Determination of Tetracyclines in Chicken by Solid-Phase Extraction and High-Performance Liquid Chromatography

Application Note

Food Safety

Authors

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Abstract

A method for the simultaneous determination of the seven antibiotic residues of minocycline, oxytetracycline, tetracycline, demeclocycline, chlortetracycline, methacycline, and doxycycline in chicken has been developed. In this method, solid-phase extraction (SPE) and HPLC/UV are used consistent with Chinese regulatory methods. Samples are prepared in EDTA-McIlvaine buffer solution (pH 4.0), the clean up is done with an Agilent SampliQ OPT cartridge, and the HPLC separation is performed with an Agilent ZORBAX column (5 µm, 250 mm × 4.6 mm id). The flow rate is 1.5 mL/min, the detector wavelength is 350 nm, and the injection volume is 100 µL. The limits of detection are between 2.5 and 5 µg/kg. Linear calibration curves are obtained over the range of 25 to 500 µg/kg. Overall recoveries range from 59.0 to 99.0%, with RSD values between 1.0 and 6.5%.

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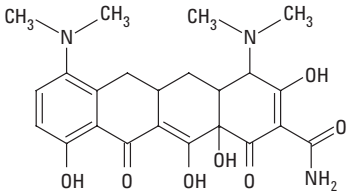
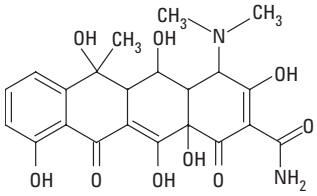
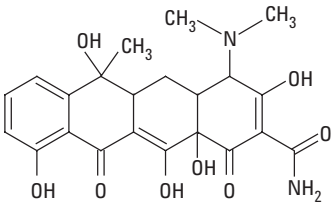
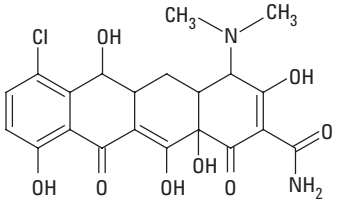
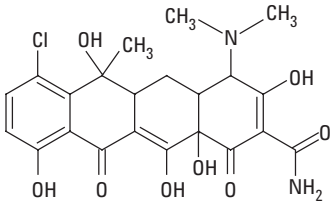
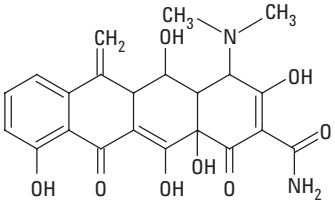
Introduction

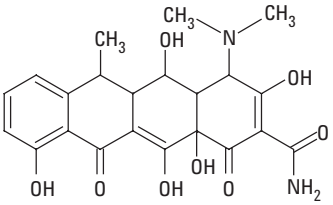
"Tetracyclines" is the common name for a group of antimicrobials with a hydronaphthacene structure (Table 1). Tetracyclines are used against a wide range of gram-negative and gram-positive microorganisms. The Chinese government has set maximum residue limits (MRLs) for tetracyclines in muscle (100 µg/kg) and promulgated a government standard (GB/T 21317-2007) that established a method for the determination of tetracyclines in animal tissues. This application note describes the implementation and optimization of the method described in GB/T 21317-2007 and the results of validation.

Table 1. Tetracyclines Used in This Study

No.	Name	pKa	log P	Structure
1	Minocycline CAS # 10118-90-8	3.3/7.2/9.3	+0.5	
2	Oxytetracycline CAS # 6153-64-6	3.3/7.3/9.1	–0.9	
3	Tetracycline CAS # 60-54-8	3.3/7.7/9.7	–1.3	
4	Demeclocycline CAS # 127-33-3	3.3/7.2/9.3	+0.2	
5	Chlortetracycline CAS # 57-62-5	3.3/7.4/9.3	–0.62	
6	Methacycline CAS # 914-00-1	3.5/7.6/9.2	–0.3	

Continued

Table 1. Tetracyclines Used in This Study (continued)

No.	Name	pKa	log P	Structure
7	Doxycycline CAS# 564-25-0	3.1/7.7/9.3	–0.02	

Experimental

Materials and Chemicals

All reagents and solvents were HPLC or analytical grade. Tetracycline standards were purchased from Sigma-Aldrich or from China's National Institute for the Control of Pharmaceutical and Biological Products (NICPBP).

