LT008

GL Sciences Inc.

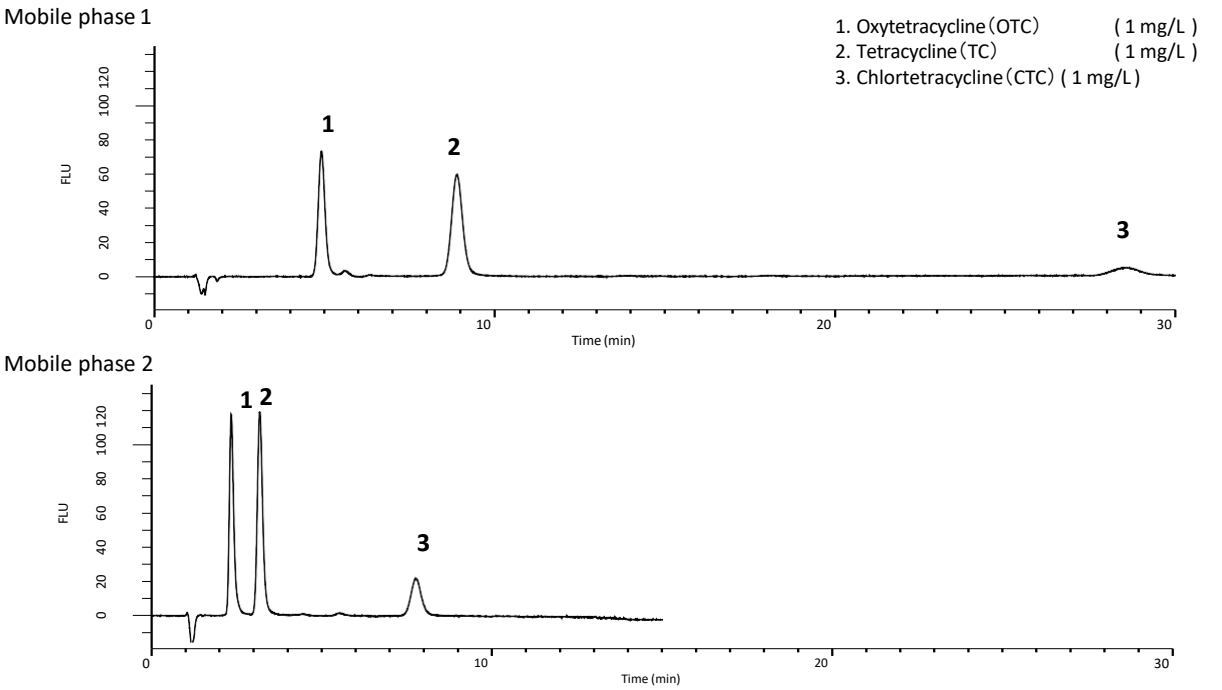
Analysis of Tetracycline Antibiotics in Foods by HPLC

This note describes a determination method for tetracyclines using HPLC-fluorescence detection system. The method is based on the analytical procedures announced from Japanese Ministry of Health, Labour, and Welfare.

Three tetracycline antibiotics (oxytetracycline, tetracycline, and chlortetracycline) are regulated, and the guideline values were established at values ranging from 0.1 to 1.2 ppm (shown in the last page).

The procedures consist of depoteination, liquid- liquid extraction, solid-phase extraction (SPE), and HPLC analysis. Two mobile phase conditions for HPLC analysis are described: one is for simultaneous determination of oxytetracycline and tetracycline, the other is for analysis of chlortetracycline.

The separation was successfully achieved, and the obtained calibration curves were linear in the range of 0.1-2.0 mg/L.



