BNA Standard Preparation

1 SCOPE AND APPLICATION

1.1 This SOP details the procedure for preparing standards used in the BNA sample extraction and analysis.

2 REAGENTS

NOTE: Record Lot Numbers of all the reagents and solvents used in the Note Book. For each new chemical standard received, enter all the information of standard into the Laboratory Information Management System (LIMS) and get the serial number, and then file the certificate of analysis in a certification folder. If no expiration date for the chemical received, call the company and if they cannot give any date then enter one (1) year from date of receipt. If any expired or expiring stock standard is re-certified, make sure the standard is still good by preparing stock, intermediate and working standard and check against valid standards. Response should agree within +/- 15%. Keep all the documents of re-certification in the certification folder. Re-certification should be limited to stable standards and do not re-certify ICAL standards, and always use un-expired ICAL standards.

2.1 Methylene Chloride, Methanol and Acetone, Optima or HPLC grade-Fisher Scientific

2.2 Commercial Stock Standards and neat standards are available from Ultra Scientific, Fisher, Accu Standard, Chem Service, Restek, O_2Si and other suppliers. Generally, one-milliliter vials of standards are purchased for making calibration curve and check standard solutions. However, larger vials (5 mL) of higher concentration standards (up to 10,000 ppm) may be used for surrogate spike solutions and matrix spike solutions since these are used up so quickly.

3 EQUIPMENT

3.1 Volumetric Flasks- 1, 2, 5, 10, 25, 50, 100, and 500 mL

3.2 Volumetric pipettes- 0.5, 1, 2, 5, and 10 mL

3.3 Micro syringes- 5, 10, 25, 50, 100, 250, 500 and 1000 µL (Required from the manufacturer, the certificate stating that the volume is accurate to < 1%, otherwise calibrate and keep the record of these gas-tight micro-syringes or volumetric pipets quarterly)

3.4 2 mL screw cap amber glass auto sampler vials (or 1.8 mL crimp top vials) with colored cap for easy identification.

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3.5 Amber glass bottle with Teflon lined screw cap - 25, 50 and 100 mL

4 STANDARDS QUALITY ASSURANCE

4.1 New standards must be checked for correctness and condition upon receipt. Enter the information for each new standard into the LIMS Electronic Standard Preparation Log which includes all pertinent information such as date of receipt, compound list, concentrations, lot number, solvent, expiration date for the standard preparation. When preparing a standard make sure that all the preparation information is entered into the Electronic Standard Preparation Log. The Preparation Log will create a serial number and barcode specific for the standard you prepare. Place the serial number or barcode onto the vial along with the standard name, concentration, solvent, date and your initials.

4.2 All prepared spike, surrogate and internal standard stock solutions must be re-made every 6-months or at the time of the standard expiration date, whichever comes first. All working standards should be re-made on a 3-month interval. Before making any standards from commercial sources, check to ensure that the expiration date has not passed. Before putting any standard into use, verify that it is acceptable by comparing against the previously used standard or an external check standard. Responses should agree within +/- 15%. If not, check for instrument problems or re-make the new standard. **DISCARD ANY OLD STANDARDS AFTER NEW STANDARDS HAVE BEEN MADE AND FOUND TO BE ACCEPTABLE!**

4.3 Any standard remaining in an open ampoule should be transferred to a 2-mL vial and capped using a Teflon-lined rubber septum. (Make sure Teflon side of septum faces the standard solution!) Replace any pierced septa before returning the standard to storage. Draw a line on the vial at the meniscus and date the vial. Always check the meniscus when you use concentrate from a vial to make sure no evaporation has taken place. If it is below the line you drew, open a fresh ampoule. Store the vial in an upright position either in the box the concentrate came in or in the rack with the standards in the refrigerator. Record all standard preparations in the electronic logbook!!

5 STANDARDS PREPARATION

NOTICE: Review relevant SOPs and refer to the Material Safety Data Sheets (MSDS) for hazard and handling instructions before preparing standards.

The BNA standard in ampules may precipitate during storage. Visually inspect all standard ampules before use. Ultra Sonication of the sealed ampules prior to preparation usually insures complete dissolution of the compounds into the solvent and is a good standard practice. Do not use any vial with an un-dissolved precipitate.

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Always print labels from LIMS each time you make standard. Do not hand write!

