USP Analysis of Tetracyclines

Application Note

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Introduction

Tetracyclines are a group of antibiotics used for the fight against infection by foreign unicellular microorganisms. Doxycycline acts by inhibiting bacterial protein synthesis through binding to ribosomes. It is used for a broad range of infections including dermatological infection as well as for more specific infections, such as cholera. There are also investigations underway into its effectiveness in combating osteoporosis. It is believed that this antibiotic may block the action of enzymes involved in the breakdown of joint cartilage as well as possibly protecting unaffected joints. Doxycycline is obtained by semi-synthesis from oxytetracycline with the intermediate metacycline produced in this process. Therefore, it is important to ascertain whether or not there is any tetracycline derivative of these compounds present in a sample to be used for drug production. There is a USP method[1] for the analysis of doxycycline and quantification of the compound, which uses a reversed phase PS/DVB PLRP-S column. These columns are ideal for USP methods, offering chemical stability, full pH range, high pressure capability and easy regeneration.

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Conditions

Sample:	20 mg Tetracycline in 25 mL 0.01 M HCl
Column:	PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5500)
Eluent:	60 g 2-methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K_2HPO_4 at pH 8 + 50 mL 10 g/L tetrabutylammonium hydrogen sulphate at pH 8 + 10 mL 40 g/L sodium edetate at pH 8, made up to 1000 mL with water (pH adjustment using dilute NaOH)
Flow Rate:	1.0 mL/min
Temperature:	60 °C
Detection:	UV, 254 nm

Results and Discussion

Good resolution of oxytetracycline, metacycline and doxycycline is obtained using the USP method (Figure 1). Results in the table reveal how values for the plates per meter, symmetry and resolution factors indicate symmetrical peaks with good resolution.

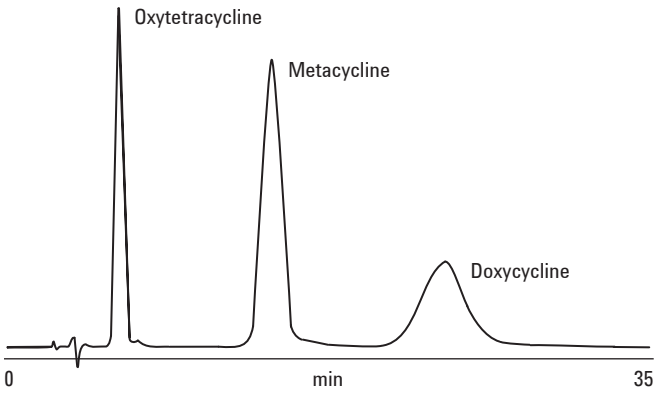

Figure 1. Tetracycline mix.

Table 1. Values for plates per meter, symmetry and resolution factors.

Sample	Plates/m	Symmetry	Resolution Factor
Oxytetracycline	5220	1.03	
Metacycline (OTC/MTC)	4794	0.95	6.77
Doxycycline (MTC/DC)	1899	1.17	2.29

Analysis of doxycycline alone shows some small early eluting peaks (Figure 2).

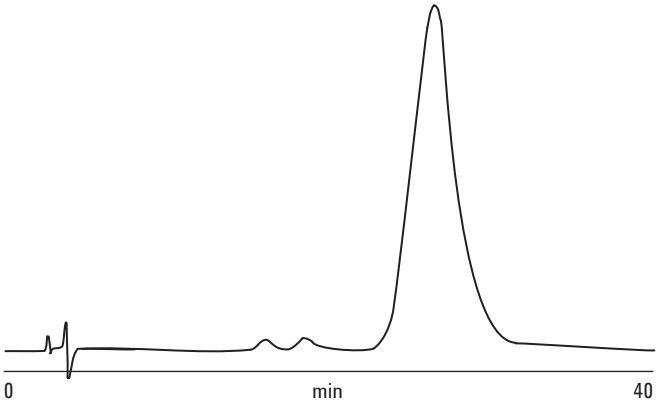

Figure 2. 20 µL injection of doxycycline.

The first peak appears to correlate with the elution position of metacycline, while the other elutes in the tail of this compound (Figure 3).

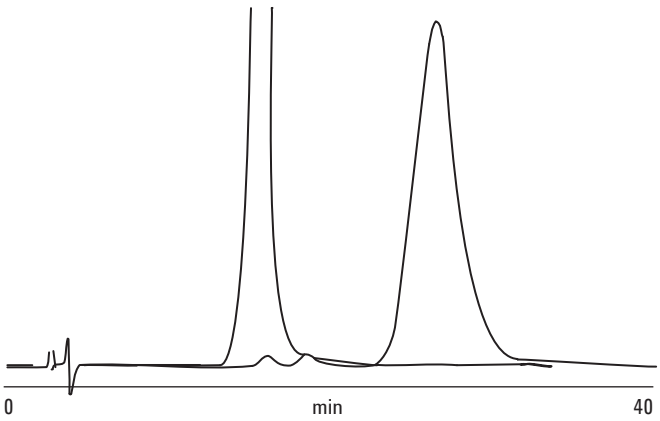

Figure 3. Overlay of metacycline and doxycycline.

This clearly shows that contaminants are present in this sample.

Conclusion

HPLC using a PLRP-S column successfully analyzed a mix of tetracyclines according to the USP method. Other international pharmacopeia commissions also produce compendia of approved, validated methods of analysis. These methods are an invaluable reference for quality assurance departments and/or analytical labs. Agilent Technologies is the only manufacturer of macroporous polystyrene/divinyl benzene HPLC materials in a full range of pore sizes from 100Å to 4000Å, and particle sizes from 3 µm to 10 µm, for use in pharmacopeia methods.

Reference

[1] US Pharmacopeia: L21, European Pharmacopeia: R

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This information is subject to change without notice.

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