Determination of aflatoxins with Agilent 1290 Infinity LC, Agilent 1260 Infinity Binary LC and Agilent ZORBAX RRHT 1.8 µm columns by FLD after electrochemical derivatization with a Coring Cell

Application Note

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Abstract

The determination of contaminants in foods requires high throughputs and high sensitivities. With the Agilent 1290 Infinity LC system and the Agilent 1260 Binary LC systems, it is possible to transfer methods with low outputs to faster applications. The determination of aflatoxins B1, B2, G1 and G2 by HPLC and post-column derivatization for fluorescence detection can be accelerated by using shorter Agilent ZORBAX RRHT 1.8 µm columns instead of conventional 5-µm columns. High resolution was achieved despite post-column derivatization with the Coring Cell and related band broadening. Sample throughput was increased by a factor of >2 with analysis times of about 10 min including column flushing.

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Introduction

Aflatoxins are naturally occurring mycotoxins produced by certain molds growing on some food crops during production and storage. Aflatoxin B1 (Figure 5) is the most dangerous, because it is carcinogenic and very small amounts can be toxic for humans.[1] For that reason, foods must be monitored, because contamination by various mildews producing aflatoxins can easily occur.

Aflatoxins B1, B2, G1 and G2 show absorption by UV, but the detection limits of food control are so low, that UV-detection is not sensitive enough. To improve detection sensitivity, the aflatoxins can be post-column derivatized with a Coring Cell.[2] After post-column electrochemical bromation of mycotoxins, sensitivity can be dramatically enhanced using fluorescence detection. The influence of matrix components is also reduced, reaching lower limits of detection.

Post-column derivatization requires a four second reaction time between Coring Cell and detector. This is usually achieved by low flows and wide capillaries.

This Application Note addressed the following question: can post-column derivatization be combined with rapid resolution LC to achieve better results in the analysis of aflatoxines, even in complex matrices such as marzipan and hazelnuts?

Agilent 1290 Infinity LC		Agilent 1260 Infinity Binary LC	
G4220A	Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser	G1312B	Agilent 1260 Infinity Binary Pump with Vacuum Degasser
G4226A	Agilent 1290 Infinity Autosampler	G1367C	Agilent 1260 Infinity Autosampler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment	G1316B	Agilent 1260 Infinity Thermostatted Column Compartment
G1321A	Agilent Fluorescence Detector	G1321A	Agilent 1260 Infinity Fluorescence Detector

Table 1
Configuration of the Agilent 1290 Infinity LC system and Agilent 1260 Binary LC system.

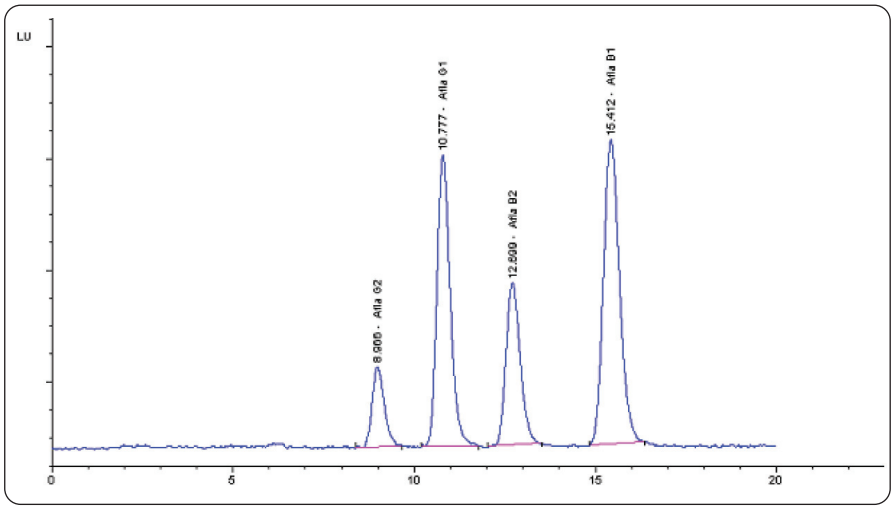

Figure 1
Separation on a standard LC column (Eurospher, Knauer, Germany), 250 mm × 4.6 mm, 5 µm, 1 mL/min, 30 °C.

Experimental

Analysis of aflatoxins can be achieved by using a fast LC configuration (Table 1).

Experimental conditions

Eluent:	Wasser/Acetonitrile/Methanol /65% Nitric Acid / KBr: 600 / 200 / 200 / 100 µL/ 119 mg/L pH = 2.79
Detection:	FLD (Ex: 362 nm; Em: 435 nm)
Flow:	1.0 mL/min
Injection volume:	20 µL
Temp:	40 °C

The setup for post-column derivatization was preset according to the recommendations of Coring for analysis of aflatoxines with conventional HPLC[2].

Results and Discussion

The first run of a standard was performed in a conventional 250 mm column (Figure 1). The parameters were transferred to a 100 mm × 4.6 mm RRHT column filled with 1.8 µm particles using the Method Translator.

Figure 2 shows the results of the aflatoxines standard analysis. The results illustrate comparable resolution, but the shorter column reduced analysis time (Table 2).

