BPATest™

Instruction Manual

VICAM®

A Waters Business

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BPATest™ Instruction Manual

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GENERAL INFORMATION

1.1 INTENDED USE

The methods described in this manual are intended for quantitative analysis of bisphenol A (BPA) in selected nonalcoholic beverages using VICAM immunoaffinity column cleanup and a High Performance or an Ultra Performance Liquid Chromatography/Fluorescence Detector. (HPLC/FLD or UPLC/FLD). These optimized instrumental methods offer commercial, research, and government laboratories a cost-effective approach to increasing the accuracy and precision of BPA test results while meeting the demand for faster turnaround times and higher throughput. The proprietary monoclonal antibodies in VICAM's immunoaffinity columns efficiently extract highly concentrated, ultra-clean test samples, minimizing the risk of errors due to chemical interferences or over-dilution, without adding multiple time-consuming steps to laboratory workflows.

1.2 PRINCIPLE

A chemical component of a vast array of thermoplastic consumer products, BPA can migrate into food and beverages from plastic packaging and other polycarbonate and epoxy-based food contact articles and materials, including storage containers, disposable tableware, sports drink and water bottles, and the inner coating of cans and water supply pipes. BPA safety studies have linked this known endocrine disruptor to numerous adverse health effects, ranging from diabetes, heart disease, and reproductive and developmental problems to breast and prostate cancers.

BPA regulations continue to evolve in response to mounting scientific and consumer concern about its potential impact on public health and the environment. Zero tolerance policies for its occurrence in infant formula are already in force the EU, the U.S., Canada, and several Asian and Latin American countries, and increasingly tight BPA limits for other food and beverages are emerging in many European countries and U.S.A. states as well. Consequently, a growing number of food and beverage manufacturers are testing BPA levels in their products not only to ensure regulatory compliance, but also to meet increasing consumer demand for BPA-free products.

1.3 APPLICABILITY

The procedures in this manual were developed specifically for HPLC and UPLC analysis of nonalcoholic beverages without insoluble solids (e.g., carbonated and noncarbonated soft drinks and orange juice without pulp) as well as for orange juice with pulp, dairy-based coffee drinks, homogenized whole milk, and other beverages with insoluble solids.

Although VICAM'S BPATest columns are also designed to work with other complex sample matrices such as environmental waters, optimized procedures for these alternative applications lie beyond the scope of this manual. For help with applications not addressed in this manual, please contact our Technical Services Department (see page 18).

1.4 LIMITATIONS

To ensure optimal results, follow the procedures described in this manual. Do not use materials after their expiration date. Deviations from these instructions may interfere with the test's performance.

1.5 STORAGE CONDITIONS AND SHELF LIFE

- Store columns in refrigerator (2–8° C). Bring to room temperature for use.
- Do not freeze columns or reagents.

1.6 PRECAUTIONS

- Use only equipment specified by VICAM.
- Use only solvents and reagents that meet the purity requirements of the application (see Materials and Equipment, page 3).
- Avoid contact of any test reagents or solutions (e.g., methanol, water, sample extract, or column eluate) with rubber or plastic. Rubber or plastic may leach fluorescent materials or BPA into the sample.
- Work in a fume hood or safety cabinet when preparing acid, base, and organic solvent solutions.

2.0 MATERIALS AND EQUIPMENT

The reagents and equipment required for the BPATest procedures described in this manual depend on the sample matrix and instrumental technique entailed in the specific application. Please consult the relevant procedure to determine which supplies are required.

2.1 MATERIALS REQUIRED

Description	Part #
BPATest columns (25/box)	176004211
Fluted filter paper, 24 cm (100)	31240
Disposable cuvettes (250)	34000
Phosphate buffered saline (PBS) BPA free	
Acetonitrile LC-MS grade*	
Sodium hydroxide (NaOH), reagent grade	
Sodium chloride (NaCl)†	
Methanol‡	
BPA free Purified water with resistivity ≥18 MΩ at 25ºC	
Glacial acetic acid, ACS grade	

2.2 EQUIPMENT REQUIRED

Description	Part #
Digital scale with AC adaptor	20100
Cuvette rack	21010
Micropipettor, 1 mL	G4033
Micropipette tips for 1 mL micropipettors (100)	20656
2-Position Pump Stand w/ Air Pump (20 mL)	G4076
or 2-Position Pump Stand w/ Air Pump (10 mL)	21040
or 4-Position Pump Stand w/2 Air Pumps (10 mL)	21045
or 12-Position Pump Stand w/6 Air Pumps (10 mL)	G1104
or 12-Position Pump Stand w/6 Air Pumps (20mL)	600001707
Vortex mixer	23040
Nitrogen evaporator or equivalent evaporator	
Glass graduated cylinder, 100mL	

*For UPLC mobile phase.
† Use reagent-grade NaCl.
‡For HPLC applications, use HPLC-grade methanol (VICAM #35016); for UPLC, use LC-MS grade.

3.0 EQUPMENT PREPARATION

3.1 CLEANING EQUIPMENT

Before Testing

Background fluorescence from contaminated equipment can affect test results. For this reason, all equipment should be cleaned before use to remove dust, fingerprints, fibers, and other fluorescent contaminants. This is particularly important when using brand-new equipment or equipment that has not been used for a long time.

