

Fractionation of Oil or Bitumen by Column Chromatography and Gravimetric Determination of Saturated, Aromatic, Resin, and Asphaltene Fractions

1. Introduction

This procedure utilizes open column chromatography for compound class separation of oils and bitumen extracts. The saturated, aromatic, resin and asphaltene (SARA) fractions are dried and the weight percent of each fraction in the oil or bitumen is determined gravimetrically. Class separation and gravimetric SARA determination typically uses 50 to 100 mg of oil or bitumen (volume equivalent).

2. Interfaces with Other Methods

Bitumen used in the procedure is obtained using **EGL Method 03**. Products obtained from this procedure can be analyzed using the following Methods:

1. **EGL Method 05** *Elemental Analysis of CHNS/S/O*
2. **EGL Method 08** *Analysis of Whole oil, Saturate, Aromatic, or Bitumen extracts by Gas Chromatography Flame Ionization Detection*

Additional Methods include

1. **EGL Method 25** *Method for Sample Login, Control, and Disposition*
2. **EGL Method 29** *Calibration of Laboratory Scales and Analytical Balances*

3. Materials and Equipment

1. Solvents: chloroform, benzene, *iso*-octane, Methanol (High purity HPLC grade, Optima¹ or equivalent).
2. Glass Wool; Pyrex brand or equivalent. Baked at 420 °C for 24 hours.
3. 7 mL clear glass vials with foil lined caps; Baked at 420 °C for 24 hours.
4. Syringe Filters; 0.45 µm PTFE, Non-Sterile
5. Glass Luer-Lok Syringe
6. Graduated Serological Pipets; 5ml, Sterile, Plugged, Borosilicate glass.
7. Glass Pasteur Pipets
8. Rotary evaporator apparatus; Buchi Rotavapor-R, Neslab RTE-101 refrigerated recirculator and a Savant Speed Vac water jet vacuum.
9. Nitrogen evaporator; Organomation N-evap., nitrogen gas cylinder
10. Labconco Centrivap Console
11. Class A volumetric pipettes

¹ Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

12. Hand held UV light
13. Alumina; 80-200 mesh
14. Silica Gel; Acid Washed for Preparatory Column Chromatography and Flash Chromatography or equivalent.
 - i. Grade 62
 - ii. Grade 923

4. Procedure

- Column Preparation
 1. Activate silica gel and alumina by baking 24 hours at 240 °C and let cool in a dessicator.
 2. Prepare benzene: methanol azeotrope – 60.5:39.5 wt/wt (benzene/methanol)
 3. Partially deactivate silica to 5 percent water (1:20 wt/wt) and alumina to 1 percent water (1:100 wt/wt). (For example, combine 20 g alumina with 0.2 g water). Combine ingredients in jar, cap lid and mix by shaking. Let stand for at least 48 hours before using. Creating one column uses approximately 2 g of silica grade 923, 1 g of silica grade 62, and 0.75 g of alumina. Use these numbers to estimate how much of each component is needed for the number of samples in the set.
 4. Plug 5 mL serological pipets with glass wool by first removing the cotton plug. Using a glass rod, pack pre-baked (420 °C for 24 hours) glass wool into a pipet tip. Optionally: using a #3 cork borer cut a small piece of a GF/A filter paper and using tweezers place the paper on top of the glass wool.
 5. Place silica gel (grade 923), silica gel (grade 62), and alumina in 3 separate small Erlenmeyer flasks and add enough *iso*-octane to completely cover the material. Thoroughly mix silica and alumina slurries to remove all air bubbles. Sonicate if necessary. Use a Pasteur pipet with a short tip for dispensing slurry.
 6. Mount the serological pipets on a stand with burette clamps. Place beaker or jar under each pipet to catch waste solvent. Fill the column with isooctane. As the isooctane is draining start adding the silica gel (grade 923) using a Pasteur pipet. Slowly add silica gel (grade 923) slurry to just above the 2 mL mark on the column. Periodically tap the column with a plastic rod or pencil to remove air bubbles and evenly distribute the silica gel and facilitate compaction. Stop tapping as soon as settling appears complete and there are no air bubbles. Avoid over packing.
 7. Slowly add the silica gel (grade 62) slurry up to just above the zero mL mark (Alternatively, continue to use silica gel (grade 923) slurry for this step). Tap the column if necessary to remove air bubbles
 8. Add the alumina slurry to about 2 to 3 cm (~ 0.5 mL) above the zero mark on the column.
 9. Temporarily store packed columns vertically, completely immersed in *iso*-octane in a capped (using foil or glass stopper) graduated cylinder or similar vessel. One 500 mL graduated cylinder holds about 24 packed columns.
- Asphaltene Precipitation

