Automated SPE Disk Extraction Method for the Analysis of Nonyl Phenol and Bisphenol A in Water Samples

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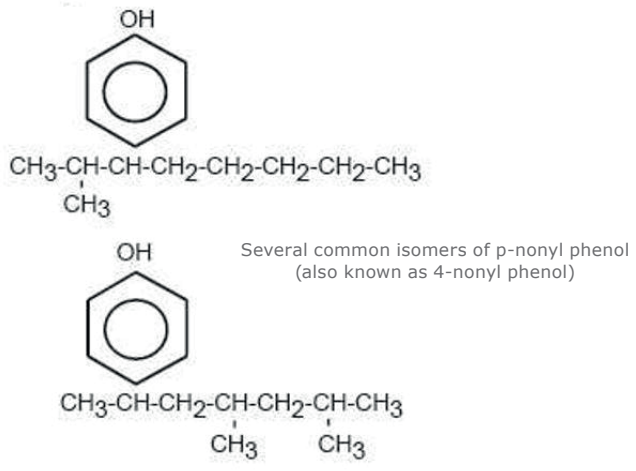
Scope

This application note will outline methods for the extraction of nonyl phenol and bisphenol A using Biotage automated or manual SPE solutions and the DryVap® Concentrator System. The first section will highlight the use of the Biotage® Horizon 5000 fully automated extraction system and the method used for this application. Additionally, there will be an Application Modification section that will highlight the use of the Biotage® Horizon 4790 (with data and discussion) and Biotage® VacMaster™ Disk for this application.

Introduction

Nonyl phenol and Bisphenol A are both of great environmental concern as they have been classified as endocrine disruptors. Endocrine disruptors are those compounds that mimic estrogen and thus could induce hormonal responses. Nonyl phenol has been banned in the European Union as a hazard to both human and environmental safety because of this concern.



Several common isomers of p-nonyl phenol (also known as 4-nonyl phenol)

Figure 1. Nonyl Phenol Chemical Structure.

Nonyl Phenol

Nonyl phenol is an organic compound formed during the alkylation process of phenols. Nonyl phenol is not a single chemical compound, but rather a term used to refer to a family of compounds, all of which have a central aromatic (or benzene) ring and a nine-carbon side chain. Because of their man-made origins, nonyl phenol is classified as xenobiotic. See Figure 1 for the chemical structure.

One use of nonyl phenol is as a surfactant, which reduces the surface tension of water forming a bridge between two compounds that normally do not mix. This is one reason nonyl phenol is commonly found in water samples. It is also used in pesticide products as "inert" ingredients, with the purpose of increasing the amount of spray solution that remains on leaf surfaces and in general to make the pesticide product more potent.

Bisphenol A

Bisphenol A was first synthesized in 1891 and evidence of its estrogenicity came from experiments in the 1930s. However, the use of bisphenol A was shelved for this role as an estrogen mimic when diethylstilbestrol was invented. Both nonyl phenol and bisphenol A are now deeply imbedded in consumer products and the concern of these compounds to mimic hormonal responses has raised the need for adequate testing of these compounds. This application note will discuss the sample preparation method used to analyze for these compounds in drinking and wastewater.

Currently, bisphenol A has many uses. It is used in the synthesis of polyesters, polysulfones, and polyether ketones, as an antioxidant in some plasticizers, and as a polymerization inhibitor in PVC. It is also a key monomer in production of polycarbonate plastic, which is used to make a variety of consumer products including baby bottles, water bottles, sports equipment, medical devices, CDs, and household electronics. See Figure 2 for the chemical structure.

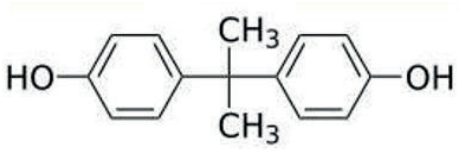


Figure 2. Bisphenol A Chemical Structure.

