Application Note

Date: No. 748002H-E

Analysis of oxytetracycline in milk

The permissible levels of and methods used to analyze oxytetracycline (OTC), an antibiotic with a fodder additive and veterinary uses, in foodstuffs are controlled according to food hygiene laws1,2. According to the official protocol, OTC was added to a milk sample at the accepted reference level (0.1 ppm) and was analyzed following the pre-processing scheme of Fig. 1. Chromatograms of a standard sample and a milk sample spiked with standard are shown in Fig. 2.

< References >
1) Ministry of health and welfare ordinance 62 (26th December, 1995)
2) Ministry of health and welfare ordinance 218 (26th December, 1995)

addition of 0.1 ppm OTC to 10.0 g milk
↓
citric acid buffer solution **) 100 ml
↓
n-hexane 100 ml
↓
shake 5 min
↓
centrifuge (3500 rpm) 5 min
↓
aqueous layer -> n-hexane layer
↓
filter → 50mL
conditioning
↓
methanol 10 ml
water 10 ml
saturated EDTA ***) 5 ml
↓
GL-Pak PLS-2 (270mg/6mL)
↓
wash with 30 ml water
↓
elute with 10 ml methanol
↓
methanol distillation (<40 degree celsius)
↓
dissolve in 2.5 ml of 1.36% phosphoric acid 1
↓
calcium solution
↓
Inject 20 ml to HPLC

) EDTA *) 1.86 g in mixture of 307 ml 0.1 M citric acid and 197 ml of 0.2 M magnesium acetate
***) ethylenediamine 4 sodium acetate

Fig. 1 sample pre-processing

Conditions:

Column:	ODS column (4.6mm ID x 150mm L)
Eluent:	Imidazole buffer*)/CH_3OH(80/20)
Wavelength:	Ex 360nm, Em 425nm
Flow rate:	0.7ml/min
Column temperature:	40 degree celsius
Sample:	Oxytetracycline STD(0.1ppm) Oxytetracycline(0.1ppm) in milk
Injection volume:	20uL

*) 1M Imidazole + 1mM EDTA + 50mM$(CH_3COO)_2Mg$, pH7.2

Keywords : 1.oxytetracycline, 2.milk, 3.ODS, 4.FL, 5.official method

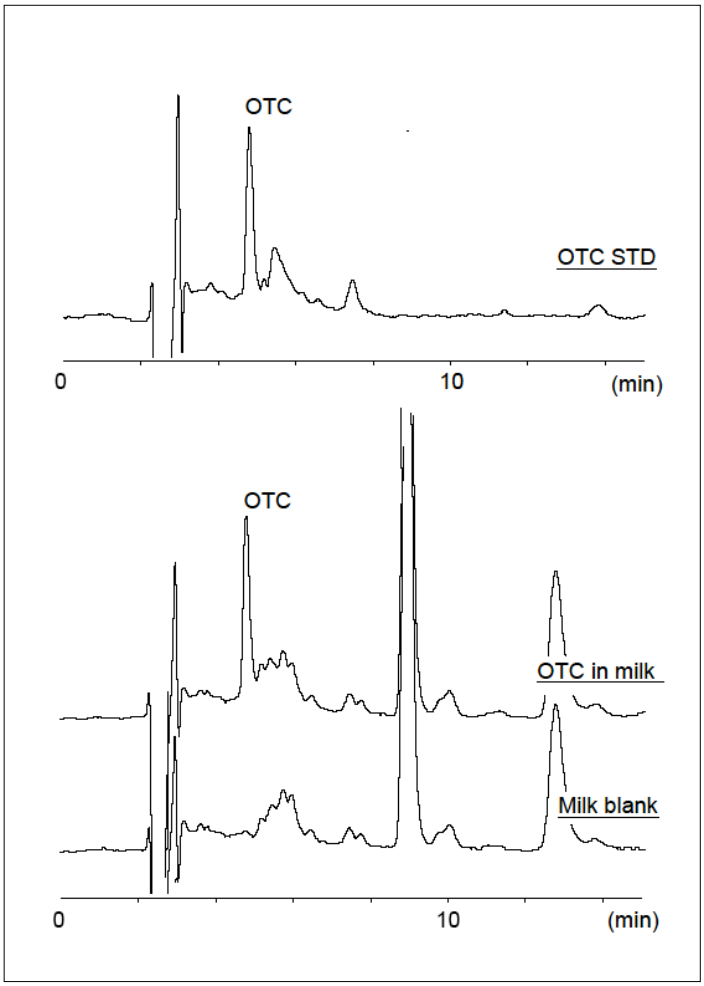

Fig. 2 Chromatograms of OTC standard sample and OTC spiked milk

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