Chromatograms obtained from standard solution

Conditions

Column :Inertsil ODS-3 (5µm, 150 x 4.6 mm I.D.)
Cat.No. 5020-01731

Col. Temp. : 40°C

Detection : FL Ex 380 nm, Em 520 nm

Inj. Vol. : 20 µL

Mobile phase 1 for analysis of OTC and TC

Eluent : A) CH_3OH B) Imidazol Buffer*
A/B = 15/85, *v/v*

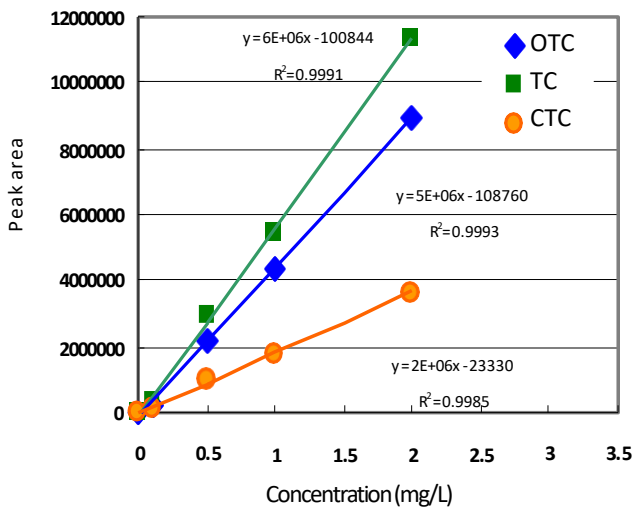
Flow rate : 1.2 mL/min

Mobile Phase 2 for analysis of CTC

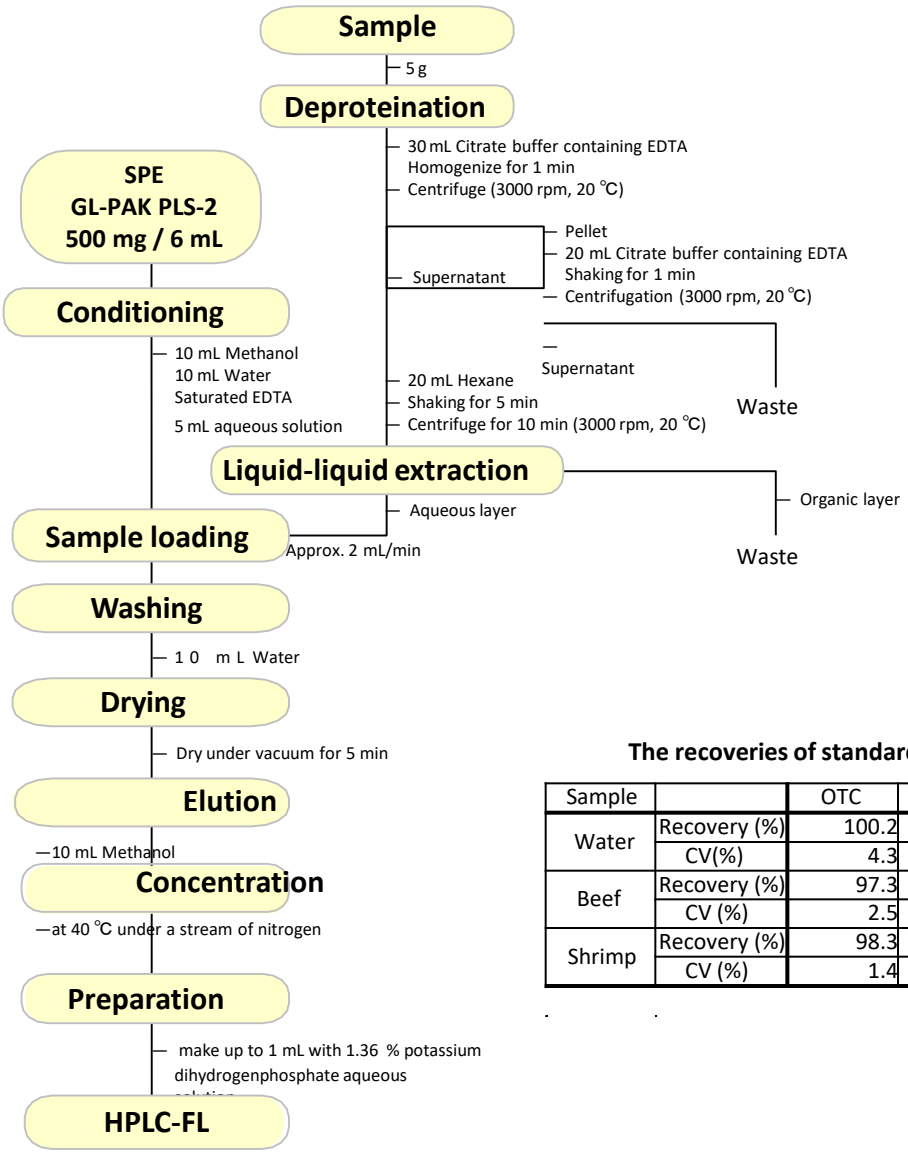
Eluent : A) CH_3OH B) Imidazol Buffer*
A/B = 25/75, v/v

