Designation: D7574 − 16

Standard Test Method for Determination of Bisphenol A in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry[1]

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 of Terms Specific to This Standard:*

3.2.1 *environmental water, n*—shall refer to water tested using this method. See Section 5.

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 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

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 *PPB, n*—parts per billion

3.3.14 *PPT, n*—parts per trillion

3.3.15 *QA, adj*—Quality Assurance

3.3.16 *QC, adj*—Quality Control

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[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.

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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.

3.3.17 *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, 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 250ºC 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 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 and an analytical column.[7]

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.

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

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

[6] A Waters ACQUITY 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 (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.

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.[11]

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 ^{13}C labeled bisphenol A where all ring carbon atoms are uniformly labeled ^{13}C.

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 ^{2}H.

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

8.14 *Solid Phase Extraction Cartridges*—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 Safety Data Sheets (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 % NH_4OH 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 0ºC and 6ºC 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*[12]—C18 column or equivalent.

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

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[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.

[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.

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 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/10 % 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*—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*—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) 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 standards with the concentrations values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with the pure materials, and correctly carrying the weights through the dilution calculations.

12.2.1 Prepare Stock Standard Solution A (Level 8) by adding to a 25 mL volumetric flask individual solutions of the following: 37.5 µL of 100 ppm BPA in methanol and 37.5 µL of 100 ppm BPA (Ring-13C12) in acetonitrile then dilute to 25 mL with 90 % water/10 % methanol to ensure the BPA remains in solution and does not adhere to the walls of the flask. The 100 ppm BPA stock solution is made in methanol to ensure solubility. The 100 ppm BPA (Ring-13C12) in acetonitrile is purchased. The preparation of the Level 8 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the stock concentrations prepared, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Stock Standard Solution A and Stock Internal Standard Solution B are then diluted with 90 % water/10 % methanol to prepare the desired calibration levels in 2 mL amber LC vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (Minutes)	Flow (µL/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

