

Method: ACEN-23 Revision: 5 Final Revision Date: 11/19/03	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 1 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

METHOD SUMMARY

The trace impurities listed below are separated and detected using a gas chromatograph with an FID detector.

	<u>Quantitation Limit (ppmw)</u>
Acetone	< 10
Acrylonitrile	< 3
Allyl Alcohol	< 10
Benzene	< 3
cis-Crotonitrile	< 3
trans-Crotonitrile	< 3
Methacrylonitrile	< 10
<u>Methanol</u>	<u>< 15</u>
Oxazole	< 3
Propionitrile	< 5
Pyridine	< 3

These limits are guidelines only. They may be different, depending on instrument sensitivity.

SAFETY

Acetonitrile is hazardous to the health and dangerous to handle. Use acetonitrile in a well ventilated hood. Review the MSDS for detailed information concerning toxicity, first aid procedures and safety precautions.

Refer to the appropriate safety section or site manual for the necessary protective equipment to use when handling any reagents or samples.

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Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 2 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

APPARATUS AND REAGENTS

1. **Carrier Gas** - Helium minimum purity 99.995 Mol. %.
2. **Hydrogen** - high purity [CAS 1333-74-0].
3. **Air** - Hydrogen free, high purity.
4. **Balance**, analytical, sensitive to ± 0.1 mg.
5. **Serum Bottles**: 50 mL capacity with crimp-top caps and TFE/silicone septa
6. **Syringe**, 10 μ l. For autosampler or manual injection.
7. **Disposable Syringes**: 1 cc capacity.
8. **Crimper and Decapper**: for serum vials.
9. **Vials**: 2 ml autosampler with snap-on septa/lids.
10. **Syringes**: gas tight glass, 100 and 500 μ l capacity (SGE Cat No. 005200 and 007200).
11. **Water**: ASTM type II or equivalent.
12. **Gas Chromatograph** - HP-5890 with an FID and split injection system or equivalent.
13. **Column**: 90 to 120m, 0.53 mm ID, 3.0 micron film of Rtx -1 Other columns may be used after it has been established that such columns are capable of separating all major impurities and the internal standard from the acetonitrile under operating conditions appropriate for the column.
14. **Integrator** - HP-3396A or equivalent data acquisition system.
15. **Stock Reagents**: Known purity acetone, acetonitrile, acrylonitrile, allyl alcohol, benzene, crotononitrile, isobutanol, methacrylonitrile, methanol, oxazole, propionitrile and pyridine.

CALIBRATION

Calibration standards are made by adding known amounts of acetone, acrylonitrile, allyl alcohol, benzene, crotononitrile, isobutanol, methacrylonitrile, methanol, oxazole, propionitrile and pyridine to low impurity acetonitrile. These standards are analyzed on the gas chromatograph to get component area counts. Calibration factors are generated for the components.

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 3 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 4 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

CALIBRATION PROCEDURE

NOTE:List all reagent lot numbers, concentrations, the balance used for weighing and the name or initials of calibrator on an appropriate calibration/response factor log document.

Stock Acetonitrile

1. Collect 2 liters of high purity (stock) acetonitrile from a suitable high quality source.
2. Inject the stock acetonitrile 3 times using the gas chromatograph (GC) that you are calibrating.
3. Determine the average area count (AC) for each known impurity. These values will be needed below in step 13 of Calibration Standards to calculate the total concentrations of compounds in the standards made with this acetonitrile.

Stock Standard

1. Purchase the following known purity reagents: acetone, acrylonitrile, allyl alcohol, benzene, crotononitrile, isobutanol, methacrylonitrile, oxazole, propionitrile, methanol and pyridine.
2. Determine the cis- and trans-crotononitrile percentages by injecting small amount of the mixture (a needle full of a 10 ul syringe may work (about 0.6 ul)), then measure the peak areas and calculate the relative percentage of each.
3. Tare a 50 mL serum bottle with septum.
4. Add 47 mL of stock acetonitrile to the bottle. Weigh to the nearest 0.1 mg and record as the initial weight.
5. Use the glass syringes to add the following amount of each component to the bottle.