Flow rate : 1.4 mL/min

*Imidazol Buffer:
To 68.08 g of imidazol, 0.37 g of EDTA and 10.72 g of magnesium acetate were added. After dissolved in water, the aqueous solution was made up to 800 mL with water. The pH was adjusted to 7.2 with acetic acid, and the solution was made up to 1000 mL with water.



The calibration curves for tetracyclines



The recoveries of standard addition

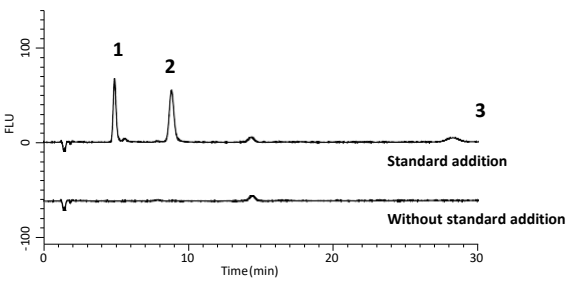
Sample		OTC	TC	CTC
Water	Recovery (%)	100.2	93.1	93.9
	CV(%)	4.3	4.0	9.2
Beef	Recovery (%)	97.3	83.5	83.0
	CV (%)	2.5	7.7	6.2
Shrimp	Recovery (%)	98.3	86.1	86.2
	CV (%)	1.4	1.6	4.7

(n=5)

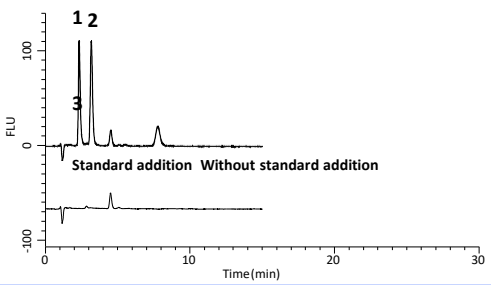
Beef extract

Concentrations of the tetracyclines in standard addition
1. Oxytetracycline (1 mg/L = 0.2 mg/kg)
2. Tetracycline (1 mg/L = 0.2 mg/kg)
3. Chlortetracycline (1 mg/L = 0.2 mg/kg)

Mobile phase 1



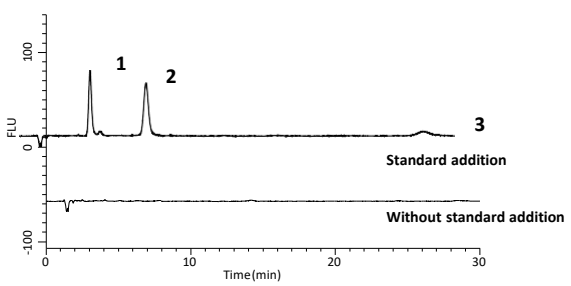
Mobile phase 2



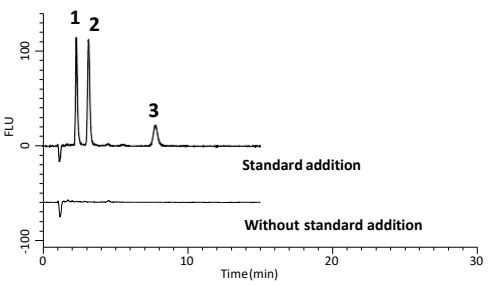
Shrimp extract

Concentrations of the tetracyclines in standard addition
1. Oxytetracycline (1 mg/L = 0.2 mg/kg)
2. Tetracycline (1 mg/L = 0.2 mg/kg)
3. Chlortetracycline (1 mg/L = 0.2 mg/kg)

Mobile phase 1



Mobile phase 2



Advantages of 3 μm particle-packed 100 mm x 3 mm I.D. columns

Since the columns with various specifications are available, Inertsil series are helpful also for downsizing, which decreases running cost of HPLC analyses.

- Solvent usage and sensitivity

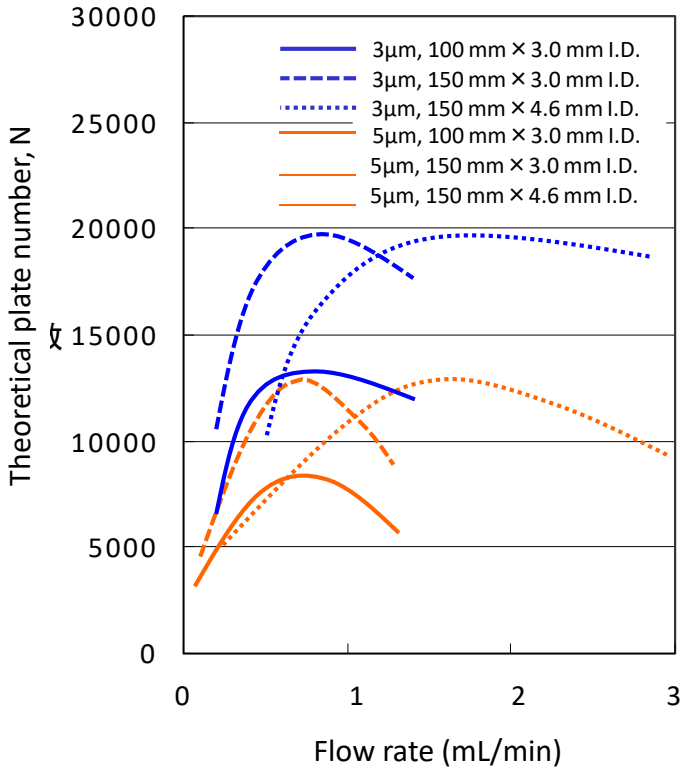
Optimum flow rate for HPLC separation decreases proportionally to the square of column inner diameter (I.D.). To use smaller I.D. columns reduces solvent consumption. Moreover, when concentrate-dependent detectors (e.g. UV-Vis detector and Fluorescence detector) were used, the sensitivity is improved with decrease in flow rate. Enhancement in sensitivity can also be expected.

- Number of theoretical plates

Smaller particle columns provide higher separation efficiency. Therefore, comparable number of theoretical plates can be obtained even with 100 mm length column.

- Price

The shorter columns are generally available for lower prices.

