

National Bureau of Standards

Certificate

Standard Reference Material 1587

Nitrated Polycyclic Aromatic Hydrocarbons in Methanol

This Standard Reference Material (SRM) consists of four vials, each containing approximately 1 mL of a methanol solution of seven nitrated polycyclic aromatic hydrocarbons (N-PAH). SRM 1587 is intended primarily for use in calibrating chromatographic instrumentation used for the determination of N-PAH but can also be used to add known amounts of these compounds to methylene chloride extracts.

Certified Concentrations of N-PAH

Certified concentrations of the seven N-PAH in SRM 1587 are given in Table 1. The concentration values are certified in $\mu\text{g/g}$ units but are also reported in $\mu\text{g/mL}$ units for user convenience. The certified values in Table 1 are derived from the concordant results obtained from high-performance liquid chromatography (HPLC) and gas chromatography (GC). These data are given in Table 2. The N-PAH used in the preparation of this SRM were obtained from Midwest Research Institute, Kansas City, MO, where they were synthesized under contract from the Coordinating Research Council, Inc., Atlanta, GA. Compound purities were determined at Midwest Research Institute. Each compound was at least 99 percent pure and, therefore, no correction was made for purity.

NOTICE AND WARNINGS TO USERS

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 NBS.

Storage: Sealed ampoules, as received, must be stored in the dark at temperatures between -25 and 0°C .

Use: Prior to use, samples for analysis should be brought to room temperature, equilibrated for one hour and ultrasonically vibrated for about 30 seconds, to ensure complete solubility of the compounds. Certified values are not applicable for ampoules stored after opening, even if resealed. Aliquots should be withdrawn from the ampoules immediately after opening and processed without delay for the certified values in Table 1 to be valid within the stated uncertainty. However, if this is impossible or impractical, we recommend that after aliquots are removed from the ampoules, they should be transferred to amber containers and not be stored at room temperature for more than 12 hours before use. The toxicity or carcinogenicity/mutagenicity of the N-PAH in this SRM has not been precisely defined; however, this material should be treated as a potential health hazard.

Preparation and analytical determinations for this standard were performed in the Organic Analytical Research Division, Center for Analytical Chemistry, by W.F. Kline, R.M. Parris, R.E. Rebbert, and L.T. Sniegowski.

Consultation on the statistical design of the experimental work was provided by R.C. Paule of the National Measurement Laboratory.

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

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

Gaithersburg, MD 20899
June 25, 1985

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

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Preparation and Analysis

The methanol solution of seven N-PAH was prepared and ampouled in the NBS Toxic Chemicals Handling Facility. Approximately 6.3 L of methanol in a 7-L glass Erlenmeyer flask were purged with argon at a flow rate of approximately 400 mL/min for 1.5 hours. The mass of the methanol was then determined using a top-loading balance. The flask was sealed with a *Teflon-covered stopper and covered with black plastic. An electrobalance was used to determine the mass of each N-PAH component added. The gold foil weighing boat containing the N-PAH sample was transferred to the flask containing the methanol. After all seven of the nitro-PAH had been transferred, the headspace in the flask was again filled with argon and the stopper was secured onto the flask. The solution was magnetically stirred for 20 hours in the dark at room temperature. Each 2-mL amber ampoule was purged with argon at a flow rate of approximately 600 mL/min just prior to the addition of approximately one mL of solution to the ampoule which was then flame sealed. The solution was not stirred during the ampouling process. An argon-filled Teflon gas bag was used as a reservoir of argon to fill the headspace of the flask created as the solution was removed during the ampouling process.

Six randomly selected ampoules were analyzed in duplicate by GC on a DB-5 (30 m x 0.25 mm I.D. x 0.25 μ m film) column using helium as a carrier gas together with flame ionization (FID) detection. The column was temperature programmed from 60 to 240 °C at a rate of 30 °C/min and from 240 to 270 °C at a rate of 10 °C/min. Quantification was accomplished using benz[a]anthracene as an internal standard. Two independently prepared calibration solutions were used to establish detector response factors. A representative chromatogram from the GC analyses is shown in Figure 1.

Sixteen randomly selected ampoules were analyzed in duplicate by HPLC on a C₁₈ column *(Zorbax ODS) using a methanol/water mobile phase with UV detection at 254 nm. Separation of the N-PAH was accomplished using an isocratic mobile phase at 70 percent methanol in water for 10 minutes, followed by a linear gradient to 100 percent methanol in 20 minutes. Column temperature was maintained at 30 °C. Quantification was accomplished using fluorene as an internal standard. Three independently prepared calibration solutions were used to establish detector response factors. A representative chromatogram from the HPLC analyses is shown in figure 2.