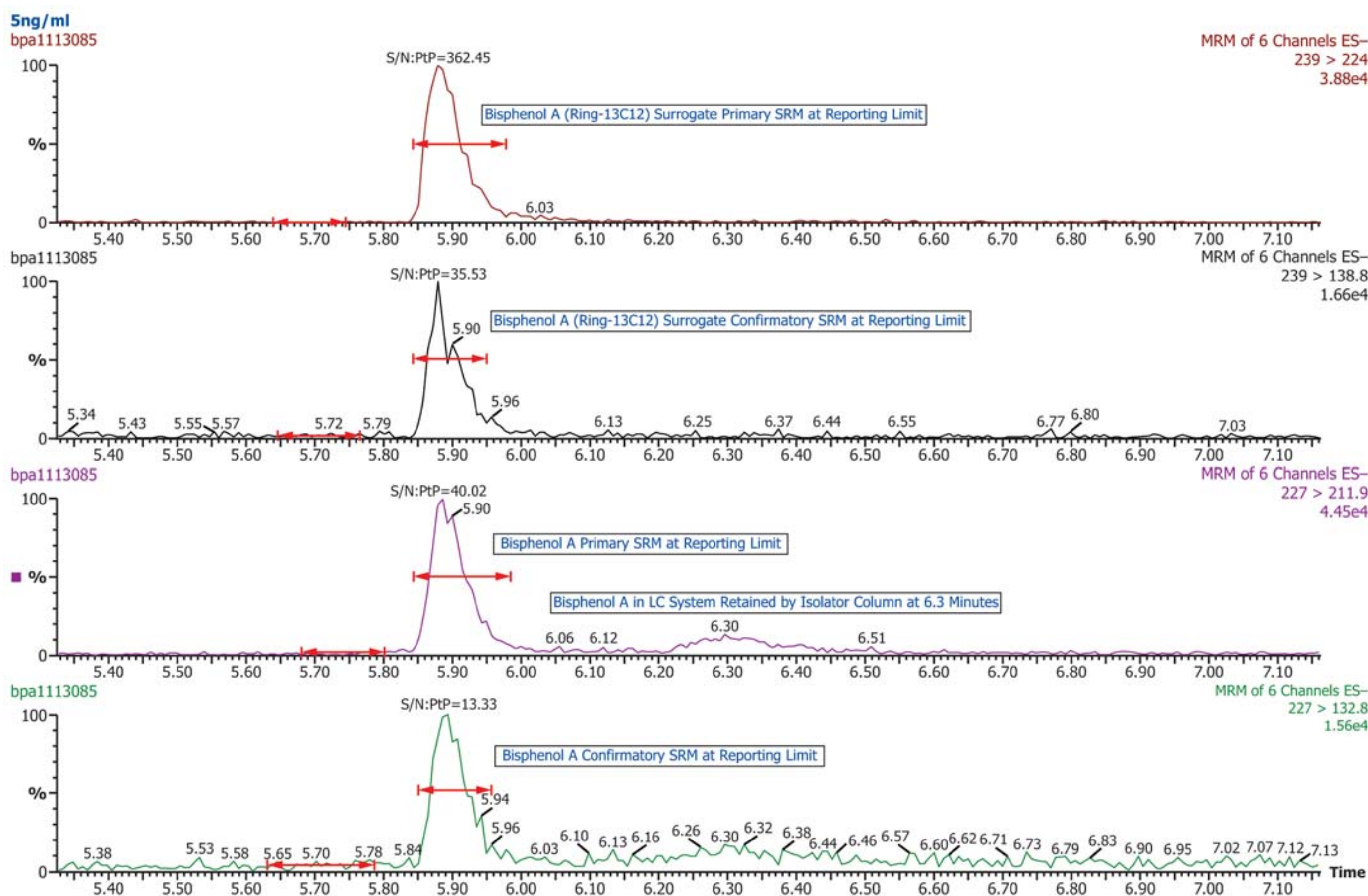

FIG. 1 Example SRM Chromatograms Signal/Noise at Reporting Limit

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

standards are routinely replaced every six months if not previously discarded for QC criteria failure.

12.2.3 Inject each standard and obtain a chromatogram for each one. An internal standard calibration technique is used monitoring the primary and confirmatory SRM transition of each analyte. Calibration software is utilized to conduct the quantitation of the target analyte and surrogate using the primary SRM transition. The ratio of the primary/confirmatory SRM transition area counts are given in Table 3. These are given as informative and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio must be within 30 % of the individual labs accepted primary/confirmatory SRM transition area ratio. The primary SRM transition of each analyte is used for quantitation and the confirmatory SRM transition for confirmation. This gives added confirmation by isolating the parent ion, fragmenting it

into two product ion fragments, and relating it to the retention time in the calibration standard.

12.2.3.1 Many laboratories may not have tandem mass spectrometry (MS/MS) capability. A single quadrupole mass spectrometer (MS) or time-of-flight mass spectrometer (TOF-MS) may be used if it is capable of meeting or exceeding the performance criteria in the standard. A mass spectrometer operated in the selected ion monitoring (SIM) mode may be able to meet the method detection limit in this standard. However, the laboratory is responsible to generate the optimum MS conditions for the parent and confirmatory ions as well as their ion area ratio acceptance criteria which must meet or exceed the criteria in this standard.

12.2.4 The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an internal standard calibration using the peak areas in ppt, ppb or ppm units as long as the analyst is consistent. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.5 An internal standard is used to account for experimental drift in the standard due to matrix interferences that result in ion enhancement or suppression. BPA (Propane-D6) internal standard is added to all calibration standards and sample extracts to obtain a concentration of 75 ppb. A calibration curve is generated using the Response of Analyte verses the known concentrations as shown in Fig. 2. The response of the analyte is calculated using Eq 1.

$$\textit{Response of Analyte} = \frac{(\textit{Peak area of Analyte})}{\left(\frac{\textit{Peak Area of Internal Standard}}{\textit{Concentration of Internal Standard}}\right)} \quad (1)$$