Stock solution (0.1 mg/mL) was prepared in methanol and kept in the freezer (–20 °C). Working solutions were prepared using the stock solution diluted with a mixture of methanol/10 mmol/L trifluoroacetic acid solution (1/19). The working solutions were prepared daily.

The SPE cartridges were Agilent SampliQ OPT 3 mL, 60 mg (p/n 5982-3036). The analysis was performed on an Agilent 1200 HPLC with DAD. The analytical column was an Agilent ZORBAX SB-C8 5 µm, 250 mm × 4.6 mm id (p/n 880975-906).

McIlvaine buffer, mix 1000 mL 0.1 mol/L citric acid with 625 mL 0.2 M disodium hydrogen phosphate. Adjust pH to 4.0 ± 0.05 with NaOH or HCl as needed.

Na_2EDTA-McIlvaine buffer (0.1 mol/L), mix 60.5g Na_2EDTA. $2H_2O$ into 1625 mL McIlvaine buffer.

HPLC Conditions

Column:	Agilent ZORBAX SB-C8 250 mm × 4.6 mm, 5 µm
Flow rate:	1.5 mL/min
Column temperature:	30 °C
Injection volume:	100 µL
Detector wavelength:	350 nm
Mobile phase:	Methanol-acetonitrile-10 mmol/L TFA solution, gradient elution

Time (minutes)	% methanol	% acetonitrile	% 10 mmol TFA
0	1	4	95
7.5	6	24	70
13.5	7	28	65
15	1	4	95

Sample Preparation

A 200-g sample of chicken was homogenized with a tissue disintegrator, placed in a clean, sealed container, and stored in a freezer below –18 °C.

A 5-g homogeneous sample (accurate to 0.01 g) was placed into a 50-mL polypropylene centrifuge tube with 20 mL 0.1 mol/L Na_2EDTA-McIlvaine buffer solution and vortex mixed for 1 minute followed by a 10-minute ultrasonic extraction in an ice bath. The sample was then centrifuged at a rotate speed of 3,000 r/min for 5 minutes (below 15 °C). The supernatant was removed and saved in a clean tube. The extraction was repeated twice with 20 mL and 10 mL successively. The combined supernatant fluid was brought to 50 mL with buffer, mixed well, centrifuged at a rotate speed of 4,000 r/min for 10 min (below 15 °C), and filtered with fast filter paper.

SPE Purification

The procedure used for the SPE extraction is shown in Figure 1. Agilent SampliQ OPT cartridges were preconditioned with 5 mL of methanol, then 5 mL of a 10 mmol/L TFA solution. A 10-mL extract (equivalent to a 1-g sample) was passed through the SampliQ OPT cartridge at a speed of 1 mL/min. After the sample effused completely, the cartridge was washed with 3 mL of water (pH adjusted to 4.5 with TFA). The entire effluent was discarded. The cartridge was dried under negative pressure below 2.0 kPa for 3 minutes. Finally, the cartridge was eluted with 10 mL of 10 mmol/L oxalic acid in methanol. The eluent was collected and dried under nitrogen below 40 °C. The resulting residue was dissolved and made to a constant volume of 0.5 mL using the methanol/10 mmol/L TFA solution (1/19). Then the residue was filtered through a 0.45-µm filter membrane (p/n 5185-5836) and analyzed.

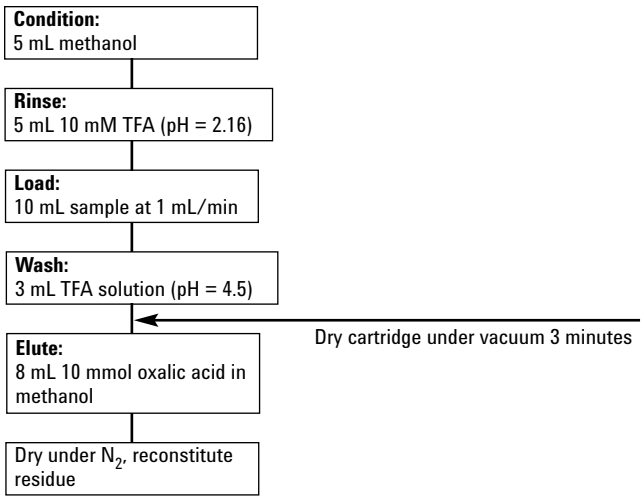


Figure 1. Tetracycline SPE procedure.

