



Designation: **D7574–09 D7574 – 16**

Standard Test Method for Determination of Bisphenol A in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry¹

This standard is issued under the fixed designation D7574; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This procedure covers the determination of bisphenol A (BPA) extracted from water utilizing solid phase extraction (SPE), separated using liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS). BPA is qualitatively and quantitatively determined by this method. This method adheres to multiple reaction monitoring (MRM) mass spectrometry.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 The method detection limit (MDL) and reporting limit (RL) for BPA are listed in **Table 1**.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*²

[D1129 Terminology Relating to Water](#)

[D1193 Specification for Reagent Water](#)

[D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water](#)

[D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents](#)

[D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water](#)

[D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis](#)

[D5905 Practice for the Preparation of Substitute Wastewater](#)

2.2 *Other Documents:*³

[The Code of Federal Regulations 40 CFR Part 136, Appendix B](#)

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology [D1129](#).

3.2 *Definitions: Definitions of Terms Specific to This Standard:*

3.2.1 *environmental water, n*—~~effluents from secondary and tertiary publicly owned treatment works (POTW); shall refer to water tested using this method. See Section 5 fresh surface and ground water.~~

3.2.2 *independent reference material, IRM, n*—a material of known purity and concentration obtained either from the National Institute of Standards and Technology (NIST) or other reputable supplier. The IRM shall be obtained from a different lot of material than is used for calibration.

3.3 *Abbreviations: Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

¹ This test method is under the jurisdiction of ASTM Committee [D19](#) on Water and is the direct responsibility of Subcommittee [D19.06](#) on Methods for Analysis for Organic Substances in Water.

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² For referenced ASTM standards, visit the ASTM website, [www.astm.org](#), or contact ASTM Customer Service at [service@astm.org](#). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, [http://www.access.gpo.gov](#).

TABLE 1 MDL and Reporting Range

Analyte	MDL ^A (ng/L)	Reporting Range ^B (ng/L)
Bisphenol A	5	20–600

^A MDL determined following the Code of Federal Regulations, 40CFR Part 136, Appendix B.

^B Lowest point of the reporting range, reporting limit, is calculated from the LV 1 concentration calibration standard in Table 4. Fig. 1 displays the signal/noise ratio at the reporting limit.

3.3.2 *IC, n*—Initial Calibration

3.3.3 *LC, n*—Liquid Chromatography

3.3.4 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.5 *MDL, n*—Method Detection Limit

3.3.6 *MeOH, n*—Methanol

3.3.7 *mM, n*—millimolar, 1×10^{-3} moles/L

3.3.8 *MRM, n*—Multiple Reaction Monitoring

3.3.9 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.10 *NA, adj*—Not Available

3.3.11 *ND, n*—non-detect

3.3.12 *P&A, n*—Precision and Accuracy

3.3.13 *ppt=PPB, n*—parts per trillion, ng/L-billion

3.3.14 *PPT, n*—parts per trillion

3.3.15 *QA, adj*—Quality Assurance

3.3.16 *QC, adj*—Quality Control

3.3.17 *RL, n*—Reporting Limit

3.3.18 *RSD, n*—Relative Standard Deviation

3.3.19 *RT, n*—Retention Time

3.3.20 *SDS, n*—Safety Data Sheets

3.3.21 *SRM, n*—Single Reaction Monitoring

3.3.22 *SS, n*—Surrogate Standard

3.3.23 *TC, n*—Target Compound

3.3.24 μM , *n*—micromolar, 1×10^{-6} moles/L

3.3.25 *VOA, n*—Volatile Organic Analysis

4. Summary of Test Method

4.1 This is a performance based method and modifications are allowed to improve performance.

4.2 Solid phase extraction is used to extract water samples.

4.2.1 *Solid Phase Extraction*—250 milliliter volume of sample adjusted to pH 2 is extracted using a solid phase extraction cartridge. The resulting methyl tert-butyl ether (MTBE) extract is solvent exchanged into methanol, concentrated to a volume of 0.2 mL, brought to a final volume of 1 mL with water and then analyzed by LC/MS/MS operated in the multiple reaction monitoring (MRM) mode.

