Application Note

Food & Beverage

The finest LC-EC applications for Food & Beverage analysis

Phenols
Bisphenol A
Catechins
Flavonoids
Phenols
Antioxidants
Resveratrol
Epicatechin
Quercetin
Other polyphenols

Carbohydrates
Monosaccharides
Lactose
Other oligo- and polysaccharides

Vitamins, minerals etc.
A, C, D, E, and K
Iodide
Q10, ubiquinols

Bisphenol A in Drinking Water

- Sensitive analysis of BPA leaching - LOD: 0.3 nM
- Gradient HPLC-ECD with automated sample pre-concentration
- Fast, robust and reproducible analysis

Summary

Concerns have been expressed about the estrogenicity of bisphenol A (BPA) and other aromatic components that leach from the plastic containers and packages into food and beverages. In this note a sensitive method is presented to analyze (canned and bottled) drinking water for the presence of BPA.

An ALEXYS system is used in combination with an in-line solid phase sample pre-concentration step. A detection limit of 0.3 nmole/L is shown.

Introduction

Bisphenol A (BPA) is an important chemical building block that is primarily used to make polycarbonate plastic and epoxy resins, both of which are used in a wide variety of materials. Common examples of polycarbonate products include eyeglass lenses, digital media (e.g., CDs, DVDs), electronic and electrical equipment housings (e.g., personal computers, appliances, power tools), automobile headlight lenses, sports safety equipment (e.g., helmets, goggles), and reusable food and drink containers. When used as a coating on the interior of metal cans, epoxy resins protect the integrity and quality of the food inside by preventing corrosion and metal contamination of the canned foods and beverages.

BPA (Fig 1) can be detected with electrochemical detection (ECD) after HPLC separation. As ECD is a very sensitive mode of detection, this can be used to analyze and quantify trace levels of BPA in drinking water. Moreover, the highly apolar feature of BPA can be used to apply pre-concentration before injection, thus increasing the detection limit even further.

This note shows the results of analyzing BPA on the ALEXYS® Analyzer using an LC-ECD method with automated in-line pre-concentration.

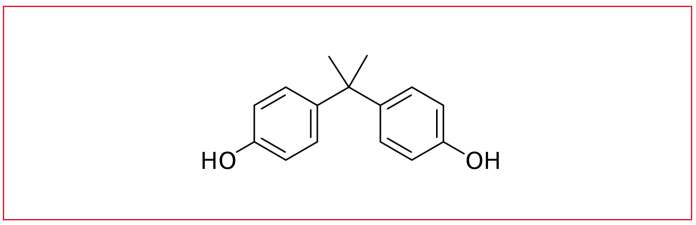

Figure 1: Molecular structure of bisphenol A (BPA).

Method

A dedicated ALEXYS Bisphenol A Analyzer was used. This LC-ECD system contains an autosampler with a 10-port valve and an additional LC pump. This facilitates running an automated sample pre-concentration step based on solid phase extraction as part of the isocratic LC-ECD analytical method (Table 1).

Automated sample pre-concentration

The advantage of applying sample pre-concentration is not only an improvement of detection limit, but it also benefits the chromatographic separation and sample clean-up. A trial experiments where 1 mL of sample was directly injected on the analytical column (without the pre-concentration step) resulted in chromatograms with a large front peak, a drifting baseline and a considerable shift in retention times.

For the sample pre-concentration step, a volume of 1 mL sample is loaded onto a pre-concentration column (Fig. 2). By first applying a solvent with low eluting strength, the (apolar) BPA is retained on the (apolar) pre-concentration column. The second more apolar mobile phase elutes the BPA onto the analytical column and separates it in isocratic mode.