Results and Discussion

Linearity, Limits of Detection

Stock solutions were diluted to different concentrations and analyzed by HPLC. Linear regressions were calculated for the tetracyclines using the areas and the solution concentrations. The limit of detection (LOD) was the injection concentration whose signal-to-noise ratio was between 2 and 3. The linear range was between 25 and 500 µg/kg. The linearity and LOD are shown in Table 2.

Table 2. Linearity and LODs of Tetracyclines

Compound	Regression equation	Correlation coefficient	LOD (µg/kg)
Minocycline	Y = 86.313 × –0.1491	0.9996	2.5
Oxytetracycline	Y = 95.965 × +0.0261	0.9999	2.5
Tetracycline	Y = 103.97 × –0.4698	0.9999	2.5
Demeclocycline	Y = 68.659 × –0.1172	0.9998	5
Chlortetracycline	Y = 51.752 × –0.0284	0.9999	5
Methacycline	Y = 98.243 × +1.2567	0.9985	2.5
Doxycycline	Y = 76.408 × +1.0756	0.9987	5

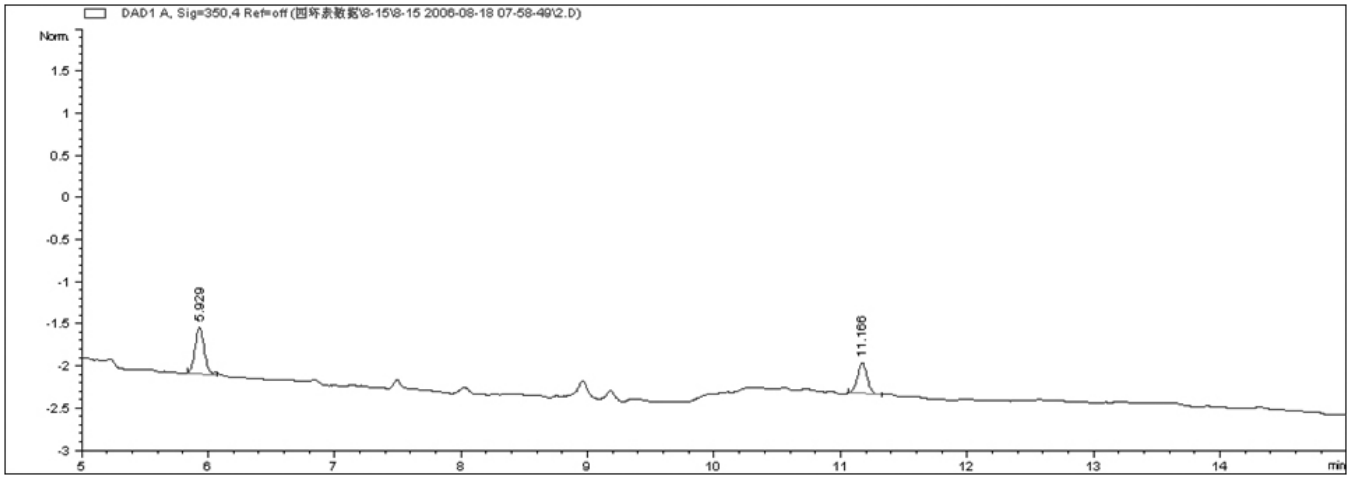


Figure 2. Chromatogram of a chicken blank.