<u>Component</u>	<u> </u> μ l <u> </u>
Acetone	50
Acrylonitrile	150
Allyl Alcohol	50
Benzene	50
Crotononitrile	250

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Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 5 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

Methacrylonitrile	250
Oxazole	50
Propionitrile	50
Methanol	50
Pyridine	50

6. Weigh the bottle after each addition and record the weight to the nearest 0.1 mg.
7. Cap the bottle and shake to mix.
8. Label the serum bottle with a title, preparation date, expiration date and preparer's initials.
9. Determine the individual component weight by subtracting the previous weight from the weight after a component is added.
10. Calculate the added concentration (mg/kg) of each component (comp.) as follows:

$$\text{Component Concentration (mg/kg)} = \frac{\text{Comp. Wt. (mg)}}{\text{Total Wt. (kg)}} \times \frac{\text{Comp. Purity (\%)}}{100}$$

Stock Internal Standard Preparation

1. Tare a 50 mL serum bottle with septum.
2. Add 50 mL of water to the bottle and record the weight to the nearest 0.1 mg (initial wt.).
3. With a 1 cc disposable syringe, add 0.25 mL of isobutanol to the bottle and record the weight to the nearest 0.1 mg (final wt.).
4. Cap the bottle and shake to mix.
5. Label the bottle with a title, preparation date, expiration date, and preparer's initials.
6. Calculate the isobutanol concentration (mg/kg) using the following formula:

Isobutanol Concentration =

$$\frac{\text{Final Wt. (gm)} - \text{Initial Wt. (gm)}}{\text{Final Wt. (gm)}} \times \frac{\text{Isobutanol purity (\%)} \times 10^6}{100}$$

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 6 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

Calibration Standards

1. Prepare three standards by diluting the stock standard with stock acetonitrile.
2. Tare a 50 mL serum bottle with septum.
3. Add about 48ml of stock acetonitrile to the bottle and weigh (all weights to the nearest 0.1 mg). This will be your Initial Wt.(A) for the internal standard calculation.
4. Use a 1 cc syringe to add 0.2 ml of the internal standard (prepared in the previous section) to the bottle. Record the weight (Final Wt.(A) for the internal standard calculation, Initial Wt.(B) for the component calculation).
5. Use a 1 cc syringe to transfer 0.25 cc of stock standard to the bottle and record the weight (Final Wt.(B) for the component calculation).
6. Cap the bottle and shake to mix.
7. Label each bottle with a title, preparation date, expiration date and preparer's initials.
8. Repeat steps 2 to 7 and make two more calibration standards by adding 0.5 and 1.0 cc of stock standard to stock acetonitrile.
9. Calculate the concentration (mg/kg) of each component added (including isobutanol) to each standard using the following formulas:

Internal Standard concentration =

$$\frac{\text{Final Wt. (A) (gm)} - \text{Initial Wt. (A) (gm)}}{\text{Final Wt. (B) (gm)}} \times \text{Isobutanol Concentration in Internal Std, mg/kg}$$

Component concentration =

$$\frac{\text{Final Wt. (B) (gm)} - \text{Initial Wt. (B) (gm)}}{\text{Final Wt. (B) (gm)}} \times \text{Component Concentration in Stock Std, mg/kg}$$

10. Analyze each calibration standard in triplicate (three injections of each standard). A chromatogram of a standard is attached as Figure 1.
11. Obtain area counts for the components and internal standard from integrator or chromatography data system.
12. Average the area counts (AC) for each component in the three injections of a standard.

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 7 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

- To compensate for the amount of each component in the stock acetonitrile used to dilute the standard, calculate the total component concentrations (mg/kg) by standard as follows:

Total Comp. Conc. (mg/kg) =

$$\frac{\text{Added Comp. Conc. mg/kg} \times \text{Avg AC Cal. Std}}{\text{Avg AC Cal Std} - \text{Avg AC Stock Aceto}}$$

- Enter the area counts and calculated total component concentrations into the data acquisition system calibration table. The data system will generate a calibration factor and curve for each component. To perform a manual calculation, refer to the Calculation section below.

Calibration Acceptance Criteria

- Calibration curve correlation coefficients should be better than 0.95. For most cleanly resolved analytes, the correlation coefficients will be better than 0.99.
- The response factors for each component from one calibration to the next should be within $\pm 10\%$ for the highly resolved components, and $\pm 20\%$ for acetone. If the response factor for a component is outside this range, then check the purity of the stock reagent by GC analysis and measure the water content by Karl Fisher analysis.