1. All 7 mL glass asphaltene collection vials should be tared and labeled with the proper LIMS identification number and as “asphaltenes” prior to continuing on to the next steps.
 2. For oil; weigh the sample (optimal weight 50 to 100 mg) directly into a clean 7 mL vial. Then add 2-3ml of isooctane and mix with a vortex mixer for 30 sec or with a sonication bath to insure adequate mixing. Asphaltenes will precipitate, and the supernatant is known as maltene. Note: The traditional operational definition of asphaltene is the portion of the oil that is insoluble in excess volumes of n-pentane at 0°C.
 3. For bitumen; Take an aliquot of the chloroform extract dissolved in chloroform with known concentration (optimal weight 50 to 100 mg of bitumen) using a class A volumetric pipet and place into a clean 7 mL vial. If the volume of extract needed to obtain the optimal weight exceeds 7 mL a 20 or 40 mL vial can be used. Reduce the volume to about 1 mL using a nitrogen evaporator. Add an equal or greater volume of isooctane to the vial and mix with a vortex mixer. Reduce the volume again to about 1 mL. Repeat adding 1 mL and reducing the volume three more times for a total of four solvent exchanges. The asphaltenes will precipitate.
- Asphaltene Separation

The asphaltene precipitate is removed from the maltene by one of two techniques:

1. For the first technique, transfer the maltene supernatant using a Pasteur pipet to a filter system consisting of a Luer-lock glass syringe (5 mL) fitted with a 0.45 micron PTFE (Teflon) syringe filter (13 mm or 25 mm diameter), that is vertically-mounted on a stand with a clamp. Allow the maltene supernatant to drip under gravity, or gently push with the syringe plunger. Collect the filtrate in a clean 7 mL vial. Rinse the asphaltene precipitate remaining in the original vial with 1 mL *iso*-octane and transfer the rinse to the filter system. Repeat the asphaltene precipitate rinse two more times for a total of 3 mL. Save the filtrate (maltene) for steps below. The asphaltene precipitate remaining in the original vial is dissolved with three 0.5 to 1 mL of chloroform and transferred to the filter system and the chloroform filtrate is collected in a baked, etched and tared 7 mL vial. Rinse the original vial and filter system with successive 1 mL chloroform rinses until the transfer is complete (usually about 3 x 1 mL rinses are sufficient). Insoluble residue on the syringe filter may be inorganic matter such as clay minerals or silt.
2. For the second technique, centrifuge the vial 2-3 minutes and decant the maltene supernatant using a Pasteur pipet into clean 7 mL vial. Rinse the asphaltenes precipitate in the initial vial with 1 mL *iso*-octane. Repeat the centrifuging and decanting steps three times or until the rinse solvent is clear. Save the rinses/supernatant (maltene) for steps below. Dissolve the asphaltenes in chloroform and transfer with a Pasteur pipe into a baked, etched, tared 7 mL vial.

Note: The second technique is better for samples with high asphaltene content. Also both techniques may be combined by passing the decanted maltene supernatant through the aforementioned filter system.

