

# Instruction sheet

## **AFFINIMIP® SPE Bisphenol A** cartridges

### **CLEAN-UP PROCEDURE OF BISPHENOL A**

Users should read all instructions before using this kit.

*For laboratory use only*

**AFFINIMIP® SPE Bisphenol A** is developed and manufactured by **AFFINISEP**

**E-mail: [contact@affinisep.com](mailto:contact@affinisep.com)**

**Technical support: [tech.support@affinisep.com](mailto:tech.support@affinisep.com)**

**[www.polyintell.com](http://www.polyintell.com)**

Version 2.4

## Table of contents

1. INTRODUCTION.....	3
2. PRINCIPLE OF AFFINIMIP® SPE.....	3
3. PRODUCT INFORMATION .....	3
4. PRECAUTIONS FOR USE.....	3
5. RECOMMENDATIONS FOR HPLC ANALYSIS .....	4
6. GENERAL INSTRUCTIONS FOR SPE .....	4
6.1. Equipments required .....	4
6.2. Flow rate .....	4
6.3. Preparation process .....	4
7. CLEAN-UP PROCEDURE OF BISPHENOL A AND BADGE:.....	4
7.1. Preparation of solutions .....	4
7.2. Preparation of the loading solution for different matrices.....	5
a. General instruction for the loading solution.....	5
b. Preparation of Water or Milk.....	5
c. Preparation of beer matrix:.....	5
d. Preparation of powdered infant formula:.....	5
e. Preparation of liquid from canned food .....	5
f. Preparation of vegetables from canned food .....	5
7.3. Protocol for clean-up:.....	6

## Method for Selective Phase Extraction of Bisphenol A using Molecularly Imprinted Polymers

### 1. INTRODUCTION

**AFFINIMIP® SPE Bisphenol A** has been developed to selectively extract Bisphenol A from a broad range of food. This product can also be used for the extraction of closely structural molecules such as BADGE.

By using **AFFINIMIP® SPE**, the expected result is a clean-up and a pre-concentration of the sample at trace level.

### 2. PRINCIPLE OF **AFFINIMIP® SPE**

**AFFINIMIP® SPE** is a solid phase obtained by a polymerisation process to create a three-dimensional network that recognizes the shape and functional group positions of a template molecule. The **AFFINIMIP® SPE** selectivity comes from the technology of molecularly imprinted polymer (MIP) used during the synthesis.

### 3. PRODUCT INFORMATION

#### Description of the kit

Each solid phase extraction (SPE) cartridge **AFFINIMIP® SPE Bisphenol A** of this kit contains 100mg of sorbent in a 3mL cartridge.

#### Information and storage

Storage: Room temperature.

Each cartridge has a single use.

### 4. PRECAUTIONS FOR USE

SPE methods developed for C18 or other sorbents are not appropriate for **AFFINIMIP® SPE Bisphenol A**. **The extraction procedure described below has been optimized for the extraction of Bisphenol A from water, beer, milk, powdered infant formula, liquid or vegetables from canned food. For the treatment of another kind of matrix, please contact us to adapt the extraction procedure.**

## 5. RECOMMENDATIONS FOR HPLC ANALYSIS

For HPLC analysis, the following conditions have been used:

Column: C18 (USP L1), 4.6 x 150mm, spherical silica gel (Type A), particle size: 3 $\mu$ m

Mobile phase: gradient Water-ACN

Flow rate: 1mL/min

Detection of Bisphenol A by fluorescence:  $\lambda_{ex}$ : 230nm  $\lambda_{em}$ : 315nm

This product is also suitable for a clean-up before GC/MS/MS and LC/MS/MS.

## 6. GENERAL INSTRUCTIONS FOR SPE

### 6.1. Equipments required

In addition to standard laboratory materials, the following equipments are required for the use of **AFFINIMIP® SPE** cartridges:

- SPE vacuum manifold
- Nitrogen Mini-vap evaporator or centrifugal concentrator to dry the collected samples

### 6.2. Flow rate

It is very important to follow the flow rate given in the protocol.

Most especially for the loading, if the sample flow rate is too high, components may not interact sufficiently with the sorbent and the analyte recovery yield will be lower.

### 6.3. Preparation process

For the preparation of the MIP, a template is required. Bisphenol A analogues were used instead of Bisphenol A to prevent false positive signals in case of bleeding.

## 7. CLEAN-UP PROCEDURE OF BISPHENOL A AND BADGE:

### 7.1. Preparation of solutions

- Solution 60/40 Water/Acetonitrile (v/v)

In a 10mL-volumetric flask, add 4mL of Acetonitrile and complete with ultrapure water.

- Solution MeOH-2% Formic acid

In a 100mL-volumetric flask, add 2mL of Formic acid and complete with Methanol.

## **7.2. Preparation of the loading solution for different matrices**

### **a. General instruction for the loading solution**

*For your loading solution, the pH of aqueous solution must be between 5 and 6 (ideally ~5).*

**In addition, users developing their own extraction method must take into considerations that the composition of the Acetonitrile – Water loading solution must contains a maximum of 40% Acetonitrile.**

### **b. Preparation of Water or Milk**

Water and Milk are loaded without previous treatments except an adjustment of pH between 5 and 6 (*ideally ~5*).

### **c. Preparation of beer matrix:**

The beer is degassed by sonicating the sample during 1 hour to form the loading solution.

### **d. Preparation of powdered infant formula:**

4.4g powdered infant milk is reconstituted in 30 mL of water and warmed up at ~ 50°C during 20 seconds using microwaves. Then 20 mL of acetonitrile are added to 20 mL of warm milk and centrifuged at 4000 rpm during 10 minutes. The supernatant is collected and filtered on filter paper (4-7µm). This extract is diluted 1:1 with water to form the loading solution.

