



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 2779

### Gulf of Mexico Crude Oil

This Standard Reference Material (SRM) 2779 is intended for use in evaluating analytical methods for the determination of selected polycyclic aromatic hydrocarbons (PAHs), hopanes, and steranes in a crude oil matrix. All of the constituents for which certified, reference, and information values are provided are naturally present in the oil. A unit of SRM 2779 consists of five ampoules each containing 1.2 mL of crude oil.

**Certified Mass Fraction Values:** Certified mass fraction values for 21 PAHs are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values are based on the agreement of results obtained at NIST using multiple analytical techniques.

**Reference Mass Fraction Values:** Reference mass fraction values are provided for an additional 22 PAHs (Table 2), alkylated PAHs (Table 3), and hopanes and steranes (Table 4). Reference values are noncertified values that are estimates of the true value. However, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

**Expiration of Certification:** The certification of **SRM 2779** is valid, within the measurement uncertainties specified, until **30 September 2020**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Handling, Storage, and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

**Maintenance of SRM Certification:** NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of technical measurements leading to certification were performed by M.M. Schantz and L.C. Sander of the NIST Analytical Chemistry Division.

Evaluation of the data was provided by N.A. Heckert, S.D. Leigh, and A.L. Pintar of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Analytical measurements were performed by B.A. Benner, Jr., J.R. Kucklick, and M.M. Schantz of the NIST Analytical Chemistry Division. Additional results for PAHs, hopanes, and steranes were used from 24 laboratories that participated in an interlaboratory study coordinated by M.M. Schantz.

Partial funding support for the preparation and certification of this SRM was provided by the National Oceanic and Atmospheric Administration and ultimately paid by BP Corporation North America, Inc. as part of the natural resource damage assessment for the 2010 Deepwater Horizon oil spill.

Stephen A. Wise, Chief  
Analytical Chemistry Division

Robert L. Watters, Jr., Chief  
Measurement Services Division

Gaithersburg, MD 20899  
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*Certificate Revision History on Last Page*

## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** This material is naturally occurring crude oil and may contain constituents of unknown toxicities; therefore, caution and care should be exercised during its handling and use.

**Storage:** Sealed ampoules, as received, should be stored in the dark at temperatures between 4 °C and 30 °C.

**Use:** Samples for analysis should be withdrawn immediately after opening ampoules and should be processed without delay for the certified values in Table 1 to be valid within the stated uncertainties. Certified values are not applicable to material stored in ampoules that have been opened, even if they are resealed.

## PREPARATION AND ANALYSIS<sup>(1)</sup>

**Sample Collection and Preparation:** The petroleum crude oil for this SRM was collected on May 21, 2010 on the drillship *Discoverer Enterprise* from the insertion tube that was receiving oil directly from the Macondo well during response operations. The oil was collected into certified cleaned 2.5 liter glass bottles and transported under chain of custody to TDI Brooks Laboratory in College Station, Texas. A portion was subsequently provided to NIST under the authority of the National Oceanic and Atmospheric Administration (NOAA). The water was separated from the oil by letting it sit in a separatory funnel for 4 h and removing the water. The resulting oil was homogenized by stirring for 18 h in a 20 L glass flask before transferring into 2 mL amber glass ampoules that had been flushed with argon.

**Analysis for PAHs, Hopanes, and Steranes:** The general approach used for the value assignment of the PAHs, hopanes, and steranes in SRM 2779 consisted of combining results from analyses using various combinations of different cleanup/isolation procedures and chromatographic separation and detection techniques [2]. Three sets of gas chromatography/mass spectrometry (GC/MS) results, designated as GC/MS (I) through GC/MS (III) were obtained at NIST.

