Aflatoxin & Ochratoxin Analysis

A Completely Automated Solution with the Freestyle, ThermELUTE, and SMART Immunoaffinity Cleanup Columns
• Pickering Labs primarily makes HPLC Post-column instruments and reagents for a variety of post-column applications.

• LC Tech has been the European distributor for Pickering Labs for about 20 years.

• Recently Pickering Labs has been distributing LC Tech products in North America along with help from Chromatographic Specialties in Canada.
supplies innovative products and methods for the preparation and analysis of food, feed and environmental samples.
Introduction

- The goal: totally automated system for Aflatoxins (B1, B2, G1, G2) and/or Ochratoxin A

From raw extract to chromatogram
=> in 15 minutes
=> without manual steps
Introduction

- The Components
Instrumentation

Sample preparation and Analysis

**FREESTYLE™**
- FREESTYLE Basic
- SPE module
- ThermELUTE module
- Racks and vials
- AflaCLEAN and/or OtaCLEAN
  SMART Immunoaffinity Cleanup Columns

Any HPLC
- Pump(s)
- Autosampler
- Column oven
- Derivatizer (e.g. UVE™)
- Fluorescence detector

**[Images]**
Sample preparation with large volume injection

**FREESTYLE Basic** Automated and unattended handling of the immunoaffinity column:
- loading of a precise volume
- washing
- drying in case of AflaCLEAN SMART
- column transfer into the ThermELUTE module

**ThermELUTE module** heating = breaking the antigen-antibody bond
- elution of the analytes with water and
- large volume injection into HPLC sample loop
**Analysis Instrumentation**

Any HPLC possible tested with DIONEX Ultimate 3000

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
<td>Isocratic mode</td>
</tr>
<tr>
<td>Autosampler</td>
<td>Only needed for standard injection</td>
</tr>
<tr>
<td>Column switching valve</td>
<td>If Aflatoxins and Ochratoxin A should be analyzed a switching valve is recommended</td>
</tr>
<tr>
<td>Column Oven</td>
<td>For two columns (Afla &amp; Ochra)</td>
</tr>
<tr>
<td>Column(s)</td>
<td>Standard guard and analytical columns</td>
</tr>
<tr>
<td>Derivatizer</td>
<td>UV derivatizer, photochemical derivatization</td>
</tr>
<tr>
<td>Fluorescence detector</td>
<td>Standard</td>
</tr>
<tr>
<td>Software</td>
<td>Standard</td>
</tr>
</tbody>
</table>
Dionex Ultimate 3000 details

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Pump</strong></td>
<td>LPG 3400a</td>
</tr>
<tr>
<td><strong>Sampler</strong></td>
<td>WPS 3000 TSL</td>
</tr>
<tr>
<td><strong>Mobile Phase</strong></td>
<td>Methanol/Water/Acetonitrile, 30/60/15</td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>1.2 mL/min</td>
</tr>
<tr>
<td><strong>Column Oven</strong></td>
<td>36 °C</td>
</tr>
<tr>
<td><strong>Column</strong></td>
<td>LCTech HPLC column (LCTech P/N 10522)</td>
</tr>
<tr>
<td><strong>Fluorescence detector</strong></td>
<td>RF 2000, FLD</td>
</tr>
<tr>
<td><strong>Fluorescence wavelength</strong></td>
<td>365 nm excitation, 460 nm emission</td>
</tr>
<tr>
<td><strong>Derivatizer</strong></td>
<td>LCTech UVE</td>
</tr>
</tbody>
</table>
The goal:
Completely automated system for Aflatoxin and/or Ochratoxin A analysis

Step 1:
Replace 1 mL or 3 mL immunoaffinity columns with SMART Immunoaffinity columns
What is so smart about SMART columns?

**Original size:**

25 mm

They are completely comparable to 1 mL or 3 mL immunoaffinity columns.

But,
- less time is needed
- less sample is needed
- less solvent is needed
- Less waste

And
- toxin concentration is equivalent to 1 mL or 3 mL format
SMART columns - advantages

What is so smart about SMART columns?

They meet AOAC requirements

**Afla CLEAN™ SMART**

- Loading capacity: 100 ng B1
- Recovery > 80%

**Ota CLEAN™ SMART**

- Loading capacity: 100 ng Ochratoxin A
- Recovery > 80%
What is so smart about SMART columns?

Reduced Cleanup time

- Incredible time reduction
  - less sample volume = less time for sample loading
  - high flow rate

Cleanup process down to less than 9 minutes
Cleanup process down to less than 13 minutes
SMART columns - advantages

What is so smart about SMART columns?

They are applicable for all regulated matrices

All kinds of nuts:
- Hazelnut
- Walnut
- Brazil nut
- Peanut
- Etc.

Pistachios, almonds, raisins, figs

All kinds of cereals, corn, rice

All common spices

etc.