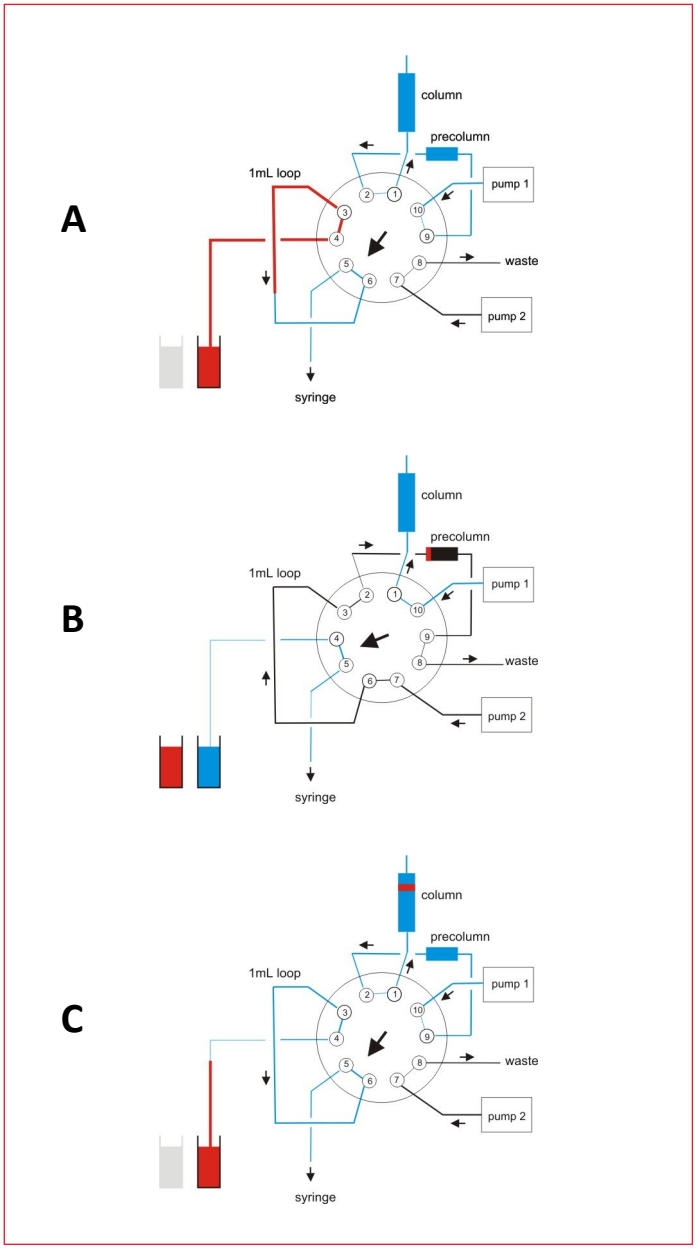

Figure 2: Schematic representation of the in-line pre-concentration program: [A] a 1 mL loop is filled with sample. [B] The 10-port valve switches and pump 2 with mobile phase A carries the sample into the pre-column.
[C] The valve switches back and pump 1 reverse-flushes the concentrated sample from the pre-column to the analytical column with mobile phase B. Meanwhile, the next sample can be loaded into the loop
The procedure is automated and programmed as part of the method settings.

ECD working potential

A hydrodynamic voltammogram was constructed to find the optimal working potential for detection of BPA (Fig. 3). A potential of 900 mV was chosen. This value also gave the highest SN ratio (not shown).

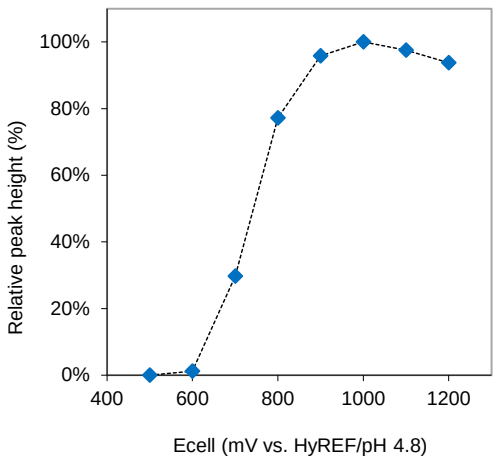

Figure 3: Hydrodynamic voltammogram of BPA.

