

Determination of Fumonisin in Corn

Fumonisin is a type of toxin produced by fusarium molds that may be detected on corn and rice plants, etc. These substances are of great concern as they are known to cause leukoencephalitis in horses, and are presumed to be causative agents in human esophageal cancer^{1,2}.

Since fumonisins contain a primary amino group in their structure, a widely used method of analysis consists of fluorescence derivatization with o-phthalaldehyde (OPA), followed by HPLC analysis¹⁻³.

Because this reaction progresses very quickly by merely mixing the sample with the reagent at room temperature, it can be performed automatically using an autosampler equipped with reagent mixing capability.

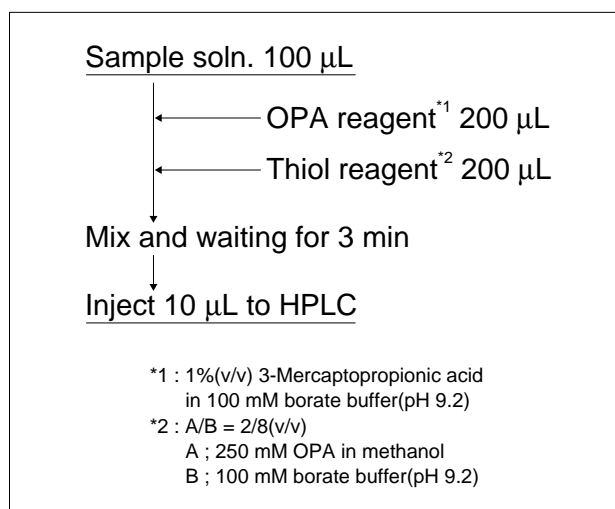
Here we introduce a pre-column derivatization-HPLC method for the determination of fumonisin B₁ and B₂ using OPA and 3-mercaptopropionic acid as the reagent.

■ Analysis of Standard Solution

The pre-column derivatization procedure is shown in Scheme 1. Although 2-mercaptoethanol is most often used as the nucleophilic reagent essential for the reaction with OPA, 3-mercaptopropionic acid was used here in consideration of the stability of the derivative substances and separation of the inherent reagent peaks.

The HPLC analytical conditions used when injecting the derivatives are shown in Table 1. As for the mobile phase solution, a pH in the neutral vicinity provides somewhat higher detection sensitivity for the derivatives, however, here we used a weakly acidic buffer solution to address the separation of the contaminant peaks and durability of the packing material.

Fig.1 shows the chromatogram obtained from analysis of the fumonisin B₁ and B₂ standards using these conditions. The detection limit with this method is about 10 ng/mL (S/N=3, fumonisin B₁).



Scheme 1 Pre-column Derivatization Procedure

Table 1 Analytical conditions

Column	: STR ODS-II (4.6 mm I.D.×150 mmL.)
Mobile phase	: 50 mM (Sodium) citrate buffer (pH4.3) /methanol (3/7,v/v)
Flow rate	: 1.0 mL/min
Temperature	: 40 °C
Detection	: RF-10AXL EX335 nm, Em440 nm

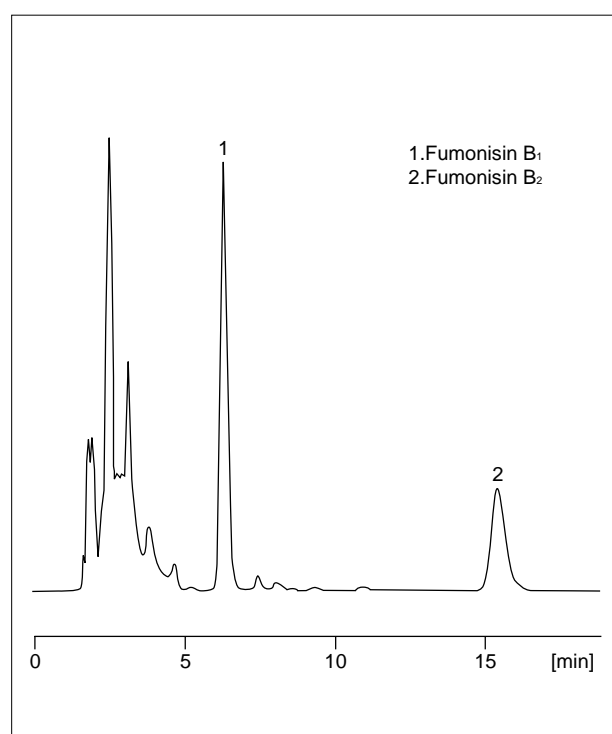


Fig.1 Chromatogram of A Mixture of Fumonisin B₁ and B₂

■ Repeatability

Table 2 shows the repeatability of the peak areas obtained when the derivatization reaction operation was conducted manually and when it was conducted automatically using an autosampler. The autosampler used here was the SIL-10A*. The peak area values of the derivatives vary according to the elapsed time from the start of the reaction to the injection into the HPLC. Therefore, it is an essential condition that this time be accurately controlled. The results of Table 2 indicate that if this condition is upheld, the repeatability results obtained are about the same, whichever the method used.

