#### **FOOD & BEVERAGE**

# Analysis of Bisphenol A in Food by Solid Phase Microextraction Using an Overcoated Fiber

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#### Introduction

Bisphenol A (BPA) is commonly used for food packaging applications such as polycarbonate bottles and the linings of metal cans used for soups, juices, etc. It is a suspected endocrine disruptor, and therefore, low level, long term exposure as a result of migration into food from packaging materials is a concern. The use of BPA in food contact applications is regulated by the Food and Drug Administration (FDA), and in 2013 was prohibited for use in the packaging materials of infant formula.<sup>1</sup> For other food contact applications, margins of safety were published by FDA as "NOAEL", which stands for "no observed adverse effect level". This NOAEL was set at 5 mg/kg body weight per day, which is well above the estimated dietary intake.<sup>2</sup> Similarly, tolerable daily intake or "TDI" was set by the European Union (EU) at 4 µg/kg body weight per day.<sup>3</sup> While exposure to BPA through diet is thought to be low, testing continues in order to assess its migration into food from can and lid linings, plastic containers, etc. In the case of the EU, the specific migration limit (SML) for BPA from packaging into food has been amended in Sept 2018 to 0.05 mg/kg (formerly 0.6 mg/kg) food. In case of contact materials for food products with intended use for infants or young children, no BPA migration from coatings or varnishes is permitted at all.<sup>4</sup>

Extraction methods for determination of BPA in food include both solvent extraction (SE) and solid phase extraction (SPE), with the latter more commonly used with liquid samples and the former for solid samples. Analysis can be done by either LC or GC, and both have been used throughout the literature. Solid phase microextraction (SPME) has been used for the determination of BPA in water, but has not been widely used for this application in food matrices due to sensitivity and fiber ruggedness issues associated with exposure to matrix components such as fats and proteins.<sup>5,6</sup>

The purpose of this application was to revisit the use of SPME to develop a quick, easy, and sensitive method for analysis of BPA in a variety of food products. The issues mentioned previously related to food matrices and SPME were addressed through the use of an overcoated (OC) divinylbenzene (DVB) fiber. The overcoating, which consists of polydimethylsiloxane (PDMS), protects the DVB layer from contamination and increases the physical robustness of the fiber.



In addition, material adhering to the overcoating is more easily removed during the wash step typically performed for such samples. SPME extraction using the OC-DVB fiber was followed by GC-MS/MS analysis for optimum sensitivity. The steps taken in method development and optimization as well as durability comparison are outlined here. For more detailed information, please refer to the online version of this article under SigmaAldrich.com/Analytix (Issue 4).

#### Experimental

The final, optimized SPME method using the OC fiber is described in **Table 1**. After extraction, the fiber was desorbed in the inlet of a 7890/7000C GC-MS/MS system, and analysis proceeded following the conditions

### Table 1. Optimized SPME procedure for extractionof BPA from food samples

sample/matrix:	10 mL vial containing 0.5 g of sample and 6.5 mL of water at pH 4 containing 25 $\%$ sodium chloride
SPME fiber:	Overcoated PDMS-DVB, 23 gauge
incubation:	10 min, 50 °C, 400 rpm
extraction:	immersion, 50 min, 50 °C, 250 rpm, vial penetration 34 mm
wash:	0.5 min, 250 rpm, vial penetration 34 mm
desorption:	3 min, 260 °C
post bake:	6 min, 270 °C

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column:	SLB®-PAHms, 30 m x 0.25 mm I.D., 0.25 µm (28340-U)
oven:	100 °C (3 min), 15 °C/min to 300 °C (10 min)
inj.temp.:	260 °C
carrier gas:	helium, 1 mL/min constant flow
detector:	MRM: BPA: 213/119, 213/91, 119/91
	BPA-d16: 224/125, 224/97, 125/97
MSD interface:	325 °C
liner:	0.75 mm I.D. SPME