The following equipment should be washed with a mild detergent solution and then rinsed thoroughly with purified water before its first use:

- Glass syringe barrels used as sample reservoirs
- Graduated cylinders
- Beakers
- Containers that will be reused to hold, collect, or transfer sample solution.

Between Assays

- Wash the beakers and graduated cylinders with a mild detergent solution and rinse thoroughly with purified water.
- Flush the syringe barrel reservoir with methanol followed by a rinse with purified water to prevent carryover between samples.
- DO NOT wash and reuse the VICAM cuvettes. These cuvettes are designed for one-time use and should be discarded.

3.2 FLUTED PAPER FILTRATION SETUP

To ensure the sample solution flows easily through the affinity column, insoluble solids should be removed from beverage matrices such as orange juice with pulp, soy and nut milks, and dairy-based coffee drinks by passing the solution by gravity through a VICAM fluted paper filter that has been fitted around the rim of a clean glass beaker or in a glass funnel in a glass beaker.

1. Carefully open the fluted filter and insert into a large, clean glass beaker.
2. Fold edges of filter over rim of cup to hold in place. Keep the fluted folds intact, maximizing the surface area of the filter, to speed filtration.
3. As soon as the desired amount of filtrate is collected, proceed to the next step.

A pump stand serves as a convenient, stable support for the BPATest column during the immunoaffinity chromatography procedures. The stand also holds a 10 mL or 20mL glass syringe barrel that attaches to the top of the column and serves as a reservoir for the sample solution and for the reagents used to wash the column and elute BPA. Section 2.2, Equipment Required section, gives a list of available pump stands. Attaching an air pump to the column pushes viscous liquids through faster if needed (see Syringe Pump Operation, below).

Column Setup

1. Remove large top cap from IA column.
2. Cut bottom 1/8 inch off the end of the top cap with scissors or sharp blade. This provides a reusable coupling for attaching the BPATest column.
3. Attach column to coupling and fill syringe barrel with extract.
4. For gravity flow, remove bottom cap.
5. For positive pressure, insert quick disconnect on end of the air pump tubing into glass syringe barrel reservoir. See Figure 1 at right.
6. Apply pressure using air pump to push all the liquid through column at a flow rate of 1drop/2 seconds.

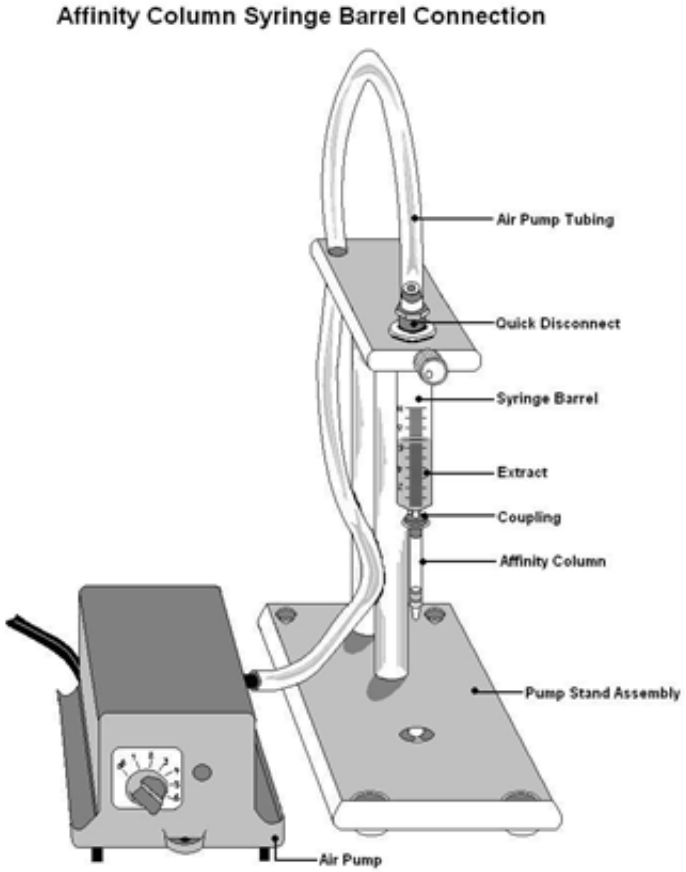


Figure 1

3.4 REMOVING BPA from BPATest™ COLUMN

Since BPA is frequently present in reagents and equipment used in manufacturing IA columns, the columns should be stripped before use to remove any contamination that could interfere with test results.

1. Connect column to the bottom outlet of the syringe barrel reservoir on the pump stand.
2. Measure 3 mL of the freshly prepared 10% BPA free acetic acid solution (see Reagent Preparation) into the syringe barrel reservoir.
3. Maintain a flowrate of 1 drop per second as gravity draws the solution through the column.
4. Wash the column with 10 mL BPA free PBS.
5. Leave about 500 µL of BPA free PBS and recap the bottom cap.

4.0 REAGENT PREPARATION

Work in a fume hood when preparing acid, base, and solvent solutions. Use **BPA free** and HPLC or UPLC grade of reagents for application (see Materials and Equipment). Solution volume may be increased or decreased as needed provided the proportions of reagents are kept consistent.

