Pretreatment and Determination of Chlortetracycline, Oxytetracycline, and Tetracyclinein Chicken and Pork by SepLine/HPLC-UV

LabTech, Inc.

Tetracyclinesantibiotics are used to prevent and cure animal diseases. However, long term usageoftetracyclinesmay cause residue accumulation in animal tissues, which affects the consumer's health. Both European Union and China have restrict rules about the amount limits of tetracyclines in animal tissues. Since fast-grown chicken, feed with certain drugs attracted great attentions recently, determination of drug residues from animaltissues is critical at this point.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 chicken and pork. The method is simple-to-use and has high recovery, which can be widely used as routine tetracyclines testing.

1. Experimental

1.1 Instrumentation and Materials

SepLine -1 Automated SPE System (LabTech)

HPLC LC600 System (LabTech)

SPE Cartridge: LabTech PLS 150 mg 6 mL

Chlortetracycline, Oxytetracycline, and Tetracyclinestandards, purity ≥ 95 %

Sodium Hydrogen PhosphateSolution (0.2 mol/L): Dissolve 28.41 g sodium hydrogen phosphate in 1000 mL water.

Citric Acid Solution (0.1 mol/L): Dissolve 21.01 g citric acid in 1000 mL water.

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

Na_2EDTA-Mcllvaine Buffer (0.1 mol/L): Dissolve 60.5 g Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) in 1625mL Mcllvaine Buffer, and shack well.

Oxalic Acid Solution (0.01 mol/L): Dissolve 0.882 g oxalic acid in 700 mL water.

Mobile Phase: Acetonitrile + Methanol + 0.01 mol/L oxalic acid solution. (20%/10%/70%)

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 (0.25 mg /L, 0.5 mg /L, 1.0 mg /L, 5.0mg/L) by mobile phase.

Methanol (AR.)

Oxalic acid (AR.)
Pure Water

1.2 Sample Preparation

Sample Extraction

- Mince and mix animal tissues, then transfer into a clean container. Weigh 6 g samples to the nearest 0.01 g, then transfer to 50 mL centrifugal tubeswith 30 mL 0.1mol/L Na_2EDTA-Mcllvaine buffer. Mix sample for 1 min on shaker and then vortex for 10 min.
- Centrifuge for 10 min at 5000 rpm. Transfer the supernatant to another centrifugal tube with additional 20 mL buffer. Centrifuge again and combine the supernatants.

Method Summary

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

Step	Solvent	Volume (mL)	Flow Rate (mL/min)	Time (Sec)
Prewet 1	Water	5.0	5.0	0
Prewet 2	Buffer	5.0	5.0	0
N_2 Purging	NULL	0.0	0.0	10
Sample Loading	Sample	10.0	2.0	0
N_2 Purging	NULL	0.0	0.0	180
Flow Path Rinse 1	Water	5.0	5.0	0
N_2 Purging	NULL	0.0	0.0	180
Elution	Methanol	10.0	2.0	0
Elution	Methanol	10.0	2.0	0
N_2 Purging	NULL	0.0	0.0	100

The eluate was driedby rotatory evaporator at 40 ^{o}C, and then reconstructed into 1 mL mobile phase.

1.3 Apparatus

HPLC Method

Column：LabTech C18 (250 mm × 4.6 mm, 5μm)
Column Temperature: 30℃
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