Table 2 Repeatability

	Manual		Auto	
	Fumonisin B ₁	Fumonisin B ₂	Fumonisin B ₁	Fumonisin B ₂
1st	901,223	438,948	828,190	390,550
2nd	958,536	462,300	864,625	406,800
3rd	938,502	453,239	884,637	419,995
4th	916,852	441,436	868,233	410,227
5th	947,335	454,586	857,297	406,022
mean	932,490	450,102	860,596	406,719
s.d.	23,222	9,724	20,698	10612
c.v.	2.49%	2.16%	2.41%	2.61%

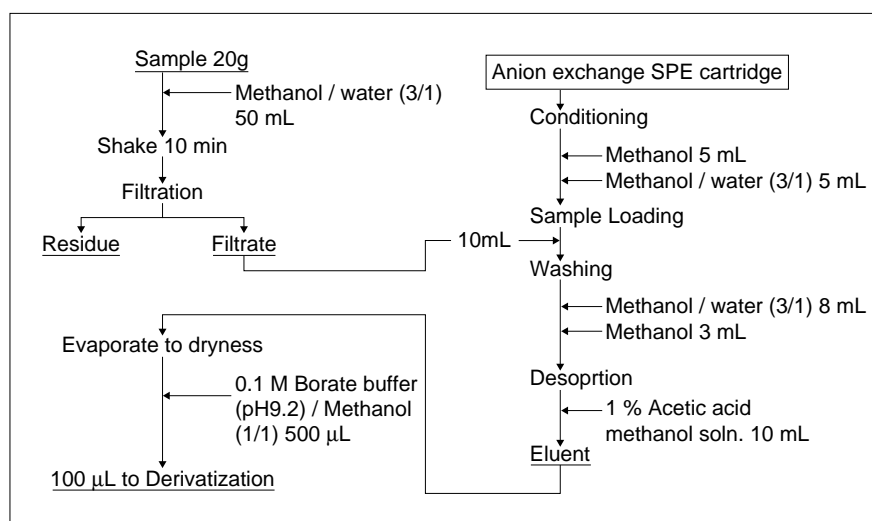
* The SIL-10AD_{VP} and the SIL-20A are not applicable for this pre-column derivatization method.

■ Analysis of Corn

Scheme 2 shows the pretreatment procedure for a corn sample. The compounds of interest were extracted using a methanol/water solution, and then cleaned up using an anion exchange resin-packed solid phase extraction cartridge. Here we used the J. T. Baker "Bakerbond spe Quaternary amine" (3 mL capacity) solid phase extraction cartridge.

After evaporating to dryness the eluate from the solid phase extraction cartridge, we re-dissolved it in a borate buffer/methanol mixed solution and submitted it to the derivatization reaction. Since the derivatization reaction using OPA proceeds quantitatively at pH9 or greater, completely removing the acetic acid by evaporation and re-dissolving it in buffer solution is recommended for the subsequent derivatization process.

Fig.2 shows the chromatogram obtained from analysis of the corn sample pretreated using the above procedure.



Scheme 2 Pretreatment of Corn Sample

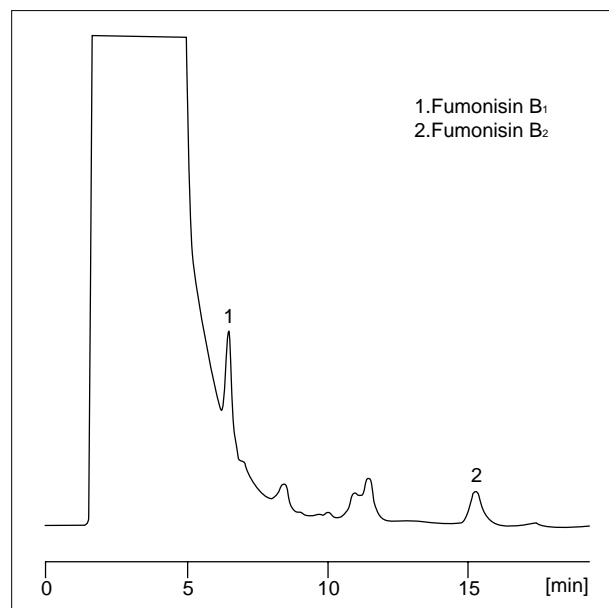


Fig.2 Chromatogram of Corn Sample

References

- 1) Kamimura: Food Chemical Monthly, 1994-1,94-103 (1994)
- 2) Kamimura: Food Chemical Monthly, 1996-4, 41-48(1996)
- 3) Shephard et al. : J. Chromatogr., 13(10),2077-2087(1990)



SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax: 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO