Cyanide (Prussic Acid) by Ion-Chromatography

Scope and Application

This method will be used to detect the presence of cyanide (prussic acid) in stone fruit (for example, cherry, plum, peach, almond) alcohol beverage products using ion chromatography with pulsed amperometric detection (PAD). This method determines prussic acid from 5 - 100 ppb on column. When samples are diluted 100x, this is 0.5 – 10.0 ppm in wine, malt beverage and distilled spirit products.

Regulatory Tolerances:

FDA lists tolerances for cherry, almond or elder tree products used in flavoring alcohol beverages not to exceed 25 ppm prussic acid (21CFR 172.510). TTB's tolerance (27CFR 24.246) is set not to exceed 1 ppm (1000 ppb) for finished wine when ferrocyanide compounds are used as wine processing aid. No tolerance has been set for distilled spirit or malt beverage products.

Levels and Limitations

Analyte	Detection Limit (on column)	Quantitation Limit (on column)	Linear Range (on column)	Calibration Range (on column)	Interferences	Instrument
Cyanide	1.0 ppb	5 ppb	5 - 110 ppb	5 – 100 ppb	matrix	ICS 6000 or equivalent

Supplemental Documents

Equipment

Instrumentation and Run Conditions:

Equivalent instrumentation may be used.

Ion Chromatograph:	Dionex ICS 6000 with EG
Detector:	Electrochemical Detector DC5
Electrode:	Ag (conventional)
Autosampler:	AS-AP
Injection Volume:	80 µL
Temperature Control: Compartment:	DC
Column Temperature:	30ºC
Gradient Pump: Flow Rate:	SP / EG with LiOH cartridge 1.0 mL/min
Mobile Phases:	63 mM (auto generated)
Gradient:	Isocratic
Run Time:	8 min
Software:	Chromeleon
Column and guard column	AS-15 4mm x 250 mm with Dionex IonPac AG15 guard

Analytical balance Mettler Toledo AG204, or equivalent

Glassware and Supplies:

2 mL autosampler vials and slit top caps
Class A volumetric flasks1 L, or as needed
Class A volumetric pipets as needed
Autopipettor capable of delivering up to 1000 µL or as needed
Polishing pad (Thermofisher #036321)

Reagent and Sample Preparation and Handling

Reagents and Standards: (Specific vendors are listed for convenience. Equivalent products may be used.)

Deionized/ MilliQ (MQ) water
Ethyl Alcohol, 200 proof, (CAS 64-17-5)
Lithium hydroxide cartridge, ThermoFisher #074534
0.1N NaOH Acros, reagent grade (CAS 1310-73-2)
Potassium cyanide, Acros >97% (CAS 151-50-8)-may be used as secondary standard when commercially prepared is unavailable
Commercially prepared certified cyanide standard diluted in water (Ex. Inorganic Ventures)
Secondary ISO Guide 34 certified cyanide standard diluted in water (Ex. Sigma)

Preparation of Solutions:

Note: Weights and volumes may be adjusted as needed; however, ensure that the ratios remain the same

40% Ethyl Alcohol (EtOH) solution (Stable for 6 months):
Add 400 mL 200 proof ethyl alcohol into a 1 L volumetric flask and bring to volume with MQ water.

Preparation of Stock and Working Standards:

Note: Weights and flask sizes may be adjusted as needed; however, ensure that the ratio of weight to volume remains the same.

Stock standard 1000 µg/mL CN-stability will depend on manufacturer date (stable for 6 months when prepared in house):
When using KCN as stock standard, molecular weights are used as a conversion factor to CN: = (26 g CN = 65.1 g KCN)

Weigh 0.251 g KCN into a 100 mL volumetric flask containing approximately 50 mL 40% EtOH, add 0.05 mL of 0.1 N NaOH (or 1 drop using disposable transfer pipet) to stabilize stock standard. Dissolve and bring to volume with 40% EtOH. This will be equivalent to 1000 µg/mL (ppm) cyanide.

Working standards (prepare fresh daily):

1. Intermediate Level 1: 100 ppb
   Transfer 100 µL 1000 ppm stock standard into a 1 L volumetric flask containing 500 mL MQ water. QS to volume with MQ water.

2. Level 2: 50 ppb

Transfer 50 µL of 1000 ppm stock standard into a 1 L volumetric flask containing 500 mL MQ water. QS to volume with MQ water.

3. Level 3: 10 ppb

Transfer 1 mL of Level 1 into a 10 mL volumetric flask containing MQ water. QS to volume with MQ water.

4. Level 4: 5 ppb

Transfer 1 mL of level 2 into a 10 mL volumetric flask containing MQ water. QS to volume with MQ water.

5. Intermediate check standard: 50 ppb (prepare fresh daily) Prepare 50 ppb intermediate check standard as described in (2) above preferably from secondary 1000 µg/mL stock standard.

Preparation of Unspiked and Spiked Samples

A characterized LCS has not been established; therefore a minimum of 1 test sample must be spiked to establish reliability of data. Prepare unspiked and spiked samples daily.

Note: TheBAL's wine LCS can be used to prepare unspiked and spiked samples.