4.1 COLUMN CONDITIONING SOLUTION

10% Acetic Acid (CH_3COOH) Solution (10:90 V/V Acetic Acid :Water)
Measure 5 mL of ACS-grade acetic acid into a glass graduated cylinder. Carefully add the acid to a glass beaker containing 45 mL of purified water, stirring until thoroughly mixed. (DO NOT add water to acetic acid. Doing this may cause the solution to boil violently.) Rinse remaining acid from the graduated cylinder into the solution in the beaker. Unused solution should be discarded at the end of the day.

4.2 4N (W/V) Sodium Hydroxide (NaOH) Solution

Stir 16 g of reagent-grade NaOH, a little at a time, into a beaker containing 50 mL of purified water. When solution has cooled, transfer to 100mL graduated cylinder or volumetric flask, rinse beaker, add rinse water to the volumetric flask, and dilute with water as necessary to bring solution volume to 100mL. Prepare weekly or as needed. Store in tightly closed hard plastic containers in a cool, dry, well-ventilated area away from acids, metals, and other incompatible materials. DO NOT prepare or store in glass vessels.

4.3 ELUTING SOLUTION

80% Methanol Solution (80:20 Methanol :Water)
Add 80 mL methanol to a 20 mL purified BPA free water; mix thoroughly. Prepare weekly or as needed. Store in tightly closed glass container in a cool, dry, well-ventilated area away from incompatible materials. CAUTION: Methanol is flammable. Keep container tightly capped when not in use.

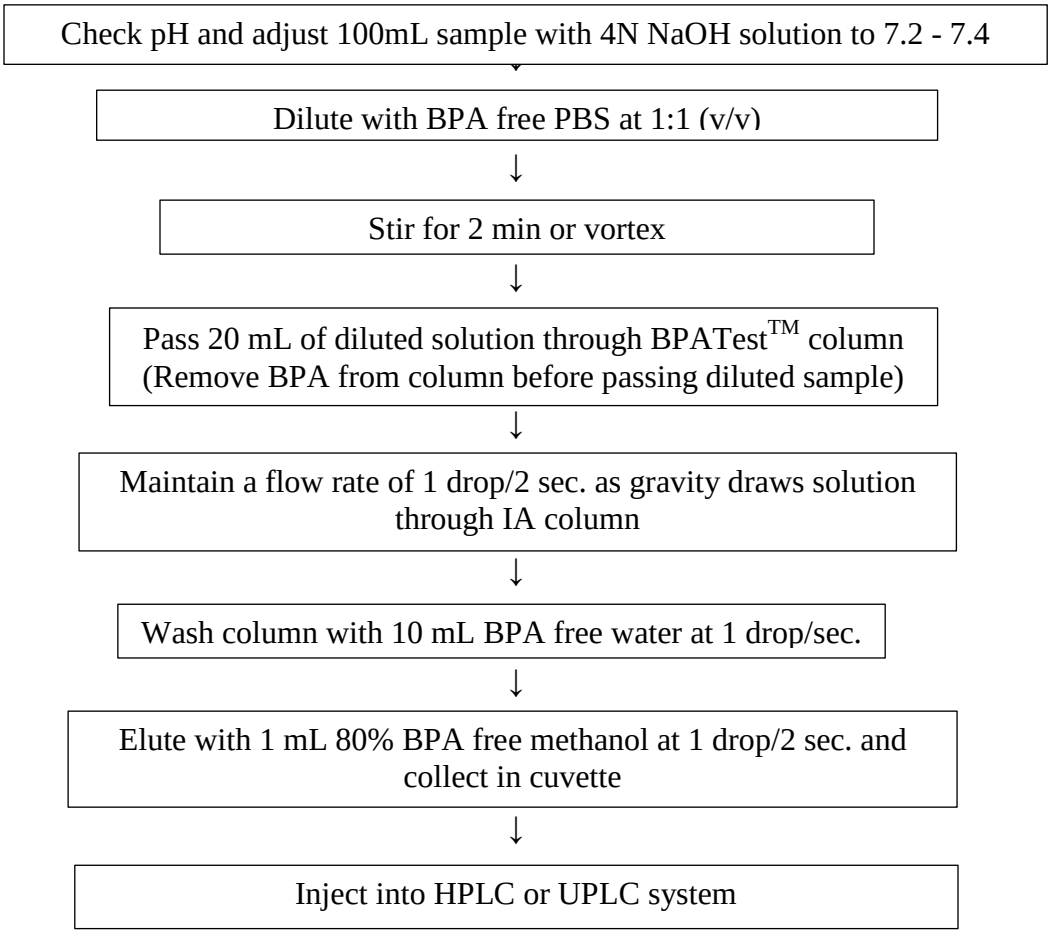
4.4 PBS (BPA free)

8.0 g NaCl
1.2 g Na_2HPO_4
0.2 g KH_2PO_4
0.2 g KCl
dissolve in approximately 990 mL purified BPA free water
adjust pH to 7.0 with concentrated HCl
bring to 1 liter with purified BPA free water