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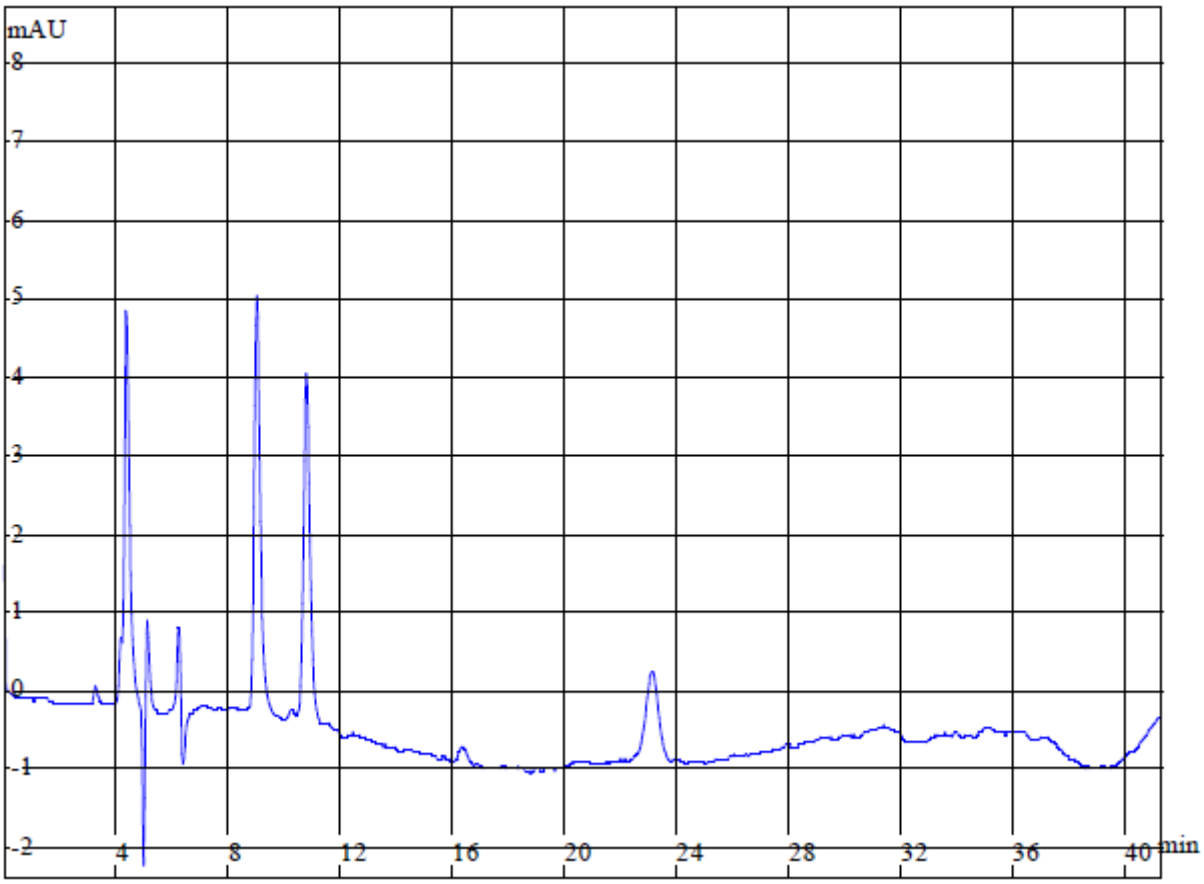

Figure 1. Chromatogram of 1 ppmChlortetracycline, Oxytetracycline, and Tetracycline Standards in Chicken after SPE.

2. Results and Discussion

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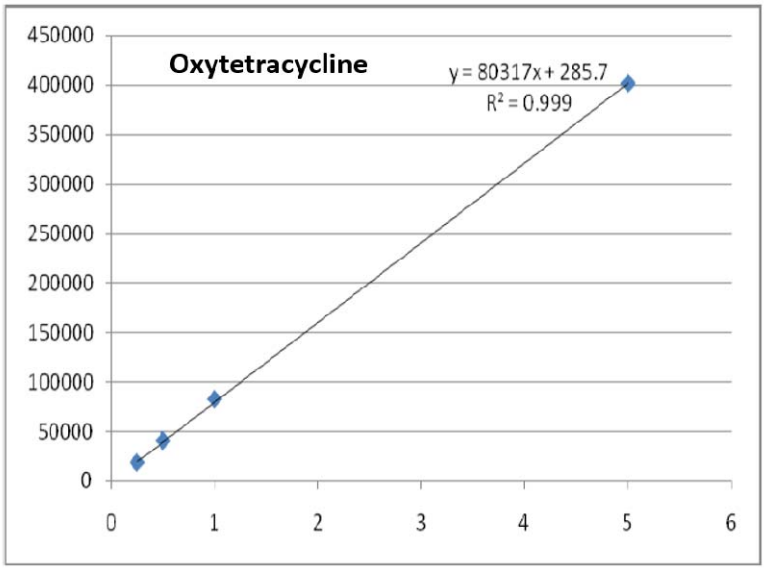

