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Organic Acids Determination in Alcoholic Beverages by Capillary Electrophoresis

Scope and Application

This method was developed to determine the concentration of organic acids found in alcoholic beverages using capillary electrophoresis (CE) with indirect UV detection. The analytes are tartaric acid, malic acid, citric acid, succinic acid, lactic acid and gluconic acid. Many acids such as tartaric, malic and citric acids occur naturally in fruits and wines. Others such as succinic and lactic acids result from fermentation. For enforcement cases, the method may assist in classifying sample products for tax purposes. The method may also be used to determine the use rate of flavors for 5010 tax credit samples. In addition, the method may be used to determine the amount of citric acid in unflavored vodkas for pre-COLA and ABSP samples.
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Regulatory Tolerances:

Refer to 27 CFR 24.182 for the use of various organic acids to correct natural deficiencies in producing wine. The TTB limit for unflavored vodkas is not more than 1000 ppm citric acid.

Levels and Limitations

Analyte	Detection Limit (mg/L or ppm)	Quantitation Limit (mg/L or ppm)	Linear Range (mg/L or ppm)	Interferences
All	~1 – 2.5 (except Citric)	5	5 – 320	None

Organic acid concentrations tend to be at various concentrations in alcoholic beverages. Therefore, starting dilutions of 1:5 and 1:100 of each sample are recommended.

Supplemental Documents

Organic Acids Solution Kit for the Agilent 7100 CE System Technical Note (Agilent Part No. 5968-9047E)

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Equipment

Instrumentation:
Capillary electrophoresis with UV detection (Agilent 7100 CE or equivalent)
Bare Fused-Silica Capillary, 75 µm i.d., l=72 cm, L=80.5 cm (Agilent Part No. G1600-62311* or equivalent)
Analytical Balance, 0.0001 g resolution
Vortex (optional)

*Note: The capillary used for this method may be purchased separately or as part of the Organic Acids Solution Kit (Agilent Part No. 5063-6510).

Glassware and Supplies:
CE vials, 2mL Glass (Agilent Part No. 5181-3375 or equivalent)
CE caps, polyurethane (Agilent Part No. 5181-1512 or equivalent)
Automatic pipets (various sizes)
15-mL and 50-mL conical tubes
Class A volumetric flasks (various sizes)
Plastic or glass transfer pipets
Plastic bottles to store standards (optional)

Reagents, Standard and Sample Preparations

Reagents: (Specific vendors and product numbers are listed for convenience. Other vendors may be also used.)

Organic acids buffer, pH 5.6 (Agilent Part No. 8500-6785*)
CE ultra-pure water (Agilent Part No. 5062-8578*) or Deionized (DI) water
Organic acids (Sigma-Aldrich, at ≥ 98% purity) – Used for primary standard solutions
Organic Acids Test Mixture (Agilent Part No. 8500-6900*) which contains 1000 ppm each of succinic, malic and lactic acids – Used for secondary source standard solution

*Note: Item may be purchased separately or as part of the Organic Acids Solution Kit (Agilent Part No. 5063-6510).

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Preparation of Primary Standard Solutions:

Note: *Other weights and volumes than indicated below for preparing the stocks and calibration standards may be used as long as the final concentrations are the same.*

Individual Stock Standards: 10,000 ppm Stock Solutions

Prepare a 10,000 ppm stock standard solution of each organic acid. For example:

1. Weigh approximately 1.0g of each compound into individual 100mL volumetric flasks and record exact weight. [Alternatively, if only citric acid analysis is performed, weigh approximately 1.0g of citric acid into a 100mL volumetric flask and record exact weight.]
2. Dilute the contents to volume with CE ultra-pure water or DI water and mix well. Sonication may be used to aid mixing.
3. Each solution may be transferred to a plastic bottle for storage.
4. Account for exact weights and purity to calculate exact concentration as ppm.

The shelf-life of the individual stock standards is 1 year stored refrigerated.

Preparation of 500 ppm Mixed Stock Standard:

Prepare a 500 ppm mixed stock standard containing each organic acid. For example:

1. Pipet 1250µL of each 10,000 ppm individual stock standard into the same 25-mL volumetric flask or into the same 50-mL conical tube. [Alternatively, if only citric acid analysis is performed, pipet 1250µL of the 10,000ppm citric acid stock standard into a 25-mL volumetric or into a 50-mL conical tube.]
2. Dilute the contents to volume with CE ultra-pure water or DI water, or add the appropriate amount of CE ultra-pure water or DI water to the conical tube to generate a final volume of 25mL. Mix well.
3. The solution may be transferred to a plastic bottle for storage.

The shelf-life of the mixed stock solution is 1 year stored refrigerated.

Preparation of 5, 10, 20 and 40 ppm Calibration Standards:

Prepare 4 calibration standards at the above concentrations using the 500 ppm mixed stock standard. For example:

1. 5 ppm: Pipet 50µL of 500 ppm mixed stock* into a 15-mL conical tube, add 4.95mL CE ultra-pure water or DI water. Mix well.
2. 10 ppm: Pipet 100µL of 500 ppm mixed stock* into a 15-mL conical tube, add 4.90mL CE ultra-pure water or DI water. Mix well.
3. 20 ppm: Pipet 200µL of 500 ppm mixed stock* into a 15-mL conical tube, add 4.80mL CE ultra-pure water or DI water. Mix well.
4. 40 ppm: Pipet 400µL of 500 ppm mixed stock* into a 15-mL conical tube, add 4.60mL CE ultra-pure water or DI water. Mix well.

The shelf-life of the calibration standards is 1 month stored refrigerated.

*Note: For citric acid determination only, the 500ppm mixed stock standard or the 500ppm citric acid stock standard can be used to prepare the calibration standards.

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Preparation of 25ppm Second Source Standard:

Prepare a 25ppm second source standard using the purchased Organic Acids Test Mixture. For example: Pipet 125µL of 1000ppm Organic Acids Test Mixture into a 15-mL conical tube, add 4.875mL CE ultra-pure water or DI water. Mix well.

The shelf-life of the second source standard is 1 month stored refrigerated.

Laboratory Control Sample (LCS) Preparation:

1. Spike a vodka (unflavored) with 100 ppm of each organic acid using the mixed 500 ppm stock solution. For example, pipet 2.0mL of the mixed 500 ppm stock solution into a 15-mL conical tube and add 8.0 mL of vodka. Mix well.
2. Dilute 1:100 in duplicate. For example, pipet 20µL of the spiked vodka solution into a two 15-mL conical tubes and add 1980µL CE ultra-pure water or DI water to each tube. Mix well.

Sample Preparation:

Since the organic acid concentrations in alcoholic samples are typically unknown, starting dilutions of 1:5 and 1:100 are recommended. For example:

1. 1:5 Dilution – Pipet 400µL sample into a 15-mL conical tube and add 1600µL CE ultra-pure water or DI water. Mix well.
2. 1:100 Dilution – Pipet 20µL sample into a 15-mL conical tube and add 1980µL CE ultra-pure water or DI water. Mix well.

Note: Sample analysis should be performed as soon as possible after dilution. Otherwise, solutions should be stored refrigerated until the time for analysis.

CE Vial Preparation (for Agilent 7100 CE or equivalent):

1. Add approximately 1.5 to 2mL organic acids buffer to a vial (flush vial).
2. Pipet 300µL CE ultra-pure water or DI water to a vial (waste vial).
3. Add approximately 1.5mL organic acids buffer to each inlet and outlet vial.
4. Add approximately 1 to 1.2mL CE ultra-pure water or DI water (blank), standard or sample to a vial.

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Procedures

Analyze the standards and samples on the CE instrument using the following parameters:

CE Operating Conditions:

CE Parameter	Setting
Replenish Execution Mode: Use Electric:	Parallel Yes
Cassette Temperature Mode: Temperature	Temperature set 20.0 °C
Pressure Mode: Pressure:	Off 2
Inlet Vial & Outlet Vial:	3 & 4 (for Instrument Method BAL_CE3) 5 & 6 (for Instrument Method BAL_CE4) 7 & 8 (for Instrument Method BAL_CE5) 14 & 15 (for Instrument Method BAL_CE6)
Power:	6.0 W
Voltage:	-25.0 KV
Current:	100 µA
Current Lower Limit Alarm Mode:	Limit set
Current Lower Limit:	2 µA
Preconditioning Program Function: Flush:	 240s (Inlet: 1 Outlet: 2)
Injection Program Function: Apply Pressure: Apply Pressure:	 50mbar for 2s (Inlet: Injection Vial; Outlet: Outlet Home Vial) 50mbar for 4s (Inlet: 3 Outlet: Home Vial)
Postconditioning Program: Stop Time Stop Time Mode: Stop Time: Post Time Post Time Mode:	 Time set 10.00 min Off
Instrument Curves Store Leak Current: Store Internal Pressure: Store Replenish Level Pressure: Store Vacuum: Store Injection Pressure: Store External Pressure: Store Tray Temperature: Store Cassette Temperature:	 No No No No No No No No

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DAD Operating Conditions:

DAD Parameter	Setting
Peakwidth: UV Lamp Required:	>0.05 min (1.0 s response time) (5 Hz) Yes
Analog Output1 Analog 1 Zero Offset: Analog 1 Attenuation:	 5% 1000 mAU
Signals Signal Table: Use Signal: Signal: Wavelength (nm): Bandwidth (nm): Use Reference: Reference Wavelength (nm): Reference Bandwidth (nm):	 Yes Signal A 350.0 20.0 Yes 200.0 10.0
Prepare Mode Margin for Negative Absorbance:	 100 mAU
Auto balance Auto balance Postrun: Auto balance Prerun:	 No Yes
Spectrum Spectrum Store: Spectrum Range WL from: Spectrum Range WL to: Spectrum Step:	 All 190.0 nm 400.0 nm 2.0 nm
Stop Time Stop Time Mode:	 As CE
Post Time Post Time Mode:	 Off
Instrument Curves Store UV Lamp Anode Voltage:	 No
Fraction Collection:	Off

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An example run sequence is given below:

Sample ID	**Sample Name**	**Example Instrument Method[1]**
Blank	CE Ultra-Pure Water	BAL_CE3
WSB-XX-0001	5ppm Standard	BAL_CE3
WSB-XX-0002	10ppm Standard	BAL_CE3
WSB-XX-0003	20ppm Standard	BAL_CE3
WSB-XX-0004	40ppm Standard	BAL_CE3
Blank	CE Ultra-Pure Water or DI Water	BAL_CE3
LCS-XX-001 #1 1:100	LCS #1	BAL_CE4
LCS-XX-001 #2 1:100	LCS #2	BAL_CE4
B-20XX-00100-001 1:100	Enforcement	BAL_CE4
B-20XX-00100-002 1:100	Enforcement	BAL_CE4
B-20XX-00100-003 1:100	Enforcement	BAL_CE4
B-20XX-00100-004 1:100	Enforcement	BAL_CE4
B-20XX-00100-001 1:5	Enforcement	BAL_CE4
B-20XX-00100-002 1:5	Enforcement	BAL_CE5
B-20XX-00100-003 1:5	Enforcement	BAL_CE5
B-20XX-00100-004 1:5	Enforcement	BAL_CE5
Blank	CE Ultra-Pure Water or DI Water	BAL_CE5
NLC-20XX-005	25ppm Second Source Standard	BAL_CE5
Blank	CE Ultra-Pure Water or DI Water	BAL_CE5

[1]Each instrument method in the sequence table above includes a different set of inlet and outlet vials for the run buffer due to its limited buffering capacity after 5 to 7 injections. Therefore, perform no more than 5 to 7 injections using each set of inlet and outlet vials.

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Quality Control

1. Analyze the LCS in duplicate. Control limits for the LCS will be established when sufficient data has been obtained.
2. Re-run the second source standard (25 ppm) as a check standard at least once after every 10 sample injections (including the LCS) as a control to check for drift. Control limits for the check standard will be established when sufficient data has been obtained.
3. Determine the linearity of the curve for each organic acid. The correlation coefficient for each organic acid should be ≥ 0.99.

Sources of Uncertainty

1. Sample preparation and/or dilution errors
2. Capillary degradation
3. Loss in CE buffer capacity

Calculations

1. Integrate each organic acid peak in the standard electropherograms. The instrument software can be used to generate linear equations using the area counts of the standard solutions.
2. Integrate each organic acid peak in the sample electropherograms. The instrument software can be used to calculate each organic acid amount using the area count of the organic acid peak.
3. Multiply this amount by the dilution factor to obtain the final organic acid concentration in ppm. Alternatively, the final organic acid concentration in the samples may also be calculated in LIMS.

Reporting Results

Report results in ppm to the nearest whole number.

Safety Notes

1. Consult the MSDS for any chemicals used that are unfamiliar. All chemicals shall be considered hazardous – avoid direct physical contact.
2. CE waste is to be disposed of in the appropriately labeled containers.
3. The instrument is operated at high voltage. Do not touch any parts of the instrument that are unfamiliar. Do not attempt any repairs if untrained to do so.

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References

Organic Acids Solution Kit for the Agilent 7100 CE System Technical Note (Agilent Part No. 5968-9047E)

High Performance Capillary Electrophoresis – An Introduction, Agilent Technologies

Required Training and Demonstration of Competence

1. Receive in-house CE training.

2. Initial certification is achieved by running 7 LCS replicates with acceptable accuracy and precision results or successful proficiency test.

3. Periodically, chemist are retested for competency (e.g. every 5 years) and/or given a proficiency test.

Revision History

Rev. 1 – Initial revision

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