APPLICATION NOTE

Liquid Chromatography

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Further Speed-up of Aflatoxin B1, B2, G1 and G2 in Vegetable Feed Stuff and Peanuts by UHPLC-FLD with Kobra Cell Derivatization without Concentration with Immunoaffinity Columns

Introduction

Based on the high interest in the market to analyze even more food samples to find mycotoxins contaminations, we developed an analytical method in UHPLC to perform Aflatoxin analysis (enhancement of: 007883_01 Aflatoxins in Corn by UHPLC-Kobra Cell Application Brief).

The main benefits using this method in UHPLC are:

- Significantly reducing the time of analysis
- Significantly reducing the injected volume
- Reduce the chemicals consumption
- Preserve the components separation
- Increase the number of samples tested per day

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Potential Market

- Agriculture (e.g. Food Lab, Custom Lab, Public Health)
- Food & Beverage (e.g. Food and Beverage companies)

Recommended HPLC conditions using a Kobra Cell

Kobra Cell:	100 µA
Analytical Column:	Brownlee™ Pinnacle DB C18 HPLC Column, 1.9 µm particle size, length × I.D. 50 mm × 2.1 mm
Mobile phase:	Water/Acn/MeOh 75-10-15+119 mg KBr + 350 µL HNO_3 4M
Flow:	1 ml/min @ 6000 psi
Fluorescence Detector:	Excitation 362 nm Emission 435 nm PMT: Super high Em Bandwidth: Wide
Injection Volume:	2 µL
Total Run Time:	3 min.

Suggested UHPLC Instrument configuration

Flexar Micro Pumps	Binary Micro pumps
Flexar VD	Vacuum degasser 5 channels
Flexar Autosampler	Autosampler
Flexar Oven	Column Oven
Flexar FL	Fluorescence detector
Chromera®	Chromatography Data System

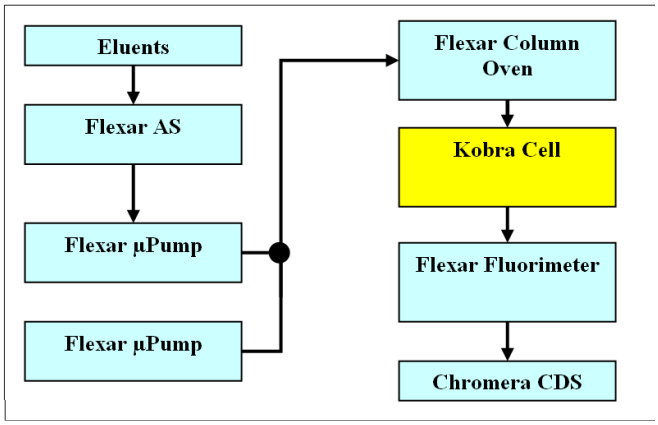
Strategic HPLC configuration

Confirmation of the presence of aflatoxins in a sample by UHPLC requires derivatization of the aflatoxins B1 and G1 in order to enhance their natural fluorescence under UV light and make them more easily detected.

Previously, the only options available for derivatizing aflatoxins involved the use of trifluoroacetic acid (TFA), pyridinium bromide perbromide (PBPB) or iodine. All of these methods are reliable, however they do have some significant limitations which can be overcome by use of the Kobra Cell.

The Kobra Cell overcomes many of the problems relating to alternative derivatization procedures. The Kobra Cell is an electrochemical cell which generates a reactive form of bromine for derivatization of aflatoxins B1 and G1, resulting in enhanced fluorescence and thus more sensitive detection. The derivatization of aflatoxins occurs rapidly (reaction time is approximately 4 seconds) at ambient temperature. The daily preparation of the derivatizing agent is not required and no extra equipment or additional maintenance of the equipment is necessary.

The key component of AFLAPREP is the immunoaffinity column which contains a gel suspension of monoclonal



antibody covalently attached to a solid support. The antibody is specific for aflatoxins B1, B2, G1 and G2.

Following extraction of the toxins, the sample extracted is passed through the immunoaffinity column. Any aflatoxin which is present in the sample is retained by the antibody within the gel suspension.

The column is washed with water to remove extraneous non-specific material; the bound toxin is released by the antibody following elution from the column with methanol or acetonitrile.