Preparation and Storage of Stock Standard Solutions

- Fresh stock acetonitrile will be used every time stock or calibration standards are prepared. However, if a very clean and pure acetonitrile sample is obtained from the unit, it will be stored and used to prepare standards for an indefinite period.
- Reagents will be kept no more than one year.
- Internal standard will not be kept for more than 6 months. After that time, the old standard will be discarded and new standard prepared.
- Calibration standards will not be kept for more than 6 months. After that time, the old standard will be discarded and a new standard prepared.

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 8 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

Calibration Frequency

1. The GC will be calibrated every 6 months with new standards, or more frequently as dictated by changes in the instrument configuration.
2. The GC calibration will be checked on a monthly basis, using a single injection, analyzing a calibration check sample that contains a minimum of two of the analytes. Acrylonitrile and propionitrile are good, stable candidates. The analyte and internal standard peak area counts, divided by their respective concentrations, in the check standard should be within 15% of the previous analysis of that check standard and the original calibration standard. If not, first troubleshoot the instrument (syringe, split ratio, septum leak, column connection leaks, detector gas flows, gas supply purity). If a re-analysis of the check standard still does not come into the accepted response range, then prepare a new check standard and analyze. If the area count/concentration values are still not within the appropriate tolerance, then perform a complete recalibration.
3. If anything unusual happens (such as an extended power outage, column change, etc.) a calibration check will be done prior to any sample analysis.

SAMPLE ANALYSIS PROCEDURE

Confirm that stable instrument conditions have been established as listed in Table 1. Add internal standard to the sample in the same manner as the Calibration Standards. Inject the sample and calculate the amount of each component.

CALCULATIONS

Component results are typically calculated by the chromatography data system. This data system calculation should be the equivalent of the following process:

1. Calculate a response factor for each component from the calibration:

$$RF = \frac{(\text{Peak area of component})}{(\text{Peak area ISTD})} \times \frac{(\text{Conc. ISTD})}{(\text{Conc. Component})}$$

where Conc. ISTD and Conc. Component are the concentrations of ISTD and component in the calibration standard solution.

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08	Organic Impurities By GC	Page 9 of 11
Next Review: 04/01/12		Reviewed by: Jennifer Young

2. Calculate the component concentration in the sample:

$$\text{Conc. Component} = \frac{(\text{Area component}) \times (\text{Conc. ISTD})}{(\text{Area ISTD}) \times \text{RF}}$$

REPORT

Impurities are report as ppmw, or as less than the quantitation limits listed in the Method Summary.

Acetone	<10 ppmw
Methacrylonitrile	150 ppmw
Acrylonitrile	25 ppmw
Propionitrile	15 ppmw
Benzene	8 ppmw
Oxazole	5 ppmw
c- crotonitrile	10 ppmw
t- crotonitrile	12 ppmw

Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08 Next Review: 04/01/12		Organic Impurities By GC

TABLE 1
Separation Conditions Using the Rtx-1 Capillary Column

Gas Chromatograph: HP-5890 with split injector, and flame ionization detector (FID), or equivalent.

Column: 90 to 120m X 0.53mm ID x 3.0 micron film of methyl silicone, preferred 105m column from Restek (part number 10189)

Injection Flows:

Column: 5 mL/min.

Split: 20 mL/min.

Septum purge: 4.0 mL/min.

Temperatures: Oven Program: Initial = 50 °C (hold 20 min.)

Rate 1 = 5 °C/min.

Final 1 = 100 °C (hold 0 min.)

Rate 2 = 15 °C/min.

Final 2 = 250 °C (hold 5 min.)

Injector: 250 °C

Detector: 280 °C

Detector Flows: Fuel Air: 360 mL/min.

Fuel Hydrogen: 33 mL/min.

Injection Size: 1 to 2 µL.