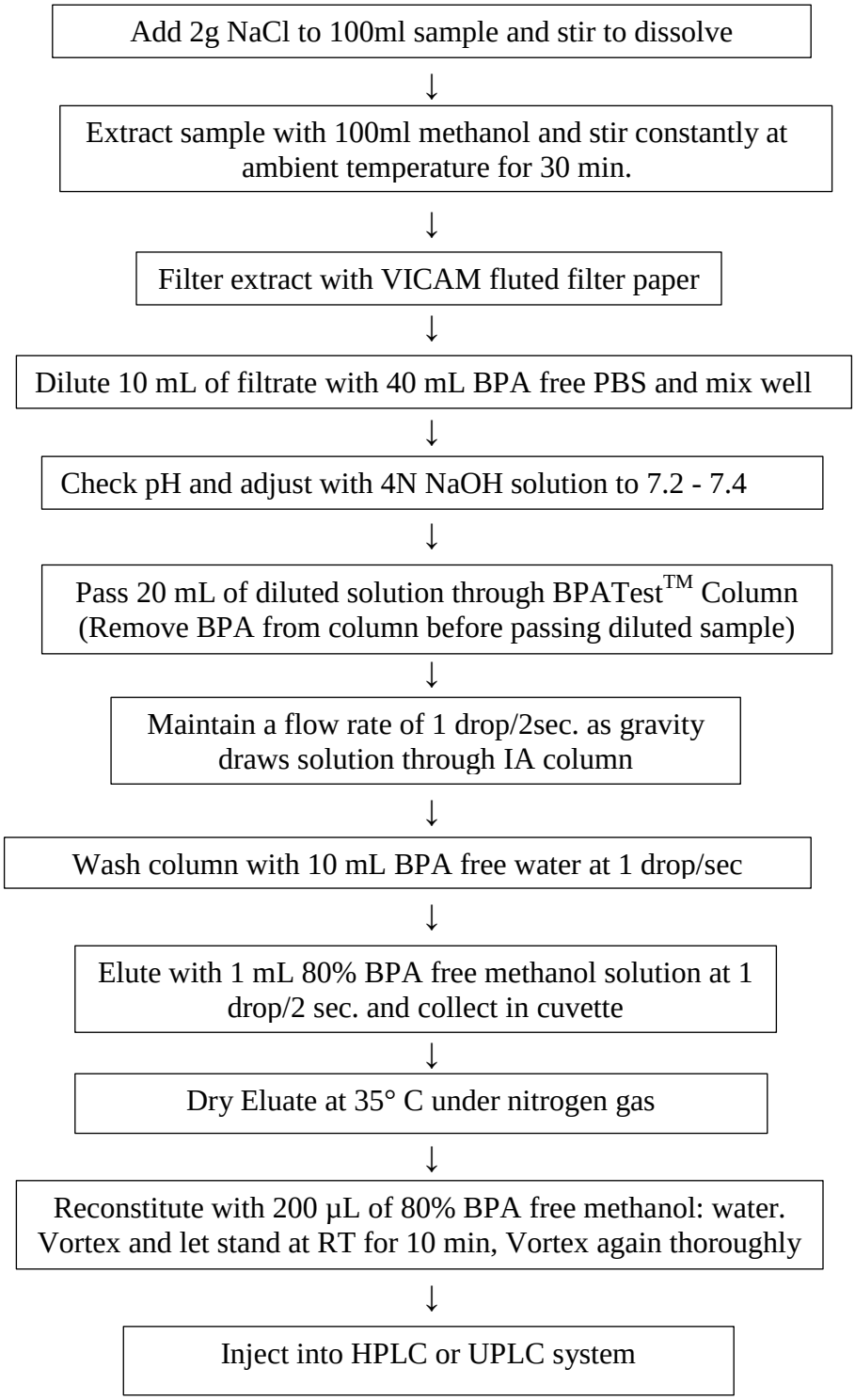
5.0 PROCEDURE OVERVIEW

The combination of IA column cleanup and HPLC/FLD or UPLC/ FLD detection offers a streamlined approach to accurately determining sub-ppb levels of BPA in complex organic matrices. Column cleanup requires no special training, takes as little time as 10 minutes, and delivers performance advantages ranging from faster run times and decreased solvent consumption to improved peak resolution. Samples prepared according to the steps shown below can be injected into either HPLC or UPLC systems; however, UPLC sample cleanup requires the use of higher-grade reagents (see Materials and Equipment, page 3) while reducing solvent consumption and injection volumes, shortening run times, and affording more sensitive detection of ultra-trace levels of BPA. The steps are described in detail in section 6.

5.1 PROCEDURE: BEVERAGES WITHOUT INSOLUBLE SOLIDS FOR EXAMPLE, CARBONATED AND NONCARBONATED SOFT DRINKS AND ORANGE JUICE WITHOUT PULP



5.2 PROCEDURE: BEVERAGES WITH INSOLUBLE SOLIDS FOR EXAMPLE, ORANGE JUICE WITH PULP, HOMOGENIZED WHOLE MILK, AND DAIRY-BASED COFFEE DRINKS



6.0 SAMPLE CLEANUP PROCEDURES

6.1 Beverages without Insoluble Solids: Carbonated and Noncarbonated Soft Drinks and Orange Juice without Pulp

EXTRACTION AND DILUTION

1. Measure 100 mL of sample into 250 mL graduated glass cylinder or beaker.
2. Check pH and slowly pipette drops of 4N NaOH solution into the sample until the pH reaches 7.2 - 7.4.
3. Transfer 10 mL of pH-adjusted sample to a clean glass vessel.
4. Dilute sample with 10 mL of BPA free PBS.
5. Stir for 2 minutes or vortex until thoroughly mixed.

