

Liquid Chromatography

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HPLC Analysis of the Semi-Synthetic Antibiotic Azithromycin Using an Epic C18 Column

Abstract

Azithromycin, discovered in 1980, is a semi-synthetic macrolide antibiotic related to erythromycin.¹ The drug is widely used to treat chest infections such as pneumonia, infections of the nose and throat such as sinus infection (sinusitis), skin infections, Lyme disease, and some sexually transmitted infections.² The mode of action of azithromycin is like other macrolide antibiotics, it inhibits bacterial protein synthesis by binding to and interfering with the assembly of the 50S large ribosomal subunit of the bacteria, thereby inhibiting translation of mRNA.³

This application brief describes the use of an Epic™ C18 column for the simple analysis of azithromycin (Figure 1).

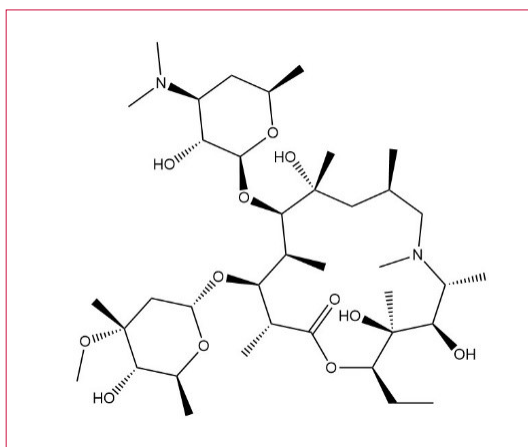


Figure 1: Chemical structure of azithromycin (ChemDraw).

Experimental Conditions

Hardware/Software

The chromatographic separation was conducted using a PerkinElmer LC 300 HPLC system and detection was achieved using a PerkinElmer LC 300 Multi-Wavelength UV/VIS detector. All instrument control, data acquisition, and data processing were performed using the SimplicityChrom™ software.

Method Parameters

All LC method parameters are shown in Table 1.

Table 1: LC method parameters.

Column	Epic C18 150 x 4.6 mm, 5 μ m (P/N: 135291-EC18)
Mobile Phase	80:20 Methanol: Phosphate Buffer pH 7.5 (premixed)
Flow Rate	2.0 mL/min
Oven Temperature	50 °C
Sample Temperature	Ambient
Detector Wavelength	Analytical Wavelength = 210 nm, Analytical Bandwidth = 10 nm, Reference Wavelength = 360 nm, Reference Bandwidth = 10 nm
Injection Volume	20 μ L, Partial Loop Injection Mode
Sampling Rate	2 pts/sec (Hz)

Solvents and Samples

All solvents were HPLC grade and samples were filtered using 0.45 μ m nylon filters, P/N 02542903.

The potassium phosphate buffer (pH 7.5, 0.03 M) was prepared by dissolving 1.079 g monobasic potassium dihydrogen phosphate (KH_2PO_4) and 3.844 g dibasic potassium phosphate (K_2HPO_4) in 900 mL of water. The pH was adjusted to 7.5 using concentrated sodium hydroxide. The solution was then made up to 1000 mL. The final mobile phase was then prepared by premixing using the buffer solution and gradient grade methanol (20/80 v/v).

A 1 mg/mL azithromycin standard was prepared in a 10 mL volumetric flask by dissolving a quantity of azithromycin dihydrate (MW= 785.02 g/mol), equivalent to 10 mg of azithromycin (MW = 748.98 g/mol), in 60/40 (v/v) gradient grade methanol/buffer solution.

Results and Discussion

Azithromycin was analysed in under 10 minutes using an Epic C18 (150 x 4.6 mm, 5 μ m) column (P/N: 135291-EC18), as demonstrated in Figure 2. The Epic C18 facilitates an efficient separation (18,656 N/m calculated using the tangential method) and displays excellent peak shape, highlighted by the low tailing factor of 1.03 (Table 2). This is due to the superior base deactivation and high-density bonding technology of the Epic C18.