5.1 **BNA Internal standard (50 µg/mL):** Prepare from the stock concentrate containing the following at 4000 µg/mL (Ultra Scientific - US-108N, Internal Standards Mixture, 12 x 1 mL of 4000 µg/mL)

1,4-Dichlorobenzene-d4	Phenanthrene-d10
Naphthalene-d8	Chrysene-d12
Acenaphthene-d10	Perylene-d12

Allow Internal Standard Stock (4000 µg/mL) to completely warm up to room temperature. The vial containing the solution should be sonicated for several minutes to insure complete dissolution of compounds into the solvent. Visually check the bottom of the vial for crystals of compound. The more heavily deuterated compounds may crystallize during cold storage and then require a warm water bath as well as sonication to re-dissolve. Pipette exactly 0.125 mL of the Internal Standard Stock into a 10-mL volumetric flask. Dilute to volume with methylene chloride. Transfer to a 15 mL centrifuge tube and store at –10 °C or less.

5.1.1 BNA Internal standard at 10 µg/mL: Take 2 mL of BNA Internal Standard (50 µg/mL) and place into a 10 mL volumetric flask. Dilute to volume with methylene chloride and mix well. Use this concentration for Instrument blank check and for sample dilutions.

5.1.2 BNA Internal standard at 1 µg/mL: Take 1 mL of BNA Internal Standard (10 µg/mL) and place into a 10 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.1.3 BNA Internal standard at 100 µg/L: Take 1 mL of BNA Internal Standard (1 µg/mL) and place into a 10 mL volumetric flask. Dilute to volume with methylene chloride and mix well. Use this concentration for Instrument blank check and for sample dilutions.

5.2 **<u>BNA Surrogate Mix for Water/Sediments (200 µg/mL acids, 100 µg/mL base neutrals)</u>:**

5.2.1 Take 5 mL each of the Acid Surrogate Spike Mix (10,000µg/mL) (Restek Corporation – 31087; Acid Surrogate Mix) and the Base-Neutral Surrogate Spike Mix (5,000 µg/mL) (Restek Corporation – 31086; B/N Surrogate Mix) and place into a 250 mL volumetric flask. Dilute to volume with methanol and mix well. Alternatively, make only 100 mL of this mix by diluting 2 mL each in methanol and store rest of the concentrate carefully.

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5.2.2 Transfer to amber glass bottles with Teflon lined screw caps. Label as "BNA Surrogate Mix for Water/Sediments (200 µg/mL acids, 100 µg/mL base neutrals), in methanol " and store at 4 °C or less.

5.2.3 For water acid and base neutral extractions Tinted methanol indicator at 2000 µg/mL of 250 mL is purchased from O_2Si, catalog number 020328-99-250. Add 0.5 mL of this solution along with surrogate.

5.3 **BNA Matrix Spike Mix for Water/Sediments – 625/8270/OFR matrix spike mixes for water/sediments (50 µg/mL for all the components):**

5.3.1 Follow sec. 5.4.2 to 5.4.5 to prepare each of the 625/8270/ matrix spike mixes from 1st source without the addition of surrogate spikes. Use this 50 µg/mL of 625/8270/OFR intermediate for spiking; add 0.2 mL for a final volume of 1 mL (For drinking water analysis add 0.2 mL of 625/8270 spike). For TCLP use 625 intermediate for spiking.

5.3.2 Transfer to 15 mL centrifuge tube with Teflon lined screw caps. Label as "625/8270/ Matrix Spike Mix for Water/Sediments (50 µg/mL), in methanol " and store at 4 °C or less.

5.4 **Mixed BNA Calibration (ICAL) & Second Source Continuing Calibration Verification (CCV) Stock Standards (for 625/8270 and OFR, GC-MS Analyses):**

5.4.1 Prepare the following mixed stock standards at 100 µg/mL (ICAL) and 50 µg/mL (CCV): The standards for ICAL were used from Ultra Scientific or Restek and Accu Standard or Supelco or O_2Si for CCV by mixing them separately. These mixes may come from sources other than Ultra Scientific and Accu Standard as deemed necessary. Store the prepared intermediate standards mix at -10 °C or less.

5.4.2 **625 ICAL Stock Mix at 100 µg/mL with Surrogates:** Add the appropriate aliquots of the following concentrated stocks to a 1 mL volumetric flask to make 100 µg/mL. Dilute to volume with methylene chloride. The usual aliquot is 0.1 mL for 1000 µg/mL and 0.05 mL for 2000 µg/mL.

