Determination of PAH Compounds from Aqueous Samples Using a Non-Halogenated Extraction Solvent and Atlantic® C18 Disks

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This application note will outline optimized methods for the extraction of polycyclic aromatic hydrocarbon (PAH) compounds from aqueous samples using Biotage automated or manual SPE solutions and 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 and Biotage® VacMaster™ Disk for this application.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, naturally occurring in coal, crude oil, gasoline, and their byproducts (e.g. coal tar or creosote). In addition, PAHs are formed in the incomplete combustion processes of all organic materials, such as wood or fossil fuels. Consequently, the EU water framework directive (WFD) lists in its annex X the whole group of PAHs as priority hazardous substances.

The traditional extraction solvents used for solid phase extraction (SPE) methods involving PAH compounds are dichloromethane (DCM) and acetone. DCM has been used in the past because of its excellent solvating properties and its low boiling point which results in higher yields after extraction, drying, and concentration. DCM however is dangerous to work with as it has been proven to be a carcinogen at very low exposure levels. As such, many laboratories have now mandated that solvent extractions in environmental methods do not use any halogenated solvents, particularly DCM.

Previous work, done by Frederick Weres[1], has demonstrated good extraction efficiencies using acetone as the eluting solvent. However, acetone creates a problem with residual water in the final extracts due to its miscibility with water.

The restriction placed on the types of solvents used has given rise to a need for an extraction method which makes use of a non-halogenated, non-polar solvent for the extraction of PAH compounds. This solvent must achieve excellent recoveries when using traditional SPE methods.

This application note was developed to demonstrate the extraction of 16 PAH compounds listed by the US Environmental Protection Agency (EPA) as priority pollutants, including all PAHs listed in the content of the EU WFD, using the Biotage® Horizon 5000 automated extraction system. It will show the efficiency of the extraction while demonstrating the excellent recoveries of PAH compounds using hexane and minimal amounts of acetone. Methods were developed and results are shown using Atlantic® C18 47 mm disks. This study examined aqueous samples with and without particulates, containing dissolved PAH compounds. The water samples with particulates were extracted using the Fast Flow Sediment Disk Holder (FFSDH). The FFSDH allows the whole sample to be extracted (including particulates and other debris in the water) without having to separately filter the water samples first.

Biotage®

Instrumentation

- Biotage® Horizon 5000 Automated Extraction System
- DryVap® Concentrator System
- DryDisk® Separation Membrane
- Atlantic® C18 Disk (47 mm)
- Atlantic® Fast Flow Prefilter (Fine and Coarse)
- 47 mm Disk Holder
- Fast Flow Sediment Disk Holder (FFSDH)

Method Summary

Extraction of Particulate Free Aqueous Samples (using standard 47 mm Disk Holder)

1. Adjust a 1 L aqueous sample to pH 2 with HCl, cap the bottle and mix.
2. Spike PAH Standard mix (100 µg/mL in MeOH) into samples (use 200 µL for a 20 µg spike).
3. Place the sample bottle on the Biotage® Horizon 5000 Extraction System and place the Atlantic® C18 disk in the standard 47 mm disk holder. Attach collection vessels to the system.
4. Start the PAH extraction method detailed in Table 2 and collect the sample extract (approximately 30 mL).
5. Dry and concentrate the extract on the DryVap® Concentrator System using the parameters listed in Table 1.
6. Analyze sample extracts by GC/MS.

Extraction of Aqueous Samples with Particulates (using the Fast Flow Sediment Disk Holder (FFSDH))

1. Adjust a 1 L aqueous sample to pH 2 with HCl, cap the bottle and mix.
2. Spike PAH Standard mix (100 µg/mL in MeOH) into samples (use 200 µL for a 20 µg spike).
3. Load the FFSDH with an Atlantic® C18 47 mm disk, a Fine Atlantic® Prefilter and Coarse Atlantic® Prefilter.
4. Place the sample bottle on the Biotage® Horizon 5000 Extraction System and attach collection vessels to the system.
5. Start the PAH extraction method detailed in Table 3 and collect the sample extract (approximately 80 mL).
6. Dry and concentrate the extract on the DryVap® Concentrator System using the parameters listed in Table 1.
7. Analyze sample extracts by GC/MS.