12.2.6 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98 this point must be re-injected or a new calibration curve must be regenerated. If the low or high point (or both) is excluded, minimally a six point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.7 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards, other than the high or low, causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. If the low or high point is excluded, a seven point curve is acceptable using a quadratic fit. An initial 8-point curve over the calibration range is suggested in the event that the low or high point must be excluded to obtain a coefficient of determination >0.99. In this event, the reporting range must be modified to reflect this

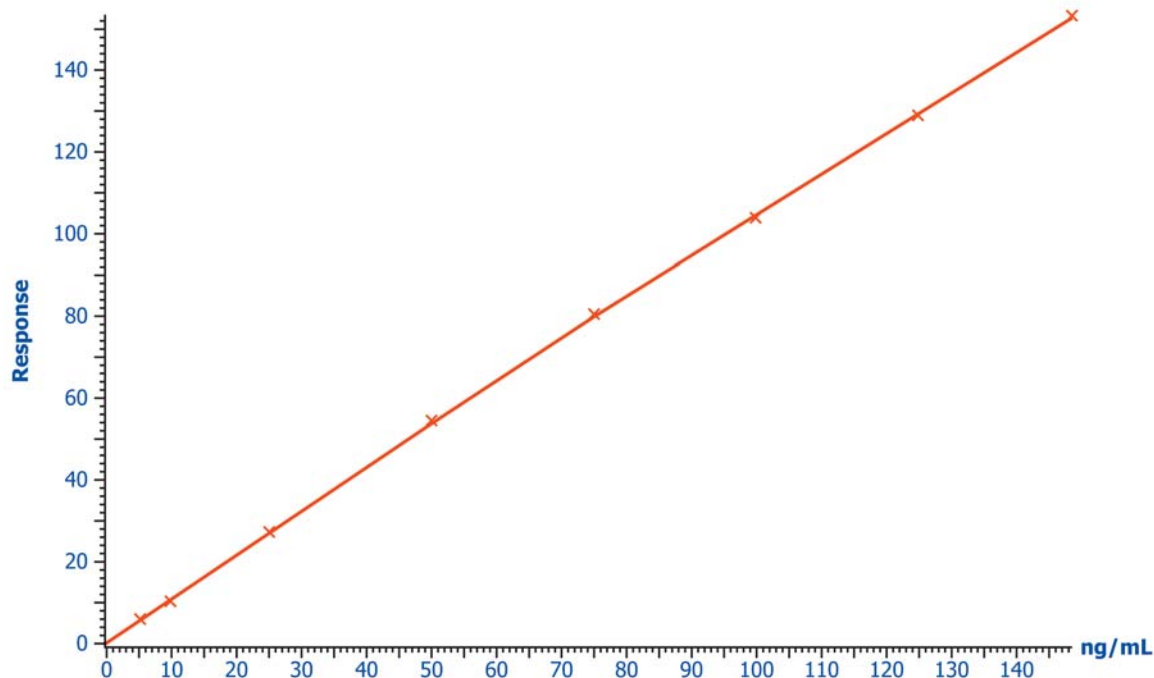

FIG. 2 Example Calibration Curve for BPA

change. Each calibration point used to generate the curve must have a calculated percent deviation less than ±25 % from the generated curve.

12.2.8 The retention time window of the MRM transitions must be within 5 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.3 A mid-level calibration check is required at the end of each batch of 20 samples or less and must be within ±25 % of the calculated concentration. Based upon the 8-point calibration curve from 5 to 150 ppb in Table 4, this mid-level check should be at 75 ppb. The same 75 ppb standard that was used to generate the calibration curve should be used as the calibration check. Instrument stability may be verified by comparing the midpoint of the calibration standard before and after the batch. If the mid-level calibration check does not meet the acceptance criteria, instrument maintenance must be performed such as cleaning the probe and sample cone(s). A new calibration curve will need to be generated as described in Section 12.2 before samples can be analyzed.

12.4 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., perform a precision and bias study to demonstrate laboratory capability.

