



Analysis of Azithromycin using ZirChrom®-PBD

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The ZirChrom®-PBD column has been designated by the USP as L49 and can be used for the analysis of azithromycin. The pH required for the stability of the azithromycin analytes requires a high pH compatible column such as the ZirChrom®-PBD phase. This application note presents the pH 11.0 analysis of azithromycin on ZirChrom®-PBD.

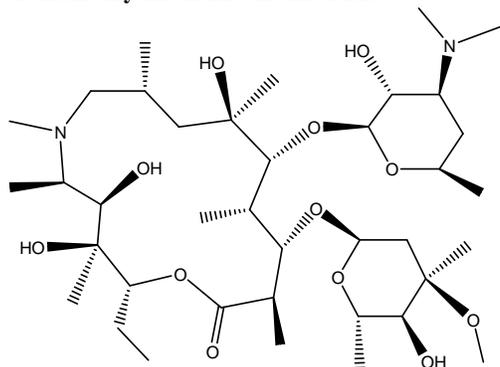


Figure 1: Structure of azithromycin.

Introduction

Azithromycin is a macrolide antibiotic that interferes with the growth of bacterial cells. Macrolides have activity against many gram-positive bacteria (excluding enterococci and methicillin-resistant *Staphylococcus aureus*), and have variable activity against respiratory gram-negative pathogens, *Mycobacterium avium* infections, gonorrhea, and *Chlamydia* infections (2). Azithromycin is used to treat bacterial infections in many different parts of the body but most often used to treat respiratory infections in children and adults.

The high pH necessary for the analysis of azithromycin prohibits the use of traditional silica based substrates and necessitates the use of the pH stable zirconia-based ZirChrom®-PBD. Azithromycin and its impurities lack good chromophores and thus any analytical study of purity must be made using an electrochemical detector.

Experimental

An analysis of Azithromycin and its analog Azaerythromycin was performed at 30°C using a ZirChrom®-PBD column (see figure 2). The separation conditions were as follows:

Column: ZirChrom®-PBD, 150 mm x 4.6 mm i.d. (Part Number: ZR03-1546)
Mobile Phase: 5.8 g monobasic potassium phosphate in 2130 mL of water, added to 870 mL of acetonitrile adjusted to pH 11.0 with potassium hydroxide
Temperature: 30 °C
Flow Rate: 1 ml/min.
Injection Vol.: 50 µl, 5 micrograms/mL
Pressure Drop: 195 bar

Detection: Electrochemical, amperometric mode, dual glassy carbon electrodes. Electrode 1:+.070V, Electrode 2:+0.82V, Background current: 85 nA.

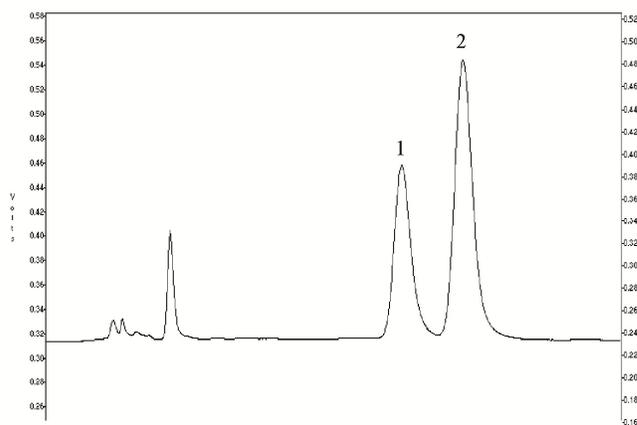


Figure 2: USP standard azithromycin. Solutes: 1=azaerythromycin, 2=azithromycin.

The ZirChrom®-PBD column gives plate counts of better than 2000 plates/column, with asymmetry factors of 1.37 and 1.45 for azithromycin and azaerythromycin respectively. Calibration curves constructed using this analysis show excellent linearity over a concentration range of 0.0 to 10.0 micrograms/mL. Studies of column life at pH 11 for ZirChrom®-PBD have shown that ZirChrom®-PBD is stable for more than 10,000 column volumes of operation. ZirChrom columns exhibit a high degree of reproducibility from column to column, making the ZirChrom®-PBD an excellent choice for the application of the azithromycin assay.

Additional suggestions and recommendations for this assay can be found in ZirChrom [Technical Bulletin #311](#).

References

- (1) USP, USP Monograph: Azithromycin (2005).
- (2) http://www.pfizer.com/download/uspi_zithromax.pdf

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