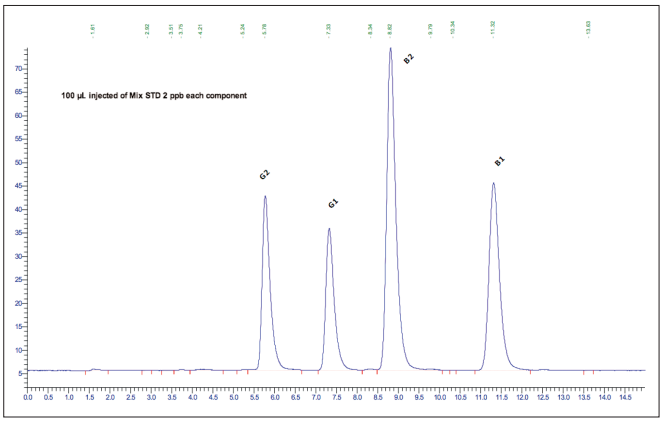
The elute is collected in a vial for the analysis by UHPLC.

Results

Standard

Aflatoxin Standard Solution with “Traditional HPLC” method = 2 ng/mL or 2 ppb

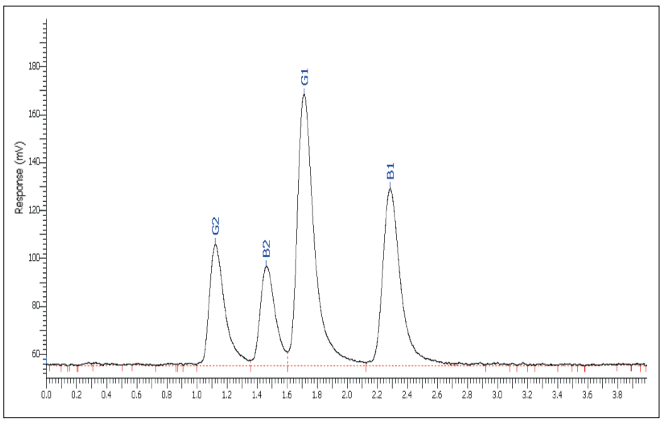
- 100 µL injected 2 ppb for each chemical



Standard

Aflatoxin Standard Solution using method UHPLC Chromatography = 2 ppb

- 2 µL injected 2 ppb for each chemical

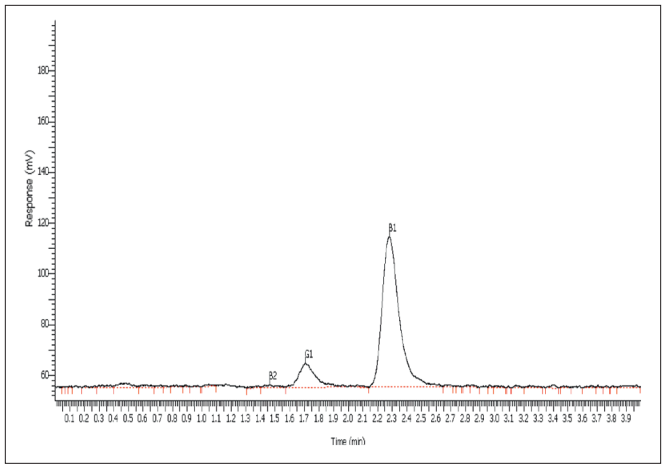


Component Name	Retention time (min)	Area (µV/s)	Conc. (ppb)
G2	1.12	359814.01	2.026
B2	1.46	290750.31	2.068
G1	1.72	883570.46	2.085
B1	2.29	609100.68	2.071

