

# Hexane Extractable Material (Oil & Grease) Analysis with Automated Solid Phase Extraction Using the Biotage® Horizon 5000

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## Introduction

Hexane extractable material (HEM), also known as Oil & Grease extraction is a simple water quality measurement. The measurement can be used to assess pollution for compliance or as a measure of influent contamination to protect a waste treatment plant from levels that might cause malfunction. This measurement is used around the world and regulatory agencies have developed methods such as US EPA 1664B, ISO 11349, and Standard Methods 5520G.<sup>1,2,3</sup> We have shown the feasibility of Oil & Grease measurement on a dedicated automated system, the Biotage® Horizon 3100 (previously known as the SPE-DEX® 3100).<sup>4</sup> In this work we will demonstrate the performance of a general purpose automated Oil & Grease and semivolatile extraction system, the Biotage® Horizon 5000.

The performance of the system for this application will be demonstrated by showing compliance with the quality control requirement of US EPA Method 1664B for small modifications. A new extraction procedure would be considered a modification to the method; therefore the laboratory is required to repeat the initial precision and recovery (IPR) study in Section 9.2.2 9 to demonstrate that the modification produces results equivalent to or superior to results produced by this method. If the detection limit is suspected to be affected by the new modification a Method Detection Limit study (MDL) would need to be demonstrated. In this case the same disk and chemistry is used, only the automation has changed.<sup>5</sup>

In this study the laboratory confirming the performance of each typical solid phase extraction (SPE) configuration on the Biotage® Horizon 5000 by conducting an IPR study to satisfy the quality control requirements for a method modification.



## Instrumentation

- » Biotage
  - » Biotage® Horizon 5000 (previously known as the SPE-DEX 5000) Extractor
  - » Oil and Grease Standard 40 mg
  - » Pacific® Premium disks (90 mm and 47 mm)
  - » Atlantic® Fast Flow Pre-Filters (90 mm and 47 mm)
  - » Dry Disk® Solvent Drying System (SDS)
  - » DryDisk® Membranes
  - » Speed-Vap® IV Automated Evaporation System
  - » Aluminum Pans
- » Ohaus® Balance
  - » Adventurer Pro Model: AV64
  - » Max: 65 g, d=0.0001, 10 °C/30 °C



## Method Summary

- Obtain sixteen 1 L samples of DI water.
- Acidify each sample with hydrochloric acid until pH <2.
- Add one Oil and Grease 40 mg Standard to each bottle (total concentration of 40 ppm).
- Extract four samples using the Biotage® Horizon 5000 with 90 mm Pacific® Premium SPE Disks using the method shown in Table 1.
- Extract four samples using the Biotage® Horizon 5000 with 47 mm Pacific® Premium SPE Disks using the method shown in Table 2.
- Extract four samples using the Biotage® Horizon 5000 with 90 mm Pacific® Premium SPE Disks with Pre-filters using the method shown in Table 3.
- Extract four samples using the Biotage® Horizon 5000 with 47 mm Pacific® Premium SPE Disks with Pre-filters using the method shown in Table 4.
- Dry the extracts using the Dry Disk® SDS with a new DryDisk for each sample (Sodium sulfate and decanting are allowed for some methods, check with your regulatory agency).
- Label and pre-weigh twenty-four aluminum pans and add the extract of one sample to each pan.
- Use the Speed-Vap® IV Evaporation System to evaporate each extract.
- Weigh each pan and record the weight difference as mg/L of HEM.

**Table 1.** Biotage® Horizon 5000 Extraction Method for 90 mm Pacific® Premium Disks (No Pre-Filters).

Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (s)	Sat. Time (s)	Soak Time (s)	Drain Time (s)	
1. Condition SPE Disk	Hexane	16	60	2	1	60	60	
2. Condition SPE Disk	MeOH	16	60	2	1	60	2	
Step		Sample Flow Rate (#)	Done Loading Sample Delay (s)					
6. Load Sample		5	45					
Step		Dry Time (s)	Pump Rate (#)	N2 Blanket				
7. Air Dry Disk Timer		180	6	Off				
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
8. Elute Sample Container	Hexane	28	35	5	Off	1	20	30
9. Elute Sample Container	Hexane	22	35	5	Off	1	45	45
10. Elute Sample Container	Hexane	22	60	6	Off	1	45	60
Step	Solvent	Solvent Vol.	Purge	Pump	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
11. Wash Sample Container	MeOH	16	60	6	Off	1	45	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
12. Elute Sample Container	Hexane	16	35	5	Off	1	45	45
13. Elute Sample Container	Hexane	16	35	5	Off	1	45	45
14. Elute Sample Container	Hexane	16	60	6	Off	1	45	60