#### Table 2. GC-MS/MS conditions

listed in Table 2. The samples analyzed (canned pumpkin, pureed carrot baby food, cream of chicken soup and canned energy drink) were obtained from a local grocery store and refrigerated prior to testing. They were prepared for SPME by weighing 0.5 g into a 10 mL autosampler vial. Spiked samples for determination of accuracy and repeatability were spiked at 10 ng/g by direct addition of 5 µL of a 1 µg/mL solution of BPA in methanol to the 0.5 g sample. Samples were then allowed to equilibrate for 30-60 minutes. 6.5 mL of SPME diluent (LC-MS/MS grade water containing 25 % NaCl by weight, and adjusted to pH=4 with  $H_3PO_4$ ) was added to each, followed by 7  $\mu$ L of a 1  $\mu$ g/mL methanolic solution of BPA-d16 internal standard. To decrease BPA background from the laboratory, all measuring glassware and pipettes used were glass, and were triple rinsed with methanol prior to use. The salt used to make the SPME diluent was treated in a muffle furnace and stored in a glass jar.

Samples (spiked and unspiked) were quantitated against matrix-matched calibration curves prepared as described previously and extracted following the method in **Table 1**.

### **Results and Discussion**

#### **Method Optimization**

A primary goal of method development was to determine a single set of SPME parameters that could be used with multiple sample types. In the following paragraphs, optimization of critical parameters is outlined (more details in the online version under SigmaAldrich.com/Analytix).

**Salt, pH and dilution.** Addition of salt and lowering of pH increased response significantly of both BPA and BPA-d16 (not shown). The samples to be analyzed were mostly very viscous, and required dilution prior to SPME; thus, a water diluent at pH 4 containing 25 % salt was chosen. After experimentation with different sample sizes/dilutions, 0.5 g diluted to 7 mL was found to work adequately for all the matrices evaluated, which included canned pumpkin, pureed carrots, condensed cream of chicken soup, and a fruit flavored energy drink (latter one probably could had been analyzed undiluted).

**Post-extraction wash.** Since the method was to be used with food samples, incorporation of a post-extraction wash step was critical in removing residual matrix on the surface of the fiber prior to desorption

in the GC inlet. Past work found this step to be more effective with the overcoated rather than the standard DVB fiber.<sup>7</sup> To maximize washing, a 30-second time was chosen for the method. Since good response was still obtained, the loss in response compared to no/ shorter wash time was not expected to severely impact the sensitivity of the method.

**Extraction & equilibration conditions.** Extraction times from 10 to 60 minutes were studied using 25 % saltwater at pH 4, spiked at 0.1 ng/mL (10 min equilibration @ 400 rpm agitation). Response steadily increased from 10 to 50 minutes and then leveled off from 50 to 60. Thus, 50 minutes was chosen as the extraction time.

Since temperature can influence the kinetics of the extraction, especially when working in heavy matrix like pureed carrot baby food, the effect of extraction temperature was studied at 30 °C, 40 °C, and 50 °C. Absolute response of BPA increased with temperature; thus 50 °C was chosen as the temperature for extraction and equilibration.

**BPA background.** BPA is a common laboratory contaminant, thus a major challenge in its analysis by any approach is managing background. SPME requires minimal sample preparation steps and materials, reducing sources of contamination compared to liquidliquid extraction or SPE. Some steps taken to reduce background for the SPME method included methanol rinsing of pipettes and glassware, muffle furnace treatment of the sodium chloride, and use of LC-MS/MS water in a glass bottle for the diluent solution. Some background was generated upon injection in the GC, and increasing the septum purge setting from 3 to 6 mL/min reduced this. Some BPA background was still present from the SPME process; however, low level detection from samples was still possible.

**Calibration.** Since SPME is an equilibrium extraction technique, quantitation must be done against standards extracted using the same method as the samples. In the case of BPA, extraction efficiency varied by matrix, thus matrix calibration had to be used for accurate quantitation. An unspiked sample for each was included as a "0" concentration point.



