Application Note

► High speed analysis of bisphenol A and bisphenol F

Category	Food
Matrix	Extract from wrapping film
Method	UHPLC
Keywords	Bisphenols, PVC/polycarbonate additives, plastics, baby bottles
Analytes	Bisphenol A, bisphenol F
ID	**VFD0015N**, 07/10

PLATIN blue

Summary

A time-optimized method for the separation and determination of bisphenol A (BPA) and bisphenol F (BPF) using a KNAUER PLATINblue UHPLC system is presented in this application note. Reduction of analysis time to less than 1 minute is achieved by employing the BlueOrchid C18 stationary phase with a 1.8 µm particle size filled in a 2 mm ID column. A binary high pressure gradient configuration is used at a flow rate of 0.6 ml/min and detection is carried out with a photo diode array (PDA) detector.

Introduction

Bisphenols and especially BPA and BPF have become well known to the public in the last years due to their negative health effects. The concerns over BPA as the best known bisphenol began with baby bottles and spread to include other types of food and drink packaging devices. Bisphenols are used by the plastic producing industry as antioxidants for softeners, fungicides and as intermediates during the production process of polycarbonates, epoxides, phenol resins and dyes. European companies consume 1.15 Mio tons every year concerning to the EU. Most often BPA is used in the production of the two most commonly used polymers polyvinyl chloride (PVC) and polycarbonate. In PVC production BPA acts as a polymerization inhibitor. After completed polymerization BPA residues may remain in the material. BPA is also an important monomer in the production of polycarbonate. Not all of the BPA is consumed in the production process and may leach out of the polymer later on. Today many applications of polycarbonate have been replaced with new copolymers such as co-polyester to eliminate BPA.[1,2]
The different uses of bisphenols in the mentioned materials have in common that they are not bound tightly to the host material, which is problematic, because these materials are often used in products for the food industry, like cans, wrapping films and baby bottles. This way they find their way into the human body by leaching into food and drinks.[1,2]
Why is it important to determine bisphenols? BPA has shown to have similar effects like the hormone estrogen and can affect the human endocrine system. If the concentration in blood is high enough, humans react with disordered development of the sexual organs, the nervous system and the behaviour. Especially before and shortly after birth the human body reacts particularly sensitive. That's why bisphenols are controversially discussed by public authorities and scientists.[2]

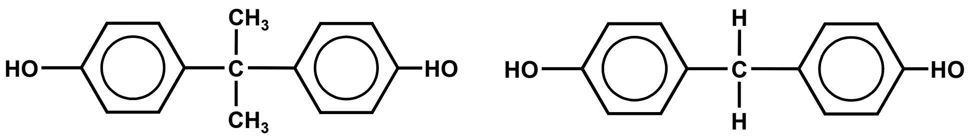
The chemical structures of the most commonly used substances BPA and BPF which are determined in this work are shown in figure 1.

Experimental sample preparation

Bisphenols can be extracted from PVC products with high extraction ratios by using the solvent acetonitrile (ACN) at slightly elevated temperatures.[3,4] A sample of 0.5 g of a wrapping film used for food packaging was cut into small pieces, 5 ml of ACN were added and the sample was put into a water bath at 40 °C for about 24 hours. After filtering through a 0.45 µm syringe filter, the sample was ready for analysis by UHPLC.

Experimental preparation of standard solution

All standard solutions were prepared with water/ACN 50:50 (v/v). 10 mg of the two substances were dissolved in 10 ml water/ACN. After mixing and diluting to a concentration of 10 µg/ml and later on to 1 and 0.1 µg/ml for the calibration the standard solution was ready for analysis by UHPLC.



bisphenol A (BPA) bisphenol F (BPF)

Fig. 1

Chemical structures

Method parameters

Column	BlueOrchid C18 1.8 µm, 50 x 2 mm		
Eluent A	Water		
Eluent B	Acetonitrile (ACN)		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	55	45
	0.70	55	45
	1.70	5	95
	4.50	5	95
	4.60	55	45
Flow rate	0.6 ml/min		
Injection volume	2 µl		
Column temperature	30 °C		
Detection	UV at 227 nm (10 mm, 2 µl flow cell; 50 Hz, 0.01 s)		
Analysis time	0.7 min		

Results

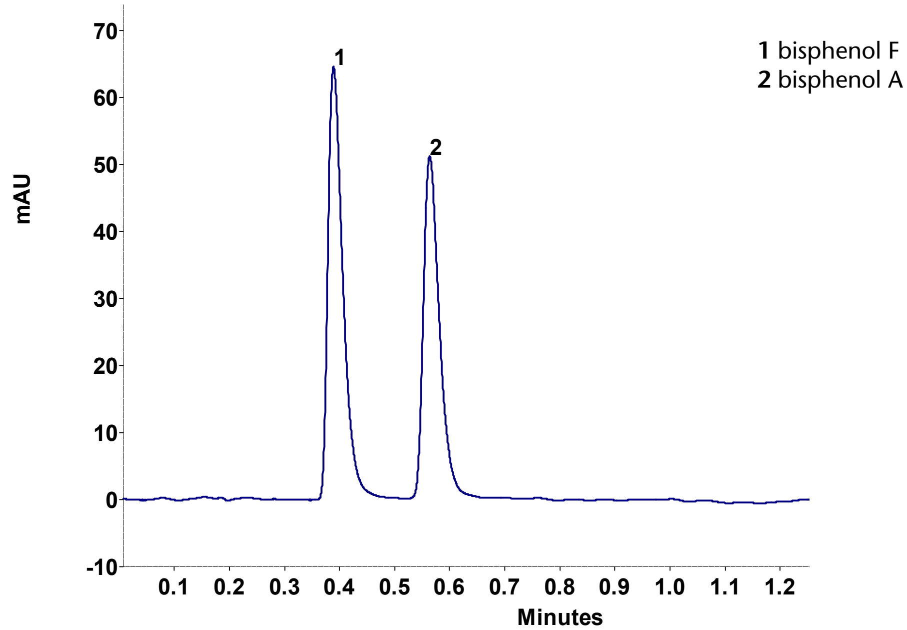


Fig. 2

Chromatogram of the bisphenol A and bisphenol F standard (10 µg/ml)

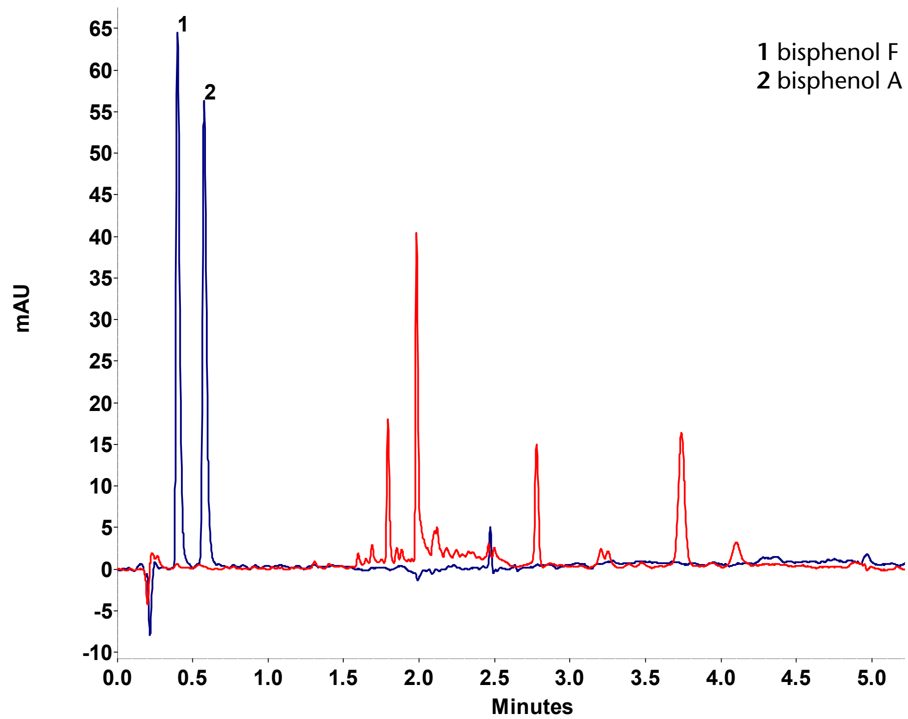


Fig. 3

Chromatogram of the wrapping film extract (red) and the bisphenol standard (blue).

With the presented UHPLC method BPA and BPF could be separated easily in less than one minute by applying BlueOrchid 1.8 C18 as the stationary phase. A chromatogram of the standard is shown in figure 2. The two substances are baseline separated with a high resolution value. Calibration was done for concentrations in the range of 0.2 – 20 ng with goodness of linearity fit (R^2) > 0.99999. Figure 3 shows that no bisphenols could be found in the sample of the wrapping film extract (red).
The number of unknown peaks in the real sample shows that the method is capable of extracting various components from the wrapping film sample.

Method performance

Limit of detection	0.2 ng (S/N = 3)
Linearity (r^2)	> 0.99999
Linearity range	0.2 – 20 ng

Conclusion

This application has shown to be a very fast method for the determination of BPA and BPF which are commonly used by the plastic producing industry. A successful separation in less than 1 minute and determination with excellent linearity over the calibration range was made possible by applying the KNAUER PLATINblue UHPLC system in combination with the BlueOrchid C18 stationary phase. In comparison to an existing well-optimized HPLC method more than 85 % of eluent and 80 % of the analysis time could be saved. That does not only save time and money, it also protects the environment. Additionally, extraction of bisphenols from PVC products can be done with high extraction ratios without using hazardous solvents like dichloromethane as described in the literature as an improved extraction method.[3]

References

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2. Umweltbundesamt, Abteilung Umwelthygiene. Telegramm: Umwelt + Gesundheit. „Bisphenol A" – Wir haben ein Problem. Ausgabe 04/2008
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4. J. López-Cervantes, P. Paseiro-Losada. Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. Food Additives & Contaminants: Part A, Volume 20, Issue 6 June 2003 , pages 596 - 606

Physical properties of recommended column

BlueOrchid C18 columns use a hydrophobic interaction separation mechanism and offer an extended pH range for analysis of acidic, basic and neutral analytes in reversed phase mode. All BlueOrchid phases feature exceptional peak symmetry and resolution. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.

Stationary phase	BlueOrchid 1.8 C18
USP code	L1
Pore size	180 Å
Particle size	1.8 µm
Form	spherical
Surface area	180 m^2/g
% C	10
Endcapping	yes
Dimensions	50 x 2 mm
Order number	05BI181BOE

Recommended instrumentation

This application requires the PLATINblue binary high pressure gradient UHPLC system equipped with degasser, autosampler, column thermostat, and PDA detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
PLATINblue UHPLC-System	A69420
PLATINblue Pump P-1	
PLATINblue Pump P-1 with Degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue Detector PDA-1	
PDA-1 flow cell (10 mm, 2 µl)	
PLATINblue modular eluent tray	
PLATINblue CG Data system	
PLATINblue CG PDA license	
PLATINblue stainless steel capillary kit	

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