Table 1. DryVap Settings

PARAMETER	SETTING
Dry Volume	100
Heat Power	5
Auto Rinse Mode	OFF
Heat Timer	OFF

References

1. Friedrich Werres, Peter Balsaa, Torsten C. Schmidt, Journal of Chromatography A, 1216 (2009) 2235–2240

Biotage® Horizon 5000 Automated Extraction System

DryVap® Concentrator System

Table 2. Standard 47 mm Disk Holder Extraction Method

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay (s)
Condition SPE Disk	Methanol	11	60	2	1	120	10	
Condition SPE Disk	Reagent water	15	60	2	1	120	10	
Condition SPE Disk	Reagent water	15	60	2	1	30	4	
Load Sample				2				45
Air Dry Disk				6			120	
Elute Sample Container	Acetone	8	15	2	1	120	240	
Elute Sample Container	Hexane	8	15	2	1	120	240	
Elute Sample Container	Hexane	8	15	2	1	60	120	
Elute Sample Container	Hexane	8	15	6	1	60	120	

Table 3. FFSDH with 47 mm Disk Extraction Method

Step	Select Solvent	Volume (mL)	Purge (s)	Vacuum	Saturate (s)	Soak (s)	Drain/Elute (s)	Sample Delay (s)
Condition SPE Disk	Methanol	20	60	2	1	120	10	
Condition SPE Disk	Reagent water	20	60	2	1	120	10	
Condition SPE Disk	Reagent water	20	60	2	1	30	4	
Load Sample				2				45
Air Dry Disk				6			120	
Elute Sample Container	Acetone	20	15	2	1	120	240	
Elute Sample Container	Hexane	20	15	2	1	120	240	
Elute Sample Container	Hexane	20	15	2	1	60	120	
Elute Sample Container	Hexane	20	15	6	1	60	120	

Application Modifications

Biotage® Horizon 4790 Methods

Biotage® Horizon 4790 Method Summary – 47 mm Disk Holder Method (for Particulate Free Aqueous Samples)

1. Adjust a 1 L aqueous sample to pH 2 with HCl, cap the bottle and mix.
2. Spike PAH Standard mix (100 µg/mL in MeOH) into samples (use 200 µL for a 20 µg spike).
3. Place an EZ-Seal over the opening of the bottle and screw on the bottle cap adaptor.
4. Place the sample bottle on the Biotage® Horizon 4790 Extraction System and place the Atlantic® C18 disk in the standard 47mm disk holder. Attach collection vessels to the system.
5. Start the PAH extraction method detailed in Table 4 and collect the extract at a high vacuum of -25 in. Hg (15.5 in. Hg at the Solvent to Waste bottle) and 15.5 psi Solvent Bottle Pressure.
6. Collect the sample extract (approximately 30 mL).
7. Dry and concentrate the extract on the DryVap® Concentrator System using the parameters listed in Table 1 above.
8. Analyze sample extracts by GC/MS.

Biotage® Horizon 4790 Method Summary – FFSDH with 47 mm Disk Method (for Aqueous Samples with Particulates)

1. Install the 8 second Elution Magnet over the IR sensor on the Biotage® Horizon 4790.
2. Install the Tefzel Plug into the Biotage® Horizon 4790 platform adjustment hole.
3. Adjust 1 L aqueous sample to pH 2 with HCl, cap the bottle and mix.
4. Spike PAH Standard mix (100 µg/mL in MeOH) into samples (use 200 µL for a 20 µg spike).
5. Load the FFSDH with an Atlantic® C18 47 mm disk, a Fine Atlantic® Prefilter and Coarse Atlantic® Prefilter.
6. Place the sample bottle on the Biotage® Horizon 4790 Extraction System and attach collection vessels to the system.
7. Start the PAH extraction method detailed in Table 4 and collect extract at a high vacuum of -25 in. Hg (15.5 in. Hg at the Solvent to Waste bottle) and 15.5 psi Solvent Bottle Pressure.
8. Collect the sample extract (approximately 80 mL).
9. Dry and concentrate the extract on the DryVap® Concentrator System using the parameters listed in Table 1 above.
10. Analyze sample extracts by GC/MS.