COLUMN CHROMATOGRAPHY

1. Remove BPA from column (see section 3.4).
2. Load 20 mL of diluted sample (10ml sample equivalent) solution into the syringe barrel reservoir.
3. Maintain a flow rate of 1 drop per 2 seconds as gravity draws the solution through the stripped BPATest™ column (see section 3.4). To accelerate a flow rate that falls below the recommended level, use an air pump.
4. Detach column from the syringe barrel and fill the column headspace with BPA free water.
5. Replace the syringe barrel on the pump stand with a clean one and reattach column. Wash the column with 10 mL of BPA free purified water at a rate of 1 drop per second until air can be blown through the column.
6. Elute BPATest™ column with 1 mL of 80% BPA free methanol : water solution at a rate of 1 drop per 2 seconds. Remove remaining solvent by blowing air through the column. Collect purified sample extract in a clean cuvette. Mix well.
7. Inject extract into HPLC or UPLC system. (see Instrument Conditions, page 13 for injection volumes and other HPLC and UPLC conditions)

6.2 Beverages with Insoluble Solids: Orange Juice with Pulp, Dairy-Based Coffee Drinks, and Homogenized Whole Milk

EXTRACTION AND DILUTION

1. Measure 100 mL of sample into a glass vessel.
2. Add 2 g NaCl to sample and stir to dissolve.
3. Dilute solution with 100 mL BPA free methanol. Mix with a magnetic stirrer at ambient temperature for 30 minutes.
4. Filter solution through VICAM fluted filter paper. Collect filtrate in clean glass vessel.
5. Transfer 10 ml of filtrate to another glass vessel and thoroughly mix with 40 mL BPA free PBS. For juice or other commodities having extremely low pH, adjust pH with 4N NaOH solution until the pH reaches 7.2 - 7.4.

COLUMN CHROMATOGRAPHY

1. Remove BPA from column (see section 3.4).
2. Load 20 mL of diluted sample solution (2ml sample equivalent) into the syringe barrel reservoir.
3. Maintain a flow rate of 1 drop per 2 seconds as gravity draws the solution through the stripped BPATest column. To accelerate a flow rate that falls below the recommended level, use an air pump.
4. Detach column from the syringe barrel and fill the column headspace with BPA free water.
5. Replace the syringe barrel on the pump stand with a clean one and reattach column.
6. Wash the column with 10 mL of BPA free purified water at a rate of 1 drop per second until air pass through the column.
7. Elute BPATest column with 1 mL of 80% BPA free methanol: water at a rate of 1 drop per 2 seconds. Remove remaining solvent by blowing air through the column. Collect purified sample extract in a clean cuvette.
8. Evaporate the eluate to dryness at 35°C under nitrogen gas.
9. Reconstitute sample in 200 µL of 80% BPA free methanol: water; briefly vortex and let stand at room temperature for 10 minutes before vortexing thoroughly.
10. Inject extract into HPLC or UPLC system. (see Instrument Conditions, page 13 for injection volumes and other HPLC and UPLC conditions)

7.0 LIMITS OF DETECTION (LOD) AND RECOVERY RATES

Tables 1 and 2 list representative LOD and recovery rates for HPLC and UPLC analyses of different types of beverage samples. The pertinent sample preparation procedure for each statistic is specified in the table.

Table 1: Limits of detection (LOD) for carbonated soft drinks, orange juice with pulp and dairy-based coffee drinks

LOD (ppb)	HPLC	UPLC
Carbonated soft drinks	0.07	0.08
Orange juice with pulp	0.06	0.03
Dairy-based coffee drinks		0.07

Table 2: Average recovery rates for carbonated soft drinks, orange juice with pulp, dairy-based coffee drinks, and homogenized whole milk spiked at 1, 4, and 16 ppb

Average Recovery Rates	HPLC	UPLC
Carbonated soft drinks	102%	96%
Orange juice with pulp	96%	97%
Dairy-based coffee drinks		96%
Homogenized whole milk		95%

8.0 INSTRUMENT CONDITIONS

8.1 HPLC Conditions

Column: Waters Nova-pak C18, 3.9x300mm (4µm)
Column temperature: 25°C
Mobile phase: methanol/water (v/v) = 60/40
Flow rate: 0.8 mL/min
Injection volume: 30 µL
Excitation: 227 nm; emission: 313 nm
Running time: 6 minutes with 3-minute delay for next sample injection
Retention time: about 5 minutes

8.2 UPLC Conditions

Column: CORTECS UPLC C18, 2.1x100mm, 1.6µm(Waters).
Column temperature: 30°C
Mobile phase: acetonitrile/water (v/v) = 50/50
Flow rate: 0.4 mL/minute
Injection volume: 5 µL
Excitation: 275 nm; emission: 313 nm
Running time: 2 minutes
Retention time: about 1.05 minutes

8.3 UPLC/MS Conditions

Before injection, standards and samples eluates should be in 50% methanol: 50% water solution. If using method 6.1, dilute standard and eluates with 600µl water before injection. If using method 6.2, reconstitute in 50% methanol:50% water solution before injection.