3. Reduce the volume of the maltene fraction to about 0.5 ml with a nitrogen evaporator for column chromatography. The asphaltenes are dried with nitrogen, centrifuge console, or under the fume hood and weighted.
- Column Chromatography
 1. All 7 mL glass saturated, aromatic, and resin collection vials should be tared, and labeled with proper LIMS identification number and as “saturated, aromatic, or resin” prior to continuing on to the next steps.
 2. Place a packed column(s) in a burette clamp on a stand. Allow the column to drain until most of the *iso*-octane above the column is gone. Without letting the column go dry, load the maltene fraction onto the column with a Pasteur pipet. Add about 1ml *iso*-octane to the vial and rinse the residual maltene.
 3. Just as the sample completely moves down onto the alumina add the first 1 mL *iso*-octane vial rinse. Repeat this step two more times for a total of three 1 mL vial rinses. Once the vial rinses are performed continue to elute with *iso*-octane. Collect 3 mL (the bed volume) in a waste beaker. Then start to collect the eluate in the appropriate tared vial, which at this point is the saturated hydrocarbon fraction. The eluate should be colorless. If it has a light yellowish tint aromatic compounds have eluted into the saturate fraction, this may be a result of improperly made columns or a result of the chemical properties of the sample. Columns should be remade and the separation should be repeated.
 4. When about 3 mL of *iso*-octane eluate (saturated hydrocarbon fraction) is collected, start to elute the aromatic hydrocarbon fraction with benzene. However, continue to collect the *iso*-octane eluate as the saturated hydrocarbon fraction. When the aromatic front can be observed to have moved to the tip of the column, switch vials and start collecting the aromatic fraction. Although the aromatic front is usually visible in normal light (pale yellow and sometimes light orange-brown), it is best observed with a hand-held UV light (blue/purple). The total volume of the saturate fraction should be 6 to 7 mL and should be colorless under normal and UV light.
 5. When about 3 mL of benzene eluate (aromatic hydrocarbon fraction) is collected, rinse the original maltene vial with three 1 mL rinses of benzene-methanol solution and add to the column, this will remove any resins stuck to the vial. Once rinses are complete continue to elute with the benzene-methanol solution. The resin front (typically dark brown) will begin to move down the column. Continue to collect the benzene eluate (aromatic hydrocarbon fraction) until the resin front is observed in the column tip. Sometimes this exceeds the 7 mL capacity of the vial so have clean spare vials ready.
 6. Collect the resin fraction (also called NSO or polar fraction) eluate (usually brown colored) in a baked, etched, tared 7 mL vial until the eluate leaving the column appears colorless - usually 5-7 mL. Sometimes water is pulled off the column

- with polar solvents containing methanol, but this should not adversely impact the resin fraction determination.
7. In the Fracs_Template_1(ver4.3) spreadsheet make a note of the column color for degree of column holdup.
 8. If requested, run gas chromatography analyses on the saturated and aromatic hydrocarbon fractions via EGL Method 08. Usually it is necessary to concentrate the aromatic fraction by gentle evaporation using the nitrogen evaporator. (Concentrating 7 mL to approximately 1 mL has been found useful.).
 9. After successful gas chromatography analyses of the saturated and aromatic fractions are complete, completely evaporate the solvent in the fractions using a nitrogen evaporator under a fume hood or using a centrifugal vacuum evaporator. Solvent evaporation is complete when the percent change in the weight is less than 1 percent/minute. The resin fraction may require low heat to evaporate water. Volatile components of the sample will be lost and the remaining fractions will be approximately C₁₅ and greater.
 10. Measure the gross weight of the vials with the dried fractions, and calculate the net weight of the fractions (C₁₅ and greater weights).
 11. To determine the percent volatile fraction (less than C₁₅) in oil samples, weigh 100 mg of oil in a tared 7mL vial.
 12. Evaporate the oil using a nitrogen evaporator for about 30 minutes. Weigh.
 13. Evaporate for additional 10 minutes and reweigh. Repeat until the percent change in weight is less than 1 percent/minute.