12.4.1 Analyze at least four replicates of a sample solution containing BPA and bisphenol A (Ring-13C12) surrogate at a concentration near the midpoint of the calibration curve. The matrix and chemistry of the solution should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

12.4.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of quality control (QC) acceptance criteria for the initial demonstration of performance in Table 5.

12.4.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5. If a concentration other than the recommended concentration is used, refer to Test Method D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.5 *Laboratory Control Sample (LCS):*

12.5.1 To ensure that the test method is in control, analyze a LCS prepared with BPA at a concentration near the midpoint of the calibration curve. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing BPA at 1.0 ppm. Spike 75 µL of this stock matrix solution into 250 mL of water to yield a concentration of 300 ppt of BPA in the sample. The result obtained for the LCS shall fall within the limits in Table 5.

12.5.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.6 *Method Blank:*

12.6.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of BPA found in the blank must be below the detection limit or significantly below the confidence limits of the known concentration of the analyte in the associated test sample. If the concentration of BPA is found above this level, analysis of samples is halted until the contamination is eliminated and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Matrix Spike (MS):*

12.7.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 20 or fewer samples by spiking the sample with a known concentration of BPA and following the analytical method. Prepare a stock matrix spiking solution in methanol containing BPA at 1.0 ppm. Spike 75 µL of this stock matrix spiking solution into 250 mL of water to yield a concentration of 300 ppt for BPA in the sample.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 8 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.7.3 Calculate the percent recovery of the spike (P) using Eq 2:

$$P = 100 \frac{|A(V_s + V) - BV_s|}{CV} \quad (2)$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = percent recovery.

TABLE 5 QC Acceptance Criteria

Analyte	Test Conc. (ng/L)	Initial Demonstration of Performance			Lab Control Sample		MS/MSD		
		Recovery (%)		Precision	Recovery (%)		Recovery (%)		Precision
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit	Lower Limit	Upper Limit	Maximum % RSD
Bisphenol A	300	43	120	20	40	120	40	120	25
Bisphenol A (Ring-13C12) Surrogate	300	45	120	16	42	120	40	120	27

12.7.4 The percent recovery of the spike shall fall within the limits in Table 5. If the percent recovery is not within these limits, a matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 5 were generated using a modified Practice D5905 artificial wastewater by a single laboratory. BPA is present at relevant concentrations to this standard in nearby POTW effluents and surface waters resulting in the use of the artificial wastewater.

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the concentration of the analyte is less than five times the detection limit for the analyte, a MSD should be tested.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 3. Compare to the RPD limit in Table 5. Relative Percent Difference (RPD):

$$RPD = \frac{|MSR - MSDR|}{(MSR + MSDR)/2} \tag{3}$$

where:

RPD	=	relative percent difference,
MSR	=	matrix spike recovery, and
MSDR	=	matrix spike duplicate recovery.

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.9 *Surrogate Spiking Solution:*

12.9.1 A surrogate standard solution containing bisphenol A (Ring-13C12) is added to all samples. A stock surrogate spiking solution is prepared in predominately methanol containing bisphenol A (Ring-13C12) at 1.0 ppm. The 1.0 ppm bisphenol A (Ring-13C12) solution is prepared by adding 250 µL of the 100 ppm BPA (Ring-13C12) acetonitrile stock solution into a 25 mL volumetric flask and diluting with methanol. Spiking 75 µL of this spiking solution into 250 mL of water results in a concentration of 300 ppt of surrogate in the sample.

12.10 *Internal Standard Spiking Solution:*

12.10.1 An internal standard solution containing bisphenol A (Propane-D6) is added to all calibration standards and sample extracts at concentration of 75 ppb before analysis. A stock internal standard spiking solution is prepared in methanol containing bisphenol A (Propane-D6) at 7.5 ppm. Spiking 10 µL of this solution into 990 µL of sample extract or calibration standard results in a 75 ppb concentration.