In addition to the chromatograms provided in figures 1 and 2, GC and HPLC retention index data are provided to assist the user in identifying the individual N-PAH compounds in this SRM. These data are *not* certified and are provided for information only. GC retention index data are listed in Table 3. HPLC retention indices for the N-PAH on both a monomeric and a polymeric C-18 column and using methanol/water and acetonitrile/water mobile phase are provided in Table 4.

*The use of a "trade name" is for identification only, and does not imply endorsement of the product by the National Bureau of Standards.

Table 1
Certified^a and Information Values for SRM 1587

	<u>($\mu\text{g/g}$)</u>	<u>($\mu\text{g/mL}$)^b</u>
2-Nitrofluorene	9.67 ± 0.39^c	7.64 ± 0.31^c
9-Nitroanthracene	5.01 ± 0.11	3.96 ± 0.09
3-Nitrofluoranthene	9.24 ± 0.06	7.30 ± 0.05
1-Nitropyrene	8.95 ± 0.28	7.07 ± 0.22
7-Nitrobenz[a]anthracene	9.27 ± 0.23	7.32 ± 0.18
6-Nitrochrysene	8.13 ± 0.11	6.42 ± 0.09
6-Nitrobenzo[a]pyrene	$(6.1)^d$	$(4.8)^d$

^aThe certified values and associated uncertainties were derived from the weighted combination of liquid and gas chromatographic data as described by Paule and Mandel in "Consensus Values and Weighting Factors," Journal of Research of the National Bureau of Standards, Vol. 87, No. 5, Sept-Oct (1982), pp 377-385.

^bThese values are provided for user convenience and were obtained from the certified concentration in $\mu\text{g/g}$. The uncertainties apply only at 23 °C. This SRM may be used between 19 and 27 °C, but a concentration change of up to 1% will occur because of the change of the density of methanol with temperature.

^cThe listed uncertainties are \pm two standard deviations of the values and include both within- and between-analytical method differences and, where observed, vial-to-vial variability.

^dConcentration data for 6-nitrobenzo[a]pyrene is provided for information only. The analytical data for 6-nitrobenzo[a]pyrene exhibited the largest uncertainty and largest between-bottle variability. Also, the solubility of this compound in the SRM solution appears to be borderline.

Table 2
 Summary of Results by Method Used for Quantification

	----- Concentration ($\mu\text{g/g}$) -----	
	<u>Gas Chromatography</u>	<u>Liquid Chromatography</u>
2-Nitrofluorene	9.51 ± 0.22^a	9.81 ± 0.16^a
9-Nitroanthracene	5.08 ± 0.08	4.96 ± 0.04
3-Nitrofluoranthene	9.30 ± 0.12	9.23 ± 0.06
1-Nitropyrene	9.09 ± 0.14	8.81 ± 0.12
7-Nitrobenz[a]anthracene	9.39 ± 0.14	9.15 ± 0.08
6-Nitrochrysene	8.21 ± 0.16	8.09 ± 0.06
6-Nitrobenzo[a]pyrene	6.09 ± 0.30	6.03 ± 0.16

^aThe listed uncertainties represent \pm two standard deviations of the average values given. The uncertainties also include, where observed, the vial-to-vial variability, but does not include the variability between the two methods.

Table 3
Gas Chromatographic Retention Indices^a

Compound	RI	RI (From Literature)
2-Nitrofluorene	(352.07)	353.06 ^b , 352.17 ^c
9-Nitroanthracene	(356.68)	357.42 ^b , 356.95 ^d
3-Nitrofluoranthene	(413.05)	413.34 ^c
1-Nitropyrene	(421.03)	421.48 ^b , 421.08 ^c
7-Nitrobenz[a]anthracene	(448.96)	
6-Nitrochrysene	(460.47)	
6-Nitrobenzo[a]pyrene	(501.71)	501.71 ^b

^aThe retention index values are not certified and are provided for information only. They were determined using the method described by Lee et al. in Anal. Chem. 51, 768 (1979).

^bD.L. Vassilaros, R.C. Kong, D.W. Later, and M.L. Lee, J. Chromatogr. 252, 1 (1982).

^cP.J. Doherty, R.M. Hoes, A. Robbat, Jr., and C.M. White, Anal. Chem. 56, 2697 (1984).

^dC.M. White, A. Robbat, Jr., and R.M. Hoes, Chromatogr. 17, 605 (1983).

Table 4
Liquid Chromatographic Retention Indices^a

Compound	----- Logarithm (RI) -----			
	Monomeric C ₁₈ (Zorbax ODS)		Polymeric C ₁₈ (Vydac TP 201)	
	A	B	C	D
2-Nitrofluorene	(2.45)	(2.33)	(2.65)	(2.64)
9-Nitroanthracene	(2.53)	(2.70)	(2.65)	(2.83)
1-Nitropyrene	(3.27)	(3.31)	(3.38)	(3.42)
3-Nitrofluoranthene	(3.35)	(3.31)	(3.47)	(3.48)
7-Nitrobenz[a]anthracene	(3.55)	(3.71)	(3.59)	(3.75)
6-Nitrochrysene	(3.75)	(3.74)	(3.76)	(3.80)
6-Nitrobenzo[a]pyrene	(4.19)	(4.77)	(4.45)	(4.40)

^a The retention index values are not certified and are provided for information only. They were determined using the procedure described by Wise et al. in J. Chromatogr. Sci. 17, 457 (1981).