5.4.3 **625 CCV at 25 µg/mL**: Add the appropriate aliquots of 100 µg/mL (sec. 5.4.1) from second source to a 1 mL volumetric flask to make 25 µg/mL. Also add appropriate aliquot of internal standard. Dilute to volume with methylene chloride.

625 CCV at 1 µg/mL: Add the appropriate aliquots of 100 µg/mL (sec. 5.4.1) from second source to a 1 mL volumetric flask to make 1 µg/mL. Also add appropriate aliquot of internal standard. Dilute to volume with methylene chloride.

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Std ID	**Catalog #**
ICAL:	
64 Compound 8270 Mega mix	31850, Restek
2 Compound Benzidine Mix	31030, Restek
Acid Surrogate Mix	ISM 290N, US
Base Neutral Surrogate Mix	ISM 280N, US
CCV:	
64 Compound	110807-01, O_2Si, Restek different lot #
2 Compound Benzidine Mix	Z-014F, Accu Std. or US-105N, US

5.4.4 8270 ICAL Stock Mix at 100 µg/mL:

Add the appropriate aliquots of the following concentrated stocks to a 1 mL volumetric flask to make 100 µg/mL. Dilute to volume with methylene chloride. The usual aliquot is 0.1 mL for 1000 µg/mL and 0.05 mL for 2000 µg/mL.

5.4.5 **8270 CCV at 25 µg/mL:** Add the appropriate aliquots of 100 µg/mL (sec. 5.4.1) from second source to a 1 mL volumetric flask to make 25 µg/mL. Also add appropriate aliquot of internal standard. Dilute to volume with methylene chloride.

8270 CCV at 1 µg/mL: Add the appropriate aliquots of 100 µg/mL (sec. 5.4.1) from second source to a 1 mL volumetric flask to make 1 µg/mL. Also add appropriate aliquot of internal standard. Dilute to volume with methylene chloride.

Std ID	**Catalog #**
ICAL:	
35 Compound SVM-8271 Mix	SVM-8271, 1000 µg/mL, Supelco
CCV:	
31 Compound M-8270 Mix	SVM-8271, 1000 µg/mL, US

5.4.6 OIL FRAKING ANALYTES (OFR): Similarly, ICAL standards and CCV were made as sec 5.4.2 and 5.4.3

List of analytes for OFR:

Benzaldehyde, 2-Butoxyethanol, 1-Butoxy-2-propanol, Benzoic acid, Alpha-Terpineol and Triethyl-2-acetylcitrate

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5.5 **DFTPP Tuning Standard Mixture (50 µg/mL and 5 µg/mL):**

5.5.1 (Pentachlorophenol, DFTPP, Benzidine and 4, 4'-DDT) (Ultra Scientific - GCM-150; Semi-volatiles GC/MS Tuning Standard; 4 x 1 mL of 1000 µg/mL)

5.5.2 Pipette exactly 0.5 mL of DFTPP solution (1000 µg/mL) into a 10 mL volumetric flask. Dilute to volume with methylene chloride. Mix well and transfer to a bottle. Store at -10 °C or less. Used for MSD 5973 and 5975.

5.5.3 Pipette exactly 0.5 mL of DFTPP solution (50 µg/mL, sec. 5.5.2) into a 10 mL volumetric flask. Dilute to volume with methylene chloride. Mix well and transfer to a bottle. Store at -10 °C or less. Used for MSD 5977.

5.6 **PAH/DPGBE Matrix Spike Mix and B/N Surrogate Spike for Water, Waste, Sediment (low level) and Puf (100, 50 and 5 µg/mL):**

5.6.1 PAH Matrix Spike Mix Intermediate at 50 µg/mL: Take 0.25 mL of PAH Mix from 1st source (2,000µg/mL) and place into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.2 DPGBE Matrix Spike Intermediate at 100 µg/mL: Take appropriate aliquot of DPGBE from stock standard (Sigma Aldrich) and place into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.3 PAH Matrix Spike Mix at 5 µg/mL: Take 1 mL of 5.6.1 (50 µg/mL) and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

