# National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material 1647b

### Priority Pollutant Polycyclic Aromatic Hydrocarbons (in Acetonitrile)

Standard Reference Material (SRM) 1647b consists of five 2 mL ampoules, each containing approximately 1.2 mL of an acetonitrile solution of 16 polycyclic aromatic hydrocarbons (PAHs). The PAHs are the 16 identified by the U. S. Environmental Protection Agency as priority pollutants. SRM 1647b is intended primarily as a calibration solution for use in chromatographic methods for the determination of PAHs. It may also be useful in recovery studies for the addition of known amounts of these PAHs to a sample; and since the solution is miscible with water, it can be used to fortify aqueous samples with known concentrations of PAHs.

### Certified Concentrations of PAHs

Certified concentrations of the 16 PAHs in µg/g and µg/mL at 23 ± 2 °C are given in Table 1. Values listed in units of µg/mL were calculated from the values in units of µg/g, using the density of acetonitrile at 23 °C. The certified values in Table 1 are derived from the concordant results obtained from liquid chromatography (LC) and gravimetry.

Table 1. Certified Values for SRM 1647b.

| Compound | Concentration (µg/g)[a] | Concentration (µg/mL)[b] |
|---|---|---|
| Naphthalene | 25.42 ± 1.15 | 19.80 ± 0.95 |
| Acenaphthylene | 20.73 ± 0.25 | 16.15 ± 0.24 |
| Acenaphthene | 25.53 ± 0.35 | 19.89 ± 0.33 |
| Fluorene | 6.16 ± 0.09 | 4.79 ± 0.09 |
| Phenanthrene | 4.49 ± 0.04 | 3.50 ± 0.04 |
| Anthracene | 0.97 ± 0.06 | 0.75 ± 0.05 |
| Fluoranthene | 9.96 ± 0.14 | 7.76 ± 0.13 |
| Pyrene | 10.76 ± 0.12 | 8.38 ± 0.11 |
| Benz[a]anthracene | 5.06 ± 0.06 | 3.94 ± 0.06 |
| Chrysene | 4.74 ± 0.08 | 3.69 ± 0.07 |
| Benzo[b]fluoranthene | 5.35 ± 0.04 | 4.16 ± 0.04 |
| Benzo[k]fluoranthene | 6.03 ± 0.07 | 4.70 ± 0.07 |
| Benzo[a]pyrene | 6.32 ± 0.09 | 4.92 ± 0.09 |
| Dibenz[a,h]anthracene | 4.67 ± 0.06 | 3.64 ± 0.06 |
| Benzo[ghi]perylene | 4.83 ± 0.09 | 3.76 ± 0.08 |
| Indeno[1,2,3-cd]pyrene | 5.60 ± 0.07 | 4.37 ± 0.06 |

[a] The certified values and associated uncertainties were derived from a statistical treatment of gravimetric and LC data. Each uncertainty is the half-width of an approximate 95% confidence interval for the certified value.

[b] Concentration values in units µg/mL were calculated using the density of acetonitrile at 23 °C (0.7789 g/mL). An allowance for the change in this density over the range 23 ± 2 °C is included in the uncertainty.

August 13, 1990
Gaithersburg, MD 20899

William P. Reed, Acting Chief
Standard Reference Materials Program

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## Notice and Warnings to User

Expiration of Certification: This certification is valid, within the limits certified, for one year from the date of purchase. In the event that the certification should become invalid before then, purchasers will be notified by NIST. Please return the attached registration card to facilitate notification.

Storage: Sealed ampoules, as received, should be stored in the dark at temperatures between 10-30 °C.

Use: Samples of the SRM for analysis should be withdrawn from ampoules and used without delay. Certified values in Table 1 listed in units of $\mu$g/mL are valid within the stated uncertainty only for aliquots removed at 23 ± 2 °C. Certified values are not applicable to ampoules stored after opening, even if resealed.

Toxicity: This SRM contains small amounts of polycyclic aromatic hydrocarbons, some of which have been reported to have mutagenic and/or carcinogenic properties; therefore, care should be exercised during handling and use. Use proper methods for disposal of waste.