4.3 The target compound, surrogate and internal standards are identified by retention time and two SRM transitions. The target analyte and surrogate are quantitated using the primary SRM transitions utilizing internal standard calibration. The final report issued for each sample lists the concentration of BPA and the bisphenol A (Ring-13C12) surrogate recovery.

5. Significance and Use

5.1 The first reported synthesis of BPA was by the reaction of phenol with acetone by Zincke.⁴ BPA has become an important high volume industrial chemical used in the manufacture of polycarbonate plastic and epoxy resins. Polycarbonate plastic and resins are used in numerous products including electrical and electronic equipment, automobiles, sports and safety equipment,

⁴ Zincke, T., 1905, "Mittheilungen aus dem chemischen Laboratorium der Universitat Marburg," *Justus Leibigs Annals Chemie*, Vol. 343, pages 75–79.

reusable food and drink containers, electrical laminates for printed circuit boards, composites, paints, adhesives, dental sealants, protective coatings and many other products.⁵

5.2 The environmental source of BPA is predominantly from the decomposition of polycarbonate plastics and resins. BPA is not classified as bio-accumulative by the U.S. Environmental Protection Agency and will biodegrade. BPA has been reported to have adverse effects in aquatic organisms and may be released into environmental waters directly at trace levels through landfill leachate and POTW effluents. This method has been investigated for use with surface water and secondary and tertiary POTW effluent samples therefore, it is applicable to these matrices only. It has not been investigated for use with salt water or solid sample matrices.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus producing discrete artifacts or elevated baselines. The use of plastic supplies and equipment must be avoided because they may contain BPA. All of these materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples.

6.2 All glassware is washed in hot water with a detergent, rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with acetone, then methanol. Detergents in plastic containers that contain BPA must not be used.

6.3 All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences can vary considerably from sample source to sample source, depending on variations of the sample matrix.

7. Apparatus

7.1 LC/MS/MS System:

7.1.1 *Liquid Chromatography System*—A complete liquid chromatography-LC system is needed in order to analyze samples.⁶ This should include a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. A system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes and requirements of the standard may be used.

7.1.1.1 *Columns*—Column to separate instrument background (isolator): A short (2.1 × 50 mm) C8 or C18 column stable at higher pH up to 12. Analytical column: ACQUITY (trademarked) 12 and an analytical column: UPLC⁷ BEH C18, 1.7 μm, 2.1 × 50 mm column or equivalent.

7.1.1.2 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.⁸ A system that is capable of performing at the requirements in this standard may be used.

7.2 *SPE Vacuum Manifold System*⁹—Supelco Visiprep solid phase extraction vacuum manifold or similar may be utilized.

7.3 Organic solvent evaporation device.

8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.¹⁰ Other reagent grades may be used, provided it is first ascertained that they are of sufficiently high purity to permit their use without affecting the accuracy of the measurement. A source for the labeled BPA standards is Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413.¹¹

⁵ Additional information about BPA is available at <http://www.bisphenol-a.org> (2008).

⁶ A Waters ACQUITY Ultra Performance Liquid Chromatography (trademarked) UPLC System was used to develop this test method. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee. UltraPerformance LC (a trademark of the Waters Corporation, Milford, MA) system, or equivalent, was found suitable for use.

⁷ A Waters ACQUITY UPLC (a trademark of the Waters Corporation, Milford, MA) HSS T3, 1.8 μm, 2.1 × 50 mm column, or equivalent, was found suitable for use.

⁸ A Waters Quattro Premier XE mass spectrometer was used to develop this test method. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee. (a trademark of the Waters Corporation, Milford, MA) mass spectrometer, or equivalent, was found suitable for use.

⁹ A Supelco Visiprep (a trademark of Sigma-Aldrich Co., LLC, St. Louis, MO) was found suitable to use, any SPE extraction manifold may be used.

¹⁰ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

¹¹ A source for the labeled BPA standards is Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,¹ which you may attend.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification **D1193**. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 2-Propanol (CAS # 67-63-0).