**Table 2.** Biotage® Horizon 5000 Extraction Method for 47 mm Pacific® Premium Disks (No Pre-Filters).

Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)	
1. Condition SPE Disk	Hexane	11	60	2	1	60	60	
2. Condition SPE Disk	MeOH	11	60	2	1	60	2	
Step		Sample Flow Rate (#)	Done Loading Sample Delay (s)					
6. Load Sample		5	45					
Step		Dry Time (s)	Pump Rate (#)	N2 Blanket				
7. Air Dry Disk Timer		180	6	Off				
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
8. Elute Sample Container	Hexane	16	35	5	Off	1	20	30
9. Elute Sample Container	Hexane	11	35	5	Off	1	45	45
10. Elute Sample Container	Hexane	11	60	6	Off	1	45	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
11. Wash Sample Container	MeOH	11	60	6	Off	1	45	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
12. Elute Sample Container	Hexane	11	35	5	Off	1	45	45
13. Elute Sample Container	Hexane	11	35	5	Off	1	45	45
14. Elute Sample Container	Hexane	11	60	6	Off	1	45	60

**Table 3.** Biotage® Horizon 5000 Extraction Method for 90 mm Pacific® Premium Disks with Pre-Filters.

Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)	
1. Condition SPE Disk	Hexane	16	60	2	1	60	60	
2. Condition SPE Disk	MeOH	16	60	2	1	60	2	
Step		Sample Flow Rate (#)	Done Loading Sample Delay (s)					
6. Load Sample		5	45					
Step		Dry Time (s)	Pump Rate (#)	N2 Blanket				
7. Air Dry Disk Timer		480	6	Off				
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
8. Elute Sample Container	Hexane	28	35	5	Off	1	25	30
9. Elute Sample Container	Hexane	22	35	5	Off	1	50	45
10. Elute Sample Container	Hexane	22	60	6	Off	1	50	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
11. Wash Sample Container	MeOH	16	60	6	Off	1	50	60
Step	Solvent	Solvent Vol. (mL)	Purge	Pump	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
12. Elute Sample Container	Hexane	16	35	5	Off	1	45	45
13. Elute Sample Container	Hexane	16	35	5	Off	1	45	45
14. Elute Sample Container	Hexane	16	60	6	Off	1	45	60

**Table 4.** Biotage® Horizon 5000 Extraction Method for 47 mm Pacific® Premium Disks with Pre-Filters.

Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (s)	Sat. Time (s)	Soak Time (s)	Drain Time (s)	
1. Condition SPE Disk	Hexane	11	60	2	1	60	60	
2. Condition SPE Disk	MeOH	11	60	2	1	60	2	
Step		Sample Flow Rate (#)	Done Loading Sample Delay (s)					
6. Load Sample		5	45					
Step		Dry Time (s)	Pump Rate (#)	N2 Blanket				
7. Air Dry Disk Timer		300	6	Off				
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
8. Elute Sample Container	Hexane	16	35	5	Off	1	23	30
9. Elute Sample Container	Hexane	11	35	5	Off	1	48	45
10. Elute Sample Container	Hexane	11	60	6	Off	1	48	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
11. Wash Sample Container	MeOH	11	60	6	Off	1	48	60
Step	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Elute Time (s)
12. Elute Sample Container	Hexane	11	35	5	Off	1	45	45
13. Elute Sample Container	Hexane	11	35	5	Off	1	45	45
14. Elute Sample Container	Hexane	11	60	6	Off	1	45	60

Table 5 shows the initial demonstration of precision and recovery for the 90 mm disks without pre-filters.

**Table 5.** Four replicates 90 mm Disk No Pre-Filters.

Sample	Recovery (mg)	Recovery (%)
1	39.2	98.0
2	38.0	95.0
3	38.0	95.0
4	38.4	96.0
Average		96.0
Standard Deviation		1.41

Table 6 shows the same information for 90 mm disks with pre-filters.

**Table 6.** Four replicates 90 mm Disk with Pre-Filters.

Sample	Recovery (mg)	Recovery (%)
1	38.2	95.5
2	38.1	95.3
3	38.0	95.0
4	38.7	96.7
Average		95.6
Standard Deviation		0.746

Table 7 shows the results for 4 samples using 47 mm disks without pre-filters.

**Table 7.** Four replicates 47 mm Disk No Pre-Filters.

Sample	Recovery (mg)	Recovery (%)
1	39.3	98.3
2	39.2	98.0
3	39.4	98.5
4	39.7	99.2
Average		98.5
Standard Deviation		0.510

In each case the recovery is better than 95%, well within the acceptable range specified in method 1664B of 83–101%. The standard deviation is well below the 11% limit specified in the method for each of the four configurations tested.

Table 8 shows the results for 4 samples using 47 mm disks with pre-filters.

**Table 8.** Four replicates 47 mm Disk with Pre-Filters.

Sample	Recovery (mg)	Recovery (%)
1	39.6	99.0
2	39.1	97.8
3	38.4	96.0
4	38.8	97.0
Average		97.5
Standard Deviation		1.27

## References

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