UHPLC Analysis of Sorbate and Benzoate in Alcoholic Beverages

Scope and Application

Sorbates and Benzoates are typically added to alcoholic beverages as preservatives.

Regulatory Tolerances:

Sorbate: may be added to wines in the acid form or as a potassium salt. The finished wine shall not contain more than 300 mg of sorbic acid per liter of wine[1]. Sorbic acid, potassium sorbate, calcium sorbate, and sodium sorbate are approved as GRAS (Generally Recognized as Safe) when used in accordance with good manufacturing practice[2].

Benzoate: may be added to prevent fermentation of the sugar in wine being accumulated as distilling material[1] in the acid form, potassium salt, or sodium salt. Benzoic acid and sodium benzoate are approved as GRAS for use at a level not exceeding 0.1 percent (w/v) in accordance with good manufacturing practice[2].

Levels and Limitations

Analyte	Detection Limit (mg/L)	Quantitation Limit (mg/L)	Linear Range (mg/L)	Interferences
Sorbic Acid	0.4	1.0	***5.0*** – 1***00***.0	
Benzoic Acid	0.4	1.0	***5***.0 – 1***00***.0	saccharin

The samples are diluted at least 1 to 5 to reduce the effects of the matrix on the chromatography.

Supplemental Documents

BAL:Form:309-1 Sorbate & Benzoate LCS Control Chart for Waters Acquity UPLC

Equipment

Instrumentation:

Liquid chromatography system: Waters Acquity UPLC with a Photodiode Array (PDA) detector or equivalent system
Column : Waters BEH C18, 1.7 µm particle size, 2.1 x 50 mm or equivalent column
PH meter
Analytical Balance

Glassware and Supplies:

Class A pipets and volumetric flasks
Pipettors: 5000 µL, 1000µL.
2 mL autosampler LC vials
0.45 µm teflon or nylon syringe filters (without GMF)
Disposable syringes

Reagent, Calibrants, and Buffer Solutions

Reagents

1) Deionized Water: 18 megaΩ or better
2) Methanol (CAS# 67-56-1; HPLC grade or high degree of purity)
3) Acetic Acid (CAS# 64-19-7; ≥99.0% or higher degree of purity)
4) Ammonium Acetate (CAS# 631-61-8; ≥99.0% or higher degree of purity)
5) Benzoic Acid (CAS# 65-85-0; ≥99.0% or higher degree of purity)
6) Sorbic Acid (CAS# 110-44-1; ≥99.0% or higher degree of purity)
7) Ethanol (CAS# 64-17-5; 200 proof)
8) 5% (v/v) Ethanol solution

Calibrants

1) **Stock Standards**- Prepare the standards stock solution consisting of 100 mg/L sorbic acid and benzoic acid. Store the stock solutions in the refrigerator for up to 12 months.

   Standards Stock solution: Weigh out 0.0200 (±.0005) g of sorbic acid and benzoic acid [Note: account for purity if the compound is not 100%], add to a 200mL volumetric flask and Q.S. with 5% ethanol.

2) **Working Standards**- Prepare the working standards as outlined below. Store the working standards in the refrigerator for up to 12 months.

   Level 3 (100.0 mg/L Sorbic and Benzoic Acid): The Standards Stock Solution is equivalent to the Level 3 standard.

   Level 2 (50.0 mg/L Sorbic and Benzoic Acid): Pipet 50.0 mL of the Standards Stock Solution into a 100 mL volumetric flask. Q.S. with 5% ethanol.

   Level 1 (5.0 mg/L Sorbic and Benzoic Acid): Pipet 5.0 mL of the Standards Stock Solution into a 100 mL volumetric flask. Q.S. with 5% ethanol.

Buffer

Ammonium Acetate Buffer, 10 mMol, pH 5.5:

1) Fill a 500mL volumetric flask about 85-95% full with DI water and then empty the contents into an appropriate sized beaker.
2) Pipet 0.05 mL of acetic acid into the beaker.