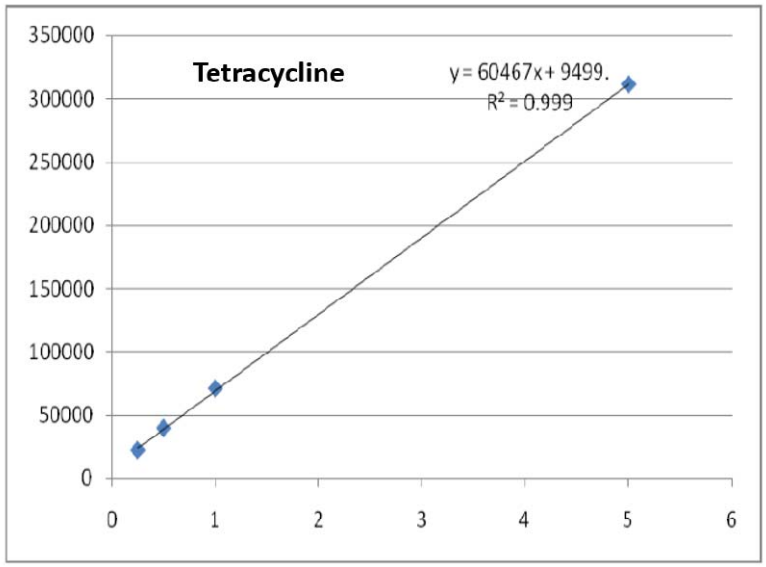

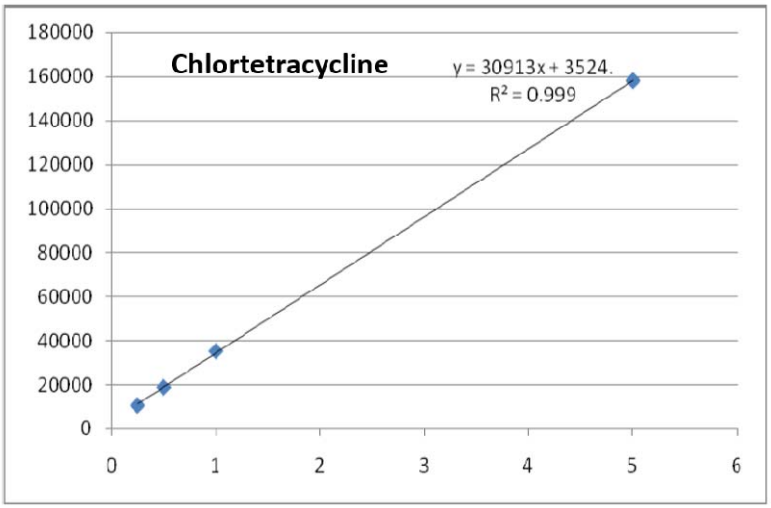

Figure 2. Calibration Curves of Chlortetracycline, Oxytetracycline, and Tetracycline.

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The linear range is between 0.25 mg/L – 5.0 mg/L. The equations are listed below, where x is sample concentration, y is peak area.

Oxytetracycline	y = 80317x + 285.7	r^2 = 0.999
Tetracycline	y = 60467x +9499	r^2 = 0.999
Chlortetracycline	y = 30913x +3524	r^2 = 0.999

9 mL animal tissue samples were spiked with 1 mL 1 ppm of standards. Then these spiked samples were purified through SepLine Automated SPE system, and then analyzed by HPLC. The mean recoveries of chlortetracycline, oxytetracycline, and tetracyclinewere listed in table 1 and table 2.

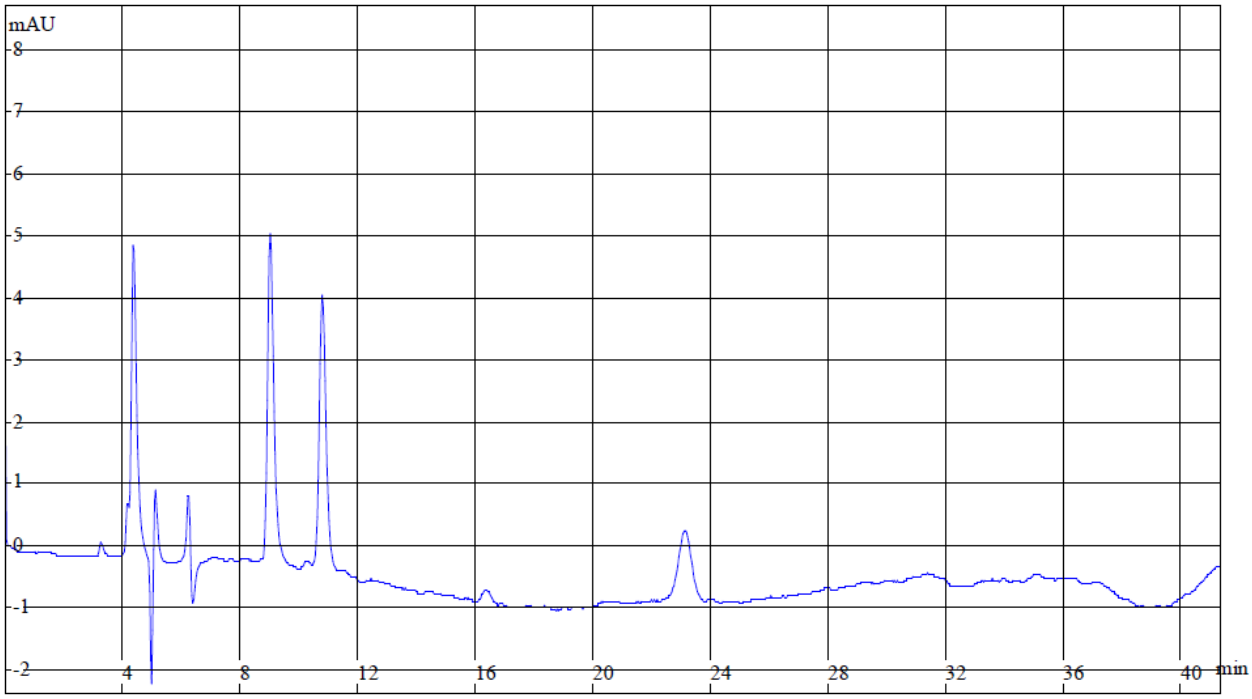

Figure 3. The LC chromatogram ofchicken samplespiked with 1 ppm after SPE.