12.11 The mass spectrometer must be calibrated per manufacturer specifications before analysis.

13. Sample Collection and Solid Phase Extraction Procedure

13.1 The water sample is acidified in the field to pH 2 with concentrated hydrochloric acid and shipped chilled between 0°C and 6°C in 250 mL amber glass bottles.

13.2 If the samples are received by the laboratory at greater than 6°C or greater than pH 2 (or both), the data is qualified estimated and noted in a case narrative that accompanies the data.

13.3 Additional acid is added if necessary in the laboratory to bring sample to a pH of 2. The samples are then appropriately spiked as required in Section 12.

13.4 *Solid Phase Extraction Procedure Steps:*

13.4.1 The solid phase extraction cartridge is placed on the vacuum manifold system under negative pressure according to the manufacturer specifications. BPA tends to adhere to surfaces of glassware, plastics and tubing and may be part of the plastic formulation. Vacuum manifold systems may use short sections of plastic tubing in order to provide a path from the SPE cartridge and fitting into the collection reservoir. This must be BPA-free tubing or replacement with BPA-free tubing or stainless steel transfer needles need to be affixed. The use of reservoirs and automatic SPE systems involving tubing should be avoided unless proven not to affect the performance of the method.

13.4.2 The cartridge is washed with 4 mL of MTBE followed by 4 mL of methanol and then with 4 mL of water at a flow rate of 5 mL/minute. These solvents can either be poured or transferred by glass disposable pipet directly to the SPE cartridge. It is best to add the solvents in small portions to the cartridge in order to minimize mixing with the previous wash solvent.

13.4.3 Once the cartridge is conditioned, the acidified water sample is poured directly from the 250 mL bottle to the SPE cartridge in order to minimize loss of BPA to other surfaces. The water sample is added to the cartridge at a rate of 10 mL/minute. High sediment content waters may reduce the flow rate.

13.4.4 Once the sample bottle is emptied, it is washed with 3 mL of 10 % methanol in water and the wash is added to the SPE cartridge, followed by a second wash of the bottle with 3 mL of water which is added to the cartridge and a third wash of the bottle with 3 mL of 10 % methanol/2 % NH_4OH in water which is also added the SPE cartridge.

13.4.5 The cartridge is then dried with vacuum for 10 minutes.

13.4.6 After drying, 4 mL of 10 % methanol in MTBE is added to the SPE cartridge and it is soaked for 1 minute. After soaking, the cartridge is eluted into a 10 mL Kuderna-Danish graduated concentrator.

13.4.7 The cartridge is then eluted into the same 10 mL Kuderna-Danish graduated concentrator tube with an additional 4 mL of 10 % methanol in MTBE at a 4 mL/minute flow rate. Ensure that the volume of the Kuderna-Danish is not exceeded if more solvent is added. It is important to fully wash the sides of the SPE cartridge with the 10 % methanol in MTBE elution solvent to remove the BPA that may have

adhered to the sides. The sides can be washed down by taking 1 mL portions of 10 % methanol in MTBE using a glass disposable pipet and rinsing the sides of the SPE cartridge while eluting into the Kuderna-Danish.

13.4.8 The 10 % methanol in MTBE extract is reduced to 0.20 mL under nitrogen blow-down at 50°C while washing the sides of the Kuderna-Danish concentrator tube using a glass pipet with approximately 5 mL of methanol.

13.4.8.1 A RapidVap N2 Evaporation System or similar nitrogen blow down device may be used.

13.4.9 The 0.20 mL methanol extract is then diluted to 1 mL with water. The 20 % methanol/80 % water extract is then transferred to a LC vial for LC/MS/MS analysis. The extract contains methanol to ensure solubility of the analyte.

13.4.10 Before LC/MS/MS analysis, 10 µL of a 7.5 ppm bisphenol A (Propane-D6) internal standard solution in methanol is added to all samples and calibration standards.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the BPA and surrogate, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. Internal standard calibration curves are used to calculate the amount of BPA and surrogate. Calculate the concentration in ppt for each analyte. BPA can be reported if present at or above the method detection limit as long as the values are accompanied by appropriate qualification codes. No qualification codes are needed if the values are at or above the respective reporting limit assuming no other QC issues apply to the values. If the concentration of BPA is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the mid-point of the calibration range and re-analyzed.