Instrumentation

- Biotage Instruments:
  - Biotage® Horizon 5000 Automated Extraction System
  - DryVap® Concentrator System
  - DryDisk® Separation Membranes
  - Atlantic® DVB SPE disk (47 mm)
- Agilent 5890/5971 GC/MS
- Column: 30 M x 0.25 mm x 0.5um HP-5ms
- Injection Liner: Splitless, double gooseneck

Method Summary

1. Obtain 1 L samples and spike any laboratory control or matrix spike samples to a concentration of 5 µg/L.
2. Place the sample bottle on the Biotage® Horizon 5000 Automated Extraction System and place the Atlantic® DVB disk in the standard 47mm disk holder.
3. Start the Biotage® Horizon 5000 using the extraction method in table 1 and collect the final sample extract.
4. Pour the final sample extract into the DryDisk® Separation Membrane reservoir on the DryVap® Concentrator System to remove the residual water in the organic solvent extract.
5. Concentrate the extract to a final volume of 1 mL.
6. The sample was analyzed by GC/MS using Selected Ion Monitoring (SIM) mode and the conditions and methods listed in tables 2, 3, and 4.

Table 2. GC/MS pressure program.

Initial Pressure	12 psi (constant flow)	
Initial Time	0 min	
Level 1	Rate	99 psi/min
	Final	50 psi
	Hold	0.13 min
Level 2	Rate	99 psi/min
	Final	12 psi
	Hold	0.0 min
Total Time		0.90 min
Remainder of run at constant pressure		

Table 3. GC/MS temperature program.

Initial Temp.	60 °C	
Initial Time	1 min	
Level 1	Rate	20 °C/min
	Final	270 °C
	Hold	0.0 min
Level 2	Rate	6 °C/min
	Final	300 °C
	Hold	0.0 min

Table 4. GC/MS conditions.

Solvent Delay	3.7 min
Injection Volume	2 µL
Injection Temperature	280 °C
Transfer Line Temperature	300 °C
Ions Monitored	107, 213, 244

Table 1. Biotage® Horizon 5000 extraction method.

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/ Elute (s)	Sample Delay (s)
Condition SPE Disk	Dichloromethane	15	60	2	1	60	60	
Condition SPE Disk	Acetone	11	60	2	1	60	60	
Condition SPE Disk	Reagent water	15	60	2	1	60	60	
Condition SPE Disk	Reagent water	15	60	2	1	0	0	
Load Sample				2				45
Air Dry Disk				6			60	
Elute Sample Container	Acetone	8	15	2	1	180	20	
Elute Sample Container	Dichloromethane	8	15	2	1	180	20	
Elute Sample Container	Dichloromethane	8	15	2	1	60	20	
Elute Sample Container	Dichloromethane	15	60	2	1	60	60	

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Application Modifications

Biotage® Horizon 4790 Method Summary

1. 1000 mL water samples were spiked to a concentration of 5 µg/L.
2. The sample was placed onto the Biotage® Horizon 4790 Automated Extraction System and the system was started using the method in table 5.
3. The Biotage® Horizon 4790 system extracted 1000 mL samples in 25 minutes with a final extract volume of 25 mL.
4. The extract was poured into the DryDisk® Separation Membrane reservoir on the DryVap® Concentrator System to remove the residual water in the organic solvent extract.
5. The DryVap Concentrator System concentrated the solvent extract to a final volume of 1.0 mL.
6. The sample was analyzed by GC/MS using Selected Ion Monitoring (SIM) mode and the conditions and methods listed in tables 2, 3,and 4 above.

Table 5. Biotage® Horizon 4790 extraction method.