Table 1. Recovery of Spiked Chicken Samples.

	Compound	Sample 1		Sample 2		Sample 3	
		Measured (ppm)	Recovery (%)	Measured (ppm)	Recovery (%)	Measured (ppm)	Recovery (100%)
1	Oxytetracycline	0.874	87.4	0.882	88.2	0.979	97.9
2	Tetracycline	0.90	90.0	0.892	89.2	0.927	92.7
3	Chlortetracycline	0.994	99.4	0.897	89.7	0.971	97.1

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Table 2. Recovery of Spiked Pork Samples.

	Compound	Sample 1		Sample 2		Sample 3	
		Measured (ppm)	Recovery (%)	Measured (ppm)	Recovery (%)	Measured (ppm)	Recovery (100%)
1	Oxytetracycline	0.839	83.9	0.963	96.3	0.979	97.9
2	Tetracycline	1.016	101.6	1.08	108	0.906	90.6
3	Chlortetracycline	0.981	98.1	1.09	109	1.09	109

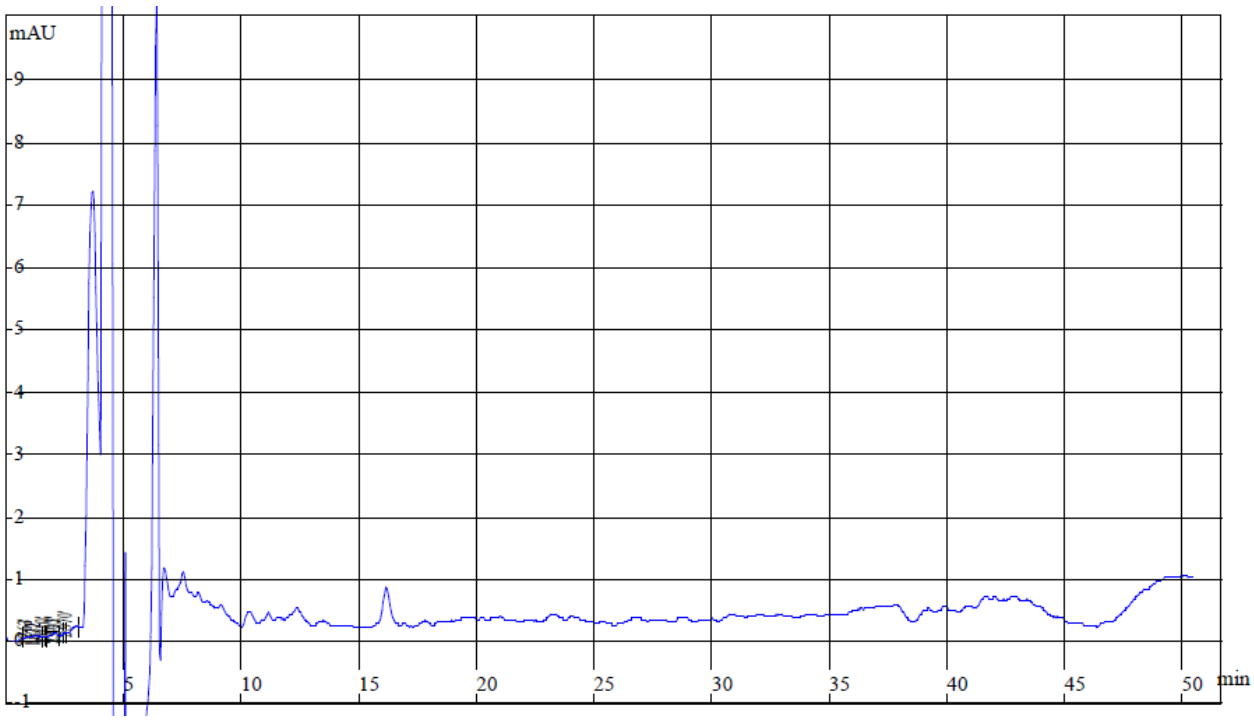

Figure 4. The LC chromatogram of chicken sample after SPE system. (Tetracyclines are not detectable)

3. Conclusion

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

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