Analysis of tetracyclines by HPLC

Rainer Schuster

Food

Abstract
Tetracyclines are used worldwide as oral or parenteral medication in the form of additives in animal feed. In food-producing animals, these drugs exhibit a high degree of activity toward a wide range of bacteria.[1, 2]

Sample preparation
After homogenization or mincing and addition of mineral acids to dissociate tetracyclines from proteins, the samples were extracted using liquid/liquid extraction followed by degreasing and/or deproteinization, purification, and concentration.[3]

Chromatographic conditions
The HPLC method presented here for the analysis of meat is based on reversed-phase chromatography and UV-visible diode-array detection.
UV spectra were evaluated as an additional identification tool.

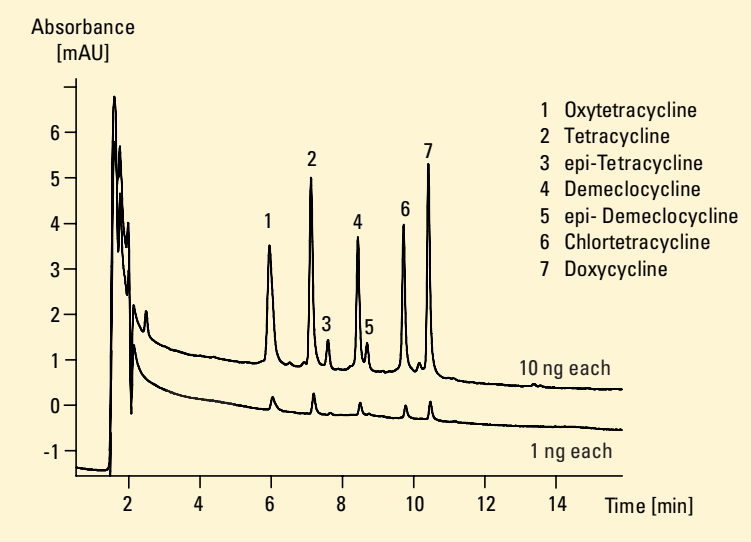

Figure 1
Analysis of tetracyclines by HPLC

Conditions

Column: 100 ˘ 4 mm Hypersil BDS, 3 µm
Mobile phase:
A = water, pH = 2.1 with sulfuric acid
B = ACN
Gradient: start with 15 % B at 10min 60% B
Flow rate: 0.5 ml/min
Column compartment: 25 ºC
Detector:
UV-DAD detection wavelength 355 nm/20 nm, reference wavelength 600/100 nm

Sample preparation

1. 1 g sample was mixed with citric acid (100 mg).
2. add 1 ml nitric acid (30 %) or 0.1 m oxalic acid
3. add 4 ml methanol 5 min in the ultrasonic bath
4. add water up to 10 ml total volume
5. centrifuge
6. inject

Agilent Technologies
Innovating the HP Way

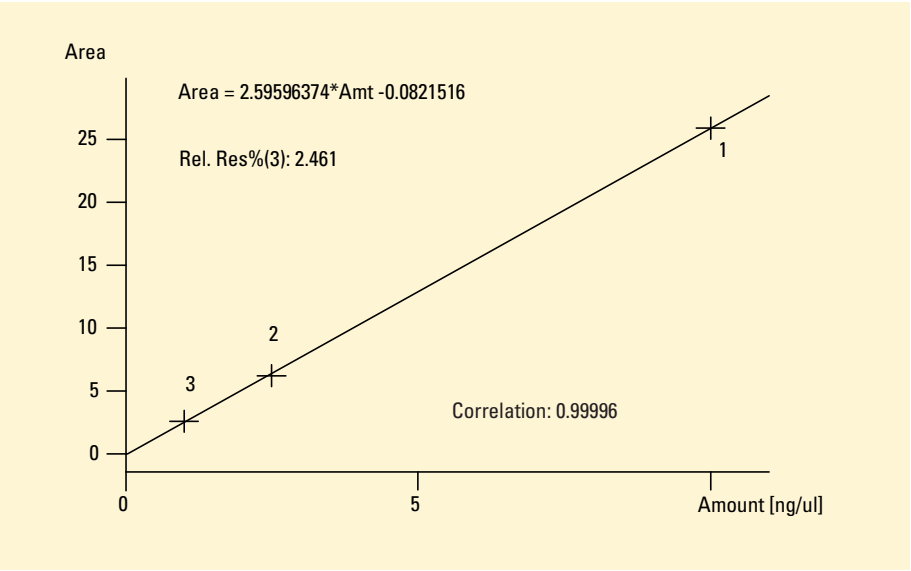

Figure 2
Linearity for oxytetracycline 1-10 ng

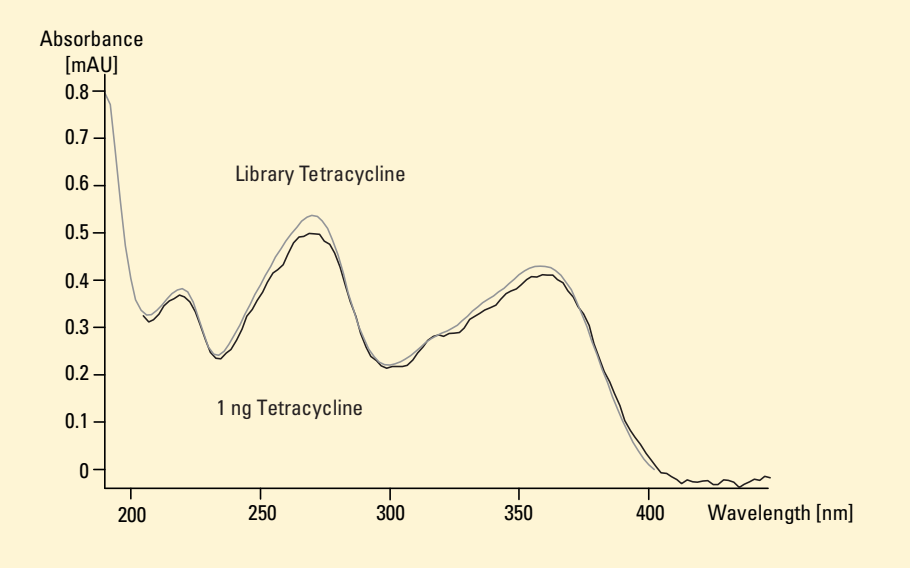

Figure 3
Analysis of tetracyclines at 100 ppb by HPLC

Rainer Schuster is application chemist at Agilent Technologies, Waldbronn, Germany.

For more information on our products and services, visit our worldwide website at http://www.agilent.com/chem

Equipment

Agilent 1100 Series
- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector, Agilent ChemStation + software

HPLC method performance

Limit of detection
for UV-DAD 100 ppb
Repeatability
of RT over 10 runs <0.2 %
of areas over 10 runs <2 %

References

1.
H. Malisch et al., "Determination of residues of chemotherapeutic and antiparasitic drugs in food stuffs of anomaly origin with HPLC and UV-Vis diode-array detection" *J. Liq. Chromatogr.*, **1988**, 11 (13), 2801–2827.14.

2.
M.H. Thomas, *J. Assoc. Off. Anal.*; **1989**, 72 (4) 564.

3.
Farrington et. al., "Food Additives and Contaminants", **1991**, Vol. 8, No. 1, 55-64.

Released 09/97
Publication Number 5966-1619E

Agilent Technologies
Innovating the HP Way