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Dichloromethane	60	30
Prewet 2	Acetone	60	30
Prewet 3	Reagent water	60	30
Prewet 4	Reagent water	0	0
Sample Process			
Air Dry			30
Rinse 1	Acetone	180	10
Rinse 2	Dichloromethane	180	10
Rinse 3	Dichloromethane	60	10
Rinse 4	Dichloromethane	60	30
Rinse 5	Dichloromethane	60	30

Biotage® Horizon 4790 Results and Conclusions

A standard was prepared in acetone at a concentration of 100 µg/mL. Of this standard, 50 µL was added to one liter of sample to give a concentration of 5 µg/L. A volume of internal standard solution of 10 µL at a concentration of 500 µg/mL (terphenyl-D14) was also added to the sample. The sample bottle was capped and mixed for 1 minute. The sample was loaded onto the Biotage® Horizon® 4790 extraction system and the system was started. For this work, 47 mm Atlantic® disks containing 0.35 g of divinyl benzene (DVB) were used. All steps of the extraction process are automated.

On average, it took about 15 minutes to process (filter) the one liter sample. The total run time (including all prewet, air dry, and solvent elution times) was roughly 25 minutes per sample. If dirty wastewater samples are to be processed, the Atlantic® glass fiber prefilter should be used. The prefilter is simply placed on top of the SPE disk and the 4790 extractor is run as normal.

Upon completion, the collection vessel containing approximately 25 mL of solvent was removed. The solvent extract was poured into the DryDisk® separation membrane holder and the DryVap® was started. Vacuum was used to pull the solvent through the DryDisk® membrane, while the membrane retains the residual water. The retention of the residual water is beneficial as the residual water can be manually washed with organic solvent, typically methylene chloride, to ensure that all organics are removed from the holder and the transfer line.

Once the DryVap® was finished concentrating the extract, which took on average 25 minutes, the final volume was brought up to 1.0 mL and the extract was transferred into a GC vial and run on the GC/MS.

The recoveries shown in Table 6 were obtained from three runs, using the technique described. The data indicates that these compounds can be adequately and quickly extracted from water samples using SPE disk technology. Figure 3 (see page 4) shows a typical chromatogram for these compounds.

Table 6. Results.

Runs	Nonyl Phenol (%)	Bisphenol A (%)
1	117.2	109.8
2	100.0	104.8
3	117.6	120.8
Ave	111.6	111.8
SD	10	8
%RSD	9.0	7.3

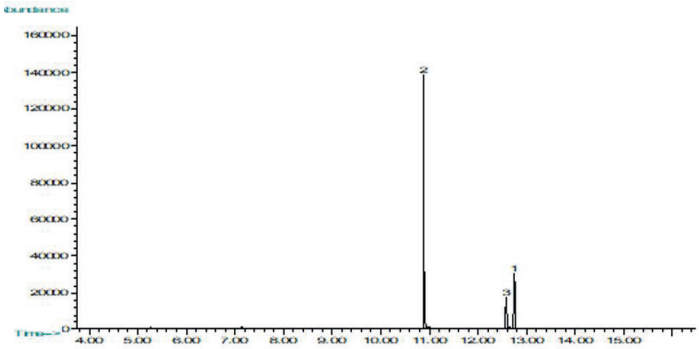

Figure 3. Typical Chromatogram (1: Terphenyl-D_{14} / 2: Nonyl Phenol / 3: Bisphenol A)

This study shows that the Biotage® Horizon 4790 Automated Extraction system, along with the Atlantic™ DVB disk, the DryDisk® separation membrane, and the DryVap® Concentrator System can be successfully used to analyze for Nonyl Phenol and Bisphenol A in water samples. This sample preparation process is fully automated, requiring minimal human intervention, and allowing for reductions in sample processing costs.

