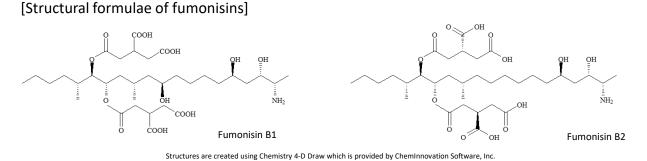
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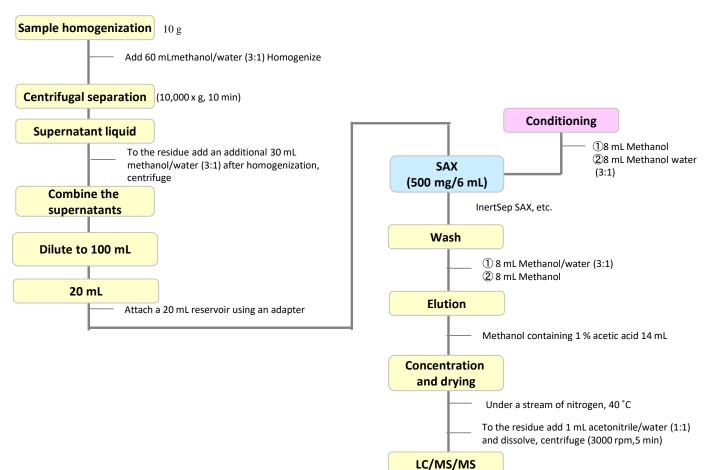
## **Analysis of Fumonisins in Cereals**

Fumonisins B1 and B2 are mycotoxins found in cereals and are typically analyzed using HPLC with fluorescence detection or LC/MS (/MS). Sample pretreatment, is commonly made using a strong anion exchange phase (SAX) because fumonisin has several carboxylic acids in its molecular structure. However, extraction with SAX alone may result in low recoveries for the samples with a high lipid content or a complex composition e.g.beer. These sample types also require pretreatment to remove lipids and other contaminants with non-polar phases such as C18 prior to treatment with the SAX phase. Two extraction methods are introduced in this application note , the first using SAX only, and the second using C18 and SAX in tandem.

## 1. Flow diagram of solid phase pretreatment



### [Example of pretreatment of samples such as corn with low lipid content]



Reference: Method 15 AOAC 995.

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[Examples of Pretreatment of Contaminated Malt, Beer and Lipid-Rich Grains]

| Sample Homogeniz   | zation 10 g   |
|--------------------|---|
|                    | Add 15 mL of water and allow to stand for 15 minutes.   |
|                    | Homogenize with the addition of methanol 45 mL  |
|                    | (After 5 minutes of deaeration,<br>Add 0.5 mL of 0.2 % ammonia-water and<br>Add 10 mL of water, mix, and transfer to a mini<br>column.  |
| Centrifugal separa | ation (10,000 x g, 10 min)  |
| Supernatant liqu   | uid   |
|                    | To the residue add an additional 30 mL of methanol/water (3:1), then homogenize and centrifuge  |
| Combine the superr | natants   |
| Dilute to 100 m    | nL  |
|                    |   |
| 20 mL              |   |
|                    | Attach a 20 mL reservoir with an adapter  |
| C18 (500 mg)       | InertSep LSC C 18, etc.   |
| SAX (500 mg)       | Used together   |
| 54X (500 mg)       | InertSep SAX, etc.  |
| Wash               |   |
| (                  | <ol> <li>10 mL Water</li> <li>10 mL of Methanol/0.2 % ammonia solution (3:1)</li> <li>Remove the C18 cartridge from the top of the SAX and attach a 6 mL reservoir</li> </ol> |
| SAX                |   |
| ЗАЛ                |   |
| Wash               |   |
|                    | ① 5 mL of Methanol/water (3:1)<br>② 5 mL Methanol   |
| Elution            |   |
|                    | Methanol containing 1% acetic acid 14 mL  |
| Concentration and  | d dry (Under a stream of nitrogen, 40 °C ).   |
|                    | Fo the residue add acetonitrile/water (1:1) 1 mL<br>Add and dissolve, centrifuge (3000 rpm, 5 min)  |
| LC/MS/MS           |   |
| ,,                 |   |

Reference: Method 15 AOAC 995.

