

### Determination of Tetracycline Antibiotics by C18 RP-HPLC

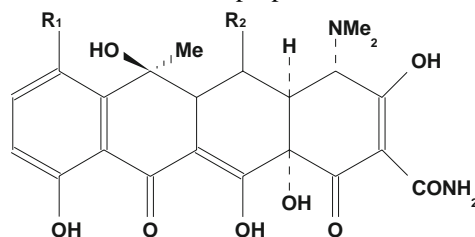
#### Abstract

A method is described for the determination of basic antibiotics, Chlortetracycline, Tetracycline and Oxytetracycline by RP-HPLC on a C18 column with UV detection. An oxalic acid buffer, tetramethylammonium chloride (TMA) and EDTA, was used. The use of TMA effectively eliminates the undesirable secondary interactions due to free silanols on the bonded silica column, providing effective and reproducible separation within 13 minutes.

Tetracycline antibiotics are widely used in modern medicine<sup>1</sup> and agricultural practice.<sup>2</sup> Chlortetracycline, oxytetracycline and tetracycline are three of the most frequently applied antibiotics in animal nutrition. Residual tetracyclines are found in muscle tissues and the organs of slaughtered animals.<sup>3</sup> Traditionally, microbiological<sup>4</sup> and fluorimetric<sup>5</sup> methods have been employed for their monitoring. These methods suffer from their inherent lack of selectivity. However HPLC offers high selectivity and sensitivity, and as a result has become the method of choice for the routine analysis of tetracyclines.

Bonded reversed phase columns have been reported to vary greatly in their suitability for the chromatography of tetracyclines. Unreacted silanols on the column interact with basic compounds, such as tetracyclines, giving poor chromatographic results.<sup>6</sup> As an alternative, more expensive polymeric RP columns have been used.<sup>7</sup>

By employing oxalic acid as a buffer, which also serves the dual purpose as a chelating



Chlortetracycline R<sub>1</sub> = Cl, R<sub>2</sub> = H  
 Tetracycline R<sub>1</sub> = H, R<sub>2</sub> = H  
 Oxytetracycline R<sub>1</sub> = H, R<sub>2</sub> = OH

agent, a method has been developed for the

#### Keywords:

Tetracycline, Oxytetracycline, Chlortetracycline, Antibiotic, Pharmaceutical, Veterinary Medicine, Animal Nutrition, Meat, Silanol blocking Agent, RP-HPLC

routine analysis of chlortetracycline, oxytetracycline and tetracycline using C18 columns. Depending on the acidity of the column, tetramethylammonium chloride could be added to further eliminate the secondary interactions of free silanols. These interactions are usually manifested as severe peak broadening and tailing.

#### Conditions

Column: Spherisorb S5 ODS2, 250 mm x 4.6 mm ID  
 Mobile Phase: 0.01 M oxalic acid, 0.01 M tetramethylammonium chloride, 3 mM EDTA (ph 2.5)/ Acetonitrile (75:25)  
 Flow Rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: UV at 280 nm  
 Injection Vol.: 20 µl

#### Preparation of Standard

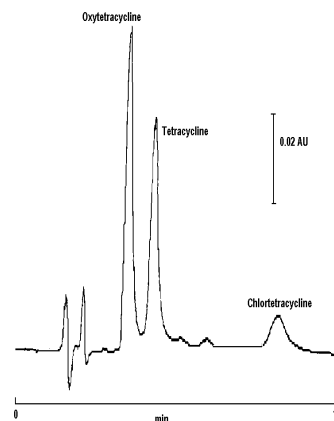


Figure 1 HPLC Separation of Tetracyclines

'...tetracycline, oxytetracycline and chlortetracycline are three of the most frequently applied antibiotics in animal nutrition...'



P1  
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Chlortetracline (1.0 mg), oxytetracycline (1.0 mg) and tetracycline (1.0 mg) were dissolved in 0.01 M HCl (10 ml).

### Acknowledgement

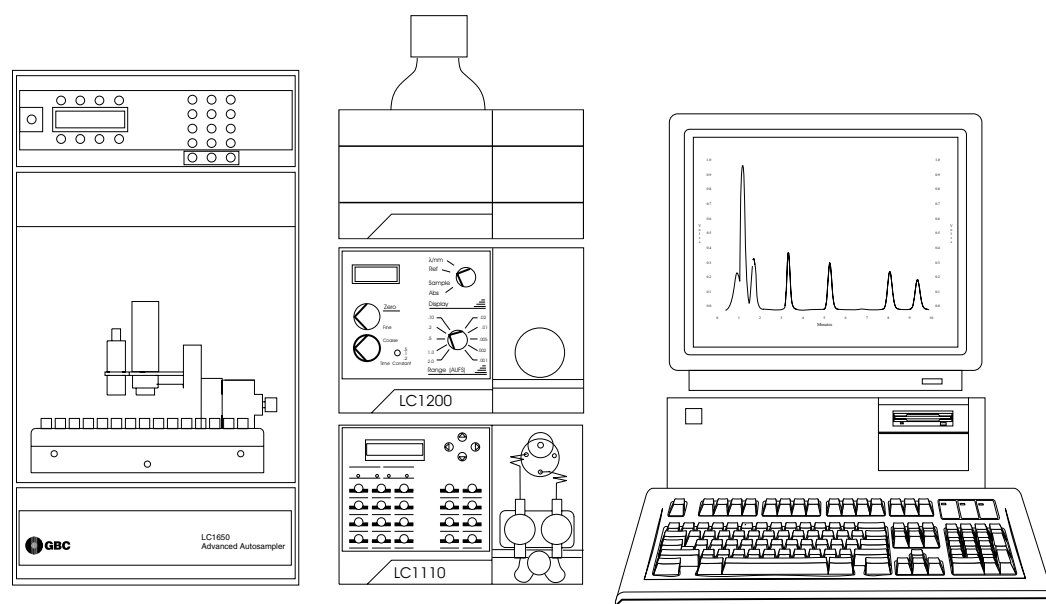
We are grateful to Mr John Walsh, Department of Agriculture and Rural Affairs (Victoria) for his technical advice and to his department for the kind gift of oxytetracycline and chlortetracycline.

### GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump  
LC1200 Variable Wavelength UV/Vis  
Detector  
LC1650 Advanced Autosampler  
WinChrom Chromatography Data  
Management System  
LC1445 System Organiser  
GBC Column Heater

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P1  
01-0372-00

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