HPLC application note

Determination of Penicillins by C18 RP-HPLC

Abstract

A method is described for the determination of Amoxicillin, ampicillin and Penicillin-G by reversed phase HPLC on a C18 column with UV detection. An oxalic acid buffer containing tetramethylammonium chloride (TMA) and DETA was employed. The use of this mobile phase has eliminated the undesirable secondary interactions between the acidic silanols on the column and the basic functionalities of the analytes, providing effective separation of the three penicillins in less than 8 minutes.

'...in the treatment of mastitis and other animal diseases, methods capable of distinguishing individual penicillins have been important from a regulatory point of view...'

Penicillins are one of the most widely used antibiotics in modern mecidine. Traditionally the detection of penicillins in biological samples, *e.g.*, milk and tissues, has been carried out by bioassay techniques.[1] However, these methods lack the selectivity in determining one penicillin from another analogue. With a variety of penicillins other than Penicillin-G being used today in the treatment of mastitis and other animal diseases, methods capable of distinguishing individual penicillins have been important from a regulatory point of view. HPLC offers the versatility necesssary, in that, standard LC procedures can be easily modified to accomodate the specific requirements of different analyses.

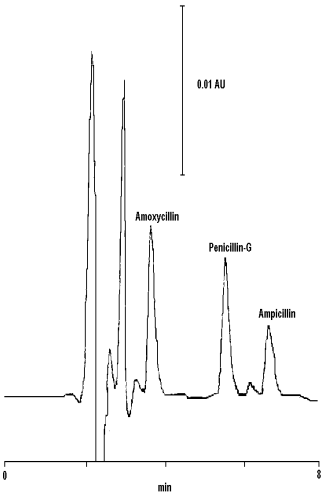
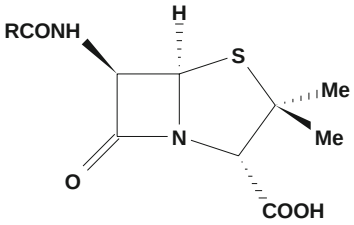


Figure 1 *HPLC Separation of Penicillins*

One of the inherent problems of the analysis of penicillins with basic functionalities on reversed-phase packings is the interactions of these moieties with the free silanols on the silica support.[2] Binding to these silanols causes broadening and distortion of chromatographic peaks of basic penicillins like Amoxicillin and Ampicillin. By employing an oxalic acid as a buffer, which also acts as a chelating agent, a method has been developed for the routine analyis of Amoxicillin, Ampicillin and Penicillin-G using C18 columns. Depending on the acidity of the column, tetramethylammonium chloride (TMA) could be added to further eliminate the secondary interactions of free silanols.

Keywords:
Penicillin, Amoxycillin, Ampicillin, Antibiotic, Silanol Blocking Agent, Pharmaceutical, Veterinary Medicine, Meat, Animal Nutrition, RP-HPLC



Amoxicillin R = -4OH-C$_6$H$_4$CH(NH$_2$)-

Penicillin-G R = -4OH-C$_6$H$_4$OCH$_2$-

Ampicillin R = -C$_6$H$_5$CH(NH$_2$)-

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Conditions

Column:	Spherisorb S5 ODS2, 250x4.6mmID
Mobile Phase:	0.01 M Oxalic Acid, 0.01 M, Tetramethylammonium Chloride, 3 mM EDTA, pH 2.5/Acetonitrile(80:20)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 265 nm
Injection Vol:	20 µl
Standard Prep.:	1.0 mg in 10 ml of 0.01

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
LC1120/LC1150 HPLC Column Oven Option

Acknowledgement

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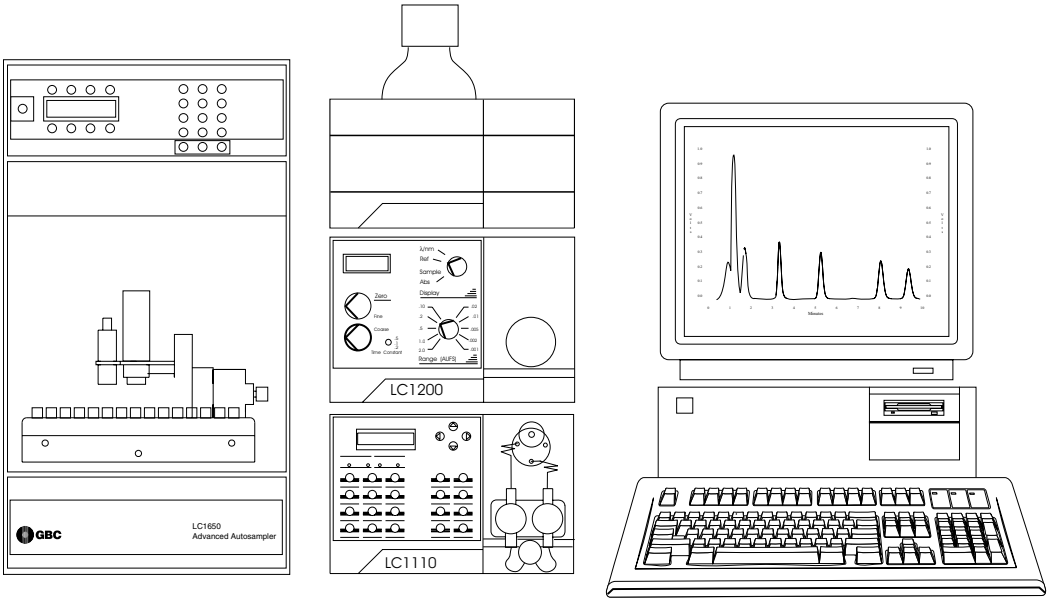
References

1. Moates, W.A., *J. Agri. Food Chem.*, 31, (1983), 1348.

2. Moates, W.A. and Leskinen, *J. Chromatogr.*, 386, (1987), 97.



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