All kinds of coffee
- Red wine
- Beer
- Raisins
- Liquorice
- Etc.
The goal:
Completely automated system
for Aflatoxin and/or Ochratoxin A analysis

Step 1:
Replace 1 mL or 3 mL immunoaffinity columns by SMART Immunoaffinity columns

Step 2:
High end Completely automated solution with FREESTYLE and ThermELUTE module
Completely automated solution

- SPE Gripper
- Rack for SMART columns
- ThermELUTE module
- Any HPLC
Completely automated solution

ThermELUTE, standard version (p/n 13572)

Heating element

Injection valve

Width: 110 mm
Completely automated solution

Rack (p/n 13497) for 60 SMART columns

Rack (p/n 13497) in tray (p/n 11915) for SMART columns
ThermELUTE with Standards

- Cooled rack for standards
- Extraction and direct injection system
- Injection port
- Injection valve
- SMART Column Gripper
ThermELUTE module

Rack for up to 60 SMART columns
SMART Column Gripper
How the system operates:

SMART Column gripper is taken up by SPE tool
How the system operates:

gripper takes up the SMART column

Unused columns

Used columns
How the system operates:

The SMART column is now tightly connected to the system and can be manipulated by positive pressure control.
How the system operates:

After handling steps (loading, washing, drying...) column is transferred into heating element.
How the system operates:

Column is transferred into heating element

The antigen-antibody bond is broken by the heat and the toxins are eluted with water. No organic solvent is required.
SMART columns - advantages

Normally you elute with organic solvent; either methanol or acetonitrile. This destroys the antigen-antibody bond and elutes the toxins. The disadvantage is that you cannot inject high volumes of methanol onto the Reverse Phase column. You usually have to evaporate your cleaned sample and re-suspend in a small volume prior to injection onto the Reverse Phase column.

When you are eluting with water you are injecting all the toxin that is bound to the SMART column (~700ul) directly onto the Reverse Phase column.

Original size: 25 mm
How the system operates:

Column is now in the heating element

The toxins are eluted into the injection valve after thermal antigen-antibody bond cleavage.

Sample was eluted as a partial loop fill with 400 µL into 700 µL sample loop
Elution followed by whole sample large volume injection

- **No loss of analytes. No storage between cleanup and analysis**
- **Large volume injection in water / all analytes are injected straight from the column**
  - No evaporation
  - No adsorption by the glass wall of storage vials
  - No loss of recovery = highest sensitivity
- **Less sample required to stay within FLD range.**
  - Less matrix on the immunoaffinity column
  - Faster sample clean-up
Advantages

Elution followed by whole sample large volume injection

- Suitable for very strict detection limits for baby food

<table>
<thead>
<tr>
<th>Maximum level according to EU Regulation 1881/2006 *</th>
<th>Detection limit with FREESTYLE + ThermELUTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1: 100 ppt</td>
<td>Aflatoxin B1: 5 ppt</td>
</tr>
<tr>
<td>Ochratoxin A: 500 ppt</td>
<td>Ochratoxin A: 30 ppt</td>
</tr>
</tbody>
</table>

* COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
Advantages

Elution followed by whole sample large volume injection

- Single-use AflaCLEAN and OtaCLEAN SMART Cleanup columns
  - 100% initial performance of SMART columns for all samples
  - No cross contamination

= Perfect analytical conditions
Increased sample throughput by parallelization of sample preparation and analysis

- FREESTYLE sample preparation time: 15 min* / sample
- HPLC analysis time: 15 min* / sample

* Ochratoxin A; Aflatoxin even faster
Completely automated solution - advantages

- Throughput at its best

  15 min / sample
  4 samples per hour
  96 samples per day

<table>
<thead>
<tr>
<th></th>
<th>Monday</th>
<th>Tuesday</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Jul</td>
<td>96 Samples</td>
<td>96 Samples</td>
</tr>
</tbody>
</table>

Monday – Sunday
Day and night
Completely automated solution - advantages

- **Loading capacity:**
  - 90 samples
  - (1 SMART column / sample
  - & samples presented in 16 mL vials)

- **Quantity of samples**
  - can be increased by reduction of sample volume and by choosing smaller vials.
Main advantages

- Completely automated
- No cross contamination
- Higher sensitivity (15 – 20x)
- Faster analysis
- Meets AOAC requirements

Shown in the chromatograms on the following slides
ThermELUTE – chromatograms

- Higher sensitivity compared to manual cleanup processing
- Excellent chromatograms (less sample needed)

**Ginger (10 ppb) with ThermELUTE, 2.8 mL sample**

**Ginger (10 ppb), manual processing, 14 mL sample**
Higher sensitivity compared to manual cleanup processing

Excellent chromatograms (less sample needed)

**Spices: Paprika**

Paprika (10 ppb) with ThermELUTE, 2.8 mL sample

Paprika (10 ppb), manual processing, 14 mL sample
ThermELUTE – chromatograms

- Higher sensitivity compared to manual cleanup processing
- Excellent chromatograms (less sample needed)

**Spices: Nutmeg**

Nutmeg (10 ppb) with ThermELUTE, 2.8 mL sample

Nutmeg (10 ppb), **manual processing**, 14 mL sample
ThermELUTE – chromatograms

- Higher sensitivity compared to manual cleanup processing
- Excellent chromatograms (less sample needed)

**Coriander (10 ppb) with ThermELUTE, 2.8 mL sample**

**Coriander (10 ppb), manual processing, 14 mL sample**
- Even low levels are detected. Not detected with manual processing

Hazelnut, not spiked

Black:
ThermELUTE detects even lowest level

Red:
Not detected with manual processing
Even low levels are detected. Not detected with manual processing.