Analytical determinations were performed at the Center for Analytical Chemistry, Organic Analytical Research Division, by L. C. Sander.

Consultation on the statistical design of the experimental work and evaluation of the data were by S. B. Schiller of the Center for Computing and Applied Mathematics, Statistical Engineering Division.

The coordination of the technical measurements leading to certification were performed under the direction of L. C. Sander, S. A. Wise, and W. E. May.

The technical and support aspects involved in preparation, certification, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by R. Alvarez.

## Preparation and Analysis

The acetonitrile solution of the 16 PAHs was prepared gravimetrically (w/w) from individual compounds. Sources and purities of the 16 PAHs are listed in Appendix A. Except for the PAHs obtained from the Community Bureau of Reference (BCR), purities of the compounds were determined by a combination of techniques including differential scanning calorimetry (DSC), gas chromatography with flame ionization detection (GC-FID), and reversed-phase liquid chromatography with ultraviolet detection (LC-UV). Purities of BCR compounds were certified by BCR, and these values are included in Appendix A. With the exception of acenaphthylene, PAH purities were ≥ 99%. Corrections for impurities were made in calculation of gravimetric values for SRM 1647b and response factor solutions. Acenaphthylene was found to contain acenaphthene and corrections were made. The SRM solution was aliquoted into 2-mL amber glass ampoules, which were purged with argon prior to addition of the solution. Samples representing early, middle, and final stages of ampouling were analyzed by LC. No evidence of sample inhomogeneity was observed.

Randomly selected ampoules (24) were analyzed in duplicate for all 16 PAHs by LC using an acetonitrile-water mobile phase. Response factors were determined from this and five separate standard solutions containing the 16 PAHs using errors-in-variables regression (1). Triphenylene was added to both the SRM and the response factor solutions for use as an internal standard. A representative chromatogram and the separation conditions are shown in Figure 1. Variations in $C_{18}$ column selectivity for PAHs are known to result from different manufacturing processes (2). Columns prepared by reaction of monofunctional $C_{18}$ silanes with silica (denoted monomeric $C_{18}$ phases) differ from columns prepared with silica substrates using trifunctional $C_{18}$ silanes in the presence of water (denoted polymeric $C_{18}$ phases). The designation "polymeric $C_{18}$ column" should not be confused with "polymer substrate columns", (which are nonsilica columns, often based on polystyrene particles). Better separations of PAH mixtures are often possible on polymeric $C_{18}$ columns (Figure 1) compared to monomeric $C_{18}$ columns. A chromatogram illustrating the separation of the components in the SRM solution using a monomeric $C_{18}$ column is provided for comparison (Figure 2). Baseline resolution of all components was not achieved with the monomeric $C_{18}$ column. The classification of monomeric and polymeric $C_{18}$ columns for the separation of PAHs has been described previously (2-9) and may be accomplished using SRM 869, "Column Selectivity Test Mixture for Liquid Chromatography, (Polycyclic Aromatic Hydrocarbons)" (10). Examples of various $C_{18}$ columns classified as "monomeric" or "polymeric" are provided with SRM 869.

Ultraviolet absorption data between 200 and 600 nm are supplied as an aid in identifying each compound certified in this SRM. The values for apparent specific molar absorbance for several prominent peaks in each spectrum are provided in Appendix B. "Specific absorbance" is defined here as absorbance per unit path length and unit concentration. The term absorptivity was avoided because it is ambiguously defined (11). The term "apparent" is used because no corrections have been applied to the data for the effects of internal multiple reflections within the cuvette. The apparent molar specific absorbances were not corrected for PAH purity. In Appendix C the apparent specific molar absorbances for each PAH at 254.0 nm are listed. The apparent specific molar absorbance at 254.0 nm should be used with caution since the absorbances measured at 254 nm do not correspond to peak maxima and very small changes in wavelength may result in significant changes in the absorbance reading. The magnitude of this change is reflected in the last column of Appendix C which gives the percent change $\varepsilon_a$ for a 1.0 nm shift away from 254.0 nm. It is important that the user check detector calibration at 254 nm.

Aqueous solubility values are summarized in Appendix D for 15 of the PAHs present in this SRM.

