Waters

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Sample Prep Notes



Rapid, Accurate Quantitation of Fumonisins in Corn, Poultry Feeds & Fusarium Culture Material Using Sep-Pak® C₁₈ Cartridges

Nasty Mold

Researchers at the U.S. Dept. of Agriculture and Iowa State University have developed an excellent HPLC method for the analysis of three fumonisins $[FB_1, FB_2, FB_3]^1$. High levels of these mycotoxins, present in corn-based feeds, cause equine leukoencephalomalacia [ELEM] and porcine pulmonary edema syndrome [PPE]. FB_1 and FB_2 induce ELEM, PPE, and liver cancer in rats. They have also been linked to human esophageal cancer.

Analytical Method

An optimized sample preparation protocol using Sep-Pak C_{18} Cartridges* isolated the analytes prior to pre-column derivatization with o-phthalaldehyde/mercaptoethanol and HPLC analysis using fluorescence detection. Method performance is indicated by the following data:

Recovery. $FB_1 - 91-94\%$; $FB_2 - 90-100\%$; $FB_3 - 81-93\%$

Precision on field samples ranged from 2% CV at 19 μ g/g of FB₁ to 9% CV at 0.17 μ g/g of FB₃. *Quantitation limits*: 0.1 μ g/g (FB₁); 0.2 μ g/g (FB₂ & FB₃)

Linear Range. 70 pg to 12 ng with $R^2 = 0.99998$

Importance of Cartridge Brand

The authors identified the SPE cartridge as the "most critical factor" in the sample prep protocol. They stated that "the brand of SPE cartridges can significantly affect performance." Recovery from a second brand of cartridge was only 60% of that obtained with Sep-Pak Cartridges. Waters cartridges "from 5 different lots, manufactured over 15 years [1978-1993!], yielded excellent agreement (n=2)" with essentially quantitative recovery and CV's ranging from 1.2% to 4.5% for FB₁ at 1.8 $\mu g/g$.

Sample Prep Protocol

Grind & weigh 10 g sample

Add 50 mL 50:50 ACN: H₂O; agitate for 30 min

Filter 10 mL of supernatant

Condition Sep-Pak C₁₈ Cartridge w/ 2 mL ACN, then 2 mL of 1% aq. KCl

Mix 2 mL of filtrate & 6 mL of 1% KCl in syringe. Push solution through cartridge.

Discard eluate.

Wash cartridge w/2 mL 1% KCI, then 2 mL 15:85 ACN:H₂O. Discard eluates.

Pass air through cartridge to expel remaining liquid.

Elute FB₁, FB₂, FB₃ w/2 mL 70:30 ACN:H₂O

Mix 0.25 mL each of eluate, borate buffer, OPA/ME solution, & H₂O in 1.5 mL autosampler vial. Shake & react for 10 min.

Inject 10µL into HPLC system.

*Sep-Pak Classic C₁₈ Cartridges (360 mg): Box of 50 - P/N WAT051910

Alternatively:

Sep-Pak Plus C_{18} Cartridges (360 mg): Box of 50 - P/N WAT020515 Sep-Pak Vac 3cc C_{18} Cartridges (500 mg): Box of 50 - P/N WAT020805

See Waters Solid Phase Extraction Applications Guide and Bibliography, Sixth Edition, P/N WAT082853, for additional references to isolation of fumonisins.

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¹ Rice, LG, Ross, PF, Dejong, J, Plattner, RD, & Coats, JR, *J. AOAC Intl*, **78(4)**(1995) 1002-1009 [SP95002].