

Method: ACEN-26 Revision: 5 Final Revision Date: 04/01/2012	HPLC Acetonitrile Specification Tests	INEOS Nitriles
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METHOD SUMMARY

This method is for evaluation of acetonitrile product after it has passed UV and other specification tests. The acetonitrile test sample is used as part of the mobile phase and mixed with water in a linear HPLC gradient program run on a specified column. No injection is done. No peaks greater than 0.005 absorbance units must be observed at 254 nm to meet the ACS specification for HPLC grade acetonitrile. The performance of the instrument and column are verified before running the product by first running a commercially purchased HPLC acetonitrile as the test sample.

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.

REFERENCES

"Reagent Chemicals," eighth edition, American Chemical Society Specifications, 1993, page 64.

APPARATUS AND REAGENTS

1. **Acetonitrile** - Gradient HPLC grade such as Fisher Optima (P/N A996-4) or equivalent.

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2. **Water** - HPLC grade such as Fisher Optima (P/N W7-4). Alternatively, lab water purified with a reverse osmosis system may be used if it has been shown to be sufficiently cleaned and filtered.
3. **Liquid Chromatograph** - The analysis is typically run with a Waters Alliance System 2690 with PDA detector and Millenium data system. However, a single wavelength UV detector could be used and other equivalent liquid chromatography systems may be used.
4. **Column** - Hibar Pre-packed Column RT 250-4 Lichrosorb® RP-18 (10u).

PROCEDURE

HPLC Method

1. The gradient program for the method is:

Time (min.)	Flow rate (mL/minute)	% H ₂ O	% aceto	Other items
0	2	0	100	
10	2	80	20	start data collection
40	2	80	20	
60	2	0	100	
70	2	0	100	

2. The detector conditions are the PDA default conditions except for 0.1 spec/sec. That is wavelength = 200 -300 nm, resolution = 1.2.

Procedure

1. Start Instrument and equilibrate:
 - a. Verify there is HPLC grade water for flowline A and a commercially purchased HPLC grade acetonitrile for flowline B. Verify the column switch is set to use the proper column (2)

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- b. Before starting the Millennium software on the computer, turn on the power to the main instrument and to the detector. Lamp and status lights on the detector will come on. Allow lamp to warm up at least one hour before starting an analysis.
 - c. Using the instrument keypad set the flow conditions: flow = 2 mL/min, 0% A, 100% B, vacuum degas on. Pressure is usually ~500 psi. Using the Direct Function key do a wet purge and an injector purge.
 - d. Then start the Millennium software on the computer (USER: system and PASSWORD: manager)
 2. Create new project folder:
 - a. Open a new project folder by going to File and clicking New. Give it a good name (like grad_date of the campaign). Make it from the Highpurity folder. Copy filters and fields and copy methods. Use the default values for size, etc. Check that the items below have been copied. If they haven't been copied then follow the procedure listed below the items.
 - b. Instrument method: ACS_COM_GR, ACS_GL_GR
 - c. Processing methods: grad_210, grad_254
 - d. Reporting methods: gradient
 - e. Method sets: ACS_COM_GR, ACS_GL_GR
 - f. Sample sets: TESTGRAD, PRODGRAD
 - g. To copy: open the Highpurity project. The first four sets of items can be copied from the Method view, the sample sets must be copied from the Sample Sets view. To copy: highlight the items to be copied and pull down the Database menu. Use copy to project.
3. Equilibrate system and run performance check:
 - a. Load Sample Set TESTGRAD
 - b. Verify there is a vial (may be empty) in position 1 of the autosampler
 - c. Press the Instrument Setup button (just to right of Stop sign button) to set up instrument. Check parameters on instrument screen. It should be flow = 2.0 mL/min, 0% A, 100% B. Pressure is usually ~800 psi.
 - d. Once the instrument is set up: method set should read ACS_COM_GR, Instrument method should read ACS_COM_GR. Set Mode to Run & Report and Continue on fault
 - e. Type sample name on sample set line one, run time is set to 70 min, and injection volume is set to 0 mL. Method set should be ACS_COM_GR,

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function = inject sample, # inj. = 1 unless you want to do more than one injection. You will have to use the arrow below the table to scroll to the right or left to see all these fields.