8.7 Acetone (CAS # 67-64-1).

8.8 Methyl tert-butyl ether (MTBE, CAS # 1634-04-4).

8.9 Ammonium Hydroxide (CAS # 1336-21-6) (ACS reagent grade or better).

8.10 Concentrated HCl (CAS # 7647-01-0).

8.11 Bisphenol A (BPA, 2,2'-Bis(4-hydroxyphenyl)propane, CAS # 80-05-7).

8.12 Bisphenol A (Ring-13C12) represents ¹³C labeled bisphenol A where all ring carbon atoms are uniformly labeled ¹³C.

8.12.1 Bisphenol A (Ring-13C12) is used as a surrogate in this standard.

8.13 Bisphenol A (Propane-D6) represents deuterium labeled bisphenol A where the 2 methyl moieties contain all ²H.

8.13.1 Bisphenol A (Propane-D6) is used as an internal standard in this method.

8.14 *Solid Phase Extraction Cartridges*—~~Oasis HLB 5cc (200 mg) LP Glass Cartridges or equivalent.~~ An SPE cartridge suitable for the extraction of BPA.

NOTE 1—If plastic cartridges are used, BPA may be found, therefore it is advisable that the cartridges be lot certified BPA-free. BPA may adhere to plastic cartridges which will cause lower recoveries. Glass cartridges have a much lower adhesive tendency to BPA and should not contain BPA in the packing or support materials, therefore glass cartridges should be used.

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves and lab coats when working with acids. Analysts should review the Material Safety Data Sheets (MSDS)(SDS) for all reagents used in this method.

10. Sample Collection, Preservation, and Storage

10.1 *Sampling*—Grab samples must be collected in 250 mL amber glass bottles. Rinsing of the bottle with 10 % methanol in water, water, and 10 % methanol/2 % NH₄OH in water is required in order to get quantitative transfer of the sample into the SPE cartridge and extraction process. BPA tends to adsorb to surfaces and rinsing will allow optimum recoveries. Conventional sampling practices should be followed. Refer to Guide **D3856** and Practices **D3694**. Pre-cleaned bottles demonstrated to be free of interferences may be used. Automatic sampling equipment should be free from plastics and tubing that contains BPA and other potential sources of contamination or adhesion.

10.2 *Preservation*—Adjust sample to pH 2 with concentrated HCl at time of collection. Store samples between 0°C and 6°C from the time of collection until extraction. Extract the sample within 7 days of collection and completely analyze within 14 days of extraction.

11. Preparation of LC/MS/MS

11.1 *LC Set Up for Liquid Chromatography BPA Isolator Column for Low Level BPA Analysis*—BPA may be a contaminant in the LC system due to the widespread use of plastic parts and tubing. Incorporating an isolator column into the LC system allows for the low level analysis of BPA and reduces the risk of high biased data. The LC conditions in this standard allow for the BPA in the system to elute 0.4 minutes later than BPA from the sample injection. The BPA concentration found in the LC system used was below the MDL of the standard but an isolator column must be incorporated to eliminate the risk of high bias. An isolator column was placed after the mixer of two solvent feeds and before port 5 of the multi-port valve which contains the injection loop. No plastics are used in the system at this point ensuring that the first eluted BPA peak is only from the BPA in the sample and not the LC system. This placement allows for the impurities in the LC, such as BPA, to be trapped by the isolator column and elute after the BPA from the sample. If a different LC system is used, consult with the instrument manufacturer for the proper placement of the isolator column for optimum results.

11.2 *LC Analytical Column*—Column¹²—ACQUITY (trademarked) UPLC BEH C18, 1.7 μm, 2.1 × 50 mm C18 column or equivalent.

¹² Waters Oasis (a trademark of the Waters Corporation, Milford, MA) HLB 5cc (200 mg) LP Glass Cartridges, or equivalent, have been found suitable for use.

11.3 *LC Operating Conditions*—Injections of all calibration standards and samples are made at a 50 μL volume using a full loop injection. If a 50 μL volume loop is installed in your LC, a “full loop” mode is the preferred technique when performing fast, quantitative analyses. This mode should be used whenever accuracy and precision are the primary concerns. Specific instrument manufacturer specifications should be followed to achieve maximum performance. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in [Table 2](#).