## References.

1. W. Nelson, **Measurement Error Models**, John Wiley & Sons (1987).

2. L. C. Sander and S. A. Wise, Evaluation of Shape Selectivity in Liquid Chromatography, **LC-GC, 8**, 378-390 (1990).

3. L. C. Sander, and S. A. Wise, Investigations of Selectivity in RPLC of Polycyclic Aromatic Hydrocarbons, **Advances in Chromatography, 25**, 139-218 (1986).

4. L. C. Sander and S. A. Wise, Determination of Column Selectivity Toward Polycyclic Aromatic Hydrocarbons, HRC CC, **J. High Resolut. Chromatogr. Chromatogr. Comm., 11**, 383-387 (1988).

5. L. C. Sander and S. A. Wise, Subambient Temperature Modification of Selectivity in Reversed-Phase Liquid Chromatography, **Anal. Chem., 61**, 1749-1754 (1989).

6. S. A. Wise and L. C. Sander, Factors Affecting the Reversed-Phase Liquid Chromatographic Separation of Polycyclic Aromatic Hydrocarbon Isomers, HRC CC, **J. High Resolut. Chromatogr. Chromatogr. Comm., 8**, 248-255 (1985).

7. L. C. Sander and S. A. Wise, Influence of Substrate Parameters on Column Selectivity with Alkyl Bonded-Phase Sorbents, **J. Chromatogr., 316**, 163-181 (1984).

8. S. A. Wise and W. E. May, Effect of $C_{18}$ Surface Coverage on Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons, **Anal. Chem., 55**, 1479-1485 (1983).

9. S. A. Wise, W. J. Bonnett, F. R. Guenther, and W. E. May, A Relationship Between Reversed-Phase $C_{18}$ Liquid Chromatographic Retention and the Shape of Polycyclic Aromatic Hydrocarbons, **J. Chromatogr. Sci., 19**, 457-465 (1981).

10. Certificate of Analysis, SRM 869 Column Selectivity Test Mixture for Liquid Chromatography, (Polycyclic Aromatic Hydrocarbons), Standard Reference Materials Program, NIST, Gaithersburg, MD 20899.

11. K. D. Mielenz, Comments on Spectrometry Nomenclature, **Anal. Chem. 48**, 1093-1094 (1976).

## Appendixes

The following supplementary information is supplied for the convenience of the user of this material. This information does not meet the requirements for certification by the National Institute of Standards and Technology.

### Appendix A. Sources and Purities of PAHs Used to Formulate SRM 1647b.

| Component | Source | Purity (weight %)[a] | |
|---|---|---|---|
| | | mean | uncertainty[d] |
| Naphthalene | commercial | 99.70 | 0.4 |
| Acenaphthylene | commercial | 95.68[b] | 0.24 |
| Acenaphthene | commercial | 99.78 | 0.25 |
| Fluorene | commercial | 99.00 | 0.3 |
| Phenanthrene | commercial | 99.60 | 0.3 |
| Anthracene | commercial | 99.73 | 0.21 |
| Fluoranthene | BCR[c] | 99.49 | 0.25 |
| Pyrene | BCR | 99.75 | 0.13 |
| Benz[a]anthracene | BCR | 99.78 | 0.15 |
| Chrysene | BCR | 99.20 | 0.2 |
| Benzo[b]fluoranthene | BCR | 99.50 | 0.3 |
| Benzo[k]fluoranthene | BCR | 99.50 | 0.3 |
| Benzo[a]pyrene | commercial | 99.50 | 0.3 |
| Dibenz[a,h]anthracene | BCR | 99.00 | 0.6 |
| Benzo[ghi]perylene | BCR | 99.00 | 0.6 |
| Indeno[1,2,3-cd]pyrene | BCR | 99.00 | 0.5 |

[a] Purity values for compounds obtained from commercial sources are consensus estimations from DSC, GC-FID, and LC-UV measurements; purity values for BCR compounds are certified by BCR.

[b] Acenaphthylene contains 3.83% acenaphthene.

[c] BCR = Community Bureau of Reference, Directorate General XII, Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels.

