affinisep

Bisphenols

Application Note

Analysis of Bisphenol A in beers by LC-Fluo with AFFINIMIP® SPE Bisphenols

Food testing

Introduction

This application note describes an efficient solid phase extraction (SPE) method using **AFFINIMIP® SPE Bisphenols** cartridges for the cleanup and analysis of bisphenol A (BPA) in beers. The analyses were carried out using HPLC with a fluorescence detector.

Beyond this application, **AFFINIMIP® SPE Bisphenols** cartridges have been found suitable for cleanup and concentration of a wide variety of bisphenol analogues in various matrices such as energy drinks[1], fish feed[2], human breast milk[3], canned food[4], and urine[5]. Furthermore, a large study, conducted by the French health agency (ANSES)[6] using a method developed by LABERCA[7] with **AFFINIMIP® SPE Bisphenols**, covering more than 1,200 food items was carried out in France in 2013.

BPA is a molecule widely found in the food packaging, and the migration of this endocrine disruptor from the packaging to food is the main source of consumers' exposure to BPA. In early 2018, European regulation 2018/213[8] amended UE regulation 10/2011, limiting the acceptable levels of BPA in plastic food contact materials. The specific migration limit (SML) of these materials was reduced from 0.6 to 0.05 mg/kg of BPA.
Since 2009, several states in the United States (U.S.) have regulated BPA in beverage containers for children under the age of three. In 2019, other bills have been introduced by some U.S. jurisdictions to regulate BPA or BPA-analogues in certain consumer goods.

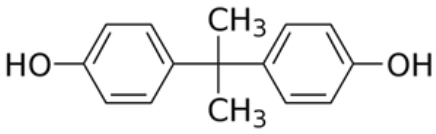

Figure 1. Chemical structure of bisphenol A.

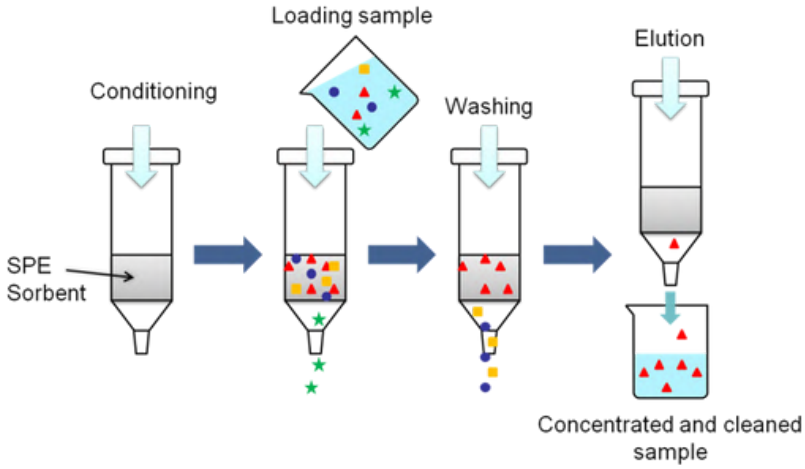

Figure 2. Principle of solid phase extraction (SPE).

Proceeding of the experiment

Sample preparation

Degas beer using sonication for 1 hour.
Adjust pH to 5–6 prior to SPE.

Purification with a 3 mL AFFINIMIP® SPE Bisphenols cartridge

EQUILIBRATION

1. 3 mL 2% acetic acid (in methanol)
2. 3 mL acetonitrile
3. 3 mL ultrapure water

LOADING

10 mL loading solution at a rate of 1–2 mL/min

WASHING

1. 9 mL ultrapure water
2. 6 mL 40% (v/v) acetonitrile (in water)
3. Dry cartridge for 30 seconds under full vacuum

ELUTION

3 mL methanol

The elution fraction is evaporated and dissolved in mobile phase prior to analysis.