Peanut, not spiked

**Black:**
ThermELUTE detects even lowest level

**Red:**
Not detected with manual processing
ThermELUTE – chromatograms

- Less sample needed, thus faster processing

Corn, 10 ppb
Black:
ThermELUTE, 2.5 mL
Red:
ThermELUTE, 1 mL
Orange:
Manual processing, 3 mL
Less sample needed, thus faster processing

**Hazelnut, 10 ppb**

**Black:**
ThermELUTE, 2.5 mL

**Red:**
ThermELUTE, 1 mL

**Orange:**
Manual processing, 3 mL
Less sample needed, thus faster processing

Raisins

- **Black**: ThermELUTE, 2.5 mL
- **Red**: ThermELUTE, 1 mL
- **Orange**: Manual processing, 3 mL
ThermELUTE – chromatograms

- Less sample needed, thus faster processing

**Pistachio, 10 ppb**

**Black:**
ThermELUTE, 2.5 mL

**Red:**
ThermELUTE, 1 mL

**Orange:**
Manual processing, 3 mL
ThermELUTE – chromatograms

- Less sample needed, thus faster processing

Nuts: Peanut

**Peanut, 10 ppb**

**Black:**
ThermELUTE, 2.5 mL

**Red:**
ThermELUTE, 1 mL

**Orange:**
Manual processing, 3 mL
Chromatogram of Peanuts

- Spiked with 10ppb total aflatoxin (G2/G1/B2/B1) (1ng G2; 4ng G1; 1ng B2; 4 ng B1) extracted according to LCTech extraction and clean-up procedure, **10 mL processed using AflaCLEAN SMART and ThermELUTE**.
- Chromatogram represents **0.28 gram matrix equivalents**.

**Clean-up time consists of**
- **10 mL sample loading** (3mL/min)
- **2 mL washing** (3mL/min)
- **6 min ThermELUTE treatment**

**Ready to inject within 10 min.**
Chromatogram of Peanuts

- Spiked with 10ppb total aflatoxin (G2/G1/B2/B1) (1ng G2; 4ng G1; 1ng B2; 4ng B1) extracted according to LCTech extraction and clean-up procedure, **2.5 mL processed using AflaCLEAN SMART and ThermELUTE.**
- Chromatogram represents **0.07 gram matrix equivalents.**

**Clean-up time consists of**
- **2.5 mL sample loading** (3 mL/min)
- **2 mL washing** (3mL/min)
- **6 min ThermELUTE treatment**

**Ready to inject within 7.5 min.**
Spiked with 10ppb total aflatoxin (G2/G1/B2/B1 (1ng G2; 4ng G1; 1ng B2; 4 ng B1) extracted according to LCTech extraction and clean-up procedure, **10 mL processed using AflaCLEAN SMART** and **ThermELUTE**.

- Chromatogram represents **0.28 gram matrix equivalents**.

**Clean-up time consists of**
- **10 mL sample loading** (3mL/min)
- **2 mL washing** (3mL/min)
- **6 min ThermELUTE treatment**

**Ready to inject within 10 min.**
Spiked with 10ppb total aflatoxin (G2/G1/B2/B1) (1ng G2; 4ng G1; 1ng B2; 4ng B1) extracted according to LCTech extraction and clean-up procedure, 2.5 mL processed using AflaCLEAN SMART and ThermELUTE.

Chromatogram represents 0.07 gram matrix equivalents.

Clean-up time consists of:
- 2.5 mL sample loading (3 mL/min)
- 2 mL washing (3 mL/min)
- 6 min ThermELUTE treatment

Ready to inject within 7.5 min.
10 mL instant coffee sample after extraction using methylene chloride partitioning, spiked at the regulated level of 10 ppb.

- The chromatogram represents 0.01 gram matrix equivalents.
- By using PICKERING post-column derivatization sensitivity could be increased by a factor of approx. 4 and chromatography (injection peak/matrix interference could be dramatically reduced).
10 mL roasted coffee sample after extraction using methylene chloride partitioning, spiked at the regulated level of 5 ppb.

- The chromatogram represents 0.05 gram matrix equivalents.
10 mL White Wine sample extracted according to LCTech OtaCLEAN SMART extraction and clean-up procedure loaded onto OtaCLEAN SMART column, spiked at the regulated level of 2 ppb.

The chromatogram represents 1 gram matrix equivalents.
10 mL Beer sample extracted according to LCTech OtaCLEAN SMART extraction and clean-up procedure loaded onto OtaCLEAN SMART column, spiked with 1 ppb. The chromatogram represents 1.42 gram matrix equivalents.
Completely automated solution - further features

- SPE Module
- GPC Module
- EVAporation Module
- ThermELUTE Module
www.freestyle-robotic.com
www.chromspec.com
sales@chromspec.com
david.mazawa@pickeringlabs.com