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LCMS-2050 High Performance Liquid Chromatograph Mass Spectrometer

Application News

Analysis of Fumonisin in Livestock Feed Blend Using a Single Quadrupole Mass Spectrometer

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User Benefits

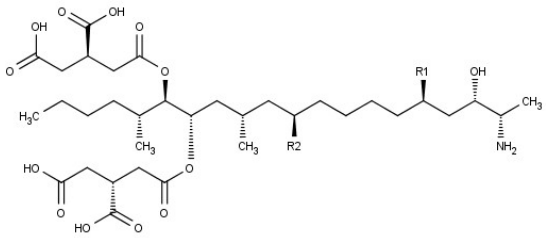
- LCMS-2050 single quadrupole mass spectrometer enables highly selective fumonisin analysis.
- All compounds of fumonisins B1, B2, and B3 can be analyzed with good accuracy and repeatability.
- Carryover can be reduced using multi-rinse function equipped in autosampler.

■ Introduction

Fumonisin[1] (Fig.1) is one of mycotoxins derived from the genus Fusarium (*Fusarium*, red mold). Four analogues of fumonisin, fumonisin B1, B2, B3, and B4 have been reported. Fumonisins B1, B2, and B3 (hereinafter referred to as FB1, FB2, and FB3, respectively) are the most natural contaminants, and have been paid attention because of their effects on livestock, such as leukoencephalopathy in horses and pulmonary edema in pigs, as well as their reported teratogenicity in the neural tube of newborn infants in areas where processed corn is the main food source[1].

The Ministry of Agriculture, Forestry and Fisheries (MAFF) has set a control standard value of 4 mg/kg for fumonisin (FB1 + FB2 + FB3) in feed as an index to confirm the effect of reducing harmful substances through GMP and other process controls by feed manufacturers[1].

This paper article describes an analysis of FB1, FB2, and FB3 in livestock feed blend using a high performance liquid chromatograph-mass spectrometer LCMS-2050.



Fumonisin B1 (FB1)	R1=OH	R2=OH
Fumonisin B2 (FB2)	R1=OH	R2=H
Fumonisin B3 (FB3)	R1=H	R2=OH

Fig.1 Structural formulae of fumonisins

■ Analysis of standard solution

Mixed standard solutions for HPLC analysis were prepared by diluting commercial fumonisin mixed standard solutions [50 µg/mL each of B1 and B2 in acetonitrile/water=1:1 solution] and fumonisin B3 solution [50 µg/mL acetonitrile/ water=1:1 solution] (FUJIFILM Wako Pure Chemicals Corporation) with dilution solution (water/acetonitrile=1:1). Eight mixed standard solutions containing 1-1000 µg/L each of FB1, FB2, and FB3 were prepared. Fig. 2 shows a chromatograms (100 µg/L) of the mixed standard solutions.

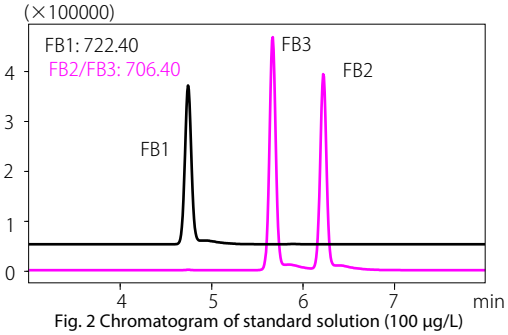


Fig. 2 Chromatogram of standard solution (100 µg/L)

■ Analytical conditions

The analytical conditions are shown in Tables 1 and 2.

In order to reduce carryover[2] of fumonisins, SIL-40C X3 autosampler, which equips a multi-rinsing function, was used to clean inside of the needle. A four-component mixture of isopropanol, methanol, acetonitrile, and formic acid aqueous solution, which has strong rinsing power against hydrophobic compounds, and sodium citrate aqueous solution, which is expected to form chelates with metals and inhibit adsorption of analytes to metals. In the blank analysis, carryover was reduced to 0.001~0.005%.

Table 1 HPLC conditions

System	:	Nexera™ X3
Column	:	Shim-pack™ GISS-HP C18 (150 mm × 2.1 mm I.D., 3 µm) *1
Mobile Phase A	:	0.1% formic acid aq.
Mobile Phase B	:	Acetonitrile
Time Program	:	30% B(0 min)→50% B(5-8 min)→95% B(8.01 -10 min)→30% B(10.01 min)
Flow Rate	:	0.2 mL/min
Column Temp.	:	40 °C
Injection Vol.	:	3 µL
Needle rinse conditions	:	R0: Acetonitrile /water=1:1 R1: 10 mM sodium citrate aq. R2: 1%formic acid aq. /Methanol/Acetonitrile/ isopropyl alcohol = 1:1:1:1 Rinse type : needle inner and outer surface rinse Needle rinse program: needle inner surface rinse: R1 –R2 – R0 needle outer surface rinse: R2

*1 P/N : 227-30084-03

Table 2 MS conditions

Ionization	:	ESI/APCI (DUIS™), Positive mode
Mode	:	SIM (FB1: *m/z* 722.40、FB2/FB3: *m/z*706.40)
Nebulizing gas flow	:	1.5 L/min
Drying gas flow	:	3.0 L/min
Heating gas flow	:	5.0 L/min
Desolvation temp.	:	500 °C
DL temp.	:	200 °C
Interface voltage	:	+1.0 kV

■ Linearity

An eight-point calibration curve (1, 5, 25, 50,100, 250, 500, 1000 µg/L) was created for FB1, FB2, and FB3. Good linearity was obtained with a coefficient of determination r2 = 0.998 or higher for each fumonisin (Fig. 3).

