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The Power of ACQUITY UPLC for the Resolution of Erythromycins

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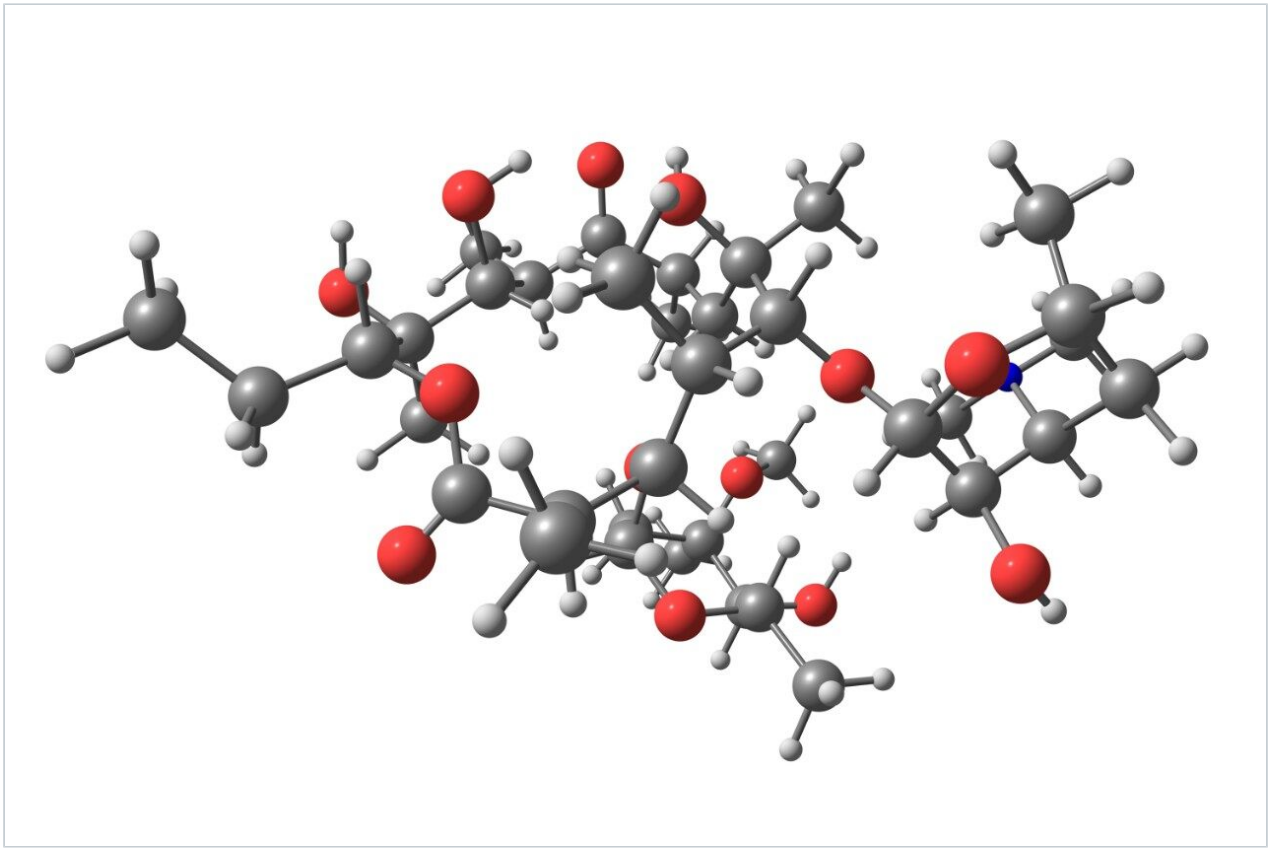

Abstract

This application note describes the efficient, high-quality analysis of erythromycins using fast, generic gradients on a C_{18} column and an evaporative light scattering (ELS) detector.

Introduction

Macrolide antibiotics are a group of compounds that are used clinically for the treatment of respiratory and soft tissue infections. Erythromycin, Clarithromycin, and Azithromycin are the three main macrolide antibiotics in clinical use. The commercially available product (marketed as erythromycin) contains primarily erythromycin A, but also small amounts of erythromycins B and C. And where erythromycin is produced by fermentation, clarithromycin and azithromycin are semi-synthetic modifications of erythromycin, and are therefore closely related to erythromycin structurally. Presently, erythromycins are analyzed using USP methods with relatively complex chromatographic procedures at low UV wavelengths, or by electrochemical procedures with obscure column chemistries. In order to facilitate a more streamlined analysis of these antibiotics (some of which are coming off patent),[1] this application note describes the efficient, high-quality analysis of erythromycins using fast, generic gradients on a C_{18} column and an evaporative light scattering (ELS) detector.

Experimental

For these experiments, an ACQUITY UltraPerformance LC (UPLC) System was coupled with an ACQUITY UPLC ELS Detector, both controlled by MassLynx 4.1 Software. The separation was achieved with an ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm Column, operating at 35 °C. The mobile phase composition was as follows: eluent A contained H_2O with 0.05% Trifluoroacetic Acid (TFA), and eluent B contained Acetonitrile with 0.05% TFA. Generic linear gradient runs were performed from (A/B) 95/5 to 5/95 over 90 seconds, with a flow rate of 0.84 mL/min and 0.5 µL injections. The ACQUITY UPLC ELS Detector was run at 20 Hz, with a time constant of 0.1 sec, a drift tube temperature of 50 °C, gas pressure of 40 psi, and the nebulizer chamber cooling on. Standards were dissolved in DMSO at a concentration of 0.5 mg/mL.