For GC/MS (I) analyses, duplicate test portions of 400 mg from 10 ampoules of SRM 2779 were transferred to a vial, spiked with a known amount of an internal standard solution (see below), and diluted with hexane. A portion of the diluted oil (0.5 mL) was fractionated using an aminopropyl solid-phase extraction (SPE) column to isolate the fraction of interest. Following a concentration step, the processed extract was then fractionated using liquid chromatography on a semi-preparative scale aminopropyl silane column. Three fractions were collected: (1) aliphatics, hopanes, and steranes; (2) naphthalene through the dimethylphenanthrenes and anthracenes; and (3) the remainder of the PAHs through molecular mass 302 g/mol. Each fraction was then analyzed by GC/MS using a 0.25 mm i.d. × 60 m fused silica capillary column with a 50 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness; DB-17MS, Agilent Technologies, Wilmington, DE), and fractions 2 and 3 were analyzed using a 0.25 mm i.d. × 15 m fused silica capillary column with a 50 % (mole fraction) liquid crystal polysiloxane phase (0.15 µm film thickness; LC-50, J&K Scientific, Milton, Ontario, Canada). The results from the DB-17MS column are denoted as GC/MS Ia and those from the LC-50 column as GC/MS Ib.

For the GC/MS (II) determination of the PAHs, one test portion (6 mg to 8 mg) from each of six ampoules was transferred to a vial, spiked with a known amount of internal standard solution (see below), and diluted with 2 % dichloromethane in hexane (volume fraction). A portion of the diluted oil (0.1 mL) was fractionated using an aminopropyl SPE column to isolate the fraction of interest. Following a concentration step, the isolated fraction was analyzed by GC/MS using a 0.25 mm i.d. × 60 m fused silica capillary column with a 50 % phenyl methylpolysiloxane phase (0.25 µm film thickness; ZB-50 column, Phenomenex, Torrance, CA).

For the GC/MS (III) determination of the PAHs, hopanes, and steranes, one test portion (1 mL, exact mass known) from each of three ampoules was transferred to a bottle and diluted with 25 mL of hexane (exact mass known) prior to adding the internal standards (see below). The extracts were fractionated into two fractions using a silica/alumina column with the majority of the aliphatics, hopanes, and steranes in fraction 1 and the majority of the PAHs in fraction 2. The analytes of interest were quantified using GC/MS on a 0.25 mm i.d. × 60 m fused silica capillary column with a 50 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness; Rxi-17sil MS, Restek, Bellefonte, PA).

For the methods described above, perdeuterated PAHs and perdeuterated aliphatics were added to the crude oil as internal standards for quantification purposes.

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<sup>(1)</sup> Certain commercial equipment, instrumentation, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

In addition to the analyses performed at NIST, SRM 2779 was used in an interlaboratory comparison exercise in 2011 [3]. Results from 24 laboratories that participated in this exercise were used as the fourth data set in the determination of the values for PAHs, hopanes, and steranes in SRM 2779. Not all of the laboratories returned data for each analyte. The laboratories participating in this exercise used the analytical procedures routinely used in their laboratories to measure the analytes of interest. For the alkylated PAHs, more than 90 % of the laboratories used the parent PAH for determination of the response factor for the corresponding alkylated group.

**Homogeneity Assessment for PAHs:** The homogeneity of SRM 2779 was assessed by analyzing duplicate test portions of 400 mg from 10 ampoules selected by stratified random sampling. Test portions were processed and analyzed as described above for GC/MS (I). No differences among ampoules were observed for the PAHs at the 400 mg test portion size.

**Certified Values:** The certified mass fraction value is a weighted mean of the mass fractions from two to five analytical methods [4]. The uncertainty listed with each value is an expanded uncertainty about the mean [4,5], with coverage factor,  $k = 2$ , calculated by combining within-method variances with a between-method variance [6] following the ISO Guide [7,8]. Sample fractionation and analysis method are denoted by the footnotes in the table.