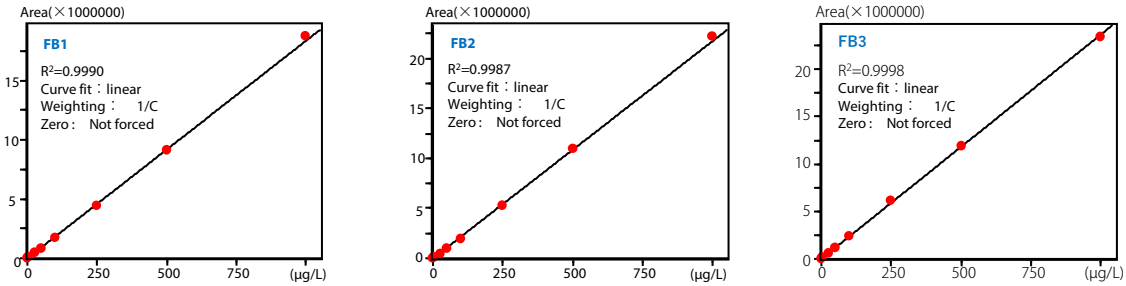


Fig.3 Calibration curves for FB1, FB2, and FB3

■ Analysis of feed blend

Livestock feed blend was pretreated as shown in Fig. 4, referring to "Analytical Standards, Simultaneous analysis of fumonisin by liquid chromatography/ mass spectrometry" [3] supervised by the incorporated administrative agency Food and Agricultural Materials Inspection Center (FAMIC). The chromatograms of the feed blend are shown in Fig. 5, and the quantitative results are shown in Table 3.

In the spike and recovery test, 40 µL of mixed standard solution (50 mg/L each) was spiked to 20 g of feed blend (spiked concentration was 0.1 mg/kg for all fumonisins), and the pretreatment shown in Fig. 4 was performed five times. In each run, obtained respective concentrations of fumonisins in the spiked feed blend were subtracted by those originally contained in the feed blend as shown in table 3. The ratios of the averages of the calculated concentrations to the spiked concentration were determined to evaluate the accuracies. In the repeatability test, respective relative standard deviations of the concentrations of five test solutions were evaluated as the repeatabilities. The results of the spike and recovery test and reproducibility test are shown in Table 4. Good results were obtained in both tests.

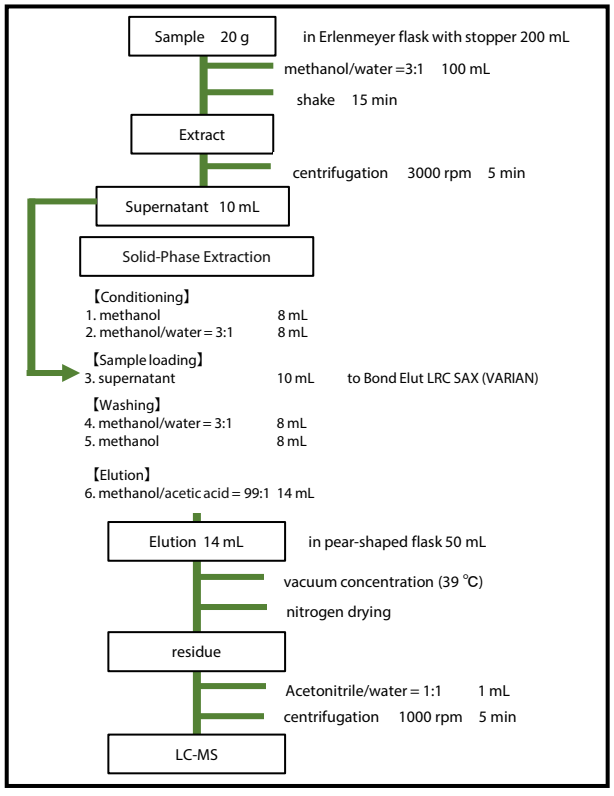


Fig. 4 Pretreatment procedures

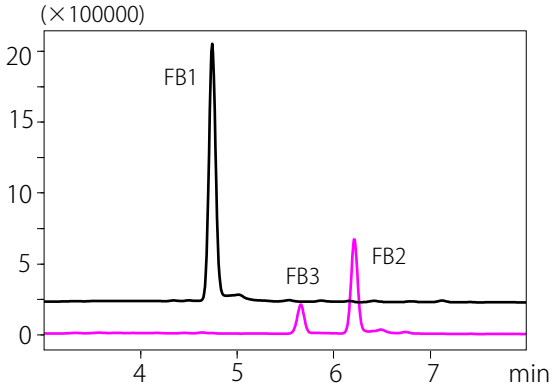


Fig. 5 Chromatogram of feed blend

Table 3 Quantitative results

	Concentration in tested solution (µg/L)	Concentration in feed blend (mg/kg)
FB1	510.4	0.26
FB2	161.2	0.08
FB3	46.9	0.02

Table 4 Results of spike and recovery test and repeatability test

	Accuracy(%)	Repeatability (%RSD)
FB1	99.2	2.9
FB2	97.2	2.9
FB3	101.9	3.2

■ Conclusion

Fumonisin analysis was performed using a single quadrupole mass spectrometer LCMS-2050. Good accuracies and repeatabilities were obtained for FB1, FB2, and FB3 in the feed blend.

The multi-rinse function of the SIL-40C X3 enabled to reduce carryover.

<References>

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2) M. Tamura, N. Mochizuki et. al., J.Sep. Sci. Vol.37（13）, 1552-1560（2014）

3) incorporated administrative agency Food and Agricultural Materials Inspection Center (FAMIC) , Analytical Standards, Simultaneous analysis of fumonisin by liquid chromatography/ mass spectrometry

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