3) Using a pH meter add ammonium acetate until the solution reaches a pH of 5.5.
4) Transfer the solution into a 500mL volumetric flask.
5) Use two or three aliquots of water to rinse out the beaker into the volumetric flask.
6) Q.S with DI water and mix thoroughly.
7) The buffer may be used for up to one week.

Procedures

1. Sample Preparation:
   i. Dilute sample at least 1:5 using DI water. The concentration of sorbate or benzoate is to be lower than the highest standard, 100 mg/L.
      a. For cream based alcoholic samples dilute the sample at least 1:5 with methanol and centrifuge the sample to remove the particulate.
   ii. Filter all samples, using at least a 0.45 µm syringe filter*, into a HPLC autosampler vial. * Use either a Teflon or Nylon (no GMF) filter with no pre-filters.

2. LC/PDA Operating Procedures:
   Analyze the standards and samples in a LC/DAD with the following parameters
   i. Organic Phase: ethanol
   ii. Aqueous Phase: 10mMol Acetate buffer pH 5.5
   iii. Detector: Quantify at wavelength 230nm. (the spectrum may be used for peak purity calculations)
   iv. Column Temperature: 40ºC
   v. Flow: 0.5 mL/min
   vi. Injection volume: 2 µL
   vii. Mobile phase gradient: (Optimized for Waters Acquity LC system, changes to the gradient may be necessary to optimize conditions on another UHPLC)

Mobile Phase Gradient Table

Time (min)	%A ***Acetate Buffer***	%B ***Methanol***	Gradient Slope
0.0	***98***	***2***	6 (linear)
3.00	***70***	***30***	6
4.00	***70***	***30***	6
5.00	***98***	***2***	6

Quality Control

1. The order of the sequence should be
   1) Blank

2) Working Standards
3) Blank
4) Up to 15 samples
5) Blank
6) 2nd Level Check

2. The Laboratory Control Sample (LCS) is either a characterized sample or is prepared by spiking a beverage alcohol sample at 200 mg/L with sorbic and benzoic acid. Store the LCS in the refrigerator. The LCS is treated as a sample and undergoes the sample preparation process as the other samples.
3. The LCS or a sample should be run in duplicate, as per laboratory policy.
4. The linearity of the calibration curves is to be greater than 0.99.
5. If the standard curve or the control samples are not within specifications then attempt tradition LC troubleshooting techniques and rerun the sequence.
6. Re-run the standards after every 15 (or less) samples in the sequence.

Sources of Uncertainty

1. Pipetting errors (especially cream liqueur samples)
2. Coeluting compounds (saccharin)
3. Column degradation

Calculations

The elution order of the compounds in the standards is as follows: benzoic acid and sorbic acid

The calibration curves are determined from the peak areas of the 3 standards at 230nm.

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
Sorbic Acid	All	mg/L (ppm)	Nearest whole #	XX
Benzoic Acid	All	mg/L (ppm)	Nearest whole #	XX

Safety Notes

UHPLC waste is to be disposed of in the appropriately labeled containers.

References

1. Code of Federal Regulations 27CFR 24.246, 24.247
2. Code of Federal Regulations: 21CFR 182.3089, 182.3225, 182.3640, 182.3795, 184.1021, 184.1733
3. *Application Note: Waters Acquity UPLC for the rapid analysis of soft drinks.* Waters Corporation, 34 Maple St. Milford, Ma 01757. 2004.

Required Training and Demonstration of Competence

1. Receive in house UHPLC training.
2. Initial certification is achieved by running 7 LCS replicates with results of precision and accuracy in agreement wit***h laboratory established values.***
3. Periodically, chemist are retested for competency (e.g. every 5 years) and/or given proficiency test.

Revision History

Rev. 1 – Added words “nylon” and “without GMF” to syringe filters Glassware and Supplies section; To Sample Preparation ii, added word “nylon” and “without GMF” filter description. Added “or equivalent” to column type (3/15/2013)
Rev. 2 – Changed linearity from 2.0 to 125.0 to 5.0 to 100.0. Removed bracket calibration. Added “with laboratory established values” for the requirements for initial certification.