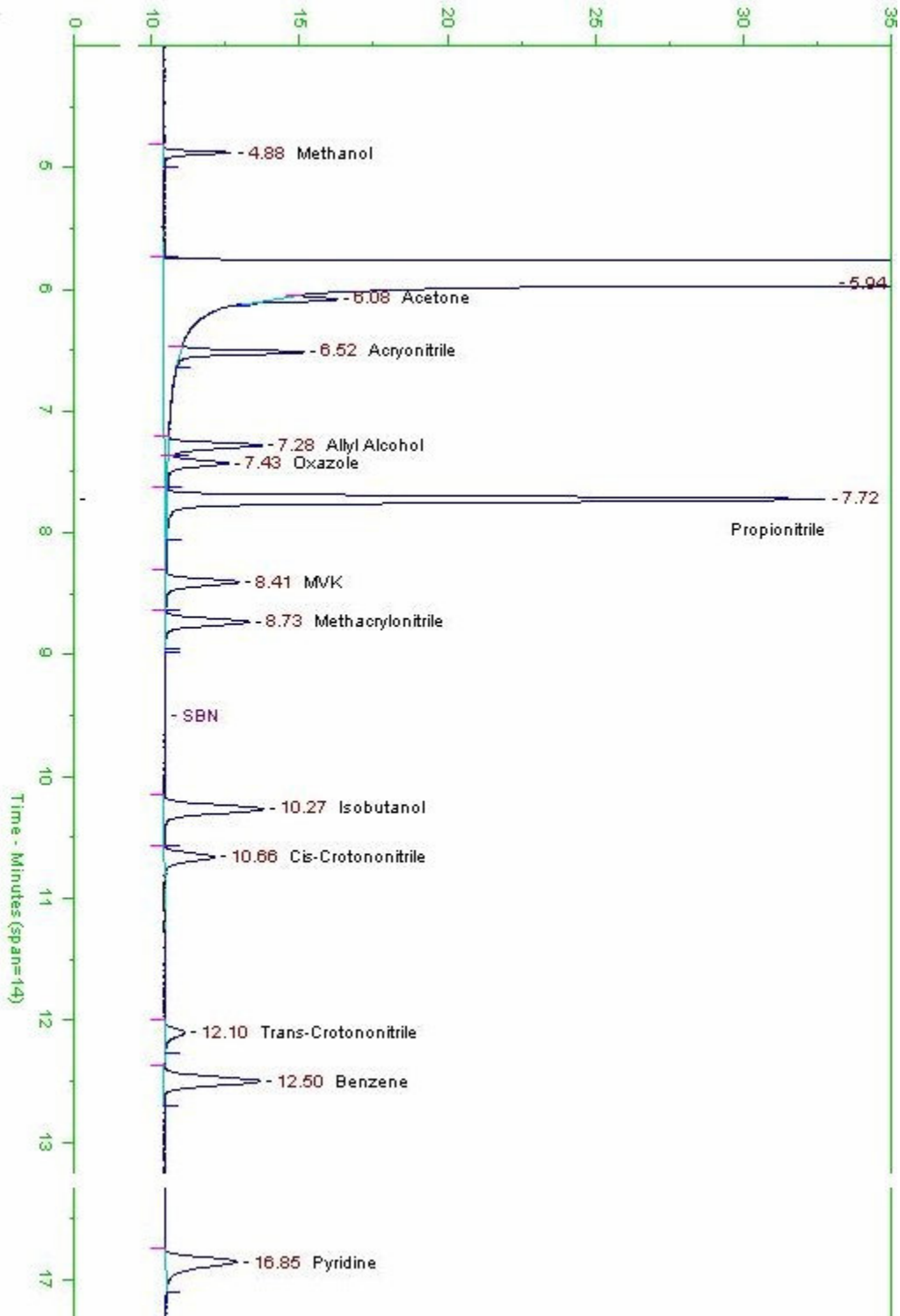
Integrator: HP-3393A or equivalent chromatography data system

Any of the above parameters may be adjusted as necessary to obtain best separation and sensitivity.

Figure 1. Chromatogram of Mid-range Calibration Standard (Attached)

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Method: ACEN-23 Revision: 5 Final Revision Date: 04/20/04	Acetonitrile Specification Tests	INEOS Nitriles
Last Review: 04/01/08 Next Review: 04/01/12	Organic Impurities By GC	Page 11 of 11 Reviewed by: Jennifer Young



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