

# EPA Method 1664B Extractions Using the Biotage® Horizon 3100 with In-Line Drying Using ISOLUTE® Sodium Sulfate Drying Cartridges

**Authors:** Deanna Bissonnette and Michael Ebitson Biotage, Salem, NH, USA.

**Keywords:** EPA, 1664B, Oil and Grease, HEM, Wastewater, Biotage® Horizon 3100, Extraction, ISOLUTE® Sodium Sulfate Cartridge, Drying, Speed-Vap®

## Introduction

The treatment and removal of oil and grease from wastewater is imperative because it can negatively affect the biological and aquatic life that encounter it. Not only that, but since the oil and grease are not miscible with water, it leaves an unappealing layer on top of the water. Even before the oil and grease can reach the aquatic life, it will solidify on the inner walls of pipes, causing blockage over time.

The purpose of this application note is to provide an in-line extraction and drying solution for measuring hexane extractable material (HEM) using solid-phase extraction. The extracts will be dried in-line during extraction using ISOLUTE® Sodium Sulfate Drying Cartridges. Using the Speed-Vap®, the extracts will be concentrated with gentle heat and consistent air flow through precisely drilled holes.

## Instrumentation

### Biotage Instruments

- » Biotage® Horizon 3100 Oil and Grease Extraction System (P/N SPE-DEX 3100)
- » Speed-Vap® Automated Solvent Evaporation System (P/N 200-1000-04)

### Biotage Consumables

- » Pacific® Premium Oil & Grease Disk, 90 mm (P/N 1664-100-PHT)
- » ISOLUTE® Sodium Sulfate Drying Cartridge (P/N 802-0250-M)
- » Oil and Grease Standards, 40 mg (P/N 50-021-HT)
- » Oil and Grease Standards, 26 mL (P/N 50-003-HT)
- » Oil & Grease Aluminum Weighing Pans, 105 mm, 125 mL (P/N 50-002-02-HT)

### Other Instruments

- » Analytical Balance: Sartorius

## Method Summary

### 90 mm Pacific® Premium Disk Extractions:

1. Obtain 17 individual liters of DI water. Acidify to a pH <2 with HCl.
2. Place a 90 mm Pacific Premium disk in a 90 mm disk holder and place on the 3100. Repeat for each sample.
3. Blank sample preparation is complete.
4. Complete the following spiking procedures for the method detection limit (MDL) and initial precision and recovery measurement (IPR).
  - a. MDL: Spike each acidified liter (10 total) with 4.0 mg/L of HEM (500 µL of the Oil and Grease Standard, 26 mL, PN: 50-003-HT).
  - b. IPR: Spike each acidified liter (4 total) with 40 mg/L of HEM (one 10 mL Oil and Grease Standard package, PN: 50-021-HT).
5. Attach the water inlet valve to each sample bottle and place them on the 3100 system. Attach an ISOLUTE® Sodium Sulfate Drying Cartridge onto the end of the check valve, being sure to secure the cartridge as far as it will go. Attach a 24/40 taper flat bottom round flask onto each active station and secure with the clip. Repeat for each sample.
  - a. Ease of flask attachment is not restricted by the cartridge.
6. Extract the samples using the extraction method found in Table 1.
7. Pre-weigh each 105 mm aluminum pan and quantitatively transfer one extract to each pan.
  - a. Pour the final extract from the 3100 system into the designated pan and rinse the collection vessel vigorously with n-hexane. Swirl slightly to collect all the HEM in the vessel and pour into the designated pan.
8. Using the Speed-Vap® Evaporation System, concentrate the extracts per the parameters in Table 2. Remove the pan from the Speed-Vap® when there is a thin layer of hexane left in the pan because the hexadecane can evaporate if heated too long.
  - b. Allow the extract to finish evaporating in the hood and transfer to a desiccator.
9. Weigh each pan and calculate the HEM recovery in mg/L for each sample.

**Table 1.** Extraction conditions using 90 mm Pacific® Disk with ISOLUTE® Sodium Sulfate cartridge on the Biotage® Horizon 3100.

Step	Step Description	Solvent	Dispense (s)	Saturate (s)	Soak (s)	Drain Solvent (s)
1:	Condition	Hexane	10	1	30	60
2:	Condition	Methanol	10	1	30	5
3:	Load Sample					
4:	Air Dry 240s					
Step	Step Description	Solvent	Rinse (s)	Saturate Elute (s)	Soak (s)	Elute (s)
5:	Rinse and Elute	Hexane	10	1	10	15
6:	Rinse and Elute	Hexane	7	1	45	45
7:	Rinse and Elute	Hexane	7	1	45	45
Step	Step Description	Solvent	Rinse (s)	Saturate (s)	Soak (s)	Drain Solvent (s)
8:	Wash	Methanol	4	1	20	180
Step	Step Description	Solvent	Rinse (s)	Saturate Elute (s)	Soak (s)	Elute (s)
9:	Rinse and Elute	Hexane	1	0	0	0
Step	Step Description	Solvent	Rinse (s)	Saturate (s)	Soak (s)	Drain Solvent (s)
10:	Wash	Methanol	0	1	10	180
Step	Step Description	Solvent	Rinse (s)	Saturate Elute (s)	Soak (s)	Elute (s)
11:	Rinse and Elute	Hexane	4	1	45	45
12:	Rinse and Elute	Hexane	4	1	45	45
13:	Rinse and Elute	Hexane	4	1	45	60