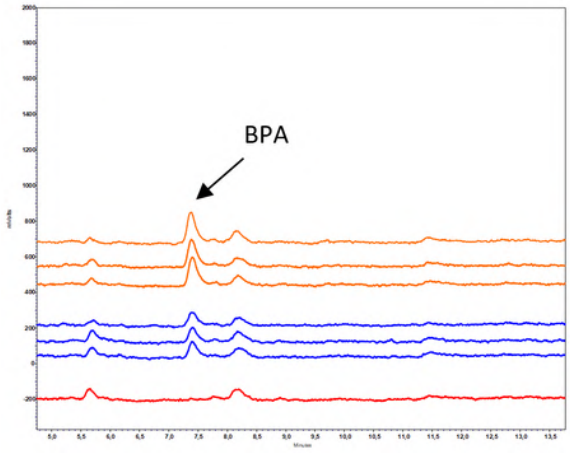

Figure 3. Fluorescence chromatograms obtained after **AFFINIMIP® SPE Bisphenols** cleanup of 10 mL of beer spiked at 2 µg/L with BPA (tested 3 times, orange) or at 1 µg/L with BPA (tested 3 times, blue), or not spiked (red).

The analysis was carried out using LC-Fluorescence. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

Compound	Concentration in blank (µg/kg)	Spike level (µg/kg)	Recovery (%)	RSD (n = 3) (%)
BPA	ND	1	106.9	1.0
		2	93.4	1.0

Table 1. Recovery of BPA in beer after **AFFINIMIP® SPE Bisphenols** cleanup. The same cleanup procedure was repeated several times (n) for each concentration, from which the percent relative standard deviation (% RSD) was calculated to determine repeatability of the method. (ND = Not detected).

The purification of spiked beer was also carried out under the same conditions (n = 9) by various operators over several days to verify the reproducibility of the procedure.

Compound	Concentration in blank (µg/kg)	Spike level (µg/kg)	Recovery (%)	RSD (n = 9) (%)
BPA	ND	1	99.3	8.9
		2	90.6	6.0

Table 2. Recovery of BPA in beer after **AFFINIMIP® SPE Bisphenols** cleanup by various operators or over several days. The same cleanup procedure was repeated several times (n) for each concentration, from which the percent relative standard deviation (% RSD) was calculated to determine reproducibility of the method. (ND = Not detected).

Finally, 50 µL sample of spiked beer was analysed by LC-fluorescence and compared to a SPE-treated beer sample using the same conditions.

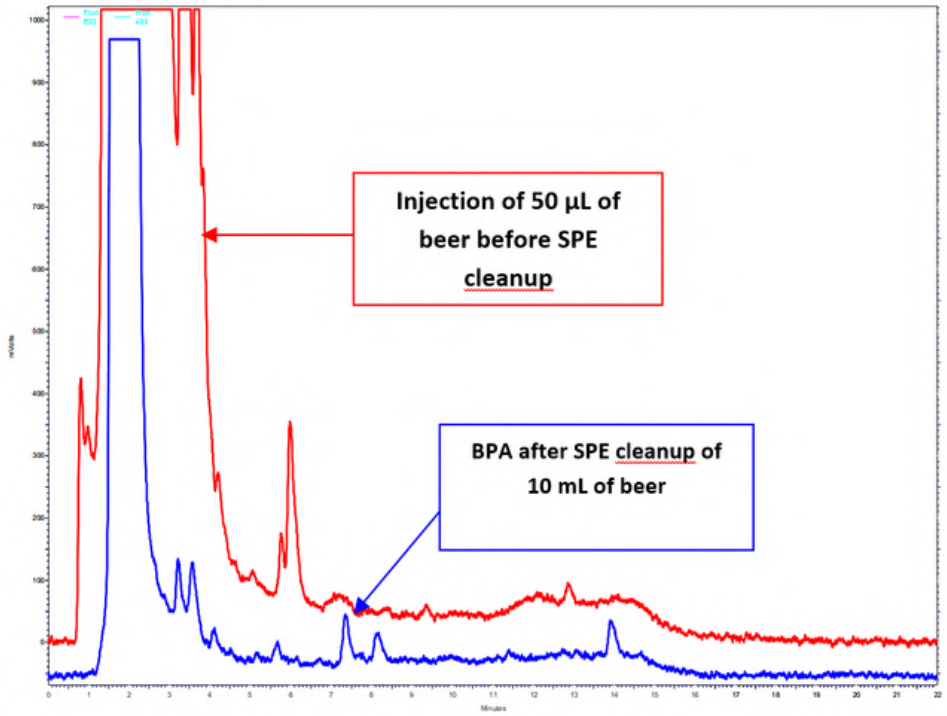

Figure 4. Fluorescence chromatograms obtained for an injection of 50 µL of beer spiked at 1µg/L with BPA (red) and after **AFFINIMIP® SPE Bisphenols** cleanup of 10 mL of beer spiked at 1 µg/L with BPA (blue).