11.4 *LC Sample Manager Conditions*—*Auto Sampler Conditions*—Wash Solvents: Weak wash is 1.2 mL of 5 % methanol in water. Strong wash is 1 mL of 30 % acetonitrile/30 % methanol/30 % 2-propanol/2-propanol/10 % 40 % water. The strong wash solvent is needed to eliminate carry-over between injections. The weak wash is used to remove the strong wash solvent. Specific instrument manufacturer specifications should be followed in order to eliminate sample carry-over in the analysis of BPA. Temperatures: Column 40°C, Sample compartment 15°C. Seal Wash: 5 minutes.

11.5 *Mass Spectrometer Parameters*—*Parameters*—Your instrument may require different settings. Variable parameters depending on analyte are shown in [Table 3](#).

The instrument is set in the Electrospray source setting.
 Capillary Voltage: 3.5 kV
 Cone: ESI Negative 40 Volts ([Table 3](#))
 Extractor: 3 Volts
 RF Lens: 0.3 Volts
 Source Temperature: 120°C
 Desolvation Temperature: 400°C
 Desolvation Gas Flow: 800 L/hr
 Cone Gas Flow: 20 L/hr
 Low Mass Resolution 1:14
 High Mass Resolution 1:14
 Ion Energy 1:1
 Entrance Energy: -1
 Collision Energy: Variable depending on analyte ([Table 3](#))
 Exit Energy: 0
 Low Mass Resolution 2:14
 High Mass Resolution 2:14
 Ion Energy 2:1
 Multiplier: 650
 Collision Cell Pirani Gauge: 7×10^{-3} Torr
 Analyser Penning Gauge: 3×10^{-5} Torr
 Inter-Channel Delay: 0.02 seconds
 Inter-Scan Delay: 0.02 seconds
 Repeats: 1
 Span: 0 Daltons
 Dwell: 0.05 seconds

11.5.1 In order to acquire the maximum number of data points per MRM channel with optimum sensitivity, the scan, delay and dwell times may be changed and optimized according to your instrument. The SRM chromatograms displayed in [Fig. 1](#) contain 17 data points across the peak at the reporting limit. Each peak requires at least 10 scans per peak for adequate quantitation. This standard contains only one target compound, one surrogate and one internal standard which are in one MRM experiment window. For details regarding retention times and SRM transitions cone and collision energies, refer to [Table 3](#).

12. Calibration and Standardization

12.1 In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following procedures described below must be followed when performing the test method.

12.2 *Calibration and Standardization*—*Standardization*—To calibrate the instrument, analyze eight calibration standards containing the eight concentration levels of BPA and BPA (Ring-13C12) surrogate with a constant concentration of BPA (Propane-D6) Internal Standard prior to analysis as shown in [Table 4](#). A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock Standard Solution A (Level 8) containing BPA and BPA (Ring-13C12)

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (Minutes)	Flow ($\mu\text{L}/\text{Minute}$)	Percent Water/5 mmolar NH_4OH	Percent Methanol/5 mmolar NH_4OH
0	300	100	0
1	300	100	0
2	250	75	25
5	250	25	75
6	250	25	75
7	300	0	100
9	300	0	100
10	300	100	0
12	300	100	0

TABLE 3 Retention Times, SRM transitions, SRM Ratios and Analyte-Specific Mass Spectrometer Parameters

Analyte	Primary/Confirmatory	Retention time (min)	Cone Voltage (Volts)	Collision Energy (eV)	SRM Mass transition (Parent > Product)	Primary/Confirmatory SRM Area Ratio
Bisphenol A	Primary Confirmatory	5.9	-40 -40	19 25	227 > 211.9 227 > 132.8	2.97
Bisphenol A (Ring-13C12) Surrogate	Primary Confirmatory	5.9	-40 -40	19 25	239 > 224 239 > 138.8	3.02
Bisphenol A (Propane-D6) Internal Standard	Primary Confirmatory	5.9	-40 -40	19 25	233 > 214.9 233 > 137.7	3.31