Biotage® VacMaster™ Disk Method Summary

1. Repeat the following steps for each active Biotage® VacMaster™ Disk station.
2. Setup the VacMaster Disk manifolds ensuring all waste lines and vacuum lines are attached. Set the vacuum pump to -24"Hg.
3. Prepare the disk holder assembly (47mm): ensure the support screen is flat in the center of the disk holder. Place the Atlantic® DVB Disk on top of the support screen with the ripples of the disk on top and add any prefilters on top of the disk. Place the disk holder assembly on the VacMaster® Disk manifold ensuring there is a tight seal with the luer fitting.
4. If using the multifunnel, place onto the disk holder assembly. If not using the multifunnel, omit those directions throughout the method.
5. Condition the SPE Disk:
   a. Guide for each conditioning step in table 7 below:
      I. Measure the appropriate VOLUME of SOLVENT into a graduated cylinder and pour into the disk holder assembly.
      II. Using a Nalgene Wash Bottle (phthalate free), rinse the multifunnel and disk holder in a circle for about 3 seconds using the same SOLVENT (approximately 5 additional mL).
      III. SATURATE the disk for the indicated time (in SECONDS). (Saturate means: quickly turn the knob to the appropriate waste destination and back to the "OFF" position. This brings the solvent into the disk media bed).
      IV. SOAK the disk for the indicated time (in SECONDS).
      V. DRAIN to the appropriate waste destination for the indicated time (in SECONDS). Switch to the "OFF" position.

Table 7. Disk Conditioning.

Solvent	Vol. (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Drain (sec.)
DCM	15	1	60	Organic	60
Acetone	11	1	60	Organic	60
Reagent Water	15	1	60	Organic	60
Reagent Water	15	1	0	Aqueous	0

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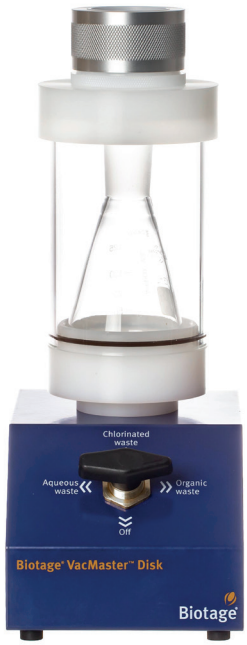
6. Load the Sample:
   a. For multifunnel: quickly and efficiently angle the bottle to rest on the multifunnel upside-down.
   b. For no multifunnel: pour a portion of the sample into the disk holder.
   c. Adjust the vacuum between -10”Hg and -15”Hg for sample load (please note, if the sample is flowing too slowly, the vacuum can be increased). Drain the sample to “AQUEOUS” waste. Continue to pour the sample into the disk holder ensuring the disk does not go dry or overflow for the duration of sample load.
7. Air Dry the SPE Disk:
   a. Return the vacuum to -24”Hg and continue to air dry the SPE disk to “AQUEOUS” waste for an additional 60 SECONDS. Switch to the “OFF” position.
   b. Remove the sample bottle from the multifunnel if it was used.



8. Elute the SPE Disk: (Please note: the elution solvent will go into the collection flask inside the chamber, not to waste containers)
   a. Place a clean 125 mL 24/40 tapered Erlenmeyer flask into the Biotage® VacMaster™ Disk collection chamber. Place the cover on the chamber. Remove the disk holder assembly and place the disk holder assembly into the lure fitting on top of the collection chamber. Attach the lure fitting of the collection chamber assembly onto the manifold.
   b. Guide for each elution step in table 8 below:
      I. Measure the appropriate VOLUME of SOLVENT into a graduated cylinder, pour into the sample bottle, and swirl around. Pour the solvent in the sample bottle into the disk holder assembly.
      II. Using a Nalgene Wash Bottle (phthalate free), rinse the multifunnel and disk holder in a circle for about 3 seconds using the same SOLVENT (approximately 5 additional mL).
      III. SATURATE the disk for the indicated time (in SECONDS) to “ORGANIC”.
      IV. SOAK the disk for the indicated time (in SECONDS).
      V. DRAIN to “ORGANIC” for the indicated time (in SECONDS). Switch to the “OFF” position.
      VI. Remove the chamber lid to release the vacuum from inside the chamber.

Table 8. Disk Elution.

Solvent	Vol. (mL)	Saturate (sec.)	Soak (sec.)	Waste Destination	Elute (sec.)
Acetone	8	1	180	Organic	20
DCM	8	1	180	Organic	20
DCM	8	1	60	Organic	20
DCM	8	1	60	Organic	60
DCM	8	1	60	Organic	60

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