HPLC	ThermoFinnigan Spectra System	HPLC gradient		
Flow rate	1mL/min	**Time (min)**	**% water**	**% Acetonitrile**
Column	Hypersil Gold C18 150 x 4.6 mm (3 µm)	0	65	35
Injection volume	50 µL	2	65	35
Detector	Jasco FP-2020 with Fluorescence detector	12	50	50
Wavelength	230 nm/315 nm (ex/em)	12.5	65	35
		22	65	35

Table 3. LC-Fluorescence conditions used for BPA analysis.

For trace analysis, we recommend using the **SilactHPLC DELAY -BPA** column as a delay column to avoid contamination of bisphenols from the HPLC device.

Conclusion

AFFINIMIP® SPE Bisphenols has been successfully used for the clean-up of bisphenol A in beers. The method offered an efficient cleanup, making suitable the analysis at low concentrations by LC-Fluorescence. Excellent recovery yields were measured from **90.6 to 106.9%** at various spike levels, and the method was shown to be reproducible with relative standard deviations of less than **9.0%**.
The cleanup method with **AFFINIMIP® SPE Bisphenols** has been shown to be efficient with a wide variety of matrices and is also suitable for bisphenol analogues and for GC-MS/MS analysis (see references [1-7]).

References

1. **Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection**, P. Gallo et al. (2017) Food Chemistry 220:406–412.

2. **Xenobiotic-contaminated diets affect hepatic lipid metabolism: implications for liver steatosis in Sparus aurata juveniles**, F. Maradonna et al. (2015) Aquatic Toxicology 167:257–264.

3. **Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry**, Y. Deceuninck et al. (2015) Anal. and Bioanal. Chem 407 (9) :2485–2497.

4. **Molecularly imprinted solid phase extraction for the selective extraction of bisphenol analogues in beverages and canned food,** Y. Yang et al. (2014) J. Agric. Food Chem. 62 (46): 11130– 11137.

5. **A high selective and sensitive liquid chromatography-tandem mass spectrometry method for quantization of BPA urinary levels in children**, C. Nicolucci et al. (2013), Analytical and Bioanalytical Chemistry, 1618-2642.

6. **Report of the French health agency (ANSES) Assessement of the health risk associated with bisphenol A (2013) full study in French.**

7. **Development and validation of a specific and sensitive gas chromatography tandem mass spectrometry method for the determination of bisphenol A residues in a large set of food items,** Y. Deceuninck et al. (2014), Journal of chromatography A, 1362, 241-249.

8. Commission Regulation (EU) 2018/213 of 12 February 2018 on the **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 (Text with EEA relevance)

Please consult the complete application notebook on our website (affinisep.com) for more results about BPA. A short video about SPE is also available.

https://www.affinisep.com/products/ready-to-use-kits-spe-and-mips/bisphenols/

Product reference

- **AFFINIMIP® SPE Bisphenols**

Catalog number: **FS106-03** for 50 cartridges 3mL

- **AFFINIMIP® SPE Bisphenols**

Catalog number: **FS106-03B** for 50 cartridges 6mL

- **AFFINIMIP® SPE Bisphenols**

Catalog number: **FS106-03G** for 50 glass cartridges 6mL

- **SilactHPLC LC-BPA** - 150x 2.1mm (3µm) for Bisphenol A analysis

Catalog number: **LC-BPA-150.2.1** for 1 pc

- **SilactHPLC DELAY -BPA** - 50x2,1mm (5µm) Delay column for Bisphenol A analysis

Catalog number: **DELAY-BPA-50.2.1** for 1 pc