Table 1. Certified Mass Fraction Values for PAHs in SRM 2779

	Mass Fraction (mg/kg)	
Naphthalene <sup>(a,b,c,d)</sup>	855	± 46
1-Methylnaphthalene <sup>(a,b,d)</sup>	1140	± 20
2-Methylnaphthalene <sup>(a,b,d)</sup>	1630	± 50
Dibenzothiophene <sup>(a,b,d)</sup>	51.8	± 2.1
Phenanthrene <sup>(a,b,c,d,e)</sup>	258	± 27
Anthracene <sup>(a,c,e)</sup>	3.42	± 0.59
1-Methylphenanthrene <sup>(a,b,c,d,e)</sup>	169	± 10
2-Methylphenanthrene <sup>(a,b,c,e)</sup>	230	± 14
3-Methylphenanthrene <sup>(a,b,c,e)</sup>	206	± 32
9-Methylphenanthrene <sup>(a,b,c,e)</sup>	232	± 19
Fluoranthene <sup>(a,b,c,d,e)</sup>	4.36	± 0.40
Pyrene <sup>(a,b,c,d,e)</sup>	14.81	± 0.39
Benz[ <i>a</i> ]anthracene <sup>(a,b,c,d,e)</sup>	7.03	± 0.85
Chrysene/Triphenylene <sup>(a,b,d)</sup>	47.4	± 1.7
Benzo[ <i>b</i> ]fluoranthene <sup>(a,b,c,d,e)</sup>	5.62	± 0.34
Benzo[ <i>e</i> ]pyrene <sup>(a,b,c,d,e)</sup>	10.78	± 0.60
Benzo[ <i>ghi</i> ]perylene <sup>(a,b,c,d,e)</sup>	2.11	± 0.26
Dibenz[ <i>a,c</i> ]anthracene <sup>(a,b)</sup>	2.03	± 0.10
Dibenz[ <i>a,h</i> ]anthracene <sup>(a,c,e)</sup>	0.574	± 0.091
Benzo[ <i>b</i> ]chrysene <sup>(a,c)</sup>	0.629	± 0.022

<sup>(a)</sup> GC/MS Ia using LC fractionation followed by analysis on a DB-17MS column.

<sup>(b)</sup> GC/MS II using SPE clean-up followed by analysis on a ZB-50 column.

<sup>(c)</sup> GC/MS III using silica/alumina fractionation followed by analysis on an Rxi-17sil MS column.

<sup>(d)</sup> Data from the interlaboratory study [3].

<sup>(e)</sup> GC/MS Ib using LC fractionation followed by analysis on a LC-50 column.

**Reference Values:** For most of the PAHs, the reference mass fraction value is a weighted mean of the mass fractions from two to five analytical methods [4] when available. The uncertainty listed with each value is an expanded uncertainty about the mean [4,5], with coverage factor,  $k = 2$ , calculated by combining within method variances with a between method variance [6] following the ISO Guide [7,8] unless otherwise indicated. Sample fractionation and analysis methods are denoted by the footnotes in the table.

For dibenzofuran, 1,2-dimethylnaphthalene, 1-methylfluoranthene, 3-methylfluoranthene, and 1,6,7-trimethylnaphthalene, the reference mass fractions value are the means of results obtained using one analytical technique. The expanded uncertainty,  $U$ , is calculated as  $U = ku_c$ , where  $u_c$  is one standard deviation of the analyte mean, and the coverage factor,  $k$ , is determined from the Student's  $t$ -distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. There are five degrees of freedom for these values except for the 1,6,7-trimethylnaphthalene (15 degrees of freedom) value and the dibenzofuran (12 degrees of freedom) value.

Table 2. Reference Mass Fraction Values for PAHs in SRM 2779

	Mass Fraction (mg/kg)
Biphenyl <sup>(a,b,c,d)</sup>	195 ± 19
Acenaphthylene <sup>(a,b)</sup>	8.09 ± 0.10
1,2-Dimethylnaphthalene <sup>(d)</sup>	173 ± 5
1,6-Dimethylnaphthalene <sup>(c,d)</sup>	1160 ± 100
1,6,7-Trimethylnaphthalene <sup>(e)</sup>	306 ± 63
Dibenzofuran <sup>(e)</sup>	25.7 ± 3.6
Fluorene <sup>(a,b,c,d)</sup>	145 ± 43
2-Methylanthracene <sup>(a,d)</sup>	23.3 ± 2.5
1,7-Dimethylphenanthrene <sup>(c,d)</sup>	110 ± 12
1-Methylfluoranthene <sup>(e)</sup>	5.77 ± 0.09
3-Methylfluoranthene <sup>(e)</sup>	1.75 ± 0.17
1-Methylpyrene <sup>(a,c,d,e)</sup>	12.1 ± 1.8
4-Methylpyrene <sup>(a,c,d,e)</sup>	21.6 ± 1.5
Chrysene <sup>(a,e)</sup>	23.3 ± 5.2
Triphenylene <sup>(a,e)</sup>	17.7 ± 6.7
6-Methylchrysene <sup>(a,c,d,e)</sup>	15.10 ± 0.56
Benzo[ <i>j</i> ]fluoranthene <sup>(a,c,e)</sup>	0.75 ± 0.29
Benzo[ <i>k</i> ]fluoranthene <sup>(a,c,e)</sup>	0.66 ± 0.28
Benzo[ <i>a</i> ]pyrene <sup>(a,b,c,d,e)</sup>	1.36 ± 0.35
Perylene <sup>(a,c,e)</sup>	0.71 ± 0.17
Indeno[1,2,3- <i>cd</i> ]pyrene <sup>(a,c,e)</sup>	0.48 ± 0.14
Picene <sup>(a,c,d)</sup>	1.92 ± 0.37