**GC-MS/MS Analysis**. A common approach to the GC analysis of BPA is derivatization using silylation or acetylation. This improves peak shape and response, allowing for better quantitation.<sup>5,6</sup> For this method, using an SLB<sup>®</sup>-PAHms column, as seen in **Figure 1**, derivatization was not necessary to obtain sufficient chromatographic performance and response.

#### **SPME Method Performance**

Analysis of spiked and unspiked samples. For all four matrices studied, both BPA and the internal standard, BPA-d16, could be detected free of interferences. An example is shown for the heaviest matrix, canned pumpkin, in Figure 2. As seen in Figure 3, the SPME method showed good linearity from the different matrices. The units are reported as ng/mL, which reflects the concentration from 0.5 g of sample diluted to a final volume of 7 mL prior to analysis, and translates to 7 to 140 ng/g BPA in the original sample. Accuracy and reproducibility of the method from these same matrices was determined by analysis of samples spiked at 10 ppb. The results of these evaluations are summarized in Table 3. Accuracy was >80% for all four matrices, with reproducibility as percent relative standard deviation (% RSD) or relative percent difference (%RPD) of <15 %. Since





matrix-matched calibration curves were used to quantitate spiked samples, the level of BPA present in each sample prior to spiking could be determined with the "0" concentration or unspiked analysis using a standard addition approach. These values are reported in Table 4. The BPA detected in the carrot/baby food in the glass jar was probably a result of leaching from the lined cap, and the level detected is in the range found by others in the analysis of baby food in glass jars with metal lids.8 In the canned samples, the highest level of BPA was detected in the cream of chicken soup. However, this level was still lower than past BPA levels determined by others in canned chicken soup.9 It should also be noted that the soup was analyzed directly without water dilution. Normal preparation for consumption requires a 1:1 dilution with water, which would essentially cut the BPA level by 50 %.

As mentioned previously, when doing immersion SPME into heavy matrices, a post extraction wash step is essential in removing residual matrix prior to the desorption step in the GC inlet. A comparison of fiber durability and method ruggedness between the OC and standard, non-overcoated versions of the PDMS/ DVB fiber was conducted by subjecting both to multiple extractions of canned pumpkin samples. Separate samples of pumpkin were weighed out and spiked at 10 ppb with BPA-d16. These were then run in a continuous sequence with 1 ng/mL BPA/BPA-d16 spiked water samples run every 6th extraction. The results

## Table 3. Accuracy and reproducibility for SPMEmethod applied to spiked samples

Sample	Spike Level	Avg. Amount Measured	Accuracy	RSD
fruit flavored energy drink	10 ng/mL	11.5 ng/mL	115 %	1 % ( <i>n</i> =3)
baby food, carrots	10 ng/g	11.7 ng/g	117 %	2 %*
cream of chicken soup (condensed)	10 ng/g	8.2 ng/g	82 %	9 %*
pumpkin	10 ng/g	11.0 ng/g	110 %	13 % ( <i>n</i> =6)

\*%RPD, 2 replicates

### Table 4. Level of BPA in unspiked samples;calculated using standard addition

Sample	Container Type	BPA Level - measured in unspiked sample
fruit flavored energy drink	can	0.8 ng/mL
baby food, carrots	glass jar with metal lid	0.65 ng/g
cream of chicken soup (condensed)	can	12.7 ng/g
pumpkin	can	1.6 ng/g

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are shown in Figure 4. For purposes of studying the trend in response, area counts were normalized to the first sample extraction. In 25 extractions of pumpkin using the OC fiber, response did not show a significant decline. The condition of the fiber after the testing sequence was fairly good, with some discoloration but no evidence of physical damage. By comparison, the standard fiber run was stopped after 18 extractions, as the coating had stripped completely off the fiber core. The response trend was erratic, as seen in Figure 4. The findings of the durability testing indicate that either the overcoating is protecting the phase from damage as a result of exposure to the pumpkin matrix, and/or the post-extraction wash step is more effective for the OC fiber at removing residual matrix. This then helps to prolong fiber life.