Table 1

Conditions	
LC system	ALEXYS Bisphenol A Analyzer
Pre-concentration column	Inertsil ODS-3 C18 (GL Sciences) 5.0 x 1.0 mm ID, 5 µm
Analytical column	Betabasic™ 18 HPLC Column (Thermo Scientific™) 150 x 2.1 mm ID, 3 µm
Mobile phase A (pre-concentration)	90% [50 mM sodium acetate, pH set to 4.8 with acetic acid];10% acetonitrile
Mobile phase B (separation)	60% [50 mM sodium acetate, pH set to 4.8 with acetic acid];40% acetonitrile
Flow rates	0.8 mL/min (pre-concentration) 0.2 mL/min (separation)
Backpressure (B)	About 90 bar
Injection volume	1000 µL
Temperature	35 °C (separation and detection)
Flow cell	2 mm GC SenCell*, HyREF, AST: position 2
$E_{cell\ 1}$	+0.9 V
I-cell	about 40 nA
ADF	0.5 Hz
Range	20 nA/V

* Original work done with a 2 mm GC VT-03, HyREF, 50 µm spacer

Electrode contamination

Analysis of relatively high concentrations of BPA (0.5 mM BPA, 10 µL injections) were associated with a near-linear decrease in peak height: after 100 injections, 50% of the original peak height was left (Fig. 4). The original response could be fully restored by polishing the working electrode. This indicates that electrode contamination is an issue at elevated concentrations of BPA; at a lower concentration (5 nM BPA, 10 µL injections) no loss of response was observed, even after 200 repeated injections (Fig. 5).

Cleaning pulse

In cases where electrode contamination is an issue, a cleaning pulse can be applied. We found that switching the potential to +0.1 V for 54 s between runs (and a few minutes of stabilization time) is sufficient to prevent electrode contamination and the associated loss of response.

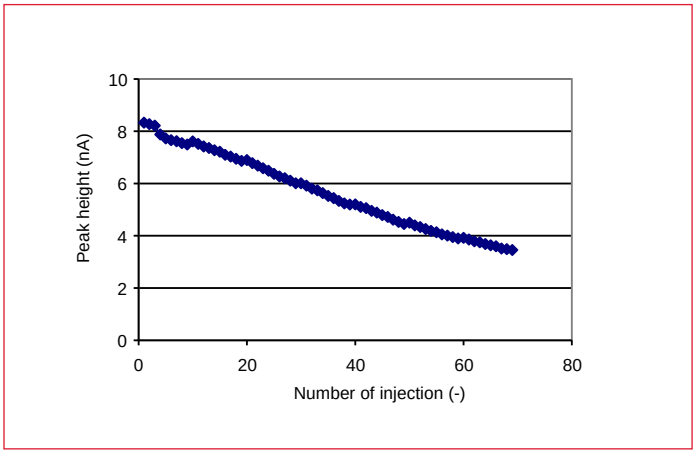

Figure 4: Response decrease due to fouling of the working electrode caused by the analysis of relatively high concentrations of BPA (500 nM BPA, 10 µL injections on a 3 mm ID column).

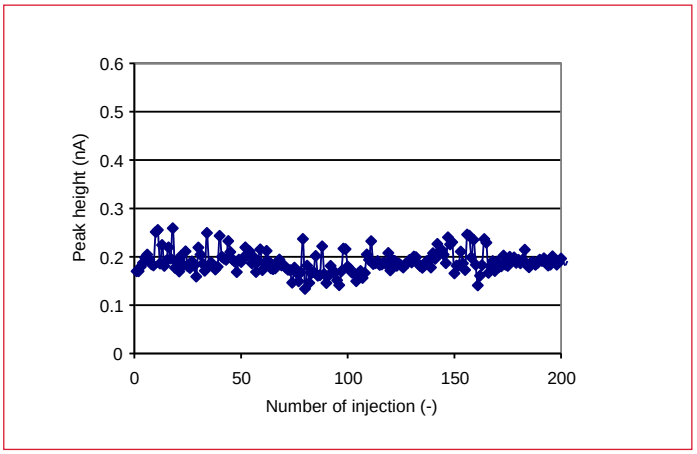

Figure 5: No response decrease is observed during 200 repeated analyses of relatively low concentrations of BPA (5 nM BPA, 10 µL injections on a 2 mm ID column).

Results

Linearity and detection limit

The response linearity was evaluated in the range of 0.1-10 nM BPA. Below 0.5 nM the results were not linear and these data points were rejected (Fig. 6). Correlation coefficient r was better than 0.999. Based on this calibration data a detection limit of 0.3 nM was calculated. Based on signal-to-noise ratio [LOD = 3 n c / s] a factor 10 better detection limit can be calculated. However, the practical detection limit is higher because of the non-linearity at lower concentrations.