[d] Uncertainties for non BCR components are expressed as the standard deviation of a single measurement. Uncertainties for BCR components are as expressed as a 95% confidence interval).

Appendix B. Apparent Specific Molar Absorbances at $\lambda_{max}$ (cont.)

| Compound | $\lambda_{max}$, nm | Apparent Specific Molar Absorbance $\varepsilon_a$, L·mole$^{-1}$·cm$^{-1}$ | Relative $\varepsilon_a$ |
|---|---|---|---|
| Benzo[b]fluoranthene | 255.6 | 44,400 | 100.% |
| | 367.5 | | 16.3 |
| | 349.2 | | 25.5 |
| | 341.1 | | 24.7 |
| | 300.4 | | 82.8 |
| | 291.7 | | 61.9 |
| | 289.7 | | 61.5 |
| | 275.7 | | 62.5 |
| | 244.8 | | 84.3 |
| | 239.2 | | 84.2 |
| | 221.3 | | 91.9 |
| Benzo[k]flouranthene | 306.7 | 58,200 | 100.% |
| | 400.0 | | 20.5 |
| | 378.4 | | 19.1 |
| | 359.4 | | 9.8 |
| | 336.0* | | 8.7 |
| | 321.4* | | 12.3 |
| | 295.2 | | 72.3 |
| | 282.9 | | 38.3 |
| | 270.3* | | 29.7 |
| | 267.0 | | 34.0 |
| | 244.3 | | 91.4 |
| | 236.9 | | 94.6 |
| | 214.6 | | 61.0 |
| Benzo[a]pyrene | 295.8 | 57,930 | 100.% |
| | 403.3 | | 6.0 |
| | 384.4 | | 45.2 |
| | 378.1 | | 41.1 |
| | 364.5 | | 40.5 |
| | 347.0 | | 20.9 |
| | 331.5 | | 8.6 |
| | 283.8 | | 76.9 |
| | 271.6 | | 51.9 |
| | 264.8 | | 87.1 |
| | 254.6 | | 72.2 |
| | 226.6 | | 46.2 |
| | 220.0 | | 42.9 |

| Compound | $\lambda_{max}$, nm | Apparent Specific Molar Absorbance $\varepsilon_a$, L mole$^{-1}$·cm$^{-1}$ | Relative $\varepsilon_a$ |
|---|---|---|---|
| Benzo[ghi]perylene | 299.0 | 56,200 | 100.% |
| | 383.0 | | 35.3 |
| | 380.8 | | 35.1 |
| | 361.8 | | 31.6 |
| | 344.7 | | 16.1 |
| | 338.9 | | 14.8 |
| | 329.3 | | 11.2 |
| | 323.9 | | 8.6 |
| | 313.4 | | 10.7 |
| | 288.6 | | 71.1 |
| | 276.5 | | 42.1 |
| | 253.7 | | 27.0 |
| | 222.2 | | 82.4 |
| Dibenz[a,h]anthracene | 296.5 | 158,400 | 100.% |
| | 393.8 | | 0.8 |
| | 372.8 | | 0.7 |
| | 348.4 | | 8.6 |
| | 332.6 | | 9.9 |
| | 319.7 | | 12.1 |
| | 287.9 | | 59.1 |
| | 285.6* | | 56.9 |
| | 277.4 | | 30.3 |
| | 274.8* | | 27.8 |
| | 229.9 | | 17.1 |
| | 221.3 | | 36.7 |
| | 215.8 | | 25.4 |
| Indeno[1,2,3-cd]pyrene | 249.6 | 71,300 | 100.% |
| | 405.6 | | 9.2 |
| | 382.8* | | 16.7 |
| | 376.9 | | 18.0 |
| | 359.2 | | 20.6 |
| | 314.7 | | 38.0 |
| | 302.4 | | 46.6 |
| | 291.4 | | 35.8 |
| | 275.2 | | 32.9 |
| | 209.8 | | 58.1 |