Sample

Aflatoxin animal feed sample using method UHPLC Chromatography

- 2 µL injected

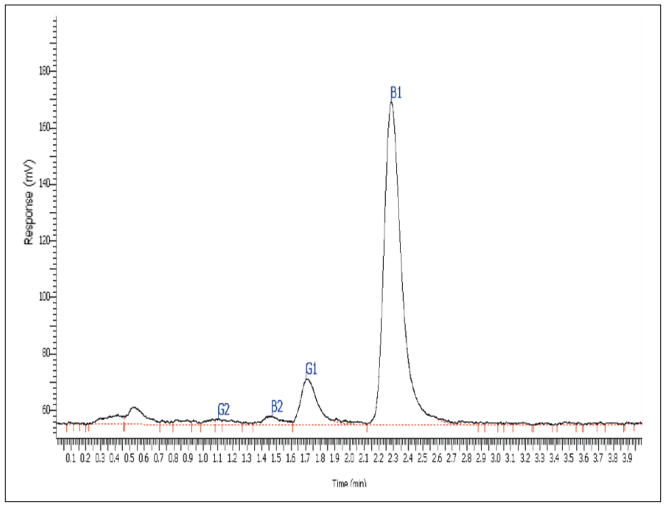


Component Name	Retention time (min)	Area (µV/s)	Conc. (ppb)
G2	n.d.	n.d.	n.d.
B2	1.47	11211.28	0.080
G1	1.71	78031.53	0.181
B1	2.28	494683.95	1.682

Sample

Aflatoxin ground corn sample using method UHPLC Chromatography

- 2 µL injected

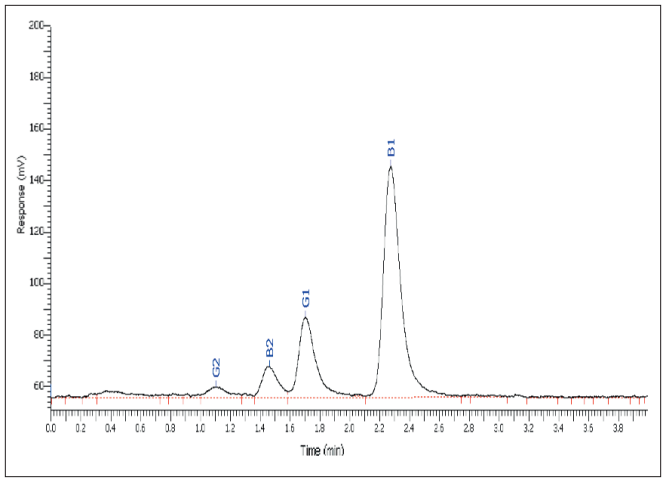


Component Name	Retention time (min)	Area (µV/s)	Conc. (ppb)
G2	1.08	9340.42	0.053
B2	1.47	17378.22	0.123
G1	1.70	117971.91	0.274
B1	2.28	929626.48	3.160

Sample

Aflatoxin peanuts sample using method UHPLC Chromatography

- 2 µL injected



Component Name	Retention time (min)	Area (µV/s)	Conc. (ppb)
G2	1.10	25619.84	0.144
B2	1.45	83608.24	0.595
G1	1.70	237513.03	0.550
B1	2.28	688163.08	2.339

R.O.I. calculator

	UHPLC vs. Traditional HPLC
Solvent consumption	Save→86.7%
Run Time	Save→71.4%
Samples/hour	Productivity→375%

Reference Material

The procedure for extraction of the analytes in Instructions for Use by AFLAPREP (R-Biopharm RHONE LTD).

Concentration: from 4 to 80 ppt for B1, B2, G1 and G2.

References

Previous Aflatoxin Method:

- PerkinElmer # 007883_01 Aflatoxins in Corn by HPLC – Kobra Cell. Application Brief.

Kobra Cell: http://www.r-biopharmrhone.com/pro/equip.html

Conclusions

The synergy of Flexar UHPLC and Kobra Cell using this method provide several advantages:

- Using the immunoaffinity preparative columns we obtain a clean sample extract without interfering substances. The result of this preparation procedure allows to have an excellent specificity of the extraction.
- The toxine derivatization is needed because the species B1 and G1 are not generally detectable in fluorescence.
- The advantage of the Kobra Cell usage is to do not pre-column derivatize the sample before run it or, use any post-column system in combination of the HPLC.
- Using the sample elute (as it is – without concentrate sample) from the immunoaffinity column, allows to have an important time saving preserving an excellent method sensitivity compared to the low limits (CE 466/2001).
- Experimental Linearity from 0.2, 0.5, 1, 2, and 4 ppb. For each solution we injected 2 µL with a real concentration of 4, 10, 20 and 80 ppt.
- This method is valid for many other vegetables matrices like: coffee, pistachios, etc. The only differences are in sample preparation before the immunoaffinity and analysis process.

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