UPLC condition		**MS condition**	
UPLC system	ACQUITY UPLC	MS system	Xevo TQD
Column	ACQUITY UPLC BEHC_{18}, 2.1 x 50mm, 1.7µm	Ionization mode	ESI negative
Column temp	40°C	Capillary voltage	3.5kV
Mobile phase A	0.5%NH_4OH in Water	Cone Voltage	30.0V
Mobile phase B	0.5%NH_4OH in Methanol	Source temp	140^0C
Elution	3min linear gradient from 5% (B) to 95% (B)	Desolvation temp	350^0C
Flow rate	0.5 mL/minute	Desolvation gas	550L/hr
Injection volume	50 µL	Cone gas	50L/hr

9.0 SPIKING SAMPLE WITH BPA AND PREPARING HPLC STANDARD CALIBRATION CURVE

9.1 Prepare BPA Spiking Solutions

Prepare a 10 µg/mL(ng/µL) BPA standard in 100% BPA free Methanol

Prepare a 1.0 µg/mL(ng/µL) BPA standard by adding 100 µL of the 10 µg/mL BPA standard to 900µL Methanol.

9.2 Spiking sample with BPA standard at 1 ppb and 5ppb level

1 ppb (ng/mL) X 100mL sample = 100 ng
100 ng ÷ 10 ng/µL BPA standard = 10 µL
Add 10 µL of the 10 ng/µL BPA standard solution to 100 mL sample

5 ppb (ng/mL) X 100 mL sample = 500 ng
500 ng ÷ 10 ng/µL BPA standard = 50 µL
Add 50 µL of the 10 ng/µL BPA standard solution to 100 mL sample

9.3 Prepare HPLC Standard Curve

Preparing the HPLC standards for **procedure 6.1** Carbonated and Noncarbonated Soft Drinks, Orange Juice without Pulp, and Other Beverages without Insoluble Solids (10mL sample equivalent)

0 ppb (ng/g) use 1ml 80% BPA free methanol

1.0 ppb (ng/mL) X 10mL sample = 10 ng
10 ng ÷ 1.0 ng/µL BPA standard = 10 µL
10 µL 1.0 ng/µL BPA standard added to 990µl 80% BPA free methanol

5.0 ppb (ng/mL) X 10mL sample = 50 ng
50 ng ÷ 1.0 ng/µL BPA standard = 50 µL
50 µL 1.0 ng/µL BPA standard added to 950µl 80% BPA free methanol

10 ppb (ng/mL) X 10mL sample = 100 ng
100 ng ÷ 1.0 ng/µL BPA standard = 100 µL
100 µL 1.0 ng/µL BPA standard added to 900µl 80% BPA free methanol

Preparing the HPLC standards for **procedure 6.2** Orange Juice with Pulp, Dairy-Based Coffee Drinks, Homogenized Whole Milk, and Other Beverages with Insoluble Solids (2mL sample equivalent)

0 ppb (ng/g) use 1ml 80% BPA free methanol

1.0 ppb (ng/mL) X 2mL sample = 2 ng
2 ng ÷ 1.0 ng/µL BPA standard = 2 µL
2 µL 1.0 ng/µL BPA standard added to 1ml 80% BPA free methanol

5.0 ppb (ng/mL) X 2mL sample = 10 ng
10 ng ÷ 1.0 ng/µL BPA standard = 10 µL
10 µL 1.0 ng/µL BPA standard added to 1ml 80% BPA free methanol

10 ppb (ng/mL) X 2mL sample = 20 ng
20 ng ÷ 1.0 ng/µL BPA standard = 20 µL
20 µL 1.0 ng/µL BPA standard added to 1ml 80% BPA free methanol

Dry all levels of standards with sample eluates in nitrogen evaporator at 35°C and reconstitute in 200 µL of 80% BPA free methanol:water.