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### 2. Products for solid-phase extraction

### [InertSep SAX]

[InertSep C18]

Mean particle

size

Carbon

End-caps Surface Area

Pore size

use

Pore volume

PH range of

Si-(CH<sub>2</sub>)17CH<sub>3</sub>

: 60 µm

: 19 %

: 450 m<sup>2</sup>/g

: 0.7 mL/g

: 60 Å

: 2-8

[Empty reservoir]

: Highly End-Capping

| Si-(CH <sub>2</sub> ) <sub>3</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>  |  |   |  |  |
|--|--|---|--|--|
| Mean particle<br>size<br>Carbon<br>Surface Area<br>Pore volume<br>Pore size<br>Ion exchange<br>capacity<br>PKa<br>PH range of use<br>Remarks |  | 45 μm<br>10 %<br>450 m²/g<br>0.7 mL/g<br>60 Å<br>0.7 meq/g<br>(-)<br>2 - 8<br>OH - ion pair |  |  |

InertSep SAX is a solid phase in which a trimethylaminopropyl group is attached to silica gel. The primary interaction has a strong anion exchange action and, as a secondary interaction, a weak nonpolar interaction. It is commonly used to extract weak anionic substances such as carboxylic acids. Syringe barrel type cartridge

| Produc  | t name        | Column size   | Qty.       | Cat.No.    |
|---|---------------|---------------|------------|------------|
| InertSep SAX Recommendation<br>for this study | 200 mg / 3 mL | 50 bottles    | 5010-61642 |            |
|   |               | 500 mg / 3 mL | 50 bottles | 5010-61643 |
|   |               | 500 mg / 6 mL | 30 bottles | 5010-61644 |
|   |               | 1 g / 6 mL    | 30 bottles | 5010-61645 |
|   | 2 g / 12 mL   | 20 bottles    | 5010-61646 |            |

#### Luer device cartridge

| Product name |                    | Column size                      | Qty.    | Cat.No.    |            |
|--------------|--------------------|----------------------------------|---------|------------|------------|
|              | InortCon Clim LCAV | Recommendation<br>for this study | 500 mg  | 50 bottles | 5010-65640 |
|              | InertSep SlimJ SAX |                                  | 1000 mg | 50 bottles | 5010-64641 |

InertSep C18 is a solid phase with non-polar interactions in which octadecyl groups are chemically bonded to silica gel. Advanced end-capping prevents cation-exchange interactions with silanol groups, resulting in reduced adsorption of basic compounds. It is suitable as a clean-up solid phase for removing lipids in the concurrent testing method for residual pesticides.

#### Large size cartridge LSC

| Product name     |                                  | Column size | Qty.       | Cat.No.    |
|------------------|----------------------------------|-------------|------------|------------|
| InertSep LSC C18 |                                  | 100 mg      | 50 bottles | 5010-63001 |
|                  | Recommendation<br>for this study | 200 mg      | 50 bottles | 5010-63002 |
|                  | tor this study                   | 500 mg      | 50 bottles | 5010-63003 |

| Product name  |                                  | Specification     | Qty.       | Cat.No.    |
|---|----------------------------------|-------------------|------------|------------|
|   | Recommendation<br>for this study | 20 mL             | 20 bottles | 5010-60104 |
| Empty reservoir (PP)<br>No frits                            |                                  | 60 mL             | 10 bottles | 5010-60105 |
|   |                                  | 150 mL            | 10 bottles | 5010-60106 |
|   |                                  |                   |            |            |
| Product name  | Specification                    | Qty.              | Cat.No.    |            |
| Solid-phase extraction cartridge<br>Connection adapter (PP) | In this study<br>Rinse           | For LSC reservoir | 12         | 5010-60004 |

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GL Sciences, Inc. Japan 22-1 Nishishinjuku 6-Chome Shinjuku-ku, Tokyo, 163-1130, Japan Phone: +81-3-5323-6620 Fax: +81-3-5323-6621 Email: world@gls.co.jp Web: www.glsciences.com GL Sciences B.V. De Sleutel 9 5652 AS Eindhoven The Netherlands Phone: +31 (0)40 254 95 31 Email: info@glsciences.eu Web: www.glsciences.eu

### <u>GL Sciences (ShangHai) Ltd.</u>

Tower B, Room 2003, Far East International Plaza, NO,317 Xianxia Road, Changning District. Shanghai, China P.C. 200032 Phone: +86 (0)21-6278-2272 Email: <u>contact@glsciences.com.cn</u> Web: www.glsciences.com.cn <u>GL Sciences, Inc. USA</u>

4733 Torrance Blvd. Suite 255 Torrance, CA 90503 Phone: 310-265-4424 Fax: 310-265-4425 Email: info@glsciencesinc.com Web: www.glsciencesinc.com

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