For the unspiked sample:

In a 100 mL volumetric flask containing 25 mL MQ water, transfer 1 mL of sample and QS to volume with MQ water.

For the spiked sample:

In a 100 mL volumetric flask containing 25 mL MQ water, transfer 1 mL of sample +10 mL of 100 ppb working standard and QS to volume with MQ water.

This gives 10 ppb cyanide on column for the fortified sample, equivalent to a 1 ppm beverage spike. Determine % recovery against the same unspiked sample using the following calculation:

$$\% \text{ recovery} = \frac{(\text{amount in spiked sample}) - (\text{amount in unspiked sample})}{\text{Amount spiked}} \times 100$$

Preparation of Samples:

Dilute all samples at least 1→100 with MQ water prior to injecting; prepare daily. Degas carbonated samples as needed.

In a 100 mL volumetric flask containing 25 mL MQ water, transfer 1 mL of sample and QS to volume with MQ water.

Procedures

Refer to instrument WG for details

1. Polish Ag working electrode using polishing pad and a few drops of MQ water.
2. Equilibrate column in recommended mobile phase for a minimum 45 min at recommended flow rate.
3. Transfer, blank (MQ water), standards and samples into autosampler vials and cap.
4. Inject a water blank and one working standard to confirm steady baseline, peak shape and retention time. An example of a typical sequence is provided below:

Injection Details				
No.	Injection Name	Position	Type	Level
1	Blank	RA1	Unknown	
2	100 ppb Std (Test)	RA2	Unknown	
3	100 ppb Std	RA2	Calibration Standard	1
4	50 ppb Std	RA3	Calibration Standard	2
5	10 ppb Std	RA4	Calibration Standard	3
6	5 ppb Std	RA5	Calibration Standard	4
7	Blank	RA1	Unknown	
8	50 ppb (Second Source Std)	RA6	Unknown	
9	Blank	RA1	Unknown	
10	Unspiked Sample	RA7	Unknown	
11	Blank	RA1	Unknown	
12	Spiked Sample	RA8	Unknown	
13	Blank	RA1	Unknown	
14	Sample (up to 10)	RB1-RB8	Unknown	
15	Blank	RA1	Unknown	
16	50 ppb (Second Source Std)	RA6	Unknown	
17	Blank	RA1	Unknown	

5. The spiked sample is treated as a sample in the sequence.
6. Use instrument software to establish calibration curve. Linearity is to be >0.99.
7. Rerun check standard and samples (after re-polishing electrode) if chromatogram is poorly resolved.
8. Rerun intermediate check standard after every 10 (or less) samples.

Quality Control

1. Ensure flat baseline. Inject intermediate check standard (50ppb concentration) in duplicate to confirm reproducibility and stability of standards. The measured value must be within 15% of the expected value or within statistically derived control limits.

2. Spike sample recovery after correcting for unspiked amount must be within 80-120%. The spike recovery value (%) is recorded in the laboratory logbook.

3. If sample results are above the calibration range, re-dilute the beverage to within range using a larger dilution factor and re-analyze.

Sources of Uncertainty

1. Standards prepared incorrectly.
2. Poor sensitivity due to fouled electrode-polish and re-inject affected standards and samples.
3. Samples not diluted appropriately.

Calculations

Use the software to calculate ppb cyanide. Correct for dilution factor (DF).

ppm cyanide (prussic acid) = (ppb*DF)/1000

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
Cyanide	Wine, Malt Bev, DSP	ppm	1 decimal	X.x

Safety Notes

- Use extreme caution when preparing and dispensing stock standard. Wear personal protective gear (Lab coat, gloves, and safety goggles). Keep stock standard in basic solution (0.1 N NaOH) to minimize volatility.

- Work in hood when handling stock standard.

- Keep all containers tightly closed.

- Dispose stock and working standard per laboratory guidelines.

- IC waste is to be disposed per laboratory guidelines.

References

Code of Federal Regulations. (2014). 27CFR§24.246 and 21CFR§172.510.

Dionex Application Note 173. (n.d.). Direct determination of cyanide in drinking water by ion chromatography with pulsed Amperometric detection (PAD). Retrieved from http://www.dionex.com

Hill, N. R., Wesemann, J. L., & Simonyi, K. (n.d.). Cyanide in stone fruit brandies/liquers by ion chromatography with electrochemical detection. *TTB (ATF) Internal Document.*

In-house instrument WG.

Required Training, Certification and Re-certification

1. Receive in house IC training.
2. Initial certification is achieved by running 7 LCS (or spike sample) replicates with results of precision and accuracy in agreement with the results of the validation package
3. Periodically, chemist are retested for competency (e.g. every 5 years) and/or proficiency test.

Revision History

Rev. 1 – Initial Revision
Rev. 2 - Extensive Revision
Rev. 3 – Updated with current instrumentation
Rev. 4 – Updated with ICS 6000, added unspiked sample preparation and sequence example.
Rev. 5 – Added note to use BAL's wine LCS to prepare unspiked and spiked samples. Deleted CL's reference. Clarified preparation of samples (sample dilution and decarbonation as needed).