Simple and rapid cleanup method for determination of aflatoxin B₁ in mixed feeds by Sep-Pak Florisil cartridge

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セップパックフロリジルカートリッジを用いた配合飼料中の
アフラトキシン B₁ 簡易迅速クリーンアップ法

Summary

A simple and rapid method is described for the determination of aflatoxin (AF) B₁ in mixed feeds. AF B₁ is extracted with a mixture of chloroform-0.1 N hydrochloric acid. The extract is cleaned up by using a Sep-Pak Florisil cartridge. AF B₁ is determined by high performance liquid chromatography (HPLC), using a μ Porasil column, a fluorescence detector, and a mobile phase of toluene-ethyl acetate-formic acid-methanol (89+7.5+2+1.5). The mean recoveries of AF B₁ spiked at levels from 10 to 40 ng/g in mixed feeds are in the range of 94.2~102.9%.

Key words: Aflatoxin B₁, アフラトキシン B₁; Mixed feeds, 配合飼料; Sep-Pak Florisil cartridge, セップパックフロリジルカートリッジ; HPLC, 高速液体クロマトグラフィー

Determination of aflatoxin (AF) B₁ in mixed feeds is already confirmed by official methods of feeds analysis in Japan,^{1,2)} that is high performance liquid chromatography (HPLC) method. However, the official method uses glass column with florisil at the cleanup. Therefore, the method requires more time and more 50 ml organic solvents as wash solvent at the column chromatography per one sample.

We developed simple and rapid cleanup method by using commercial florisil cartridge. At the same time, we could decrease the organic solvents volumes at the cleanup. This paper describes a simple and rapid HPLC method with purification on Sep-Pak Florisil cartridge for the determination of AF B₁ in various mixed feeds.

Materials and Methods

Materials An AF B₁-free swine, layer and broiler mixed feed was used in this study.

Experimental procedure Weigh 50 g sample ground fine enough to pass 1 mm mesh sieve, and place in 500 ml Erlenmeyer flask. Add 70 ml 0.1 N hydrochloric acid and 250 ml chloroform, and shake vigorously for 30 min. Filter extract through ca. 10 g diatomaceous earth filter aid in folded Toyo paper No. 2S (185 mm diameter) and place in 300 ml Erlenmeyer flask. Add ca. 10 g anhydrous

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sodium sulfate, and filter extract through folded Toyo paper No. 2 (185 mm diameter), and place in 200 ml Erlenmeyer flask.

Connect a Sep-Pak Florisil cartridge (Waters Associates, original type) to 50 ml glass syringe. Apply 50 ml filtrate to the cartridge (prewash the cartridge with 10 ml chloroform). Wash the cartridge with 10 ml chloroform-methanol (9+1), and elute with 50 ml acetone-water (99+1) into 200 ml round-bottom flask.

Evaporate in rotary evaporator to near dryness, and to dryness perfectly under stream of nitrogen. Dissolve residue in 2.0 ml benzene-acetonitrile (98+2). Filter through membrane filter (pore size 0.45 μ m) and transfer to 3 ml vial. (Scheme 1)

High performance liquid chromatography Analytical HPLC was carried out using μ Porasil (3.9 mm id $\times$ 300 mm long, Waters Associates).

As mobile phase, we used toluene-ethyl acetate-formic acid-methanol (89+7.5+2+1.5) that Manabe et al.³⁾ had published. Stabilize instrument with the mobile phase at flow rate of 1 ml/min for ca. 30 min.

Set excitation wavelength to 365 nm and emission wavelength to 425 nm.

Scheme 1. Analytical procedure for AF B₁ in mixed feeds.

Inject each 20 μ l AF B₁ working standard solutions (0.025, 0.05, 0.1, 0.2, 0.4 μ g/ml) and sample solution with accurate syringe.

Detection limit of AF B₁ is 1 ng/g.

Results and Discussion

Cleanup Kamimura et al.⁴⁾ reported that 30 ml chloroform-hexane (1+1) and 20 ml chloroform-methanol (9+1) were sufficient for removing oil and interferences from the florisil column. However by using Sep-Pak Florisil cartridge, we could get good separative peak of AF B₁ from interferences with only 10 ml chloroform-methanol (9+1). We could decrease the wash solvents volumes to only 10 ml by using a florisil cartridge. Therefore, we could determine AF B₁ more rapidly.

Elution pattern 50 ml acetone-water (99+1) quantitatively eluted AF B₁ from the florisil cartridge. Fig. 1 presents elution pattern of AF B₁ from the cartridge.

Recovery tests Broiler feeds were prepared by adding each 0.25, 0.5 and 1 ml AF B₁ standard solution (2000 μ g/ml) in chloroform. The AF B₁ concentrations in samples were each 10, 20 and 40 ng/g, respectively.

Fig. 1. Elution pattern of AF B₁ from the florisil cartridge.

AF B₁, 40 ng/g was spiked to a layer mixed feed.

Table 1. Recovery of AF B₁ added to mixed feed

Mixed feed	Added (ng/g)	Av. rec. (%)	CV (%)
Broiler	10	102.9 (99.4, 113.7, 95.6)	9.3
	20	94.2 (109.3, 93.3, 80.0)	15.6
	40	95.3 (96.6, 98.5, 90.8)	4.2

Table 1 gives recoveries of AF B₁ based on 3 determinations for 10, 20 and 40 ng/g added. The mean recoveries of AF B₁ added at levels from 10 to 40 ng/g are in the range of 94.2~102.9%.

Fig. 2 shows typical chromatograms of 4 ng AF B₁ standard and of a broiler mixed feed sample to which AF B₁ was added at the 40 ng/g level in recovery tests.

Finally, this investigation was carried out in Thailand as Japan International Cooperation Associate's National Animal Health and Production Institute project.

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Fig. 2. Typical chromatograms of AF B₁ standard (4 ng) and a broiler mixed feed extract with 40 ng/g added AF B₁.
See text HPLC conditions.

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