Table 4. Biotage® Horizon 4790 Extraction Method

Step	Solvent	Soak Time (s)	Dry Time (s)
Prewet 1	Methanol	120	5
Prewet 2	Reagent Water	60	5
Prewet 3	Reagent water	30	2
Sample Process			
Air Dry			60
Rinse 1	Acetone	120	120
Rinse 2	Hexane	120	120
Rinse 3	Hexane	60	60
Rinse 4	Hexane	60	60

Biotage® Horizon 4790 Results and Conclusions

Extractions of aqueous samples were carried out using 47 mm C18 extraction disks with standard disk holder, and with FFSDH equipped with fine and coarse prefilters. Results of this series of extractions are shown in Table 5 and Table 6. Extraction results in Table 5 showed very good recoveries with a low of 81% and a high of 94 %. The data also shows very good reproducibility with RSDs between 0.5% to 7%.

The data in Table 6 shows very good recoveries using the FFSDH. This setup has been optimized for use with high particulate samples, and recoveries are consistently high for the PAH analytes from 79% to 92%. The FFSDH method resulted with a low of 79% for naphthalene and a high of 92% for both dibenz(ah)anthracene and benzo(ghi)perylene (average RSD was 4.69%).

This application note demonstrates an efficient SPE disk extraction scheme for PAH compounds in aqueous samples that are clean or dirty. The method demonstrated excellent recoveries for an extraction scheme which uses acetone and hexane in place of chlorinated solvents. This safe, efficient extraction scheme utilizes Atlantic® C18 SPE disks and Fast Flow Sediment Disk Holder to extract the whole sample (sediment and liquids) in one step without a separate filtration and extraction of the solids contained in the sample. This combination of products and consumables aids sample flow rates through the disk allowing for faster extraction times while yielding excellent recoveries.

Table 5. Recovery Data from Standard 47 mm Disk Holder Method

Compound	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Average (%)	RSD (%)
Naphthalene	84	77	81	81	4.35
Acenaphthylene	89	84	87	87	2.89
Acenaphthene	92	86	88	89	1.60
Fluorene	88	87	89	88	1.43
Phenanthrene	88	89	89	89	0.56
Anthracene	89	91	87	89	2.27
Fluoranthene	92	100	91	94	5.23
Pyrene	91	101	90	94	6.62
Benz(a)anthracene	86	90	88	88	2.00
Chrysene	94	93	92	93	0.82
Benzo(b)fluoranthene	88	85	90	88	3.18
Benzo(k)fluoranthene	89	88	89	88	0.86
Benzo(a)pyrene	91	92	95	93	2.25
Indeno(1,2,3-cd)pyrene	87	86	86	86	1.01
Dibenz(ah)anthracene	89	88	85	87	2.17
Benzo(ghi)perylene	85	89	91	88	3.22

Table 6. Recovery Data from FFSDH Method

Compound	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Average (%)	RSD (%)
Naphthalene	77	78	84	79	4.78
Acenaphthylene	77	85	86	83	5.84
Acenaphthene	82	87	88	85	3.77
Fluorene	79	87	88	85	5.71
Phenanthrene	80	85	87	84	4.32
Anthracene	80	88	87	85	5.13
Fluoranthene	84	88	91	88	4.31
Pyrene	84	89	92	88	4.55
Benz(a)anthracene	85	92	91	89	4.26
Chrysene	83	87	90	86	4.08
Benzo(b)fluoranthene	88	90	90	89	1.49
Benzo(k)fluoranthene	84	90	93	89	5.24
Benzo(a)pyrene	85	91	91	89	3.75
Indeno(1,2,3-cd)pyrene	83	92	93	89	6.17
Dibenz(ah)anthracene	86	94	98	92	6.68
Benzo(ghi)perylene	87	93	96	92	5.01