5. Calibration and Quality Control Samples

1. In-house standard; Clifford Causey 04049-001 crude oil
2. Perform column chromatography on in-house crude oil standard with every batch of constructed columns (typically made for each sample set). Check performance as follows: the asphaltene precipitate should be black or very dark colored; the saturated fraction (dissolved in 7 mL *iso*-octane) should be colorless under normal and UV light; The aromatic fraction (dissolved in 7 mL benzene) is typically a shade of yellow and the resin fraction (dissolved in 7 mL benzene-methanol) is typically a shade of dark brown. Confirm saturated and aromatic hydrocarbons by gas chromatography EGL Method 08, R0.
3. Mass balance for the standard should meet the criteria stated in Acceptance of data.
4. Deviations from the SOP or unusual results are recorded in laboratory notebook and in the LIMS

6. Limits, Precautions and Interferences

- Health, safety and waste
 1. This procedure requires the use of chloroform, benzene, methanol, and *iso*-octane. Chloroform is a suspected carcinogen and has proven to have mutagenic effects on mammalian somatic cells. Long term exposure of

chloroform vapor can cause liver and kidney damage. The Department of Health and Human Services has determined that benzene causes cancer in humans. Long-term exposure to high levels of benzene in the air can cause Leukemia. Isooctane may cause defatting and dermatitis if exposed to the skin repeatedly or for prolonged periods of time.

2. Always wear proper laboratory personal protective equipment such as nitrile gloves, safety goggles, lab coat and always work in a ventilated fume hood.
3. Disposal of all organic solvents and samples must be performed in accordance with the current waste disposal procedure.
4. The oven is a heat source and possible source of ignition.

- Interferences

1. Care should always be taken to minimize exposure of the activated and partially deactivated silica and alumina to any moisture in the air and it should always be kept in the dessicator, in a capped jar, or in a capped isooctane slurry when not in use during column preparation.
2. It is important to minimize vapor loss while measuring the initial weight of the oil for column chromatography or for determining volatiles. Always perform these tasks with a capped vial.
3. Do not “over dry” the saturated and aromatic fractions, this may lead to loss of C₁₅₊ compounds. If a low total % recovery is observed, an aliquot of the fraction can be analyzed by GC-FID to determine if any C₁₅₊ compounds have been lost during the drying process.
4. Balance drift may occur during the procedure due to variability in ambient conditions in the laboratory. This can lead to unusually high or low total percent recoveries. The drift can be minimized by taking the final gross weight, re-dissolve and remove the extract with the appropriate solvent and record the tare weight of the vial in the same day.
5. If water or sediment is present or thought to be present in the sample, the oil is centrifuged or passed through a 0.45 µm syringe filter to remove the water prior to taking the initial weight of the oil.

7. Acceptance of Data

1. The mass balance for an oil is the sum of the weights of the four fractions (C₁₅ and greater) plus the weight of the volatiles (less than C₁₅) should ideally equal the initial oil weight. Acceptable recovery ranges are 105 percent to 75 percent of the initial oil weight.
2. The mass balance for a bitumen extract is the sum of the weights of the four fractions (C₁₅ and greater) should ideally equal the initial bitumen weight based on the calculated concentration of the extract dissolved in the chloroform. Acceptable recovery ranges are 105 percent to 75 percent recovery of the initial bitumen weight.

8. Data handling and Transfer

The mass balance for the oil and bitumen is calculated using an Excel™ spreadsheet Fracs_Template_1 (Ver4.3), transferred to the proper spreadsheet template for submittal into the LIMS via a shared network folder. Once in the LIMS, results are validated by the analyst and approved by an appropriate QA official before being released to the submitter.

9. References

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10. Attachments

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11. History of Changes

R0: Initial Issue