Note: For low level, PAH Matrix Spike Mix at 100 µg/L: Take 0.2 mL of 5.6.3 (5 µg/mL) and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.4 DPGBE Matrix Spike at 5 µg/mL: Take 0.5 mL of 5.6.2 (100 µg/mL) and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.5 B/N Surrogate Spike at 50 µg/mL: Take 0.5 mL of B/N Surrogate 1000 µg/mL and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

B/N Surrogate Spike at 5 µg/mL: Take 1 mL of B/N Surrogate 50 µg/mL (sec 5.6.5) and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

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5.6.6 Field Surrogate Spike for Puff at 50 µg/mL: Take appropriate aliquot of field surrogate and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.7 Laboratory and Field Surrogate Mix Spike for puff at 50 µg/mL: Take appropriate aliquot of field surrogate, laboratory surrogate, B/N surrogate and placed into a 10 mL volumetric flask. Dilute to volume with methanol and mix well.

5.6.8 PAH Mix Intermediate for CCV at 50 µg/mL: Take 0.125 mL of PAH Mix from 2nd source (2,000µg/mL) and place into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

PAH Mix Intermediate for CCV at 5 µg/mL: Take 0.5 mL of PAH Mix from 5.6.8 (50µg/mL) and place into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.6.9 DPGBE Intermediate for CCV at 50 µg/mL: Take appropriate aliquot of DPGBE from stock standard (Accu Standard) and place into a 10 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.6.10 PAH CCV Mix for low level analysis at 5 µg/mL: Take 0.5 mL of 5.6.8 (50 µg/mL), 0.5 mL of 50 µg/mL (5.6.5) B/N Surrogate and 0.5 mL of BNA Internal standard of 10 µg/mL and placed into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

Note: For SIM mode, PAH CCV Mix for low level analysis at 100 µg/L: Take 0.1 mL of 5.6.8 (5 µg/mL), 0.1 mL of 5 µg/mL (5.6.5) B/N Surrogate and 0.5 mL of BNA Internal standard of 1 µg/mL and placed into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.6.11 DPGBE CCV for low level analysis at 5 µg/mL: Take 0.5 mL of 5.6.9 (50 µg/mL), 0.5 mL of 50 µg/mL (5.6.5) B/N Surrogate and 0.5 mL of BNA Internal standard of 100 µg/mL and placed into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.6.12 PAH CCV Mix for puff analysis at 0.5 µg/mL: Take 0.05 mL of 5.6.8 (50 µg/mL), 0.05 mL of 50 µg/mL (5.6.7) and 0.05 mL of BNA Internal standard of 50 µg/mL and placed into a 5 mL volumetric flask. Dilute to volume with methylene chloride and mix well.

5.7 **PAH/DPGBE and Surrogates (Laboratory and Field) Calibration Intermediate Standard Mix (For Puf, 100 and 10 µg/mL, GC/MS analyses):**

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5.7.1 PAH and Surrogate Calibration Intermediate Standard mix at 100 µg/mL: Add 0.5 mL of the PAH stock mix (2,000µg/mL) and 1 mL of Base Neutral surrogate stock mix (1,000µg/mL) to a 10 mL volumetric flask. Dilute to volume with methylene chloride.

5.7.2 DPGBE Calibration Intermediate Standard at 100 µg/mL: Take appropriate aliquot of DPGBE from stock standard (Sigma Aldrich) to a 10 mL volumetric flask. Dilute to volume with methylene chloride.

5.7.3 PAH and Surrogate Calibration Intermediate Standard mix for puff at 10 µg/mL: Add 0.05 mL of the PAH stock mix (2,000µg/mL) and 2 mL of 5.6.7 to a 10 mL volumetric flask and dilute to volume with methylene chloride.

5.7.4 PAH 7 (analytes) stock standard of 1000 µg/mL purchased from two different sources, ultra scientific and O_2Si (used for ICAL and CCV).

6 CALIBRATION STANDARDS

6.1 In general, five-point calibration curves need to be made for every compound on the identification list. The behavior of some compounds such as thermally labile and active site sensitive may limit the curve for that compound to only three or so points. The upper limit to the calibration curve is usually determined by the column's capacity for that compound. A DB-5 or similar column will take a sample load of about 100 ng before column overloading occurs, but this phenomenon may occur at as little as 50 ng for some compounds. Therefore, a significant drop in response factors (RF) values at the high end of the curve will warrant a deletion of that point on the curve for that individual compound. The lower end of the curve is determined statistically as the PQL for that compound. For most compounds this is 1 ppm.