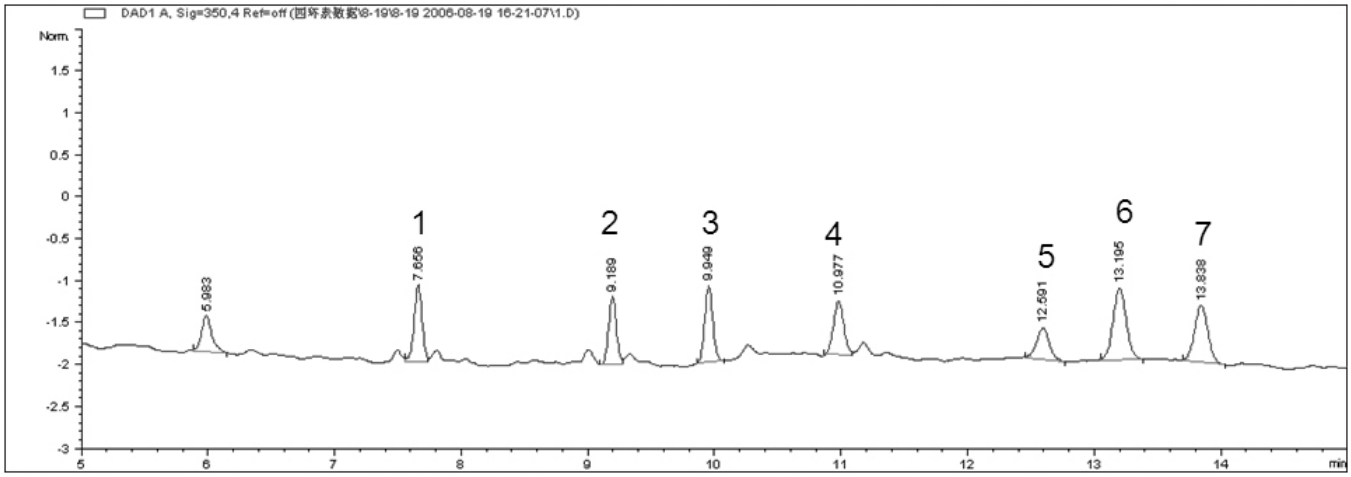


*Figure 3. Chromatogram of a chicken sample spiked at 50 µg/kg. (*1-Minocycline, 2-Oxytetracycline, 3-Tetracycline, 4-Demeclocycline, 5-Chlortetracycline, 6-Methacycline, and 7-Doxycycline)

Recovery and Reproducibility

The precision of the method was determined as recoveries of spiked tetracycline standards in chicken at 50 µg/kg, 100 µg/kg, and 200 µg/kg levels. The analysis was performed in replicates of six at each level. The chromatograms of the blank and spiked standard (50 µg/kg) are shown in Figure 2 and Figure 3. The recovery and reproducibility data are shown in Table 3.

Table 3. Recoveries and RSDs of Tetracyclines in Chicken by SPE

Compound	Spiked level (µg/kg)	Recovery (%)	RSD (%)
Minocycline	50	87.6	4.13
	100	80.8	5.68
	200	81.3	4.19
Oxytetracycline	50	68.8	6.49
	100	63.0	4.87
	200	59.4	4.35
Tetracycline	50	81.0	4.46
	100	70.0	3.47
	200	72.3	4.38
Demeclocycline	50	92.0	2.06
	100	94.8	3.78
	200	92.9	1.92
Chlortetracycline	50	93.3	3.16
	100	92.4	4.01
	200	87.7	2.54
Methacycline	50	93.3	2.89
	100	91.9	2.51
	200	86.6	3.39
Doxycycline	50	95.6	4.38
	100	96.4	1.00
	200	92.0	3.02

Conclusions

Agilent SampliQ provides a simplified and effective single-cartridge method for the purification and enrichment of multiple tetracycline compounds in chicken. The recovery and reproducibility results based on solution standards are acceptable for tetracycline residue determination in chicken under the Chinese regulation. The impurities from chicken were minimal and did not interfere with any of the tetracyclines analyzed. The LODs of the seven tetracyclines were significantly lower than the MRL (of 100 ug/kg).

Part number	Description
5982-3013	OPT Polymer - Box, 100 × 1 mL tubes, 30 mg
5982-3036	OPT Polymer - Box, 50 × 3 mL tubes, 60 mg
5982-3067	OPT Polymer - Box, 30 × 6 mL tubes, 150 mg
5982-3096	OPT Polymer - 96 Well Plate, 10 mg

References

GB/T 21317-2007, Determination of tetracyclines residues in food of animal origin-LC-MS/MS method and HPLC method.

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