^A Mobile phase: 80 percent methanol in water

^B Mobile phase: 75 percent acetonitrile in water

^C Mobile phase: 80 percent methanol in water

^D Mobile phase: 60 percent acetonitrile in water

Figure 1. GC SEPARATION OF SRM 1587 MONO-NITRATED PAH IN METHANOL

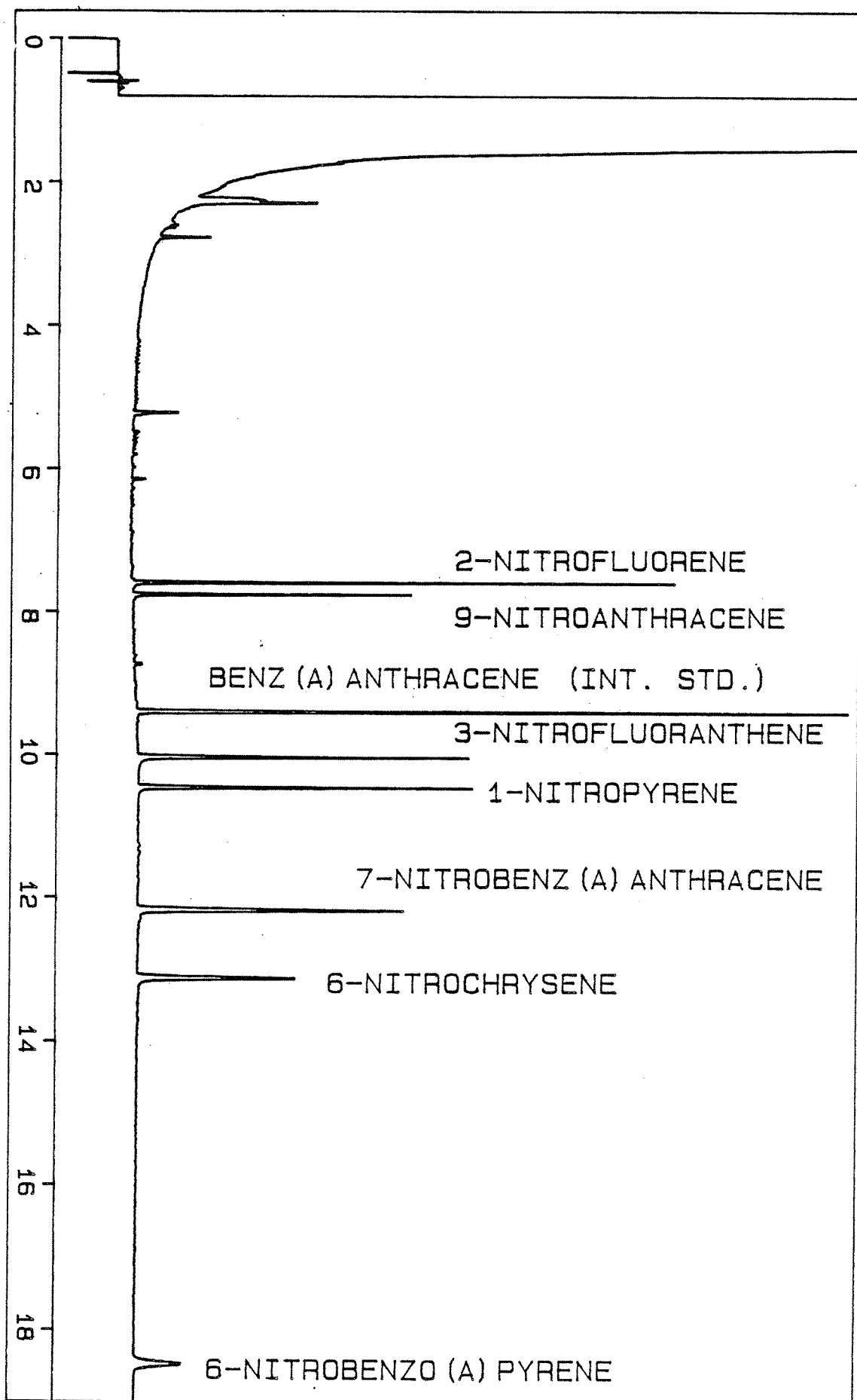


Figure 2. REVERSED-PHASE LC SEPARATION OF SRM 1587 MONO-NITRATED PAH IN METHANOL