*Shoulder

# Appendix B. Apparent Specific Molar Absorbances at $\lambda_{max}$

| Compound | $\lambda_{max}$, nm | Apparent Specific Molar Absorbance $\varepsilon_a$, $L \cdot mole^{-1} \cdot cm^{-1}$ | Relative $\varepsilon_a$ |
|---|---|---|---|
| Naphthalene | 220.4 | 98,000 | 100.% |
| | 285.7 | | 4.0 |
| | 283.4 | | 3.9 |
| | 275.8 | | 5.9 |
| | 266.2 | | 5.3 |
| | 258.2* | | 4.0 |
| Acenaphthene | 227.0 | 84,100 | 100.% |
| | 320.7 | | 2.4 |
| | 312.4 | | 1.3 |
| | 306.4 | | 3.8 |
| | 300.6 | | 4.8 |
| | 289.2 | | 7.6 |
| | 280.7 | | 6.6 |
| Acenaphthylene | 229.0 | 51,800 | 100.% |
| | 338.8 | | 7.7 |
| | 321.6 | | 19.5 |
| | 310.6 | | 15.4 |
| | 274.6 | | 4.5 |
| | 264.6 | | 5.0 |
| Fluorene | 260.7 | 18,810 | 100.% |
| | 299.6 | | 46.7 |
| | 292.0* | | 27.8 |
| | 288.4 | | 32.3 |
| | 270.8 | | 71.1 |
| | 263.4 | | 99.6 |
| | 219.9 | | 88.0 |
| Anthracene | 251.5 | 186,000 | 100.% |
| | 375.9 | | 4.0 |
| | 356.9 | | 4.2 |
| | 339.8 | | 2.8 |
| | 324.1 | | 1.5 |
| | 221.2 | | 5.8 |
| | 218.2 | | 5.6 |
| Phenanthrene | 250.7 | 63,500 | 100.% |
| | 292.7 | | 21.3 |
| | 281.2 | | 16.1 |
| | 273.9 | | 20.2 |
| | 244.1 | | 76.8 |
| | 219.8 | | 31.7 |
| | 211.3 | | 51.4 |

| Compound | $\lambda_{max}$, nm | Apparent Specific Molar Absorbance $\varepsilon_a$, $L \cdot mole^{-1} \cdot cm^{-1}$ | Relative $\varepsilon_a$ |
|---|---|---|---|
| Fluoranthene | 235.6 | 50,800 | 100.% |
| | 358.3 | | 16.3 |
| | 342.0 | | 15.9 |
| | 322.0 | | 12.1 |
| | 308.6 | | 7.0 |
| | 286.2 | | 81.2 |
| | 281.1 | | 35.1 |
| | 275.5 | | 45.4 |
| | 271.4 | | 23.5 |
| | 260.5 | | 23.6 |
| | 209.6 | | 77.6 |
| Pyrene | 240.1 | 83,100 | 100.% |
| | 334.4 | | 56.2 |
| | 318.9 | | 33.1 |
| | 305.4 | | 13.1 |
| | 294.1 | | 5.3 |
| | 272.2 | | 60.1 |
| | 261.8 | | 29.0 |
| | 251.7 | | 13.3 |
| | 231.0 | | 51.5 |
| | 207.0* | | 15.2 |
| Benz[a]anthracene | 287.0 | 93,000 | 100.% |
| | 384.3 | | 1.1 |
| | 374.8 | | 0.8 |
| | 357.8 | | 5.2 |
| | 341.0 | | 7.4 |
| | 327.8 | | 6.5 |
| | 314.2 | | 5.0 |
| | 299.7 | | 8.4 |
| | 276.5 | | 79.6 |
| | 266.7 | | 42.9 |
| | 256.1 | | 40.8 |
| | 227.7 | | 36.2 |
| | 221.7 | | 39.4 |
| Chrysene | 267.1 | 133,600 | 100.% |
| | 360.7 | | 0.5 |
| | 319.8 | | 9.2 |
| | 306.4 | | 9.2 |
| | 294.3 | | 8.6 |
| | 281.0 | | 8.9 |
| | 257.6 | | 55.4 |
| | 241.2 | | 16.1 |
| | 220.6 | | 24.1 |