#### **Summary and Conclusions**

An immersion SPME-GC-MS/MS method using an overcoated PDMS/DVB fiber was developed for the low level analysis of BPA from various food products. Method linearity from different matrices - a fruit flavored beverage, canned pumpkin, pureed carrot baby food, and cream of chicken soup - was in the range of 0.9871 (carrots) to 0.9995 (beverage). Method accuracy and reproducibility at a 10 ppb spiking level was between 80-110%, with RSD/RPD values of <15%. Durability testing showed the OC fiber to be more physically robust, with more consistent response compared to a standard fiber; the SPME method had only a few steps and was easy to automate. In addition, it was highly sensitive, and when combined with GC-MS/MS, provided the selectivity necessary to be used with different matrices.

#### References

- 1. Indirect Food Additives: Adhesives and Components of Coatings. Fed. Regist. 2013, 78 (134), 41840-41843.
- Aungst, J. Division of Food Contact Notifications, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Dept. of Health & Human Services, Memorandum regarding 2014 updated safety assessment of Bisphenol A for use in food contact applications, 6/17/2014; accessed via FDA .gov.
- Bisphenol A, European Food Safety Authority Site, efsa.europa.eu/ topics/topic/bisphenol (status 23.11.2018).
- 4. Commission Regulation (EU) 2018/213 on use of bisphenol A in varnishes and coatings intended to come into contact with food and amending regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials.
- Ballesteros-Gómez, A.; Rubio, S.; Pérez-Bendito, D. Analytical methods for the determination of bisphenol A in food. J. Chrom. A. 2009, 1216, 449-469.
- Chang, C.; Chou, C.; Lee, M. Determining leaching of bisphenol A from plastic container by solid-phase microextraction and gas chromatography-mass spectrometry. Anal. Chim. Acta, 2005, 539, 41-17.
- Stenerson, K.K.; Young, T.; Shirey, R.; Chen, Y.; Sidisky, L. Application of SPME Using an Overcoated PDMS-DVB Fiber to the Extraction of Pesticides from Spaghetti Sauce: Method Evaluation and Comparison to QuEChERS. LCGC North America, 2016, 34 (7), 500-509.
- Cao, X.; Corriveau, J.; Popovic, S.; Clement, G.; Beraldin, F.; Dufresne, G. Bisphenol A in Baby Food Products in Glass Jars with Metal Lids from Canadian Markets. J. Agric. Food Chem, 2009, 57, 5345-5354.
- Noonan, G.O.; Ackerman, L.K.; Begley, T.H. Concentration of Bisphenol A in Highly Consumed Canned Foods on the U.S. Market. J. Agric. Food Chem., 2011, 59, 7178-7185.

#### **Featured Products**

Description	Cat. No.
SPME OC Fiber Assembly (PDMS/DVB), Pk.3	57439-U
SPME fiber holder for use with CTC autosampler	57347-U
SLB®-PAHms capillary GC column, 30 m x 0.25 mm I.D., 0.25 μm	28340-U
Bisphenol A, certified reference material, TraceCERT <sup>®</sup> , 100 mg	42088
Bisphenol A-d16, analytical standard, 50 mg	442876

#### **Related Products**

Description	Cat. No.
Sodium chloride, ACS reagent grade	746398
Clear vial, screw top, 10 mL, for CTC autosampler, Pk.100	SU860099
Magnetic screw cap, with 1.3 mm septa, for autosampler vial, Pk.100	SU860101
0.75 mm I.D. direct (SPME) liner for Agilent®	2637501
Molded Thermogreen <sup>™</sup> LB-2 septa with injection hole, 11 mm, Pk.50	28336-U

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