10.0 REPRESENTATIVE CHROMATOGRAMS

1. HPLC Chromatogram for BPA in Homogenized Whole Milk

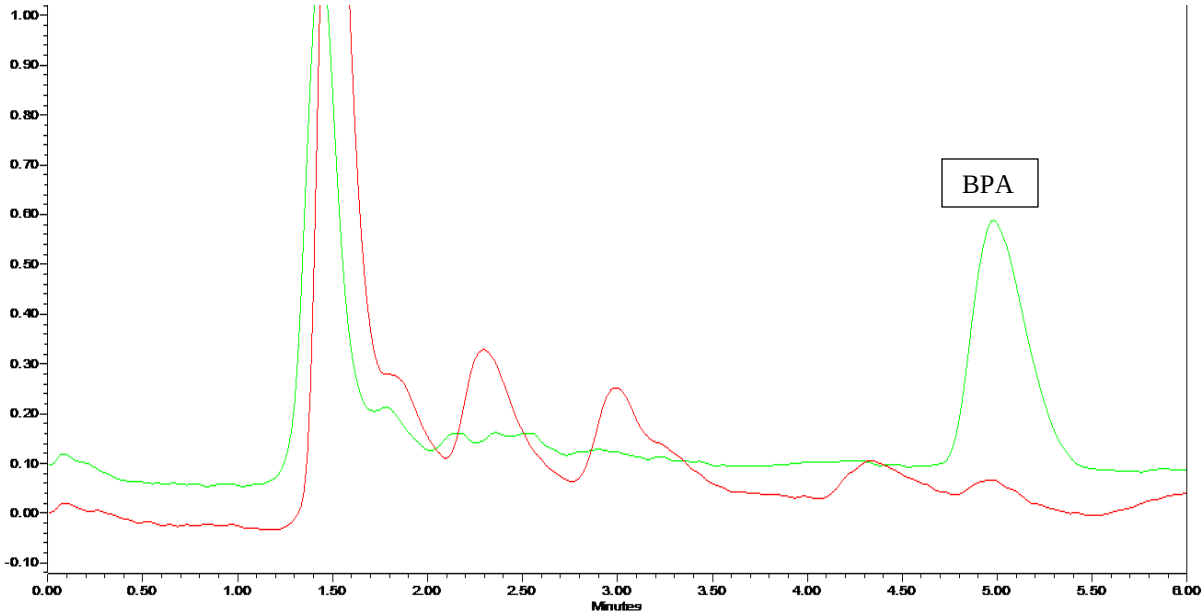


Figure 1: The red line was obtained from BPA free whole milk sample; the green line was obtained from 1 ppb BPA spiked whole milk sample.

2. UPLC Chromatogram for Carbonated Soft Drink, Orange Juice with Pulp, and Dairy-Based Coffee Drink

Chromatograms obtained for carbonated soft drink, orange juice with pulp and dairy based coffee drinks, are shown in Figures 1, 2, and 3 respectively. All of these representative samples were naturally contaminated with low levels of BPA ranging from 0.23 to 0.45 ng/mL. The chromatogram of each contaminated sample was compared with the same sample spiked with 1ng/mL BPA, and 0 standard blank was also included in the same graph.

Carbonated Drink

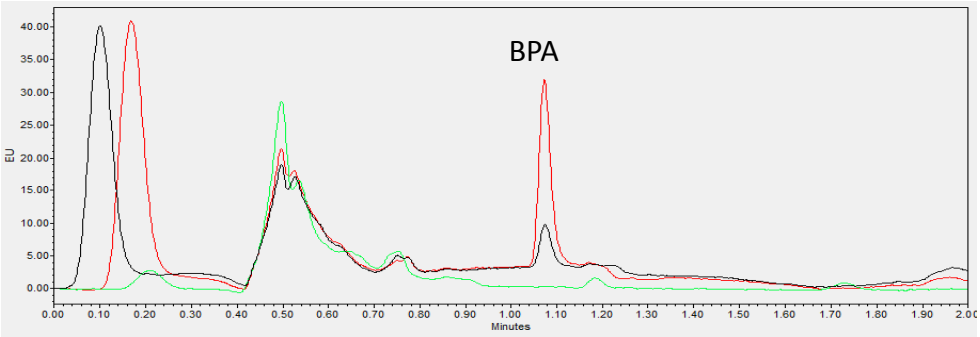


Fig. 2. Zero standard blank (green line), BPA contaminated (0.23ng/mL) carbonated drink blank (black line) and its 1 ng/mL spiked sample (red line).

Orange Juice with Pulp

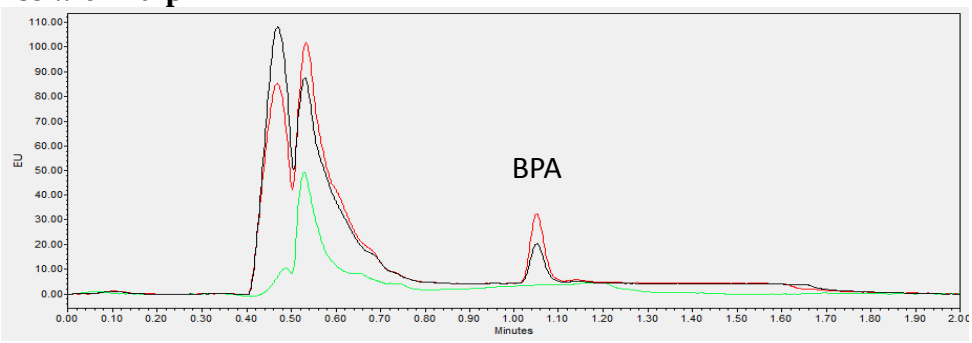


Fig. 3. Zero standard blank (green line), BPA contaminated (0.45ng/mL) orange juice with pulp (black line) and its 1 ng/mL spiked sample (red line).

