratio (Additive: Sorbic Acid)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic Acid</td>
<td>600</td>
<td>200</td>
<td>-3.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2000</td>
<td>667</td>
<td>-5.3</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>600</td>
<td>200</td>
<td>+6.0</td>
</tr>
<tr>
<td>Tartaric Acid</td>
<td>150</td>
<td>50</td>
<td>+3.7</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>600</td>
<td>200</td>
<td>+4.3</td>
</tr>
</tbody>
</table>

was injected in triplicate into the FI system (Figure 1). The average peak height found was compared with a calibration graph based on standard solutions of sorbic acid.

RESULTS AND DISCUSSION

In the proposed method, sorbic acid is oxidized by K₂Cr₂O₇/H₂SO₄ to malonaldehyde, which reacts with 2-thiobarbituric acid to form a reddish-brown product with a single absorption maximum at 532 nm. The reagent blank had practically zero absorbance at
TABLE 2. Recovery Achieved after Addition of Sorbic Acid to Wine Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sorbic Acid (µg mL⁻¹)</th>
<th>Addition of 12.5 µg mL⁻¹</th>
<th>Addition of 25.0 µg mL⁻¹</th>
<th>Addition of 37.5 µg mL⁻¹</th>
<th>Addition of 50.0 µg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Wine 1</td>
<td>85.7</td>
<td>94.0</td>
<td>100.0</td>
<td>106.7</td>
<td>99.5</td>
</tr>
<tr>
<td>White wine 2</td>
<td>8.5</td>
<td>104.0</td>
<td>99.0</td>
<td>100.0</td>
<td>99.0</td>
</tr>
<tr>
<td>White Wine 3</td>
<td>7.3</td>
<td>102.0</td>
<td>94.0</td>
<td>95.3</td>
<td>102.5</td>
</tr>
<tr>
<td>Red Wine 1</td>
<td>58.8</td>
<td>99.0</td>
<td>95.0</td>
<td>94.7</td>
<td>101.5</td>
</tr>
<tr>
<td>Red Wine 2</td>
<td>9.3</td>
<td>96.0</td>
<td>96.0</td>
<td>98.7</td>
<td>103.0</td>
</tr>
</tbody>
</table>

TABLE 3. Determination of Sorbic Acid in Wines

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sorbic Acid/µg mL⁻¹</th>
<th>Proposed Method</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Wine 1</td>
<td>85.7 ± 0.6</td>
<td>85.8 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>White Wine 2</td>
<td>8.5 ± 0.5</td>
<td>8.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>White Wine 3</td>
<td>7.3 ± 1.0</td>
<td>8.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Red Wine 1</td>
<td>58.8 ± 1.3</td>
<td>58.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Red Wine 2</td>
<td>9.3 ± 0.3</td>
<td>8.8 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

this wavelength. Because the reaction proceeds slowly at room temperature, it must be performed at 100°C, but before measuring the reaction mixture, it must be cooled in an ice bath, rather than in cold water.

In all experiments described here, two standard solutions of 3 and 7 µg mL⁻¹ were used.

**Optimization of Chemical Variables**

The first chemical variable that was optimized was the concentration of thiobarbituric acid. This concentration varied between 0.4 and 0.8%. The absorbance increased as the thiobarbituric acid concentration increased, but when this concentration was higher than 0.5%, precipitation of the reagent appeared in the tubes. An optimal concentration of 0.5% was finally established.

The optimized concentration of oxidizing mixture was 0.01 N K₂Cr₂O₇ and 0.3 N H₂SO₄, the ratio 1:1 always remaining constant. The absorbance decreased at lower and higher concentrations of both reagents.

**Selection of FI Variables**

The effect of flow rates, reaction coil characteristics, and sample injection volume was studied. The reactors length were selected as a compromise between sensitivity, time of analysis, and pressure problems that resulted when the reactors were too long. The optimum values for R1, R2, and R3, respectively, were 200, 500, and 200 cm, with a internal diameter of the tubes of 0.5 mm.

The optimized sample injection volume was 225 µL. The flow rates of the reagents and sample were optimized only by variation of the peristaltic pump speed because the reagent concentrations and sample injection volume were already determined. These flow rates varied between 0.42 and 1.40 mL min⁻¹. Pressure problems resulted from flow rates higher than 0.7 mL min⁻¹, so a flow rate of 0.7 mL min⁻¹ was chosen for all streams.

Under these final experimental conditions, a sampling rate of 40 samples per hour was achieved.

**Calibration Graph, Sensitivity, and Precision**

Under the optimum experimental conditions, a linear calibration graph was obtained from 0 to at least 15 µg mL⁻¹. The figures of merit and the calibration graph are shown in Figure 2. The detection and determination limits, defined as the concentration of analyte that gave signals equivalent to three and ten times the standard deviation respectively of the calibration graph intercept were 0.14 µg mL⁻¹ and 0.46 µg mL⁻¹, respectively. The relative standard deviation of ten replicate determinations of 6 µg mL⁻¹ of sorbic acid was 1.58%.

**Interferences**

The effects of ethanol and a number of common organic acids in wines were assessed, and the results obtained are given in Table 1. The criterion for an interference was an absorbance varying by ±6% from the expected value.

As can be seen, the tolerance limits found show that sorbic acid can be determined in the presence of a variety of species commonly present in wines.

The usefulness of the method in this type of matrix was further corroborated by a recovery study in-
volving the addition of 12.5, 25.0, 37.5, and 50.0 µg mL\(^{-1}\) of sorbic acid to wine samples. The recoveries obtained are given in Table 2; the average recovery is 99.1% with an average deviation of ±3.6%.

**Determination of Sorbic Acid in Wines**

The sorbic acid contents of various white and red wines were determined and the results checked by HPLC [13]. The results, given in Table 3 as the average of four separate determinations, show close agreement between the proposed method and the reference method.

**CONCLUSION**

A simple, rapid, and accurate spectrophotometric FI method was developed for the determination of sorbic acid in wine samples. The procedure is not subject to interference from many substances commonly found in wines and in other drink samples, such as ethanol and benzoic acid. Moreover, it can be used for the analysis of intensely colored samples, such as red wines, without steam distillation. Therefore, compared with the official spectrophotometric methods, this method is more selective, convenient, and rapid.

**REFERENCES**