6.2 Calibration curve solution preparation: Prepare ten calibration levels, for details see the table. Follow this procedure for the 8270 calibration solution. Using a clean transfer pipette for each flask, transfer the contents of the flask to an amber screw top sample vial. Place printed label and with a permanent ink marker, mark the meniscus level on the side of the vial so that in the future, any gross loss of the contents will be evident. Store at -10 °C or less.

Calibration Level (µg/mL)	Final Volume (mL)	Internal Std. (10µg/mL) Aliquot (mL)	Stock Std. (100µg/mL) Aliquot (mL)
L10- 50	1	0.1	0.5
L9- 25	1	0.1	0.25
L8- 10	1	0.1	0.1
L7- 5	1	0.1	0.05
L6- 2.5	1	0.1	0.025

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L5- 1	1	0.1	0.01
L4-0.5	1	Diluted with IS 1ppm	0.1 mL of L7
L3-0.2	1	Diluted with IS 1ppm	0.08 mL of L6
L2-0.1	1	Diluted with IS 1ppm	0.1 mL of L5
L1-0.05	1	Diluted with IS 1ppm	0.1 mL of L4

6.3 **Calibration curve solution preparation for Puf analysis:** Using five 1 mL volumetric flasks, add 0.05 mL of 10 ppm BNA internal standard solution to each flask. To prepare the following calibration standard, 2.5, 1.25, 0.5, 0.25and 0.1, known aliquot of 5.7.2 (10 µg/mL) is added to each flask using appropriate syringe. Make up to the volume with methylene chloride. Mix the contents thoroughly. Label the flask and transfer to an amber screw top sample vial. With a permanent ink marker, mark the meniscus level on the side of the vial so that in the future, any gross loss of the contents will be evident.

6.4 **Calibration curve solution preparation for Low Levels PAH/DPGBE analysis (GC/MS):**

Using seven 1 mL volumetric flasks, add 0.1 mL of 100 ppm BNA internal standard solution to each flask. To prepare the following calibration standard, 50, 25, 10, 5, 2, 1, 0.2 and 0.05 µg/mL, known aliquot of 5.7.1 (100 µg/mL) is added to each flask using appropriate syringe. Make up to the volume with methylene chloride. Mix the contents thoroughly. Label the flask and transfer to an amber screw top sample vial. With a permanent ink marker, mark the meniscus level on the side of the vial so that in the future, any gross loss of the contents will be evident.

Using seven 1 mL volumetric flasks, add 0.1 mL of 100 ppm BNA internal standard solution to each flask. To prepare the following calibration standard, 50, 25, 10, 5, 2, 1 and 0.2 µg/mL, known aliquot of 5.7.2 (100 µg/mL) is added to each flask using appropriate syringe. Make up to the volume with methylene chloride. Mix the contents thoroughly. Label the flask and transfer to an amber screw top sample vial. With a permanent ink marker, mark the meniscus level on the side of the vial so that in the future, any gross loss of the contents will be evident.

Note: For low level of PAH's in SIM mode: Using seven 1 mL volumetric flasks, add 0.1 mL of 1 ppm BNA internal standard solution to each flask. To prepare the following calibration standard, 1000, 500, 100, 25, 10, 5 and 2 µg/L, known aliquot of 5.6.3 and 5.6.5 (5 µg/mL) is added to each flask using appropriate syringe. Make up to the volume with methylene chloride. Mix the contents thoroughly. Label the flask and transfer to an amber screw top sample vial. With a permanent ink marker, mark the meniscus level on the side of the vial so that in the future, any gross loss of the contents will be evident.

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7 SECOND SOURCE CALIBRATION MIX (CCV):

7.1 Prepare a CCV from second source Std. 625/8270 at 25 µg/mL and 1 µg/mL. See details sec. 5.4.2 to 5.4.5.

Appendix of Changes

04/26/2010 Minor revisions to the text were made for format and clarity.

04/26/2010 Updated standards to reflect current use.

05/10/2011 Updated standards to reflect current use.

10/24/2013 Updated standards to reflect current use.

12/06/2013 Updated standards to reflect current use.

07/29/2015 Updated standards to reflect current use.

06/07/2016 Updated standards to reflect current use.

08/15/2016 Updated standards to reflect current use.

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