**Table 2.** Speed-Vap® Evaporation Parameters.

Parameter	Value
Temperature (°C)	40
Compressed Air Inlet Pressure (psi)	80

## Results and Discussion

The extraction process for quality control samples takes approximately 25 minutes and the evaporation time takes approximately 30 minutes. With one system, three quality control samples can be extracted and evaporated in about an hour.

Below are the results from all the extractions completed in the method summary of this application note.

**Table 3.** 90 mm Disk IPR results.

Sample	HEM (mg/L)	Recovery (%)
1	35.0	87.50
2	34.9	87.25
3	35.4	88.50
4	35.5	88.75
<b>Average Percent Recovery (X)</b>		88.00
<b>Precision (s)</b>		0.74

**Table 4.** 90 mm Disk MDL results.

Sample	HEM (mg/L)
1	2.5
2	2.2
3	2.4
4	3.0
5	3.2
6	3.0
7	2.2
8	2.5
9	2.7
10	2.6
<b>Precision (s)</b>	0.3406
<b>MDL Value &lt; 1.4 mg/L</b>	0.9608

**Table 5.** Blank results.

Sample	HEM (mg/L)
1	0.1
2	0.7
3	0.6

All blanks < 0.9608 mg/L

The ten replicate samples used for the MDL calculation show little variation keeping the calculated MDL below 1.4 mg/L, the required MDL per EPA Method 1664B. The MDLs were calculated using 9 degrees of freedom and a student t-value of 2.821 with a 99% confidence interval.

The recoveries for the four IPRs are well within the acceptable range of 83–101% recovery per EPA Method 1664B as well. The IPR resulted in a precision value of 0.3406. This is well below the method requirement of 11.0.

The blank extractions also pass EPA Method 1664B requirements all recovering below the calculated MDL.

## Conclusion

This application note demonstrates that HEM can be effectively recovered within the guidelines of EPA Method 1664B with the use of Oil and Grease product solutions from Biotage. In addition to that, the blanks indicate virtually no contamination of HEM from consumables or instrumentation.

Automated solid-phase extraction improves the precision of HEM recoveries by eliminating operator bias. With the option of the ISOLUTE® Sodium Sulfate Drying Cartridge, installed in-line with extraction, it reduces operator interaction with the sample extracts, but also stays within compliance of EPA Method 1664B.

## References

EPA Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry, available at [www.epa.gov](http://www.epa.gov), (2010).



### EUROPE

Main Office: +46 18 565900  
Toll Free: +800 18 565710  
Fax: +46 18 591922  
Order Tel: +46 18 565710  
Order Fax: +46 18 565705  
[order@biotage.com](mailto:order@biotage.com)  
Support Tel: +46 18 56 59 11  
Support Fax: +46 18 56 57 11  
[eu-1-pointsupport@biotage.com](mailto:eu-1-pointsupport@biotage.com)

### NORTH & LATIN AMERICA

Main Office: +1 704 654 4900  
Toll Free: +1 800 446 4752  
Fax: +1 704 654 4917  
Order Tel: +1 704 654 4900  
Order Fax: +1 434 296 8217  
[ordermailbox@biotage.com](mailto:ordermailbox@biotage.com)  
Support Tel: +1 800 446 4752  
Outside US: +1 704 654 4900  
[us-1-pointsupport@biotage.com](mailto:us-1-pointsupport@biotage.com)

### JAPAN

Tel: +81 3 5627 3123  
Fax: +81 3 5627 3121  
[jp\\_order@biotage.com](mailto:jp_order@biotage.com)  
[jp-1-pointsupport@biotage.com](mailto:jp-1-pointsupport@biotage.com)

### CHINA

Tel: +86 21 68162810  
Fax: +86 21 68162829  
[cn\\_order@biotage.com](mailto:cn_order@biotage.com)  
[cn-1-pointsupport@biotage.com](mailto:cn-1-pointsupport@biotage.com)

### KOREA

Tel: +82 31 706 8500  
Fax: +82 31 706 8510  
[korea\\_info@biotage.com](mailto:korea_info@biotage.com)  
[kr-1-pointsupport@biotage.com](mailto:kr-1-pointsupport@biotage.com)

### INDIA

Tel: +91 22 4005 3712  
[india@biotage.com](mailto:india@biotage.com)

Distributors in other regions are listed on [www.biotage.com](http://www.biotage.com)

### Literature Number: AN948

© 2020 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. A list of all trademarks owned by Biotage AB is available at [www.biotage.com/legal](http://www.biotage.com/legal). Other product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe.