

Determination of Tetracyclines in Milk

LabTech, Inc.

Tetracyclines antibiotics are well applied in therapy of dairy cow diseases and feed additive. These accumulated tetracyclines in cows may appear in milks and threaten public health. This present study describes the an analytical methodology by using SepLine-1 automated solid phase extraction (SPE) system and high-performance liquid chromatography (HPLC) coupled with ultraviolet detector for the sensitive determination of antibiotics chlortetracycline, oxytetracycline, and tetracycline in milk.

1. Experimental

1.1 Instrumentation and Materials

SepLine-1 Automated SPE System (LabTech)

HPLC LC600 System (LabTech)

EV311 VAC rotatory evaporator (LabTech)

SPE Cartridge: LabTech PLS 150 mg 6 mL

Chlortetracycline, Oxytetracycline, and Tetracycline standards, purity $\geq 95\%$

Mcllvaine Buffer: Mixture of 1000 mL 0.1 mol/L Citric Acid and 625 mL 0.2 mol/L Sodium Hydrogen Phosphate Solution

Na₂EDTA-Mcllvaine Buffer (0.1 mol/L): Dissolve 60.5 g Ethylenediaminetetraacetic acid disodium salt (Na₂EDTA) in 1625 mL Mcllvaine Buffer, and shake well.

Chlortetracycline, Oxytetracycline, and Tetracycline Stock Mixed Standard Solution: Weigh 10 mg of each standards and dissolve into 100 mL Methanol.

Chlortetracycline, Oxytetracycline, and Tetracycline Mixed Working Standard Solution: Dilute Stock Mixed Standard Solution into different concentration (0.2 mg /L, 0.4 mg /L, 2.0 mg /L, 4.0 mg/L, 8.0 mg/L) by mobile phase.

Methanol (AR.)

Oxalic acid (AR.)

Pure Water

1.2 Sample Preparation

Sample Extraction

- Take 20 mL milk and 20 mL Mcllvaine Buffer into one 50 mL-centrifuge tube and vortex for 2 min. Then centrifuge for 10 min at > 4000 rpm.
- Take the supernatant for analysis.

Method Summary

Table 1. Extract method of tetracyclines determination in milk by SepLine-1 Automated SPE system. (SPE Cartridge: LabTech PLS 150 mg 6 mL)

LabTech, Inc.

Address: 114 South Street, Hopkinton,
MA 01748, U.S.A

Tel: (508) 435-5500

Fax: (508) 435-5595

Step	Solvent	Volume (mL)	Flow Rate (mL/min)	Time (Sec)
Prewet	Water	5.0	5.0	0
Sample Loading	Sample	20.0	5.0	0
Flow Path Rinse 1	Water	5.0	5.0	0
Flow Path Rinse 2	Methanol	5.0	5.0	0
N ₂ Purging	NULL	0.0	0.0	300
Elution	Methanol	5.0	5.0	0
N ₂ Purging	NULL	0.0	0.0	2.0

The eluate was dried by rotatory evaporator at 40 °C, and then reconstructed into 1 mL mobile phase.

1.3 Apparatus

HPLC Method

Column: LabTech C18 (150 mm × 4.6 mm, 5µm)

Column Temperature: 30°C

Mobile Phase: Acetonitrile + Methanol + 0.01 mol/L Oxalic Acid (v/v/v = 20%/10%/70%)

Flow Rate: 0.5 mL/min

Sample Volume: 20µL

Wavelength: 350 nm

LabTech, Inc.

Address: 114 South Street, Hopkinton,
MA 01748, U.S.A

Tel: (508) 435-5500

Fax: (508) 435-5595

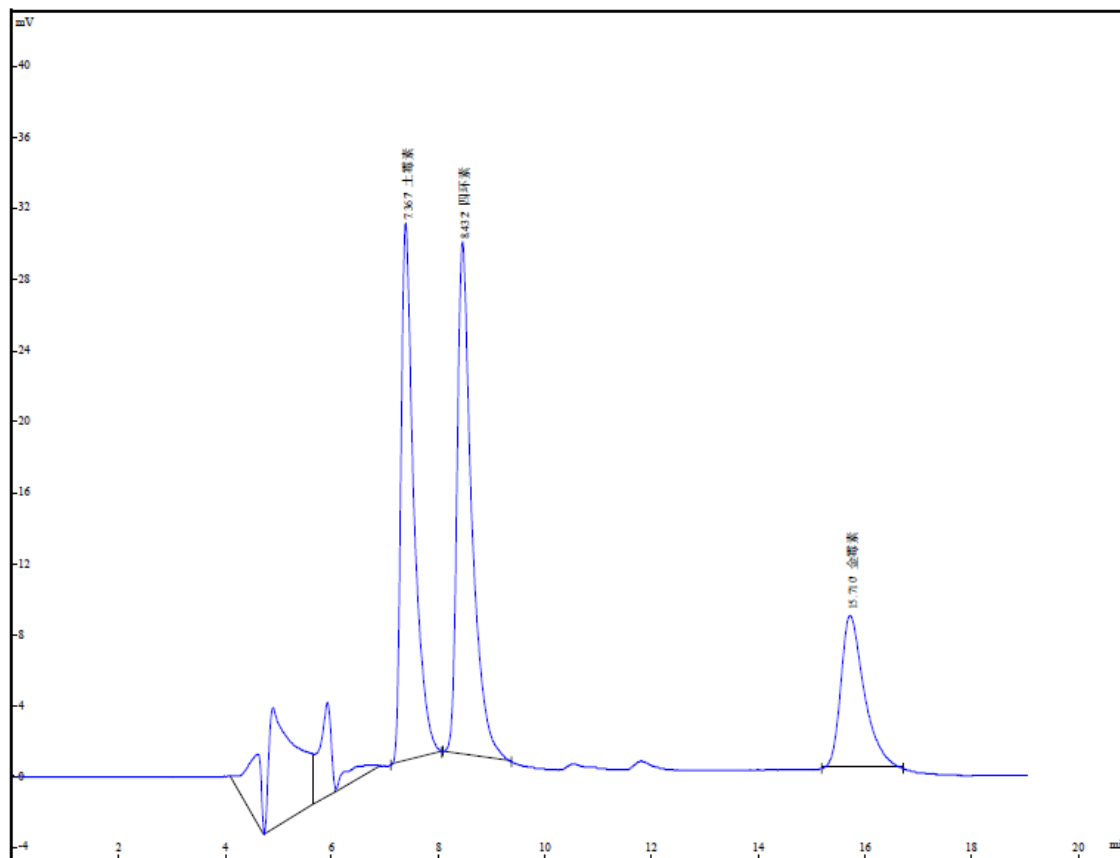


Figure 1. Chromatogram of Chlortetracycline, Oxytetracycline, and Tetracycline Standards.

2. Results and Discussion

The linear range is between 0.2 mg/L – 8.0 mg/L. The equations are listed below, where x is sample concentration, y is peak area.

Oxytetracycline	$y = 63.33x + 429.4$	$r^2 = 0.999$
Tetracycline	$y = 62.26x + 1121$	$r^2 = 0.999$
Chlortetracycline	$y = 33.08x + 214.5$	$r^2 = 0.999$

LabTech, Inc.

Address: 114 South Street, Hopkinton,
MA 01748, U.S.A

Tel: (508) 435-5500

Fax: (508) 435-5595

Standards spiked in Milk after SPE.

The milk samples were spiked with three different concentration of standards. Then these spiked samples were purified through SepLine-1 Automated SPE system, and then analyzed by HPLC (Figure 3). The mean recoveries of chlortetracycline, oxytetracycline, and tetracycline were listed in table 1.

Table 1. Recovery of Spiked Milk Samples.

