Utilizing 250 mL Sample Volumes for EPA Method 1664B Extractions with Biotage® Horizon 5000 and Biotage® Horizon 3100

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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.

EPA Method 1664B was developed for measuring oil and grease in wastewater with Hexane Extractable Material (HEM) concentrations ranging from 5 to 1000 mg/L. However, samples containing high oil and grease content (› 500 mg/L HEM) are challenging to extract and therefore the method allows for a smaller sample to be collected. To run reduced sample volumes, laboratories must be able to demonstrate the ability to meet the method detection limit (MDL) requirements at the reduced sample volume level. This application note provides a full solution for EPA method 1664B that meets the method detection limits at 250 mL sample volumes.

Instrumentation

- Biotage Instruments:
  - Biotage® Horizon 5000 (P/N SPE-DEX 5000)
  - Biotage® Horizon 3100 (P/N SPE-DEX 3100)
  - Speed-Vap® Automated Solvent Evaporation System (P/N 200-1000-04)
- Biotage Consumables:
  - Pacific® Premium Oil & Grease Disk, 47 mm (P/N 1664-47-PHT)
  - Oil and Grease QC 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

1. The same sample extraction procedure was completed on the Biotage® Horizon 5000 as well as the Biotage® Horizon 3100.
2. Obtain fourteen 250 mL sample bottles and one 1 L sample bottle and fill them with DI water. Acidify to a pH <2 with HCl. Set aside 3 of the 250 mL sample bottles and the 1 L sample bottle. These will be used for the method blanks and sample preparation for these acidified samples is complete.
3. Place a 47 mm Pacific® Premium disk in a 47 mm disk holder and place on the Horizon 5000 or Horizon 3100. Repeat for each sample.
4. Complete the following spiking procedures into the 250 mL samples for the method detection limit (MDL) and initial precision and recovery measurement (IPR).
   a. MDL: Spike seven 250 mL samples with 1.0 mg/250 mL of HEM (125 µL of the Oil and Grease QC Standard, 26 mL, PN: 50-003-HT).
   b. IPR: Spike four 250 mL samples with 4.0 mg/250 mL of HEM (1,250 µL of the Oil and Grease QC Standard, 26 mL, PN: 50-003-HT).
5. Attach the proper cap adapter onto the 250 mL sample bottles. Then, attach the water inlet valve to each sample bottle and place them on the Horizon 5000 or Horizon 3100. Attach the 19/22 taper 125 mL separatory funnel on the Horizon 5000 or a 24/40 taper 125 mL separatory funnel on the Horizon 3100 for each active station and secure with the retaining clip.
6. Extract the samples for the Horizon 5000 using the extraction method found in Table 1.
7. Extract the samples for the Horizon 3100 using the extraction method found in Table 2.
8. Dry the extracts with the DryDisk® Solvent Drying System in conjunction with the DryDisk-R or sodium sulfate.
9. Place each pre-weighed 125 mm aluminum pan into the Speed-Vap® and quantitatively transfer one extract to each pan.
   a. Pour the dried extract into the designated pan and rinse the collection vessel vigorously with n-hexane three times. Swirl slightly to collect all the HEM in the vessel and pour into the designated pan.
10. Using the Speed-Vap® Evaporation System, concentrate the extracts per the parameters in Table 3. 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.
    a. Allow the extract to finish evaporating in the hood and transfer to a desiccator.
11. Weigh each pan and calculate the HEM recovery in mg/L for each sample.

Table 1. Reduced Sample Volumes on the Biotage® Horizon 5000 with 47 mm Pacific® Premium Disks.

Step	Solvent	Volume (mL)	Purge (s)	Pump Speed	Saturate (s)	Soak (s)	Drain/Elute (s)	Done Loading Delay (s)
Condition SPE Disk	Hexane	11	60	2	1	60	60	
Condition SPE Disk	Methanol	11	60	2	1	60	2	
Load Sample				5				45
Air Dry				6			180	
Elute Sample Container	Hexane	16	35	5	1	10	15	
Elute Sample Container	Hexane	10	35	5	1	45	45	
Elute Sample Container	Hexane	10	60	6	1	45	60	
Wash Sample Container	Methanol	8	60	6	1	20	60	
Elute Sample Container	Hexane	9	35	5	1	45	45	
Elute Sample Container	Hexane	9	35	5	1	45	45	
Elute Sample Container	Hexane	9	60	6	1	45	60	

Table 2. Reduced Sample Volumes on the Biotage® Horizon 3100 with 47 mm Pacific® Premium Disks.