Biotage® VacMaster™ Disk Methods

Biotage® VacMaster™ Disk Method Summary (using a Standard 47mm Disk Holder with 47 mm Disk)

1. Repeat the following steps for each active Biotage® VacMaster™ Disk station.
2. Set up 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 (47 mm): ensure the support screen is flat in the center of the disk holder. Place the Atlantic® C18 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 time indicated (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 time indicated (in SECONDS).
      v. DRAIN to appropriate waste destination for the time indicated (in SECONDS). Switch to the "OFF" position.

Table 7. Disk Conditioning

Solvent	Volume (mL)	Saturate (sec)	Soak (sec)	Waste Destination	Drain (sec)
Methanol	11	1	120	Organic	5
Reagent Water	15	1	60	Organic	8
Reagent Water	15	1	60	Organic	5

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 120 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 or 40 mL VOA vial (using the VOA vial insert) into the VacMaster™ Disk collection chamber. Place the cover on the chamber. Remove the disk holder assembly and place the disk holder assembly into the Luer fitting on top of the collection chamber. Attach the Luer 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 time indicated (in SECONDS) to "ORGANIC".
      iv. SOAK the disk for the time indicated (in SECONDS).
      v. DRAIN to "ORGANIC" for the time indicated (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	Volume (mL)	Saturate (sec)	Soak (sec)	Waste Destination	Elute (sec)
Acetone	8	1	120	Organic	240
Hexane	8	1	120	Organic	240
Hexane	8	1	60	Organic	120
Hexane	8	1	60	Organic	40

Biotage® VacMaster™ Disk Method Summary (using a Fast Flow Sediment Disk Holder with 47 mm Disk)

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 (FFSDH): place the Atlantic® C18 Disk into the narrow part on the bottom of the FFSDH disk holder so the ripples of the disk are on the top, then place the riser with the SPE disk support screen under the disk, and attach the 47 mm ring cap to create a seal. On the wider portion of the disk holder, place the support screen (ensuring it is flat and in the center of the disk holder) and add any prefilters on top of the screen that will be used. Place the disk holder assembly onto 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 9 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 time indicated (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 time indicated (in SECONDS).
      v. DRAIN to the appropriate waste destination for the time indicated (in SECONDS). Switch to the "OFF" position.

Table 9. Disk Conditioning

Solvent	Volume (mL)	Saturate (sec)	Soak (sec)	Waste Destination	Drain (sec)
MeOH	20	1	120	Organic	5
Reagent Water	20	1	60	Organic	8
Reagent Water	20	1	30	Aqueous	5

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 120 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 VacMaster™ Disk collection chamber. Place the cover on the chamber. Remove the disk holder assembly and place the disk holder assembly into the Luer fitting on top of the collection chamber. Attach the Luer fitting of the collection chamber assembly onto the manifold.
   b. Guide for each elution step in Table 10 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 time indicated (in SECONDS) to "ORGANIC".
      iv. SOAK the disk for the time indicated (in SECONDS).
      v. DRAIN to "ORGANIC" for the time indicated (in SECONDS). Switch to the "OFF" position.
      vi. Remove the chamber lid to release the vacuum from inside the chamber.

Table 10. Disk Elution

Solvent	Volume (mL)	Saturate (sec)	Soak (sec)	Waste Destination	Elute (sec)
Acetone	20	1	120	Organic	240
Hexane	20	1	120	Organic	240
Hexane	20	1	60	Organic	120
Hexane	20	1	60	Organic	40

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