

**CONFIDENTIAL**

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Heavy Polynuclear Aromatics (HPNA) in Petroleum Fractions by Fluorescence Spectroscopy

UOP Method 860-07

Scope

This method is for estimating total aromatic hydrocarbons having 11 or more (11+) aromatic rings (heavy polynuclear aromatics, or HPNA) in petroleum fractions using fluorescence spectrophotometry. It is often used in support of commercial HPNA removal (HPNA RM) units. The method has been applied to petroleum products; e.g., hydrocracking fractionator bottoms (recycle oil). This method is not applicable to heavy feedstocks; e.g., vacuum gas oils, because of suspected interference from aromatic nitrogen compounds (see *Note 1*). This method may also be applicable to extracts from catalysts and plant deposits (see *Note 2*). The estimated value, in $\mu\text{g/mL}$, is reported for total HPNA and for distributed HPNA at specific wavelengths that are attributable to HPNA. The lower limit of detection for total HPNA is approximately $2.5 \mu\text{g/mL}$. The upper limit is 1000 to 2000 $\mu\text{g/mL}$ depending on the solubility of the HPNA and possible interferences (see *Note 1*).

Reference

UOP Method 999, "Precision Statements in UOP Methods," www.astm.org

Outline of Method

The sample is diluted in chlorobenzene. The synchronous fluorescence spectrum of the sample is obtained and quantitatively compared to the spectrum of an appropriate reference standard of Rhodamine B. A correlation has been established between the response of Rhodamine B and the response of known concentrations of HPNA. The reference standard can be either a primary standard solution of Rhodamine B dissolved in tetraethylene glycol and diluted in 1-pentanol, or a Fluorescence Intensity Sample (FIS). An FIS is a commercially available polymethylmethacrylate (PMMA) block containing Rhodamine B, sized to fit into the spectrofluorometer in place of the sample cell. Analysis of the spectra is achieved by using non-linear curve fitting to compare the emission intensity of the sample spectrum to that of the reference solution at five wavelengths attributed to HPNA (see *Note 3*).

Apparatus

References to catalog numbers and suppliers are included as a convenience to the method user. Other suppliers may be used unless otherwise indicated.

Balance, readability 0.1-mg

IT IS THE USER'S RESPONSIBILITY TO ESTABLISH APPROPRIATE PRECAUTIONARY PRACTICES AND TO DETERMINE THE APPLICABILITY OF REGULATORY LIMITATIONS PRIOR TO USE. EFFECTIVE HEALTH AND SAFETY PRACTICES ARE TO BE FOLLOWED WHEN UTILIZING THIS PROCEDURE. FAILURE TO UTILIZE THIS PROCEDURE IN THE MANNER PRESCRIBED HEREIN CAN BE HAZARDOUS. MATERIAL SAFETY DATA SHEETS (MSDS) OR EXPERIMENTAL MATERIAL SAFETY DATA SHEETS (EMSDS) FOR ALL OF THE MATERIALS USED IN THIS PROCEDURE SHOULD BE REVIEWED FOR SELECTION OF THE APPROPRIATE PERSONAL PROTECTION EQUIPMENT (PPE).

Previous editions 1985, 1987, 1989, 1993, 1998, 2005

Beakers, borosilicate glass, 50-, 150- and 250-mL, Fisher Scientific, Cat. Nos. 02-539G, J and K, respectively

Bottle-top dispenser, 5- to 25-mL, 2 required (3 are required if serial dilutions of samples are required, see *Note 4*), Fisher Scientific, Cat. No. 13-688-134

Constant temperature bath (circulator), optional (see *Note 5*), 0.01°C temperature control, Fisher Scientific, Cat. No. 13-873-301; with Bead Bath Accessory, Fisher Scientific, Cat. No. 11-715-45BB

Dry bath incubator, Fisher Scientific, Cat. No. 11-715-145D

Flask, volumetric, Class A, 1000-mL, Fisher Scientific, Cat. No. 10-210-5G

Fluorescence cell, 10-mm path length, polished quartz all sides, Wilmad/Lab-Glass, Cat. No. WG-3-Q-10

Funnel, long stem, Fisher Scientific, Cat. No. 10-326A

Micro spatula, Fisher Scientific, Cat. No. 21-401-15

Mixing cylinder, 10-mL, Fisher Scientific, Cat. No. 08-531A

Pipetter, digital microdispenser, adjustable dispensing volume, 50-μL capacity, Drummond, Fisher Scientific, Cat. No. 21-169-20C

Pipetter, digital microdispenser, adjustable dispensing volume, 1000-μL capacity, Drummond, Fisher Scientific, Cat. No. 21-169-10A (see *Note 4*)

Regulator, air, two-stage, high purity, Matheson Tri-Gas, Model 3122-590

Rhodamine B in PMMA (polymethylmethacrylate) Block, Fluorescence Intensity Sample (FIS), a minimum of 3 are required, Varian, Cat. No. 6610021900

Software, OriginPro™ with Peak Fitting Module, non-linear curve fitting software available from OriginLab, no substitution

Spectrofluorometer, having dual monochromators, equipped with individual and dual scan control, PerkinElmer, Model LS-55

Ultrasonic bath, 3.2-L capacity, Fisher Scientific, Cat. No. 15-335-30

Weighing funnel, Fisher Scientific, Cat. No. 14-353B

Reagents and Materials

References to catalog numbers and suppliers are included as a convenience to the method user. Other suppliers may be used.