15. Report

15.1 Determine the results in units of ng/L (ppt) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Single Laboratory Precision and Bias

16.1 Methods under the jurisdiction of the ASTM committee D19 may be published for a maximum of five years to the completion of a full collaborative study validation. Such standards are deemed to have met all other D19 qualifying requirements but have not completed the required validation studies to fully characterize the performance of the methods across multiple laboratories and matrices. Publication of standards that have not been fully validated is done to make current technology accessible to users of standards, and to solicit additional input from the user community.

16.2 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on reagent water to determine precision and bias. The samples were spiked with target compounds and surrogates to obtain a 300 ppt concentration of each as described in Section 12. Table 6 contains the recoveries and standard deviation for the surrogate and target compound.

16.3 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on substitute wastewater and was prepared in accordance with Practice D5905 with modifications. Triton X-100 was not used in the formulation because secondary and tertiary POTW effluents and surface water does not contain the one percent level of octylphenol ethoxylates that is present in the artificial mixture. The octylphenol and octylphenol ethoxylates have been found at the ppt level in POTW effluent and surface water. A brewery suggested the use of bottled light beer because BPA is present in their beer can linings. The resulting wastewater was diluted by 25 times to better represent a POTW effluent to determine QC

TABLE 6 Single-Laboratory Recovery Data in Reagent Water

Precision and Accuracy Samples	Measured ppt from 300 ppt Spikes	
	Bisphenol A	Bisphenol A (Ring-13C12) Surrogate
1	228	209
2	209	189
3	202	186
4	182	168
5	204	186
Average Recovery	205	188
Average Percent Recovery	68 %	63 %
Standard Deviation	16	15
Percent Relative Standard Deviation	8 %	8 %

TABLE 7 Single-Laboratory Surrogate Recovery Data in Modified and Diluted Practice D5905 Substitute Wastewater

Samples	Measured ppt from 300 ppt Spikes ASTM Substitute Wastewater Bisphenol A (Ring-13C12) Surrogate
Blank	223
1	224
2	196
3	215
4	225
5	197
6	186
Average Recovery	209
Average Percent Recovery	70 %
Standard Deviation	16
Percent Relative Standard Deviation	8 %

TABLE 8 Single-Laboratory Recovery Data in Modified and Diluted Practice D5905 Substitute Wastewater

Sample	Youden Pair	Bisphenol A Spike (ppt)	Bisphenol A Measured (ppt)	Bisphenol A Percent Recovery
Blank		0	0	-
Sample 1	1	40	34	84
Sample 2		48	37	76
Sample 3	2	250	185	74
Sample 4		300	237	79
Sample 5	3	450	314	70
Sample 6		540	364	67

acceptance criteria. The samples were spiked with the surrogate to obtain a 300 ppt concentration as described in Section 12. Table 7 contains the recoveries for the surrogate in the artificial wastewater. Table 8 contains the Youden Pair spiking levels and recoveries for BPA in the artificial wastewater.

17. Quality Control

17.1 A crucial part of a test method is quality control. A laboratory should follow their in-house QA/QC procedures and should meet or exceed the criteria given in this test method. The quality-control criteria are given in the various test method sections. Section 10 contains the sampling and preservation requirements and Section 12 contains the majority of quality control requirements when following this test method. Section 12 includes requirements for calibration, precision and bias study to demonstrate laboratory capability, initial demonstration of performance, surrogate, method blank, reporting limit check, laboratory control, matrix spike and duplicate sample requirements. An IRM should be incorporated into the analysis periodically to verify that standard concentrations are comparable between sources. The IRM criteria should be based upon the laboratories QA/QC policies and the individual data quality objectives.

18. Keywords

18.1 Bisphenol A; liquid chromatography; solid phase extraction; tandem mass spectrometry; water

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