### **e. Preparation of liquid from canned food**

The liquid is collected and filtered on filter paper (4-7µm) to form the loading solution.

### **f. Preparation of vegetables from canned food**

100g of drained canned food and 200mL of Water /ACN (50/50) are blended during 2 min and centrifuged during 10min at 4000rpm. The supernatant solution is collected, filtered (4-7µm) and diluted 1:1 with water to give the loading solution.

### 7.3. Protocol for clean-up:

In order to quantify concentration level as low as 1ng of BPA/mL of matrix, we advise to load with the maximum volume allowed by this protocol.

Step (Flow rate)	AFFINIMIP® SPE Bisphenol A (100mg/3mL)
Equilibration with (2 drops/s)	<ul style="list-style-type: none"> <li>▪ 3mL MeOH-2% Formic Acid</li> <li>▪ 3mL Acetonitrile</li> <li>▪ 3mL Water</li> <li>▪ Do not allow the cartridge to dry during conditioning</li> </ul>
Loading (L)	<p>Up to 15mL of <b>Milk matrix</b> (1 drop every 2 seconds) or Up to 10mL of <b>Beer Matrix</b> (1 drop every 2 seconds) or Up to 10mL of <b>liquid from canned food</b> (1 drop every 2 seconds) or Up to 20mL of <b>vegetables from canned food</b> (1 drop every 2 seconds) or Up to 40mL of <b>powdered infant formula</b> (1 drop/s) or Up to 50mL of <b>Water matrix</b> (1 drop/s)</p>
Washing of interferents (1 drop/s)	<ul style="list-style-type: none"> <li>• 9mL ultrapure Water</li> <li>• 6mL 60/40 Water/Acetonitrile</li> </ul>
Drying :	<p><b>Apply vacuum 3 to 5 minutes in order to remove remaining water residues to decrease the evaporation time of the elution solution</b></p> <p><i>This step is not necessary if you don't evaporate elution solution</i></p>
Elution (E1) for extraction of <b>BPA AND BADGE</b> (1 drop/s)	<ul style="list-style-type: none"> <li>• 3mL Methanol</li> </ul>
Elution (E2) For extraction of <b>remaining BADGE</b> (1 drop/s) (only for	<ul style="list-style-type: none"> <li>• 3mL Acetonitrile</li> </ul>

Elution E1 is required to extract Bisphenol A. For the analyses of Bisphenol A AND BADGE, E1 and E2 are required and gathered for the analysis. For the analyses of BADGE only, just use E2.

The elution (E) is diluted to a known volume or evaporated until dryness under nitrogen with a mini-vap evaporator (or a centrifugal concentrator). The residue is dissolved in 0.5mL of mobile phase for further analysis.

## PRODUCTS LIST

AFFINIMIP® SPE Products	Designation	Description
Multimycos10	AFFINIMIP® SPE Multimycos10	selective SPE cartridges 3mL for ZON, OTA, HT-2, T-2, Aflatoxins and Fumonisin
Zearalenone	AFFINIMIP® SPE Zearalenone	selective SPE cartridges 3mL for ZON
Ochratoxin A	AFFINIMIP® SPE Ochratoxin A	selective SPE cartridges 3mL for OTA
Patulin	AFFINIMIP® SPE Patulin	selective SPE cartridges for Patulin
	AFFINIMIP® SPE Patulin & Pectinase kit	kit of selective SPE cartridges for Patulin + 50mL pectinase enzyme solution
Deoxynivalenol	AFFINIMIP® SPE Deoxynivalenol	selective SPE cartridges 6mL for DON
Phenolics	AFFINIMIP® SPE Phenolics	selective SPE cartridges for Phenolic compounds
Estrogens	AFFINIMIP® SPE Estrogens	selective SPE cartridges for Estrogens
Zeranol Residues	AFFINIMIP® SPE Zeranol Residues	selective SPE cartridges for Zeranol Residues
Bisphenol A	AFFINIMIP® SPE Bisphenol A	selective (PP or Glass) SPE cartridges for Bisphenol A
FumoZON	AFFINIMIP® SPE FumoZON	selective SPE cartridges for Fumonisin and Zearalenone
Chloramphenicol	AFFINIMIP® SPE Chloramphenicol	selective SPE cartridges for Chloramphenicol
Tamoxifen	AFFINIMIP® SPE Tamoxifen	selective SPE cartridges for Tamoxifen
Catecholamines	AFFINIMIP® SPE Catecholamines	selective SPE cartridges for Catecholamines
	AFFINIMIP® SPE Catecholamines	selective SPE cartridges for Catecholamines
Metanephrines	AFFINIMIP® SPE Metanephrines	selective SPE cartridges for Metanephrines
Amphetamines	AFFINIMIP® SPE Amphetamines	selective SPE cartridges for Amphetamines
PECTINASE	Pectinase solution	50 mL pectinase enzyme solution
AttractSPE™ Products	Designation	Description
W/O	AttractSPE™ W/O	HLB SPE cartridges sorbent
SCX	AttractSPE™ SCX	Strong Cation Exchange SPE cartridges sorbent
WCX	AttractSPE™ WCX	Weak Cation Exchange SPE cartridges sorbent
SAX	AttractSPE™ SAX	Strong Anion Exchange SPE cartridges sorbent
WAX	AttractSPE™ WAX	Weak Anion Exchange SPE cartridges sorbent
DVB	AttractSPE™ DVB	Reversed Phase Copolymer SPE cartridges sorbent
Anionic & Cationic AttractSPE polymeric cartridges	AttractSPE™ KIT	Kit of 10 cartridges of each sorbent (SAX, WAX, WCX, SCX)

**For more information:**

For more information on our products & services, please visit [www.polyintell.com](http://www.polyintell.com)