Air, compressed, oil-free

Bottles, amber wide-mouth, Teflon™-lined closure, 5 required, Fisher Scientific, Cat. No. 03-320-8D

Chlorobenzene, 99.9% minimum purity, Aldrich, Cat. No. 270644-1L

Gloves, N-DEX, Fisher Scientific, Cat. Nos. 11-395-19A to D, depending on size

Pasteur pipette bulbs, 2-mL, Fisher Scientific, Cat. No. 14-065B

Pasteur pipette, glass, Fisher Scientific, Cat. No. 13-678-20B

1-Pentanol, 99+% purity, Aldrich Chemical, Cat. No. 398268-1L

Pipetter, disposable glass bores, Fisher Scientific, Cat. No. 21-169D, for use with digital microdispenser, Cat. No. 21-169-20C

Pipetter, disposable plastic bores, Fisher Scientific, Cat. No. 21-169-10G, for use with digital microdispenser, Cat. No. 21-169-10A

Rhodamine B, 99+% purity, suitable for dye laser, Acros Organics, Cat. No. 41900-0010, Fisher Scientific

Tetraethylene glycol, 99% minimum purity, Aldrich Chemical, Cat. No. 11017-5

Vials and caps, 22-mL, glass, molded screw cap with polyvinyl liner, Fisher Scientific, Cat. No. 03-338H

Wiper, Kimwipes™, 110- x 220-mm, Fisher Scientific, Cat. No. 06-666A

Procedure

The analyst is expected to be familiar with general laboratory practices, the technique of fluorescence spectroscopy, and the equipment being used.

Preparation of Apparatus

The instrument settings shown in Table 1 have been found to be satisfactory for the spectrofluorometer listed in *Apparatus*. Other settings may be used if equivalent performance is obtained.

Table 1
Instrument Settings for Synchronous Fluorescence

$\Delta\lambda$	6 nm
Monochromator bandpass	2.5 nm
Photomultiplier voltage	600 V
Scan rate	1 nm/sec
Step	1.0 nm
Wavelength range	
Excitation	294 to 594 nm
Emission	300 to 600 nm

Preparation of Primary HPNA Calibration Standard (PCS)

The HPNAs are quantitated by comparing the HPNA synchronous fluorescence peak areas of the sample to the synchronous fluorescence peak area of a standard solution of Rhodamine B. The solution of Rhodamine B in tetraethylene glycol is considered to be the Primary HPNA Calibration Standard, and is not intended to be analyzed each time the analysis is performed.

1. Weigh 0.16 ± 0.02 g, to the nearest 0.1 mg, of Rhodamine B into the weighing funnel.
 - Rhodamine B is a very strong dye, do not let it contact any stainable materials. Use appropriate personal protective equipment.
2. Using the long stemmed funnel, transfer the Rhodamine B to the dry 1000-mL volumetric flask directly from the weighing funnel.
 - Do not let any Rhodamine B contact the neck of the volumetric flask.

3. Wash the residual Rhodamine B that may stick to the weighing funnel through the long stemmed funnel into the volumetric flask by dropwise addition of tetraethylene glycol (TTEG).
4. Using the long stemmed funnel, finish filling the volumetric flask with approximately 950 mL of TTEG.
 - Fill the volumetric flask to just below the base of the neck. When the solution is heated by sonication and expands, the top of the solution should always remain below the graduation line. For best quantitation, do not allow the TTEG to wet the neck of the volumetric flask near or above the graduation line. Do not shake the volumetric flask.
5. Place the volumetric flask into the ultrasonic bath and sonicate the solution for 15 to 30 minutes with the ultrasonic bath at ambient temperature. If the Rhodamine B is not fully dissolved after 30 minutes, remove the volumetric flask from the ultrasonic bath and let it cool for 15 to 30 minutes. After cooling, re-insert the volumetric flask into the ultrasonic bath for another 15 to 30 minute period. Repeat the ultrasonic treatment/cooling until particles of Rhodamine B are no longer visible.
 - The ultrasound treatment will typically warm the solution slightly; this aids the dissolution of the Rhodamine B and is acceptable.
7. Allow the TTEG solution in the volumetric flask to cool to ambient temperature while on the bench top.
 - This may take 2 to 4 hours depending on the laboratory conditions, or it may be left overnight to eliminate any concerns about whether it has reached equilibrium.
8. Use the long stemmed funnel to fill the volumetric flask to just below the graduation line with TTEG. Do not allow the TTEG to wet the neck of the volumetric flask above the graduation line. Use the Pasteur pipette to fill the flask to the graduation line.
9. Mix the TTEG solution of Rhodamine B by inverting the flask several times.
10. Transfer the standard solution of Rhodamine B in TTEG into five amber, wide-mouthed bottles. Cap the bottles tightly.
11. Calculate the concentration of Rhodamine B in the PCS using Equation 1:

Concentration of Rhodamine B (called "T" in subsequent equations), moles/L

$$= \frac{G}{(479.02)(1)} \quad (1)$$

where:

G = number of grams of Rhodamine B weighed into the weighing funnel

1 = number of liters used to prepare primary standard solution, L

479.02 = molecular weight of Rhodamine B

12. Label the bottles with the concentration of Rhodamine B and all appropriate safety information, and store in a safe place. Under typical storage conditions, the PCS is stable for more than one year. After one year, prepare a new PCS.

Standardization of the Fluorescence Intensity Sample (FIS)

From the known concentration of Rhodamine B in the PCS, the equivalent concentration of the Rhodamine B in the PMMA (polymethylmethacrylate) block FIS is calculated. The FIS is analyzed and used to calculate the HPNA concentrations in the samples. It is also used to confirm correct instrument operation.

1. Obtain a background spectrum as described in *Sample Analysis*, Steps 4 through 6.

2. Using the bottletop dispenser, transfer 10 mL of 1-pentanol into a 22-mL sample vial.
 - Confirm the delivery volume of bottletop dispensers using the 10-mL mixing cylinder, drying the cylinder before each delivery volume experiment. Do not let solvent wet the side of the cylinder above the 10-mL graduation.
3. Shake the PCS solution and, using a microdispenser, withdraw a 40- μ L aliquot of the primary standard. A 40- μ L aliquot will produce a 1 in 251 dilution. Wearing gloves, wipe the outside of the glass tip of the microdispenser carefully with a wiper to remove any adhering primary standard solution.
4. Transfer the contents of the microdispenser to the vial containing the 10 mL of 1-pentanol and shake well. Immerse the tip of the microdispenser and rinse the interior of the dispenser 5-6 times with the 1-pentanol solution. Dispense the last 1-pentanol solution rinse of the microdispenser into the sample vial and allow the 1-pentanol solution to fully drain from the microdispenser into the sample vial. Shake again.
5. Using a new Pasteur pipette, transfer 3 to 5 mL of the diluted primary standard Rhodamine B solution into the fluorescence sample cell and acquire the synchronous spectrum. See Figure 1.
6. Remove the sample cell from the instrument and clean with chlorobenzene after data acquisition is complete.
7. Place an FIS in the instrument and acquire the synchronous spectrum. Then remove the FIS from the instrument. If more than one FIS is to be calibrated, repeat for each FIS.
8. Fit the instrument background spectrum with an appropriate polynomial and subtract the polynomial expression of the instrument background from the Rhodamine B PCS solution and/or the FIS. Fit the polynomial from 496 to 600 nm. The LS-55 instrument background is typically a second order polynomial over the 496 to 600 wavelength region. Correct the primary standard and/or the FIS fluorescence spectrum per the instructions supplied with the instrument. Create a data file for the primary standard and FIS data that can be loaded into the non-linear curve fitting software. Import the data into the curve fitting software. Curve fit the 496 to 600 nm region of the primary standard and the FIS using non-linear least squares (see Note 6) and calculate the peak area of the primary standard solution and the FIS spectrum. Use 1 or 2 peaks (Voigt) to fit the primary standard and FIS spectrum as needed. See Figure 2.
 - The LS-55 data system is capable of automatic background correction. It may be used in lieu of the correction described above.
9. Calculate the equivalent HPNA concentration in the FIS using Equation 2:

$$\text{Equivalent concentration of HPNA in calibrated FIS, } \mu\text{g/mL} = 5.1512 \times 10^6 \frac{\text{TS}}{\text{B}} \quad (2)$$

where:

- B** = sum of the peak areas of the PCS
- S** = sum of the peak areas of the FIS
- T** = concentration of Rhodamine B in the PCS, moles/L, Equation 1
- 5.1512×10^6** = conversion factor for converting Rhodamine B dissolved in TTEG diluted 1 in 251 with 1-pentanol fluorescence intensity to HPNA fluorescence intensities, $\mu\text{g/mL}/(\text{mole/L})$. The value of the conversion factor was determined from a series of experiments comparing the Rhodamine B fluorescence to previously analyzed reference solutions and samples.

It is recommended that several FISs be calibrated in case any are damaged during use. One calibrated FIS may be used routinely with the others in reserve.