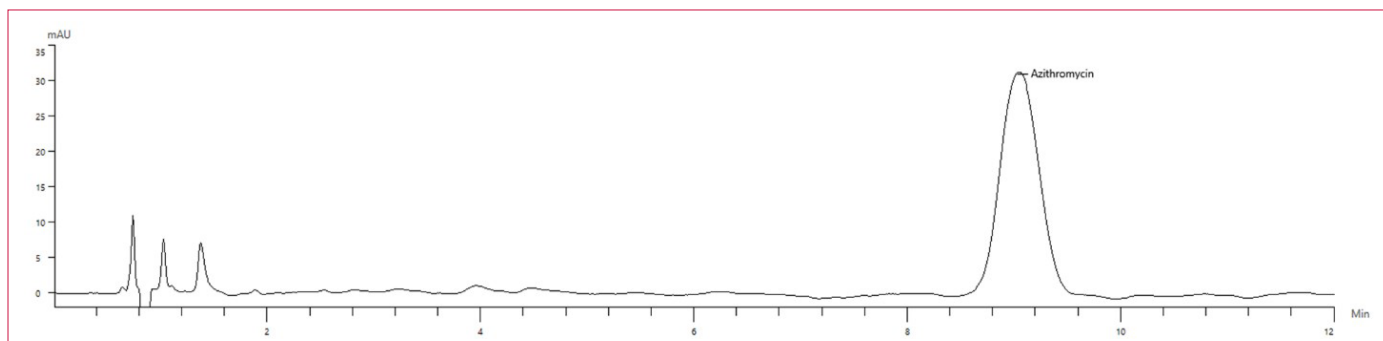


Figure 2: Analysis of azithromycin using an Epic C18 column (150 x 4.6 mm, 5 μ m).

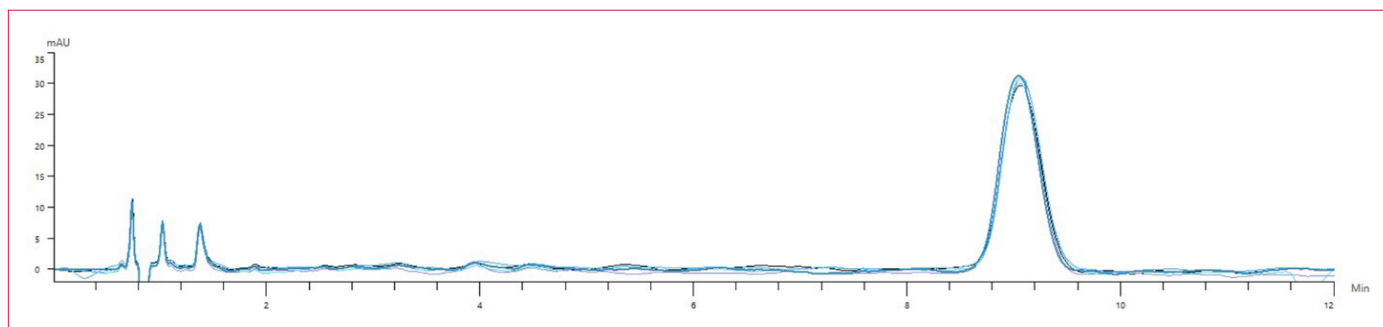


Figure 3: Overlay of 5 replicate injections of azithromycin using an Epic C18 column (150 x 4.6 mm, 5 μ m).

Table 2: Results summary.

Suitability Parameter	Epic C18 Column
Efficiency (N/m)	18,656
Tailing Factor (5% Peak Height)	1.03
Peak Area RSD (%) *	1.72

* RSD calculated from 5 successive injections.

Conclusion

- The Epic C18 column facilitates an efficient and repeatable separation of azithromycin.
- The superior base deactivation of the Epic C18 column allows for excellent peak shape, even for azithromycin which contains some polar functionality.
- Run time could be reduced by using a shorter Epic C18 column.

References

1. Fuad Al-Rimawi, Maher Kharaof, Analysis of Azithromycin and Its Related Compounds by RP-HPLC with UV Detection, Journal of Chromatographic Science, Volume 48, Issue 2, February 2010, Pages 86–90.
2. Nhs.uk, <https://www.nhs.uk/medicines/azithromycin/>, (accessed: 20/07/21).
3. Michael J. Parnham, Vesna Erakovic Haber, Evangelos J. Giamarellos-Bourboulis, Gianpaolo Perletti, Geert M. Verleden, Robin Vos, Azithromycin: Mechanisms of action and their relevance for clinical applications, Pharmacology & Therapeutics, Volume 143, Issue 2, 2014, Pages 225-245, ISSN 0163-7258.

Consumables

Component	Description	Part Number
Column	Epic C18 150 x 4.6 mm, 5 µm	135291-EC18
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (Polypropylene) Cap with PTFE/Silicone Pre-Slit Septa (100/pack)	N9306203
Syringes	1 mL Luer-Lock Tip Disposable Syringe (100/pack)	02542890
Syringe Filters	0.45 µm Particle Size Nylon Syringe Filter (100/pack)	02542903
PEEK Fittings	Finger-tight for 1/16" OD PEEK Tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301