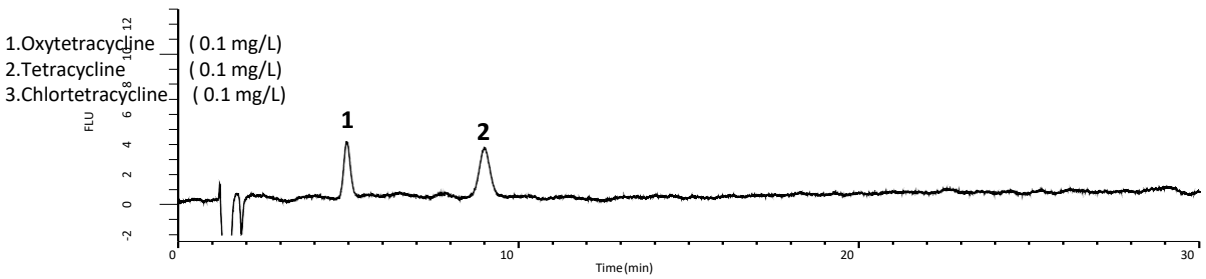


Effect of the flow rate on the number of thoretical plates

A chromatogram shown below was obtained with a 3 μm particle-packed 150 mm x 3.0 mm I.D. column. The flow rate was adjusted to maintain the same linear velocity※1. Good separation and reduction in solvent consumption were achieved. The run time may also be saved because sufficient theoretical plate number should be obtained even with 100 mm length column.

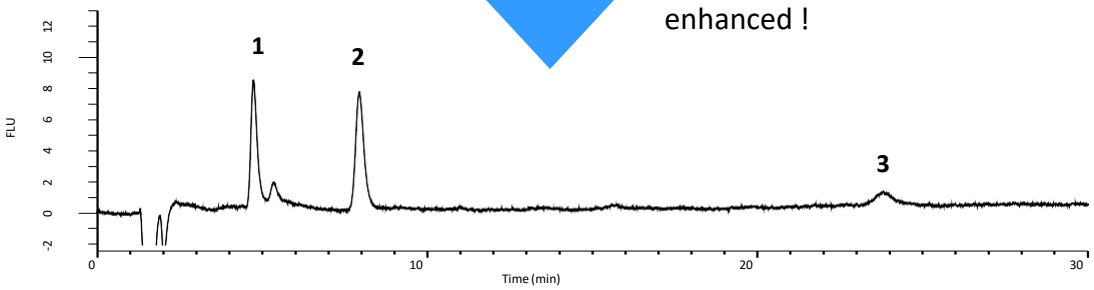
・4.6 mm I.D. column

（150 x 4.6 mm I.D., particle size: 5μm, flow rate: 1.2 mL/min）



・ 3.0 mm I.D. column

（150 x 3.0 mm I.D., particle size: 3μm, flow rate: 0.5 mL/min※2）



Solvent usage was reduced to 40 % !

The sensitivity was approximately 2-fold enhanced !

※1 Linear Velocity（L.V.）
＝ (Flow rate) ÷ (Cross-sectional area of the column)
※2 Composition of the mobile phase is the same.

Table of maximum residue levels (MRLs) for tetracyclines

MRLs for oxytetracycline, chlortetracylcine, and tetracylcine are established for the sum of the three antibiotics by Japan Food Chemical Research Foundation.

Muscle	MRLs (ppm)
Cattle, pig, and sheep	0.2
Other terretrial mammals*	0.1
Chicken, duck, and turkey	0.2
Other poultry**	0.2

Kidney	MRLs (ppm)
Cattle, pig, and sheep	1.2
Other terretrial mammals*	0.6
Chicken, duck, and turkey	1.2
Other poultry**	1.2

Fat	MRLs (ppm)
Cattle, pig, and sheep	0.2
Other terretrial mammals*	0.3
Chicken	0.2
Other poultry**	0.2

Edible offal	MRLs (ppm)
Cattle, pig, and sheep	0.6
Other terretrial mammals*	0.3
Chicken	0.6
Other poultry	0.6

Liver	MRLs (ppm)
Cattle, pig, and sheep	0.6
Other terretrial mammals*	0.3
Chicken	0.6
Other poultry**	0.6

Others	MRLs (ppm)
Chicken eggs	0.4
Other poultry eggs	0.4
Milk	0.1
Honey (including royal-jelly)	0.3

* except sheep and horse

** except duck and turkey

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