Precaution: The PMMA used in the FIS is not compatible with the chlorobenzene solvent used for sample dilution. If the FIS comes in contact with the chlorobenzene fumes, the PMMA will craze and lose its transparency. If the FIS comes in direct contact with chlorobenzene, it will dissolve. Also, the FIS should be handled using the standard spectrophotometric practices; i.e., do not touch the surfaces of the FIS where the light beam strikes and exits the FIS, do not scratch the surfaces, place into the instrument in the same orientation, etc.

If the PCS is to be used for instrument calibration for sample analysis instead of the FIS, calculate the equivalent HPNA concentration in the PCS using Equation 3:

$$\text{Equivalent concentration of HPNA in the PCS, } \mu\text{g/mL} = 5.1512 \times 10^6 T \quad (3)$$

where:

T = concentration of Rhodamine B in the PCS, moles/L, Equation 1
 5.1512×10^6 = conversion factor for converting Rhodamine B dissolved in TTEG diluted 1 in 251 with 1-pentanol fluorescence intensity to HPNA fluorescence intensities, $\mu\text{g/mL}/(\text{mole/L})$

Sample Preparation

1. Warm hydrocarbon samples in the incubator bead bath accessory at 80°C for 30 minutes. The incubator must be located in an exhaust hood to minimize operator exposure to vapors. Loosen the caps of sample vials as needed to avoid pressure build up inside the vials.
2. Transfer 10 mL of chlorobenzene to a 22-mL sample vial using the bottletop dispenser.
 - Confirm delivery volume of bottletop dispensers using the 10-mL mixing cylinder, drying the cylinder before each delivery volume experiment. Do not let solvent wet the side of the cylinder above the 10-mL graduation.
 - **CAUTION:** Chlorobenzene has a low flash point. Do not allow vapors to come in contact with a source of heat. Avoid exposure to chlorobenzene vapors by working in a hood or other controlled environment. Use appropriate personal protective equipment.
3. Shake the warm sample and, using a digital microdispenser, withdraw an aliquot of the sample.
 - A typical sample will require a 40- μL aliquot that will produce a 1 in 251 dilution. The aliquot size is determined by the HPNA concentration in the sample. On the recommended spectrometer, the actual concentration of HPNA after dilution must be between 0.05 $\mu\text{g/mL}$ and 0.1 $\mu\text{g/mL}$. The upper limit is determined by self absorption and filter effects and the lower concentration by signal-to-noise limitations.
4. Wearing gloves, wipe the outside of the glass tip of the microdispenser carefully with a wiper to remove any adhering sample. Wipe the outside of the microdispenser glass tip without letting any of the sample be drawn out of the glass tip by the wiper.
5. Transfer the contents of the microdispenser to the vial containing the 10 mL of chlorobenzene and shake well. Immerse the tip of the microdispenser into the chlorobenzene when transferring the sample. Rinse the interior of the dispenser 5 to 6 times with the chlorobenzene solution. Dispense the last chlorobenzene solution rinse of the microdispenser into the sample vial and allow the chlorobenzene solution to fully drain from the microdispenser into the sample vial.
6. Let the sample stand from 30 minutes to 12 hours to ensure complete dissolution of the HPNA. To determine the time required for dissolution, prepare a sample in duplicate. Analyze one sample after 30 min and the second after 12 hours. If the results agree within 6% there is no solubility problem. If the 12-hour sample intensity exceeds the 6% limit, the samples must be allowed to stand overnight to ensure complete dissolution.

Sample Analysis

The sample analysis procedure is written with the expectation that an FIS will be used for calibration. If the Primary HPNA Calibration Standard (PCS) is to be used instead, dilute the PCS as described in *Standardization of FIS*, Steps 2-4, and substitute the diluted PCS for FIS in the steps below. Use the peak area of the diluted PCS solution for the concentration calculations in place of the peak area for the FIS.