Additional evidence of a successful transfer was the determination of the aflatoxines in food samples such as marzipan or hazelnuts to verify the influence of the matrix.

All aflatoxins are well separated (resolution >1.5 for all peaks) with reduced analysis time of 8.0 minutes. In Figure 3 the separation of the aflatoxins in a hazelnut sample (a complex matrix compared to marzipan in Figure 4) shows a low influence of matrix components. By analyzing strong matrix contaminated samples, it is proven that a short flushing gradient with acetonitrile (to 80% in B) can be used to flush the column.

Conclusions

All Agilent systems show excellent performance regarding aflatoxin analysis. Methods with separations of conventional materials can be easily transferred to achieve shorter analysis times with comparable chromatographic resolution even with the robust 4.6 mm-columns filled with 1.8 µm particles. Both Agilent 1260 Infinity Binary LC and the Agilent 1290 Infinity LC showed significant time reduction resulting from the transfer to shorter, high resolution columns.

The extended power range of the Agilent 1290 Infinity LC systems allows the use of RRHD columns. Therefore an Agilent StableBond 100 mm × 2.1 mm, 1.8 µm particle column can be used at the same flow rate, producing a shorter cycle time with the same resolution.

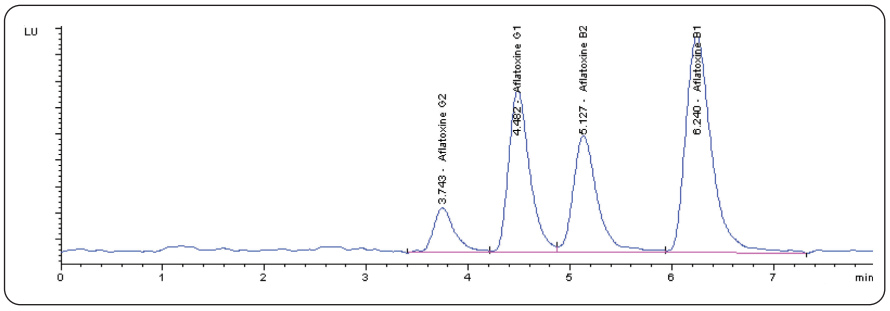

Figure 2
Separation with Agilent SB C18, 100 mm × 4.6 mm, 1,8 µm, 40 °C, 1 mL/min.

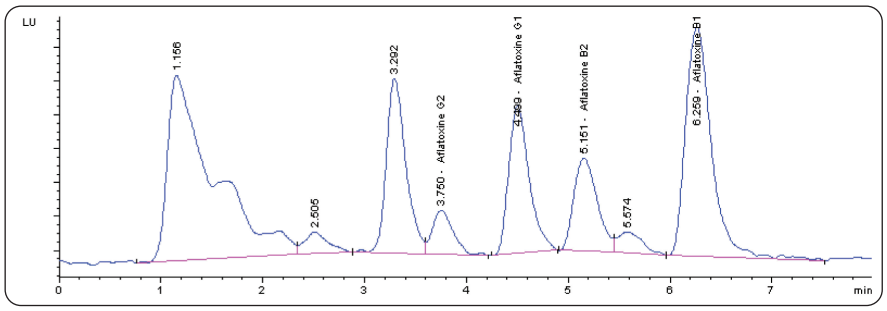

Figure 3
Determination of aflatoxins in hazelnuts.

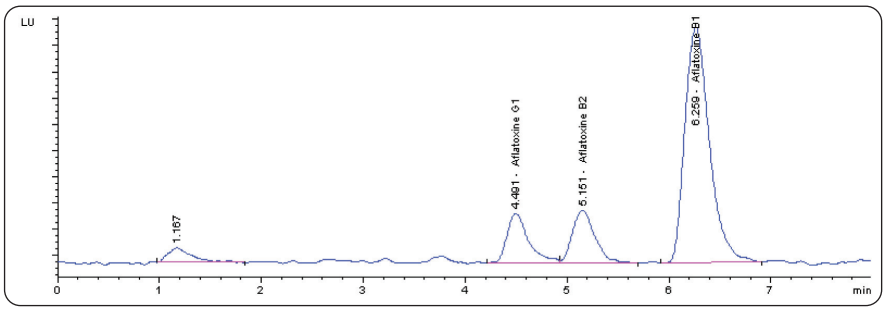

Figure 4
Determination of aflatoxins in marzipan.

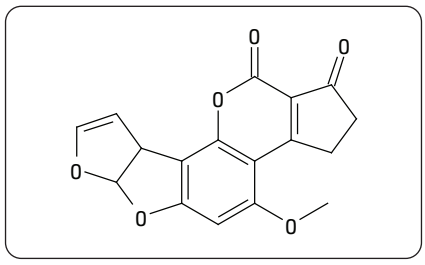

Figure 5
Structure of aflatoxin B1.

Mycotoxin	Retention time (min)
Aflatoxin G2	3.743
Aflatoxin G1	4.482
Aflatoxin B2	5.127
Aflatoxin B1	6.240

Table 2
List of aflatoxin compounds and retention times.

References

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Aflatoxins in nuts survey: http://www.food.gov.uk/news/newsarchive/2004/jun/aflatoxinsurvey

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www.agilent.com/chem/infinity

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