* Shoulder

## Appendix C. Apparent Specific Molar Absorbances at 254.0 nm for PAH's in SRM 1647b

| Compound | Apparent Specific Molar Absorbance $\varepsilon_a$, $L \cdot mol^{-1} \cdot cm^{-1} \times 10^{-3}$ | %Relative $\varepsilon_a = \frac{\varepsilon_a \cdot 100,\ 254.0\ nm}{\varepsilon_a,\ \lambda max}$ | % error in $\varepsilon_a$ for 1 nm error |
|---|---|---|---|
| Naphthalene | 3.1 | 3.2% | 11% |
| Acenaphthene | 1.2 | 1.4 | 18 |
| Acenaphthylene | 2.2 | 4.1 | 2 |
| Fluorene | 17 | 88 | 1 |
| Anthracene | 96 | 52 | 52 |
| Phenanthrene | 43 | 68 | 16 |
| Fluoranthene | 13 | 25 | 6 |
| Pyrene | 10 | 12 | 5 |
| Benz[a]anthracene | 33 | 36 | 3 |
| Chrysene | 52 | 39 | 15 |
| Benzo[b]fluoranthene | 43 | 96 | 4 |
| Benzo[k]fluoranthene | 28 | 48 | 15 |
| Benzo[a]pyrene | 42 | 72 | 2 |
| Benzo[ghi]perylene | 16 | 27 | 0.7 |
| Dibenz[a,h]anthracene | 11 | 7 | 6 |
| Indeno[1,2,3-cd]pyrene | 38 | 53 | 22 |

## Appendix D. Aqueous Solubility Data for the Individual PAH Compounds Present in SRM 1647b

| Compound | Aqueous Solubility at 25 °C[a] (ng/mL) |
|---|---|
| Naphthalene | 31700 |
| Acenaphthylene | --------- |
| Acenaphthene | 3930[b] |
| Fluorene | 1685 |
| Phenanthrene | 1000 |
| Anthracene | 45 |
| Fluoranthene | 206 |
| Pyrene | 132 |
| Benz[a]anthracene | 9.4 |
| Chrysene | 1.8 |
| Benzo[b]fluoranthene | 1.5 |
| Benzo[k]fluoranthene | 0.8 |
| Benzo[a]pyrene | 1.6 |
| Benzo[ghi]perylene | 0.7 |
| Dibenz[a,h]anthracene | 0.5[c] |
| Indeno[1,2,3-cd]pyrene | 0.2 |

[a] Unless noted otherwise, all other solubility values were determined at NIST using Dynamic Coupled Column Liquid Chromatographic Technique [W. E. May, S. P. Wasik, and D. H. Freeman, Anal. Chem. 50, 175-179 (1978) and Anal. Chem. 50, 997-1000 (1978)].

[b] D. MacKay and W. Shiu, J. Chem. Eng. Data. 22, 4 (1977).

[c] W. Davis, M. Krahl and G. Clowes, J. Am. Chem. Soc. 64, 108-14 (1942).