1. Connect the circulator (if used) to the spectrometer. Start the circulator according to the manufacturer's directions. Maintain the spectrometer sample cell mount at constant temperature, 25°C. Confirm proper water circulation.
2. Start the fluorescence spectrometer. Allow the instrument to warm up for 30 minutes.
 - The instrument must be warmed up and thermally stable before use. However, there is no need to wait for the samples to come to thermal equilibrium in the instrument; a spectrum may be obtained immediately upon placing the sample in the instrument.
 - The circulator may be maintained at constant temperature while the instrument is not being used.
3. Set all instrument scan conditions as listed in Table 1.
4. Fill the fluorescence sample cell with fresh chlorobenzene, and acquire the instrument background synchronous spectrum.
 - **CAUTION:** Use all appropriate precautions and appropriate personal protective equipment when handling chlorobenzene, Rhodamine B, TTEG and 1-pentanol. Avoid exposure to chemical vapors by working in a hood or other controlled environment. Dispose of all samples and solvents in a safe manner.
5. Empty the sample cell. The fluorescence sample cell may be dried using a gentle flow of air.
6. Inspect the spectrum to determine that the instrument baseline is smooth and that the fluorescence sample cell is clean.
7. Place the calibrated Rhodamine B FIS into the instrument and acquire the synchronous spectrum. The spectrum of the calibrated FIS must be acquired before samples are analyzed.
 - The primary standard solution of Rhodamine B in tetraethylene glycol can also be used. However, it is much safer and more practical to use the calibrated FIS. Therefore, use of the calibrated FIS is preferred. If the primary standard solution of Rhodamine B is used, continue through the procedures using the peak area of the Primary HPNA Calibration Standard solution for the concentration calculations in place of the peak area for the FIS.
8. Compare the FIS spectrum to previous FIS spectra, when available, and confirm that it has similar intensity and peak shape. If there are significant differences, the operation of the instrument needs to be examined. Either the instrument settings are wrong or there may be instrument problems. Make the necessary adjustments or repairs and repeat this step.
9. Shake the diluted sample well. Using a new Pasteur pipette transfer the diluted sample to the fluorescence sample cell and acquire the synchronous fluorescence spectrum. Typical spectra are shown in Figures 3 and 4.
10. Remove the sample cell from the instrument and clean with chlorobenzene after data acquisition is complete.
 - If the intensity of the sample fluorescence is very intense (greater than about 40 to 50 units peak height on the LS-55 instrument, or about 4 to 5 times the intensity of the calibration), there is a possibility that inner filter and self-absorption effects are occurring. If these effects are suspected, dilute the sample by a factor of about 5 and rerun. If the calculated values for the HPNA differ by more than 6%, the sample should be run at about 5:1 further dilution (see Note 4). A series of dilutions may be required to confirm

that there are no inner filter or self absorption effects occurring and that the fluorescence is linear with concentration.

11. Turn off the spectrometer after all samples are run following the manufacturer's directions. If the fluorescence sample cell is being maintained at constant temperature, the circulator may be left running continuously.

Calculations

Fit the instrument background spectrum with an appropriate polynomial and subtract the polynomial expression of the instrument background from the FIS and sample data files. Fit the polynomial from 496 to 600 nm for the FIS and 496 to 580 nm for the samples. The LS-55 instrument background typically is a second order polynomial over the 496 to 600 nm wavelength region. Instrument correct the FIS and sample fluorescence spectrum, per the instructions supplied with the instrument. Create a data file for the FIS and sample runs that can be loaded into the non-linear curve fitting software. Use the ASSCI export software in the LS-55 data system. Import the data into the curve fitting software. Curve fit the 496 to 600 nm region of the FIS and the 496 to 580 nm region of the sample data using non-linear least squares (see *Note 6*) and calculate the peak area of each of the FIS spectrum and the HPNA fluorescence peaks (see Figures 2 and 4).

Calculate the total 11+ HPNA concentration in the liquid sample using Equation 4.

$$\text{Total concentration of HPNA in the sample, } \mu\text{g/mL} = 1000 \left(\frac{UWV}{SD} \right) \left(\frac{Y}{Z} \right) \quad (4)$$

where:

- D** = volume of sample used in sample dilution, μL (typically, 40 μL)
- S** = sum of the calculated peak areas of the Rhodamine B FIS
- U** = sum of the calculated peak areas of the HPNA (510, 522, 536, 545 and 555 nm peaks) in the sample solution
- V** = volume of chlorobenzene used in sample dilution, mL (typically, 10 mL)
- W** = equivalent concentration of HPNA in calibrated FIS, $\mu\text{g/mL}$, Equation 2 (if using PCS in place of FIS, use the equivalent concentration of HPNA in the diluted PCS, Equation 3)
- Y** = if further diluted, total volume of aliquot of sample to be further diluted (**Z**) and the added chlorobenzene diluent
- Z** = if further diluted, volume of aliquot of sample to be further diluted
- 1000** = factor to convert μL of sample to mL

Calculate the individual 11+ HPNA peak concentrations using Equation 5.

$$H = \frac{AC}{U} \quad (5)$$

where:

- A** = peak area of the individual peak
- C** = total concentration of HPNA in the sample, Equation 2
- H** = concentration of an individual HPNA peak, $\mu\text{g/mL}$
- U** = sum of the calculated peak areas of the HPNA (510, 522, 536, 545 and 555 nm peaks) in the sample solution (defined in Equation 2)

Report the total HPNA concentration and the individual HPNA peak concentrations to the nearest $\mu\text{g/mL}$.