Dairy-Based Coffee Drink

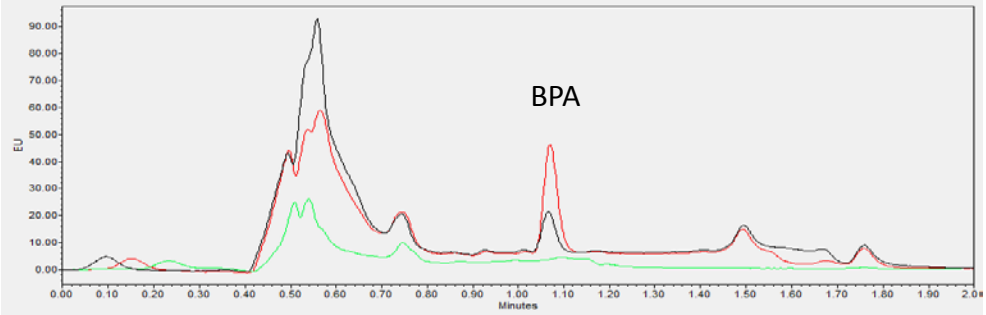
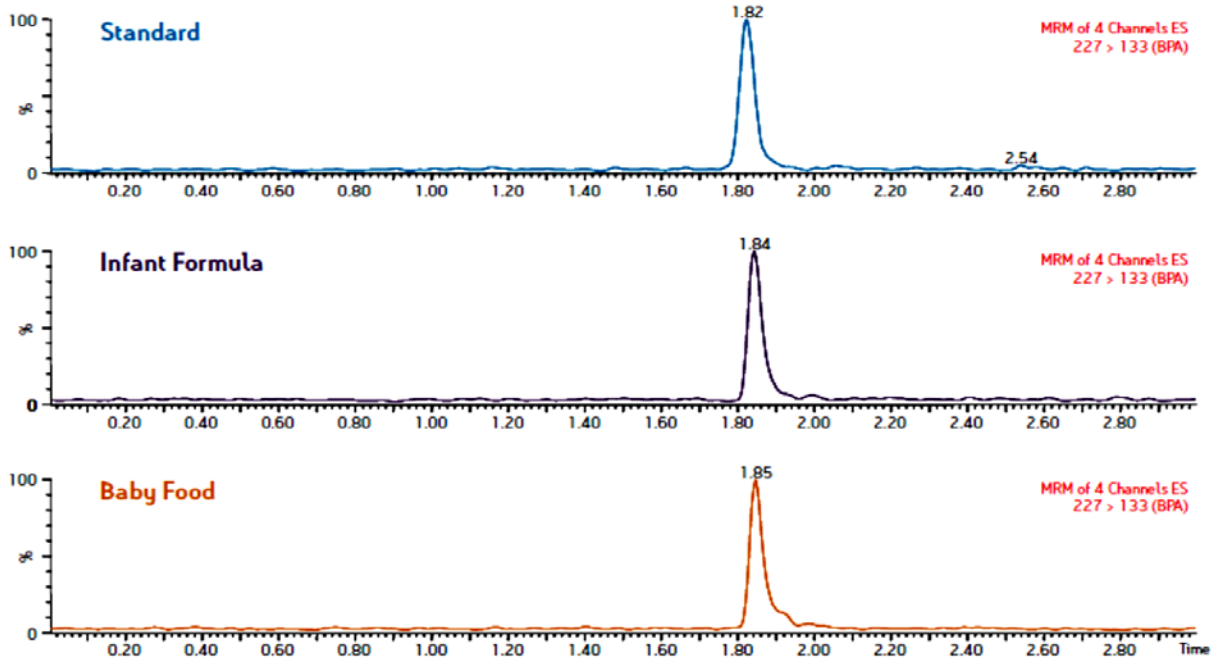


Fig. 4. Zero standard blank (green line), BPA contaminated (0.28 ng/mL) dairy-based coffee (black line) and its 1 ng/mL spiked sample (red line).

3. **MRM Chromatograms of BPA for Infant formula and Baby Food Sample Spiked at 1ppb**



11.0 TROUBLESHOOTING

1. **Problem:** False high readings

   Solution:

   Check calculation for spiked sample and standard curve.

   BPA can be found in many plastics. Make sure reagents and sample extracts are not in contact with plastic materials.

   Make sure to use BPA free water and methanol.

2. **Problem:** False low readings

   Solution:

   Maintain the recommended flow rates through the affinity column during sample passing, washing, and elution. Passing the extract too fast through the column may lead to low recoveries. Flow rate may need to be slowed down with a stopcock.

   Check calculation for spiked sample and standard curve.

12.0 TECHNICAL ASSISTANCE

For assistance please contact your local distributor or VICAM Technical Services:

Phone: +1-800-338-4381 United States

+1-508-482-4935 International and United States customers

E-mail: techservice@vicam.com

13.0 LIABILITY

The analytical methods described in this manual have been developed by VICAM to be used exclusively with the reagents in this test. The user assumes all risk in using BPATest analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that BPATest products conform to VICAM's printed specifications and quality control standards. VICAM will at its option repair or replace any product or part thereof that proves to be defective in workmanship or material. VICAM's undertaking to repair or replace such products is exclusive and is in lieu of all warranties whether written, oral expressed, or implied, including any implied warranty of merchantability or fitness for a particular purpose. VICAM shall have no liability for anticipated or lost profits or any loss, inconvenience, or damage whether direct, incidental, consequential or otherwise, to person or property, or for strict liability or negligence arising from or in connection with the use of these assay procedures or BPATest product.

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VICAM shall not be liable or responsible for any unsatisfactory or faulty results or performance involving the use of VICAM protocols or products if the testing or sampling in question is not conducted properly. Customers are solely and fully responsible for educating themselves about the proper testing and sampling procedures using VICAM protocols and products.

14.0 ORDERING INFORMATION

To place an order, contact your local VICAM distributor or contact the company directly by phone, email, or fax:

In the United States:

Phone:	+1-877-228-4244	Canada and the United States
	+1-417-725-6588	International and United States customers
Fax:	+1-417-725-6102	
E-mail:	vicam@vicam.com	

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+1 417 725 6588
Fax: +1 417 725 6102

Technical Service and Support:

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