<sup>(a)</sup> GC/MS III using silica/alumina fractionation followed by analysis on an Rxi-17sil MS column.

<sup>(b)</sup> Data from the interlaboratory study [3].

<sup>(c)</sup> GC/MS Ia using LC fractionation followed by analysis on a DB-17MS column.

<sup>(d)</sup> GC/MS II using SPE clean-up followed by analysis on a ZB-50 column.

<sup>(e)</sup> GC/MS Ib using LC fractionation followed by analysis on a LC-50 column.

The reference mass fraction values for the alkylated PAH groups are the means of results obtained using one analytical technique. The data are from the interlaboratory study [3]. The expanded uncertainty,  $U$ , is calculated as  $U = ku_c$ , where  $u_c$  is one standard deviation of the analyte mean, and the coverage factor,  $k$ , is determined from the Student's  $t$ -distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte.

Table 3. Reference Mass Fraction Values for Alkylated PAH Groups in SRM 2779

	Mass Fraction (mg/kg)	Degrees of Freedom
C1-Decalins	1040 ± 410	5
C2-Decalins	1060 ± 470	5
C3-Decalins	1460 ± 600	5
C2-Naphthalenes	2170 ± 360	22
C3-Naphthalenes	1380 ± 270	21
C4-Naphthalenes	700 ± 130	22
C2-Benzothiophenes	36 ± 13	6
C4-Benzothiophenes	30 ± 4	5
C1-Fluorenes	300 ± 60	22
C2-Fluorenes	380 ± 30	19
C3-Fluorenes	270 ± 40	21
C1-Phenanthrenes/anthracenes	670 ± 90	22
C2-Phenanthrenes/anthracenes	630 ± 60	21
C3-Phenanthrenes/anthracenes	400 ± 50	20
C4-Phenanthrenes/anthracenes	200 ± 30	19
C1-Dibenzothiophenes	130 ± 20	21
C2-Dibenzothiophenes	160 ± 20	21
C3-Dibenzothiophenes	110 ± 10	19
C4-Dibenzothiophenes	56 ± 10	17
C1-Fluoranthenes/pyrenes	67 ± 7	19
C2-Fluoranthenes/pyrenes	130 ± 20	18
C3-Fluoranthenes/pyrenes	120 ± 20	19
C4-Fluoranthenes/pyrenes	87 ± 21	11
C1-Naphthobenzothiophenes	57 ± 15	7
C2-Naphthobenzothiophenes	70 ± 19	7
C3-Naphthobenzothiophenes	48 ± 12	7
C4-Naphthobenzothiophenes	31 ± 10	6
C1-Benzanthracenes/chrysenes/triphenylenes	110 ± 7	20
C2-Benzanthracenes/chrysenes/triphenylenes	130 ± 10	18
C3-Benzanthracenes/chrysenes/triphenylenes	93 ± 12	17
C4-Benzanthracenes/chrysenes/triphenylenes	71 ± 16	12

The reference mass fraction values for hopanes and steranes, where data are available from three analytical methods, are weighted means [4]. The uncertainty listed with each value is an expanded uncertainty about the mean [4,5], with coverage factor,  $k = 2$ , calculated by combining within-method variances with a between-method variance [6] following the ISO Guide [7,8].

For analytes where the only available data are from the interlaboratory study, the reference mass fractions and associated uncertainties are calculated using the method means. The expanded uncertainty,  $U$ , is calculated as  $U = ku_c$ , where  $u_c$  is one standard deviation of the analyte mean, and the coverage factor,  $k$ , is determined from the Student's  $t$ -distribution corresponding to eight degrees of freedom and a 95 % confidence level for each analyte.