- f. Pull down the Mode pull-down menu and check that the system is in SampleSet mode, Interactive, and Allow Sample Set Changes (check marks in front). Click on any of them that are not already checked. Save SampleSet with the same name or a new name by selecting Save as sample set under the File pull-down menu. The system automatically appends the date and time to the sampleset name.
 - g. Monitor the baseline (optional): Click on the button showing the monitor (3rd from left). When traces to right of table are reasonably stable, click the red stoplight button to stop monitoring.
 - h. Run the SampleSet: Click on the carousel button (2nd from right) to start the SampleSet.
 - i. After the sample is run, reports are automatically printed for the 254 nm and at 210 nm results.
4. Running the sample:
- a. Verify there is HPLC grade water for flowline A and the sample product acetonitrile for flowline B. Verify the column switch is set to use the proper column (2)
 - b. With Quickset closed, use the instrument keypad to set the flow conditions: flow = 2 mL/min, 0% A, 100% C, vacuum degas on. Pressure is usually ~800 psi. Using the Direct Function key do a wet purge and an injector purge.
 - c. Then start Quickset, load Sample set PRODGRAD, set up the instrument and start the analysis. The analysis should be started soon after the product acetonitrile sample starts flowing through the column.
 - d. After the sample is run, reports are automatically printed for the 254 nm and at 210 nm results.

REPORT

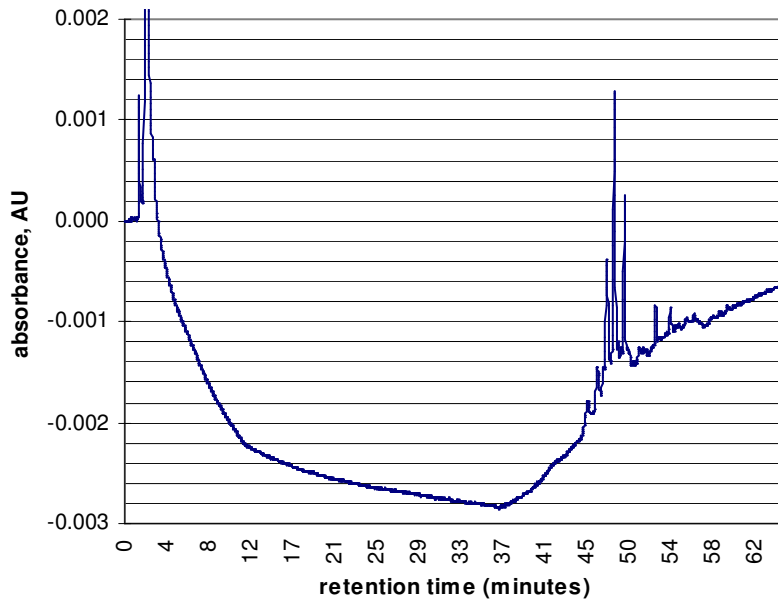
The acetonitrile passes the test if no peaks are greater than 5,000 height units (0.005 Au) at 254 nm.

Sample gradient runs are appended.

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APPENDIX

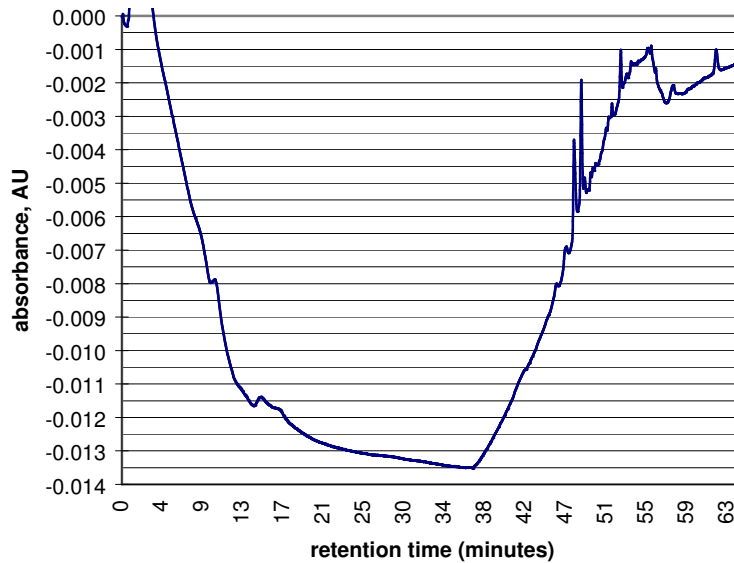
Gradient test for optima acetonitrile at 254 nm



Ignore any peaks at time < 10 min. Note peak at ~50 min is slightly larger than 2 mAU.

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210 nm HPLC gradient test for optima acetonitrile



Ignore any peaks before 10 min. Peak at ~48 min is about 5 mAU.