Notes

1. Solution species that interfere with the 11+ ring aromatic fluorescence in the 586 nm region have been observed in crude oils and heavy VGO samples. The interfering emission appears to be due to aromatic nitrogen species, since the problem is observed in high nitrogen content samples. The interference is easily recognized by poor resolution of the synchronous spectra.
2. The method is written for the analysis of liquid product samples. Analysis for HPNA on catalysts or in plant deposits requires unique sample preparation techniques, which are beyond the scope of this method. Liquid extracts of catalysts or plant deposits can be analyzed for HPNA. The estimated concentration in an extract can be used in conjunction with information about the way in which the extract was acquired to calculate the concentration of the HPNA on the catalyst or plant deposit, but such calculation is also beyond the scope of this method.
3. The different HPNA types are reported on the basis of the wavelength at which their peak fluorescence intensity occurs. There are no known molecular structures that relate to the wavelengths. It is known that the different HPNA types that are distinguished in this method have different solubilities. This method can be used to monitor changes in the HPNA concentrations, but no correlations have been made between the HPNA concentrations and catalyst stability or equipment fouling.
4. If serial dilutions are routinely required, a second bottle-top dispenser/chlorobenzene bottle and a 1000- μ L digital microdispenser may be used to minimize the labor required. A typical dilution would be to add 1000 μ L of the diluted sample to 5 mL of chlorobenzene in a 22-mL vial using the same procedure as was used to make the first dilution.
5. The spectrofluorometer sample cell mount can be held at constant temperature with a water circulator; the addition of the circulator significantly increases the cost of the apparatus (>15%). For the precision of the method reported herein, a constant temperature of 25°C was used for the sample cell mount. If the circulator is not used, the repeatability will be decreased by 2 to 4% relative. If the lower precision is acceptable, no temperature control of the sample cell mount is needed.
6. The suggested software uses the Marquardt-Levenberg algorithm for non-linear least-squares fitting. The fit should be made with Voigt peak functions. The peak widths should be a dynamic variable in the fit, but all of the peaks should have the same width ("fixed/share" in OriginPro). It has been found that most data sets require the use of 4 to 6 peaks. The lowest wavelength peak is not reported because it is an artifact from the data truncation. Peak apexes are typically within the wavelength windows shown in Table 2.

The 495, 510, 536 and 545 peaks are nearly always required to calculate a good fit, the 522 and the 555 peaks are not always required. Whether a peak is required or not to create a good mathematical fit is determined from the statistical analysis of the calculated fit.

Table 2
Peak Identification

490 to 500 nm	495 peak (not reported)
505 to 514 nm	510 peak
514 to 526 nm	522 peak
528 to 538 nm	536 peak
540 to 550 nm	545 peak
550 to 580 nm	555 peak

Precision

Precision statements were determined using the constant temperature bath and UOP Method 999.

Repeatability and Intermediate Precision

A nested design was carried out for HPNA analysis on two samples with two analysts in one laboratory. Each analyst carried out tests on two separate days, performing two tests on each day. The total number of tests for each sample was eight. The precision data are summarized in Table 3.

Two tests performed by the same analyst on the same day should not differ by more than the repeatability allowable difference with 95% confidence. Two tests performed in one laboratory by different analysts on different days should not differ by more than the intermediate precision allowable difference with 95% confidence.

Table 3
Repeatability and Intermediate Precision, $\mu\text{g/mL}$

Sample/Peak Identification, nm	Value	Repeatability		Intermediate Precision	
		Within- Day esd	Allowable Difference	Within- Lab esd	Allowable Difference
Sample 1					
510	112.3	2.70	10.6	3.04	10.5
522	22.7	1.51	5.9	3.02	11.9
536	38.3	0.94	3.7	1.95	6.5
545	23.2	0.38	1.5	0.58	1.9
555	10.0	0.89	3.5	1.02	3.4
Total	206.5	1.25	4.9	4.78	16.6
Sample 2					
510	5.1	0.46	1.8	0.99	3.6
522	3.2	0.53	2.1	1.53	5.6
536	5.6	0.18	0.7	0.30	1.0
545	1.4	0.10	0.4	0.15	0.5
555	0.6	0.06	0.2	0.11	0.4
Total	15.9	0.52	2.0	1.04	3.8

The data in Table 3 are a short-term estimate of repeatability and intermediate precision. When the test is run routinely, a control standard and chart should be used to develop a better estimate of the long-term intermediate precision.

Reproducibility

There is insufficient data to calculate the reproducibility of the test at this time.

Time for Analysis

The labor requirement to prepare a Primary HPNA Calibration Standard solution of Rhodamine B is about 8 hours. The labor requirement to calibrate an FIS from a primary standard solution of Rhodamine B or from a previously calibrated FIS is about 4 hours.

The elapsed time for a single analysis is from 2 to 12 hours, depending on the time required for complete dissolution of the sample in Step 6 in *Sample Preparation*. The labor requirement for a single analysis is 1.1 hour.