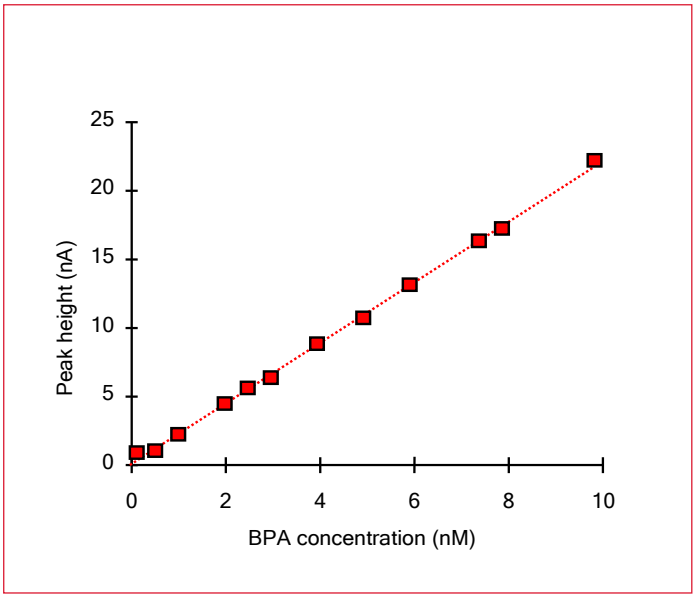

Figure 6: Calibration curve of BPA. Linear regression: Y = 0.0843 + 2.2072 X.

Reproducibility

Reproducibility was studied for analysis of different concentration (0.5, 1 and 2.5 nM BPA), based on 1 mL injections including automated pre-concentration. The results are summarized in Table 2, and Fig. 7 and 8 show the overlay of the chromatograms of 0.5 nM and 2.5 nM BPA. The method was shown to give reproducible results with an RSD of about 2% for peak area and peak height.

Table 2

Averages and %RSD of retention time and height measured on the basis of 1 mL injections (and in-line pre-concentration) of various concentrations of BPA

		Retention		*Height*	
BPA conc. (nM)	*n*	*tr (min)*	*RSD (%)*	*H (nA)*	*RSD (%)*
0.5	5	5.52	0.1	0.33	1.9
1.0	8	5.52	0.1	0.64	1.2
2.5	9	5.51	0.1	1.45	1.8