Step	Step Discription	Solvent	Despense (s)	Saturate (s)	Soak (s)	Drain Solvent (s)
1	Condition	Hexane	6	1	30	30
2	Condition	Methanol	6	1	30	3
3	Load Sample					
4	Air Dry 180 s					
Step	**Step Discription**	**Solvent**	**Rinse (s)**	**Saturate Elut (s)**	**Soak (s)**	**Elute (s)**
5	Rinse and Elute	Hexane	9	1	10	15
6	Rinse and Elute	Hexane	6	1	45	45
7	Rinse and Elute	Hexane	6	1	45	30
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate (s)**	**Soak (s)**	**Drain Solvent (s)**
8	Wash	Methanol	2	1	20	60
Step	**Step Description**	**Solvent**	**Rinse (s)**	**Saturate Elute (s)**	**Soak (s)**	**Elute (s)**
9	Rinse and Elute	Hexane	2	1	45	45
10	Rinse and Elute	Hexane	2	1	45	30
11	Rinse and Elute	Hexane	2	1	45	60

Results and Discussion

The extraction process for quality control samples on the Biotage® Horizon 5000 takes approximately 25 minutes, drying time for samples takes about 10 minutes, and the evaporation time takes approximately 25 minutes. With one system, three quality control samples can be extracted and evaporated in about an hour. When using the Biotage® Horizon 3100, the extraction time is reduced to about 15 minutes while all the other steps remain the same. Both instruments yielded about 40 mL of extract.

On both instruments, all results are well within the passing range for each test completed. The blank extractions pass EPA Method 1664B requirements all recovering below the minimum level of quantitation (5.0 mg/L), indicating clean instrumentation.

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 1.50 for the Biotage® Horizon 5000 and 2.22 for the Biotage® Horizon 3100, which are both well below the method requirement of 11.0.

The seven replicate samples on each instrument used for the MDL calculation show little variation, keeping the calculated MDL below the method requirement of 1.4 mg/L. The MDLs were calculated using 6 degrees of freedom and a student t-value of 3.142 with a 99% confidence interval.

Each table below contains the results for all of the Biotage® Horizon 5000 and Biotage® Horizon 3100 extractions outlined in the method summary.

Table 3. Speed-Vap® Evaporation Parameters.

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

Table 4. Blank results on the Biotage® Horizon 5000.

Sample	HEM (mg/L)
1 L Blank	0.2
250 mL Blank (1)	0.0
250 mL Blank (2)	0.0
250 mL Blank (3)	0.0
All blanks < 5.0 mg/L	

Table 5. IPR Results on the Biotage® Horizon 5000.

Sample	HEM (mg/L)	Percent Recovery (%)
1	39.6	99.0
2	38.4	96.0
3	39.6	99.0
4	39.6	99.0
Average Percent Recovery (X)		**98.2%**
Precision (s)		**1.50**

Table 6. MDL Results on the Biotage® Horizon 5000.

Sample	HEM (mg/L)
1	3.2
2	3.2
3	2.8
4	3.6
5	3.2
6	3.6
7	2.8
Precision (s)	**0.33**
MDL Value < 1.4 mg/L	**1.03**

Table 7. Blank results on the Biotage® Horizon 3100.

Sample	HEM (mg/L)
1 L Blank	0.4
250 mL Blank (1)	1.2
250 mL Blank (2)	1.2
250 mL Blank (3)	1.2
All blanks < 5.0 mg/L	

Table 8. IPR Results on the Horizon 3100.

Sample	HEM (mg/L)	Percent Recovery (%)
1	37.2	93.0
2	35.2	88.0
3	36.0	90.0
4	36.8	92.0
Average Percent Recovery (X)		**90.7**
Precision (s)		**2.22**

Table 9. MDL Results on the Biotage® Horizon 3100.

Sample	HEM (mg/L)
1	2.8
2	2.8
3	3.2
4	3.2
5	3.2
6	2.8
7	3.2
Precision (s)	**0.21**
MDL Value < 1.4 mg/L	**0.67**

The results are consistent across the board, resulting into little variability between instrument runs.
Please note: the dilution factor of 4 was taken into account for calculating the 250 mL sample blanks.

Conclusion

This application note demonstrates that HEM can be effectively recovered from 250 mL samples within the guidelines of EPA Method 1664B with the use of the use of Biotage® automated extraction systems and the Speed-Vap®. In addition to that, the blanks indicate virtually no contamination of HEM from consumables or instrumentation.

The route of extracting 100 mL samples was explored with this application note, however, it was deemed unsuccessful. When the samples were weighed to determine recovery, the accuracy of a decimal place were not existent. For example, values for the MDLs would return as 0.0004 g or 0.4 mg. Since the sample was reduced 10x, that value needed to be multiplied by 10, equating to a value of 4 mg. This occurred with the IPR samples as well. In order to get an accurate measurement of HEM including a decimal place for 100 mL samples, a balance capable of measuring to 0.00001 g is required. Due to this discovery, the reduced sample volume was increased to 250 mL. When the recoveries using a 250 mL sample were measured and multiplied by 4, the dilution factor, the recovery yielded more accurate measurements of HEM.

Automated solid-phase extraction improves the precision of HEM recoveries by eliminating operator bias and the use of the Speed-Vap® provides an even evaporation over each extract. When the Horizon 5000 and Horizon 3100 are used in conjunction with the Speed-Vap® to extract samples, especially 250 mL samples, the productivity within the laboratory greatly improves.

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, (2010).

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