Suggested Suppliers

Aldrich Chemical Co., 1001 W. Saint Paul Ave., Milwaukee, WI 53233 (414-273-3850)
www.sigma-aldrich.com

Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219-4785 (412-490-8300)
www.fishersci.com

Matheson Tri-Gas, 166 Keystone Drive, Montgomeryville, PA 18936 (215-648-4000)
www.mathesontrigas.com

OriginLab Corp., One Roundhouse Plaza, Northampton, MA 01060 (413-586-2013)
www.originlab.com

PerkinElmer Analytical Instruments, 710 Bridgeport Avenue, Shelton, CT 06484-4794 (203-925-4600) www.perkinelmer.com

Varian, Inc., 2700 Mitchell Drive, Walnut Creek, CA 94598 (800-926-3000) www.varianinc.com

Wilmad/Lab-Glass, 1002 Harding Hwy., Buena, NJ 08310 (856-697-3000) www.wilmad-labglass.com

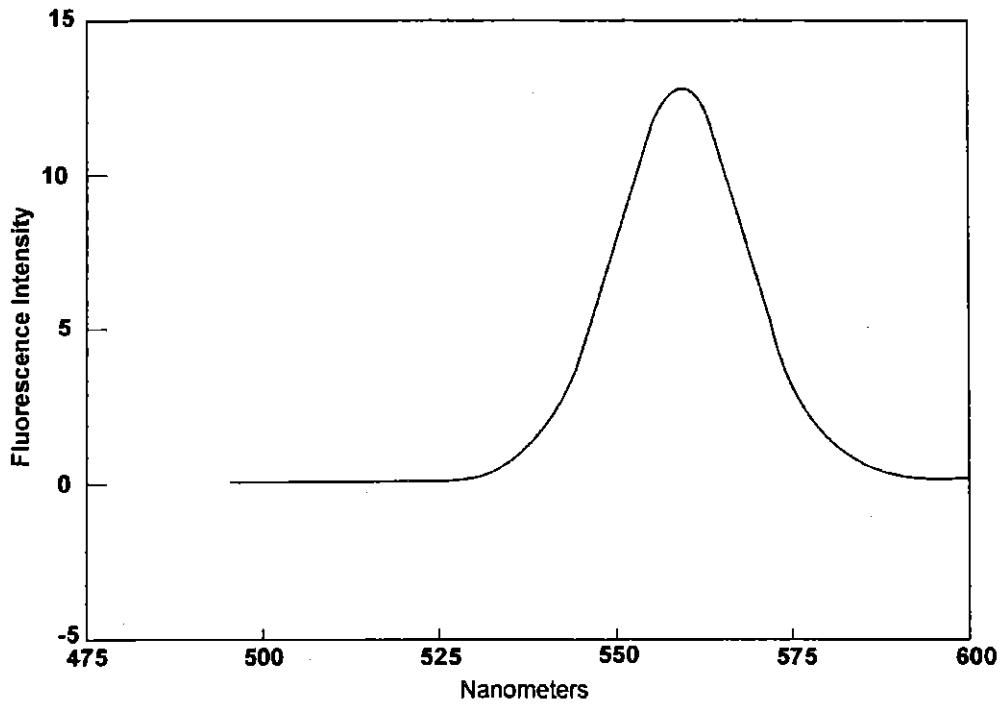


Figure 1
Typical Rhodamine B in TEG Spectrum

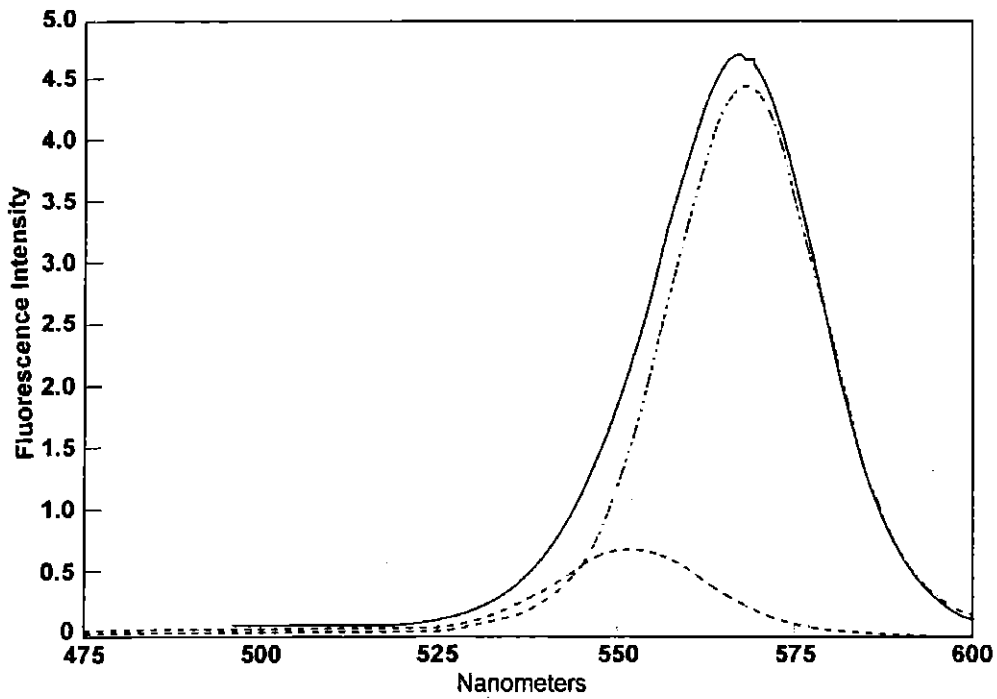


Figure 2
Typical FIS Block Spectrum, Showing Fitted Curves

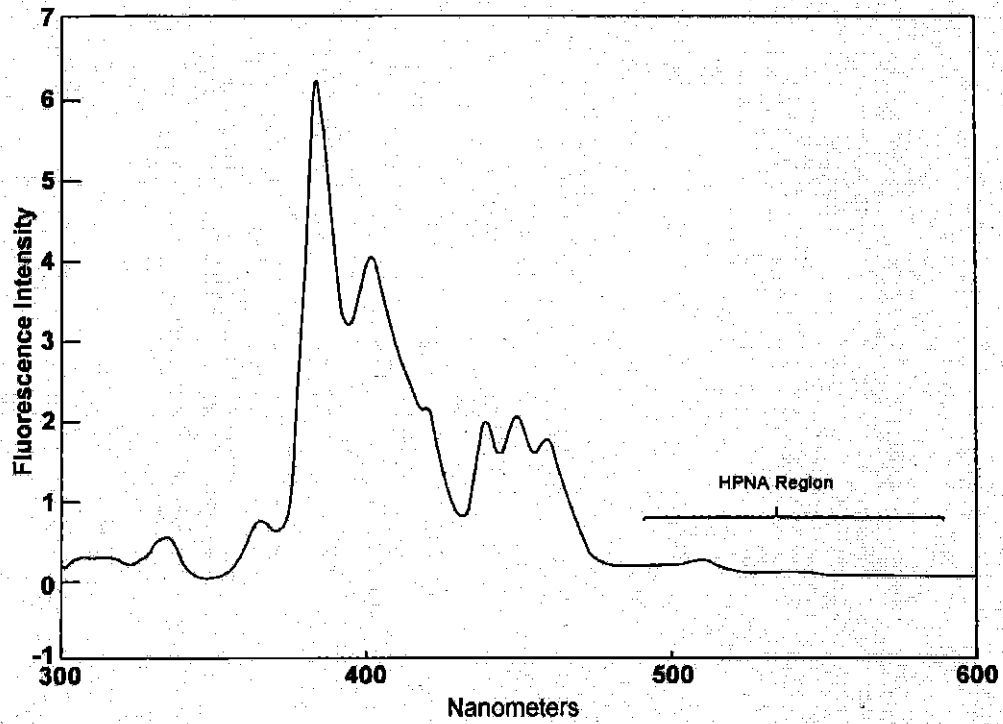


Figure 3
Typical Recycle Oil Spectrum

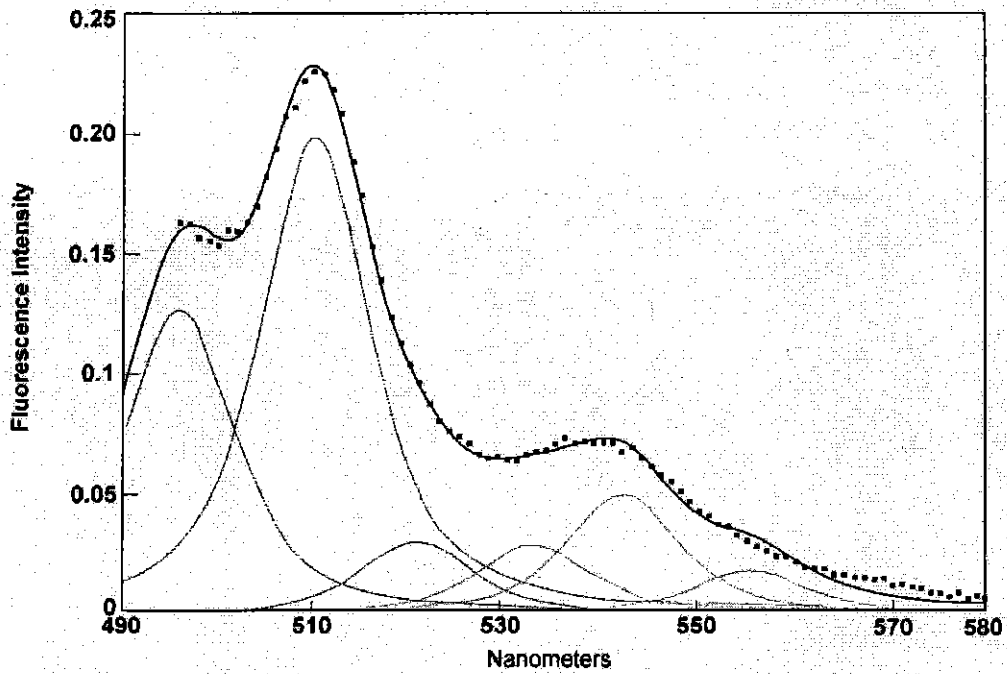


Figure 4
Typical Recycle Oil HPNA Region

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