Table 4. Reference Mass Fraction Values for Hopanes and Steranes in SRM 2779

	Mass Fraction (mg/kg)
17 $\alpha$ (H),21 $\beta$ (H)-30-Norhopane <sup>(a,b,c)</sup>	17.0 $\pm$ 4.6
17 $\alpha$ (H)-22,29,30-Trisnorhopane <sup>(a,b,c)</sup>	7.29 $\pm$ 0.79
18 $\alpha$ (H)-22,29,30-Trisnorhopane <sup>(c)</sup>	6.9 $\pm$ 1.1
17 $\alpha$ (H),21 $\beta$ (H)-30-Hopane <sup>(a,b,c)</sup>	42.1 $\pm$ 9.9
17 $\alpha$ (H),21 $\beta$ (H)-22R-Homohopane <sup>(a,b,c)</sup>	13.8 $\pm$ 3.6
17 $\alpha$ (H),21 $\beta$ (H)-22S-Homohopane <sup>(a,b,c)</sup>	17.3 $\pm$ 4.3
17 $\alpha$ (H)-Diahopane <sup>(c)</sup>	4.5 $\pm$ 1.2
5 $\alpha$ (H), 14 $\beta$ (H),17 $\beta$ (H)-Cholestane 20S <sup>(c)</sup>	22.3 $\pm$ 7.5
5 $\alpha$ (H), 14 $\beta$ (H),17 $\beta$ (H)-Cholestane 20R <sup>(a,b,c)</sup>	23.7 $\pm$ 2.7
13 $\beta$ (H),17 $\alpha$ (H)-Diacholestane 20S <sup>(c)</sup>	41.2 $\pm$ 6.7
5 $\alpha$ (H), 14 $\alpha$ (H),17 $\alpha$ (H)-24-Ethylcholestane 20R <sup>(a,b,c)</sup>	16.9 $\pm$ 5.0
5 $\alpha$ (H), 14 $\beta$ (H),17 $\beta$ (H)-24-Ethylcholestane 20R <sup>(a,b,c)</sup>	21.3 $\pm$ 8.2
5 $\alpha$ (H), 14 $\beta$ (H),17 $\beta$ (H)-24-Ethylcholestane 20S <sup>(c)</sup>	23.1 $\pm$ 6.4

<sup>(a)</sup> GC/MS Ia using LC fractionation followed by analysis on a DB-17MS column.

<sup>(b)</sup> GC/MS III using silica/alumina fractionation followed by analysis on an Rxi-17sil MS column.

<sup>(c)</sup> Data from the interlaboratory study [3].

The laboratories listed below participated in the interlaboratory comparison exercise for the determination of PAHs, hopanes, and steranes in SRM 2779 [3].

Alpha Analytical, Inc., Mansfield, MA

ALS Environmental Division, Edmonton, AB, Canada

Battelle Analytical & Environmental Chemistry Laboratory, Duxbury, MA

Columbia Analytical Services at Jacksonville, FL, Rochester, NY, and Kelso, WA

Florida International University, North Miami, FL

New York State Department of Health, Albany, NY

NOAA/NCCOS/NOS, Charleston, SC

NOAA/NMFS/Alaska Fisheries Science Center, Juneau, AK

NOAA/NMFS/NW Fisheries Science Center, Seattle, WA

Pace Analytical Services, Inc., Minneapolis, MN

RJ Lee Group, Inc., at Monroeville, PA and Pasco, WA

TDI/B&B Laboratories, Inc., College Station, TX

TestAmerica Laboratories at Mobile, AL, West Sacramento, CA, University Park, IL, Pittsburgh, PA,

Knoxville, TN, South Burlington, VT, and Tacoma, WA

Texas A&M University, College Station, TX

University of Iowa, Iowa City, IA

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- [8] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_101\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_101_2008_E.pdf) (accessed June 2012).

<b>Certificate Revision History:</b> 04 June 2012 (Editorial changes); 22 May 2012 (Corrected names in Table 4; editorial changes); 30 January 2012 (Original certificate date)
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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.