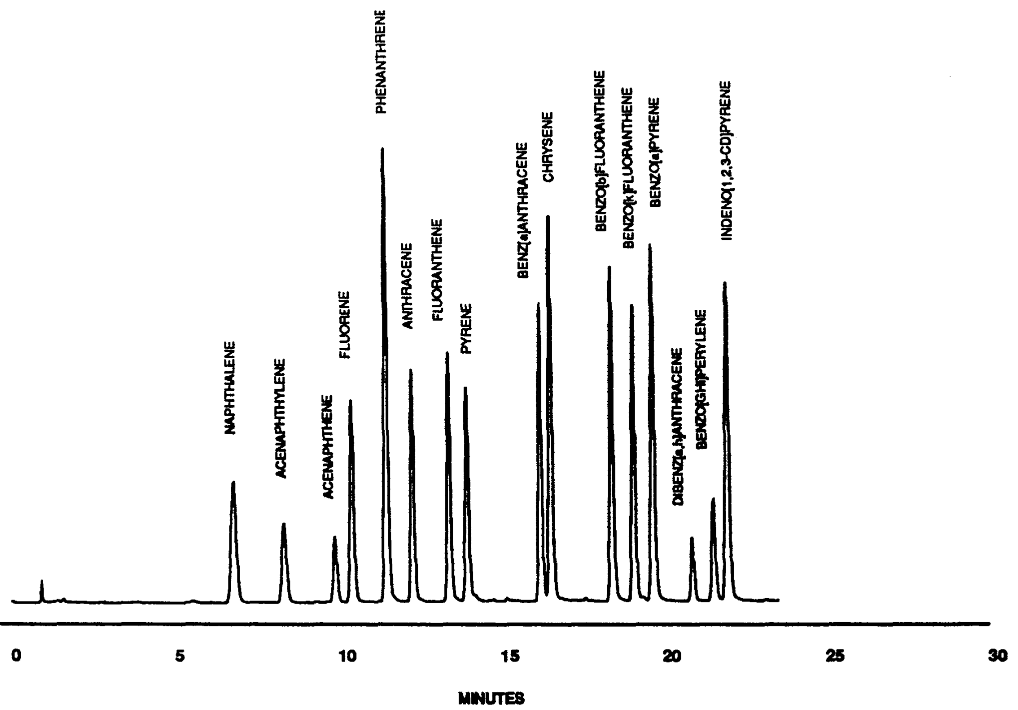

Figure 1. Separation of the 16 components of SRM 1647b. A polymeric $C_{18}$ column (Vydac 201 TP column, 5 µm, 4.6 mm x 25 cm) was used with a gradient elution program: 3 minutes hold at 50% water : 50% acetonitrile; 15 minutes linear gradient to 100% acetonitrile; and 15 minutes hold at 100% acetonitrile.

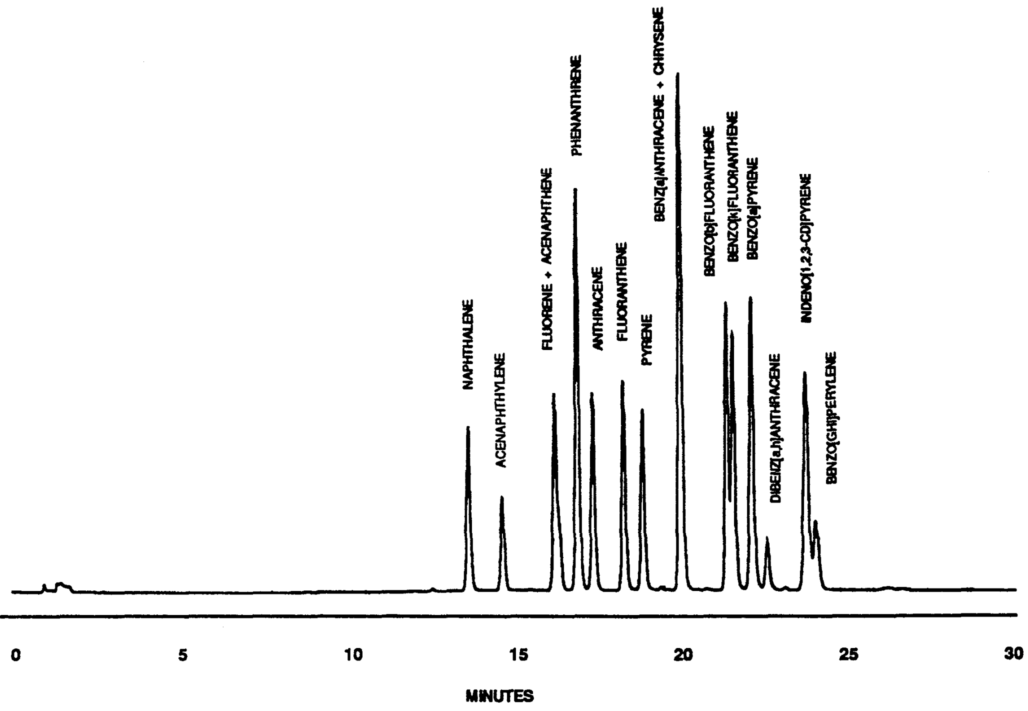

Figure 2. Separation of the SRM solution using a monomeric $C_{18}$ column (Zorbax $C_{18}$ column, 5 µm, 4.6 mm x 25 cm) and the same gradient elution program as in Figure 1.