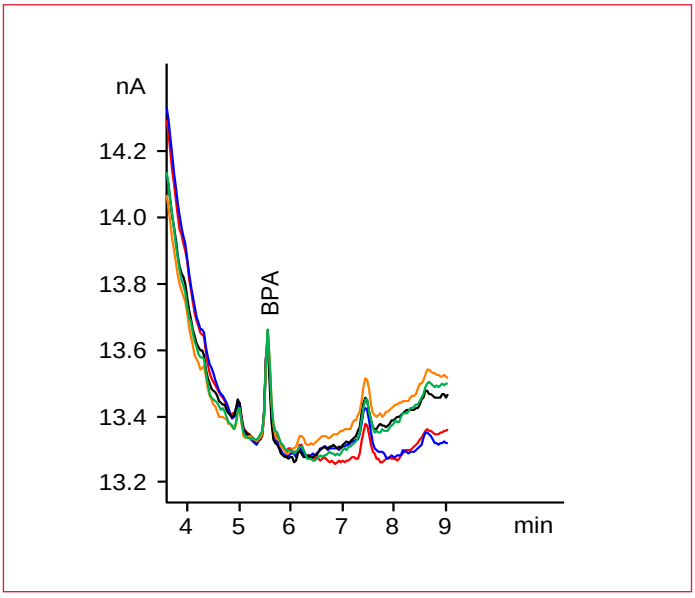

Figure 7: Overlay of 5 chromatograms of 0.5 nM BPA

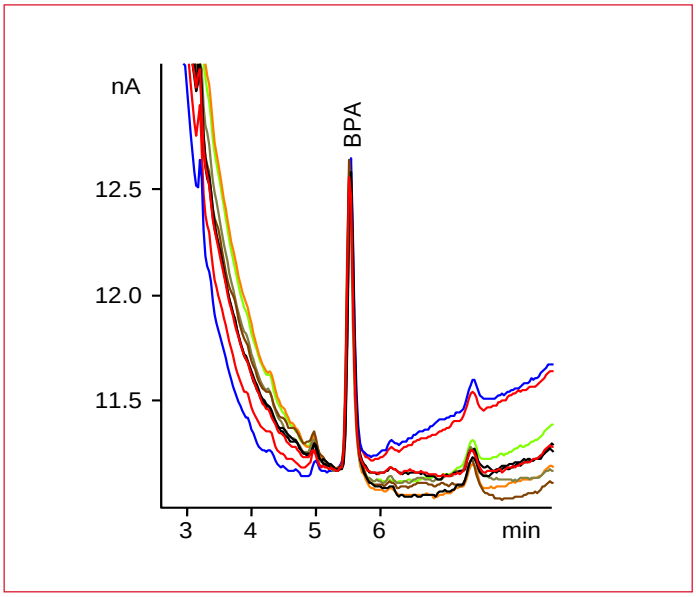

Figure 8: Overlay of 9 chromatograms of 2.5 nM BPA

Analysis of drinking water

Bottled drinking water from 3 different sources were analyzed:
A - water from a polycarbonate container (Nestlé), which is mounted on a dispensing machine
B - water from a PET bottle (Spa)
C - water from a PET bottle (Sourcy)

Chromatograms are shown in Fig. 9. Apparently, the water from the PET bottles were free of BPA (levels below the detection limit of the method). The water from the aqua machine contained a concentration of 1.5 nM BPA.

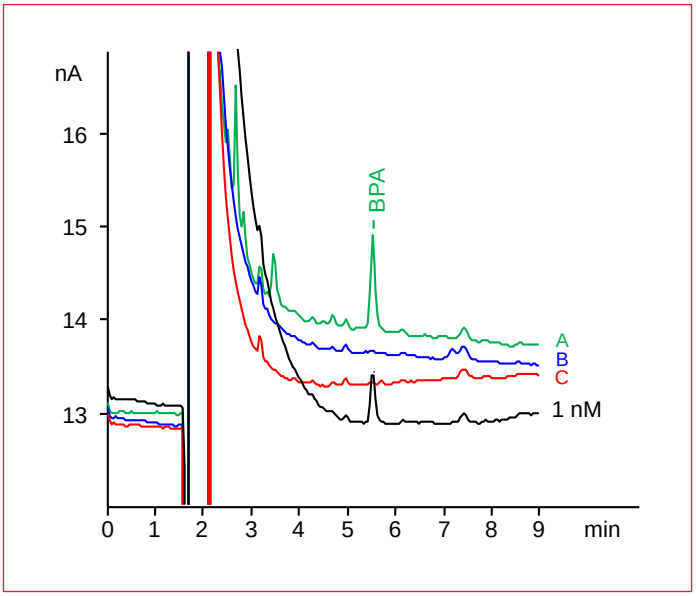

Figure 9: Chromatogram overlay of a 1 nM BPA standard and 3 different bottled drinking water samples (A, B and C). Water sample A contained 1.5 nM BPA, and the other 2 samples had undetectable levels of BPA.

References

1. Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J., Olea, N. (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. Environmental health perspectives, 108(1), 21-27.
2. Sajiki, J., Takahashi, K., & Yonekubo, J. (1999). Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications, 736(1-2), 255-261.

Conclusion

The ALEXYS Bisphenol A Analyzer is used for the analysis of BPA in drinking water. This LC-ECD system includes automated sample pre-concentration. The method was shown to give reproducible results (2% RSD for response). A detection limit of 0.3 nM has been obtained.

Figure 10: Recommended instrument configuration for this application: the ALEXYS Bisphenol A Analyzer
The system consists of two P6.1L pump swith integrated degasser, an AS6.1L autosampler with 10-port valve, and the DECADE Elite electrochemical detector. The ALEXYS Bisphenol A Analyzer can be fully controlled by the Clarity™ Chromatography Data System (CDS) software from DataApex™ (version 8.3 and up).

Ordering information

180.0090B	ALEXYS Bisphenol A Analyzer
116.4320	SenCell 2 mm GC HyREF
Software	
195.0035#	Clarity CDS single instr. incl LC, AS module

#) optional: Antec ECD drivers for use with Chromeleon CDS , OpenLAB CDS or OpenLAB Chemstation CDS are available.

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