No.	Compound	Spiked (mg/L)	Measured (mg/L)	Recovery (100%)
1	Oxytetracycline	0.4	0.39	97.4
		2	1.44	72.2
		4	3.48	87.0
2	Tetracycline	0.4	0.31	77.4
		2	1.68	83.8
		4	3.60	90.1
3	Chlortetracycline	0.4	0.33	82.6
		2	1.31	65.3
		4	2.48	62.1

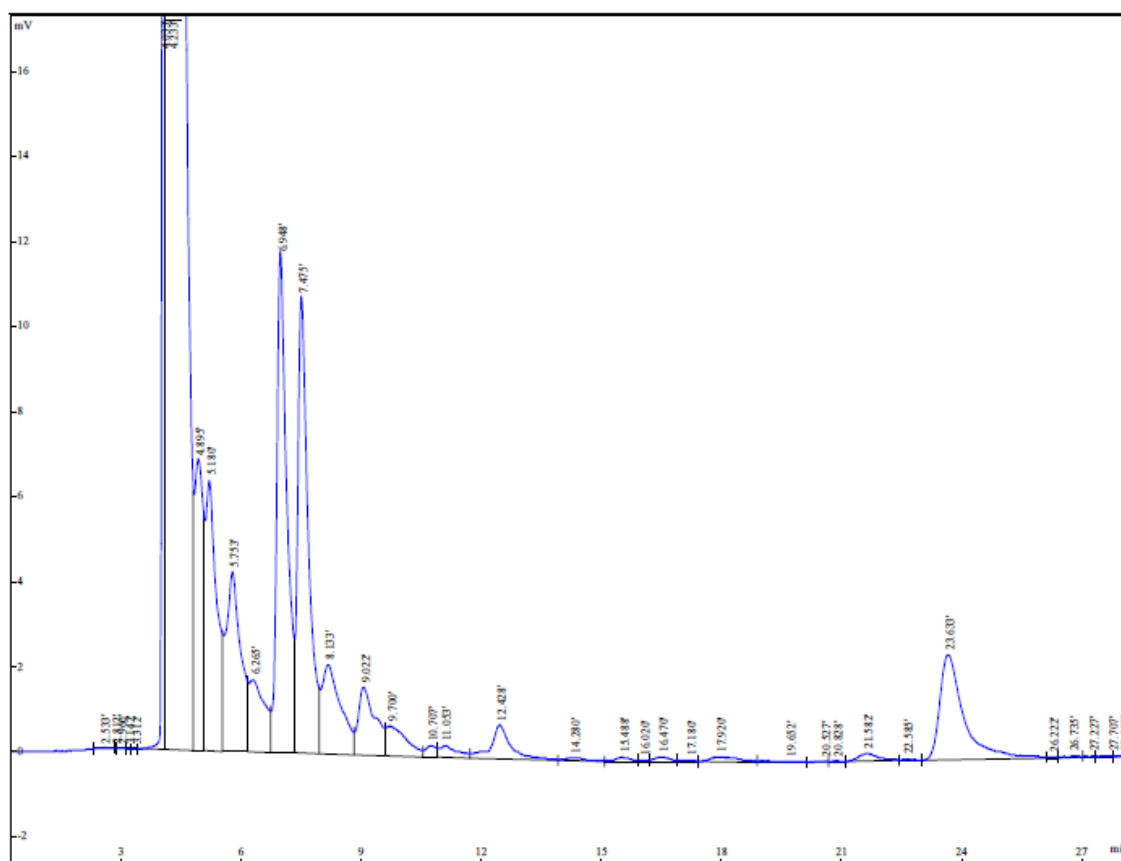


Figure 4. The LC chromatogram of real milk sample after SPE system.

3. Conclusion

LabTech, Inc.

Address: 114 South Street, Hopkinton,
MA 01748, U.S.A

Tel: (508) 435-5500

Fax: (508) 435-5595

This study develops the method by using SepLine Automated SPE system and HPLC to determine chlortetracycline, oxytetracycline, and tetracycline in milks. The results demonstrated that this method is reliable and precise for chlortetracycline, oxytetracycline, and tetracycline determination in milks.

LabTech, Inc.

Address: 114 South Street, Hopkinton,
MA 01748, U.S.A

Tel: (508) 435-5500

Fax: (508) 435-5595