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Technical Bulletin #239

Recommendations for the Application of a Quantitative Assay for Azithromycin Using a ZirChrom®-PBD Column

HPLC Column

- A 15 cm x 4.6 mm i.d. ZirChrom®-PBD (part # ZR03-1546) column is recommended for this application.
- The ZirChrom®-PBD column is nearly identical in chromatographic selectivity to the L-29 (Alumina-PBD) column.
- The use of a guard column is strongly recommended to extend column life and enhance column performance (Holder part # 850-00, Insert part # ZR03-G40).

Mobile Phase

- The use of highly basic buffers with ZirChrom®-PBD is recommended to enhance the performance of the assay.
- The recommended mobile phase is 29/71 Acetonitrile/14mM Potassium phosphate, pH 11, at a flow rate of 1.0 ml/min.

Detection Parameters

- Electrochemical detection may be used in aperometric mode with dual glassy carbon electrodes.
- Electrode 1 should be set at +.070V, electrode 2 at +0.82V.
- The background current should be set at 85 nA.

Column Loading Capacity

Using a 15 cm x 4.6 mm i.d. column, the following column capacities are indicated by a 10% reduction in k'.

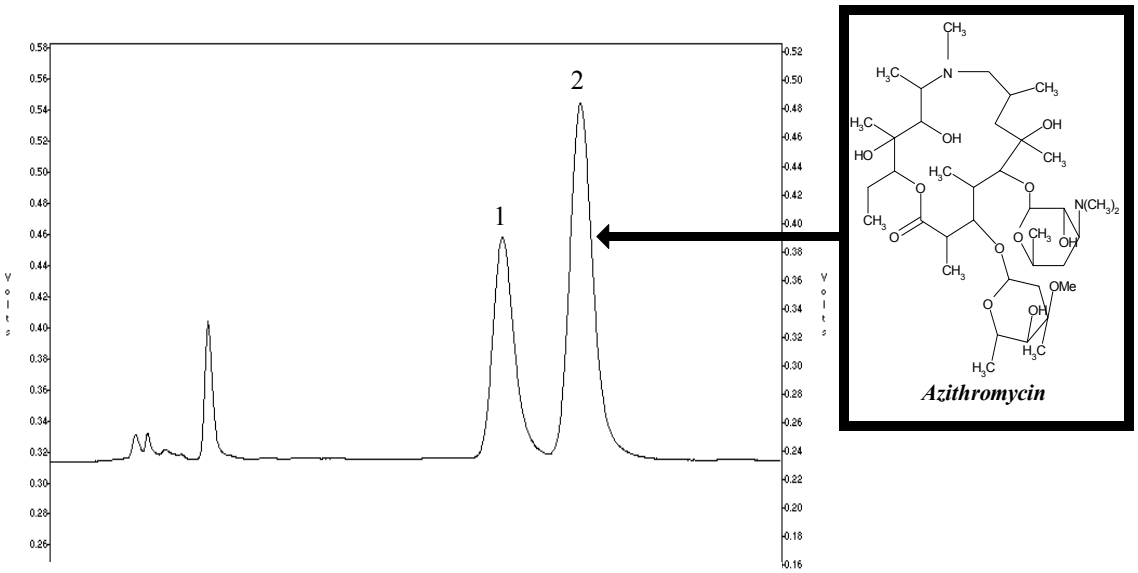
- Azithromycin — 2.0 mg/ml
- Azaerythromycin — 1.6 mg/ml

Analyte Detection Limits

Using the electrochemical detection scheme given above, the minimum detection limits are given below.

- Azithromycin — 4.7 ng/ml
- Azaerythromycin — 8.5 ng/ml

An analysis of Azithromycin and its analog Azaerythromycin is shown below.



LC Conditions: Column, ZirChrom®-PBD, 150 x 4.6 mm i.d.; Flow Rate, 1.0 ml/min.; Mobile Phase, 29/71 acetonitrile/14 mM potassium phosphate monobasic at pH 11; Column Temperature = ambient; Detector, amperometric electrochemical; Injection volume, 50 microliters; Sample concentration, 5 micrograms/ml. Solutes: 1 = azaerythromycin, 2 = azithromycin.

Analytical Performance

- 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.
- This level of performance is similar to what is reported for L-29 (Alumina-PBD).
- Studies of column life at pH 11 for ZirChrom-PBD and Alumina-PBD have shown that ZirChrom®-PBD is stable for more than 10,000 column volumes of operation, while the Alumina-PBD fails after 2500 column volumes of use.
- ZirChrom columns exhibit a high degree of reprodubibility from column to column, making them an excellent choice for the application of the Azithromycin assay.

ZirChrom column are available worldwide. For on-line ordering and for a listing of distributors, please visit our website at http://www.zirchrom.com.

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