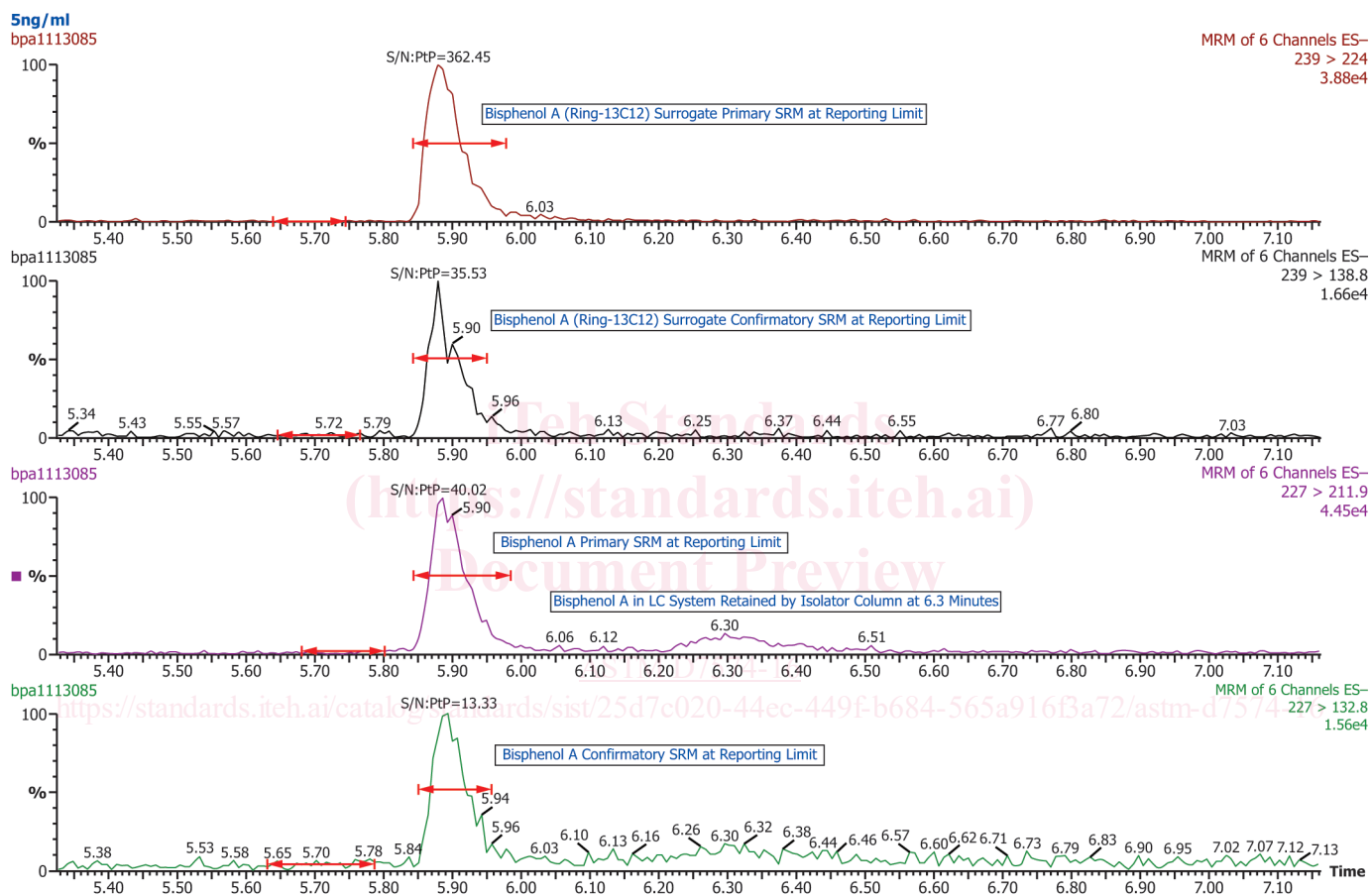


FIG. 1 Example SRM Chromatograms Signal/Noise at Reporting Limit

TABLE 4 Concentrations of Calibration Standards (ppb)

Analyte/ Surrogate/ Internal Standard	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7	LV 8
BPA	5	10	25	50	75	100	125	150
BPA (Ring-13C12) Surrogate	5	10	25	50	75	100	125	150
BPA (Propane-D6) Internal Standard	75	75	75	75	75	75	75	75

is prepared at Level 8 concentration and aliquots of that solution are diluted to prepare Levels 1 through 7. Stock Internal Standard Solution B is made at a concentration of 7.5 ppm bisphenol A (Propane-D6) in methanol. The following steps will produce