Results and Discussion

Macrolide antibiotics analysis are relevant in discovery and development laboratories, as well as in facilities that support manufacture of these clinically-administered substances. In these multi-tasking environments, efficiency of analyses is crucial. Figure 1 depicts the chromatographic results for the analysis of several macrolide antibiotics using the UPLC/ELS platform, illustrating the rapid (90-second) linear gradient analysis of azithromycin, erythromycin A, and clarithromycin dissolved in DMSO.

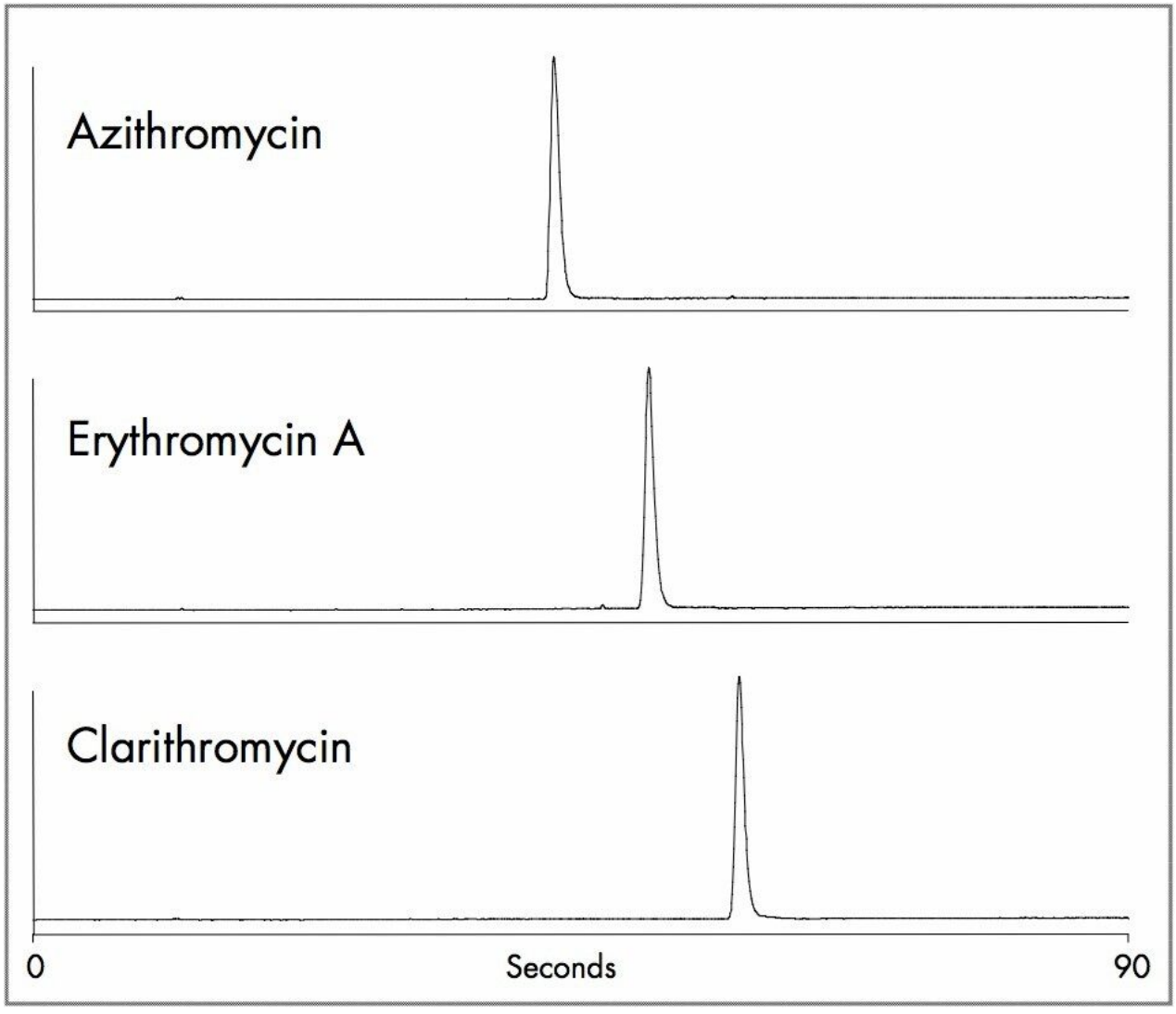

Figure 1. ELS detector traces of the three macrolide antibiotics: azithromycin, erythromycin A, and clarithromycin, using an ACQUITY UPLC System with an ACQUITY UPLC ELS Detector.

The ACQUITY UPLC ELS Detector conditions were such that the DMSO peak was not detected - a benefit for users that do not wish to confuse their sample solvent with potential early eluters. As seen in Figure 2, compounds that are very similar structurally can still elute at distinct retention times within this fast gradient run. In this separation of a mixture of erythromycins standards using a 90-second linear gradient, erythromycins A, B, and C are very closely related, yet baseline resolution is easily achieved.

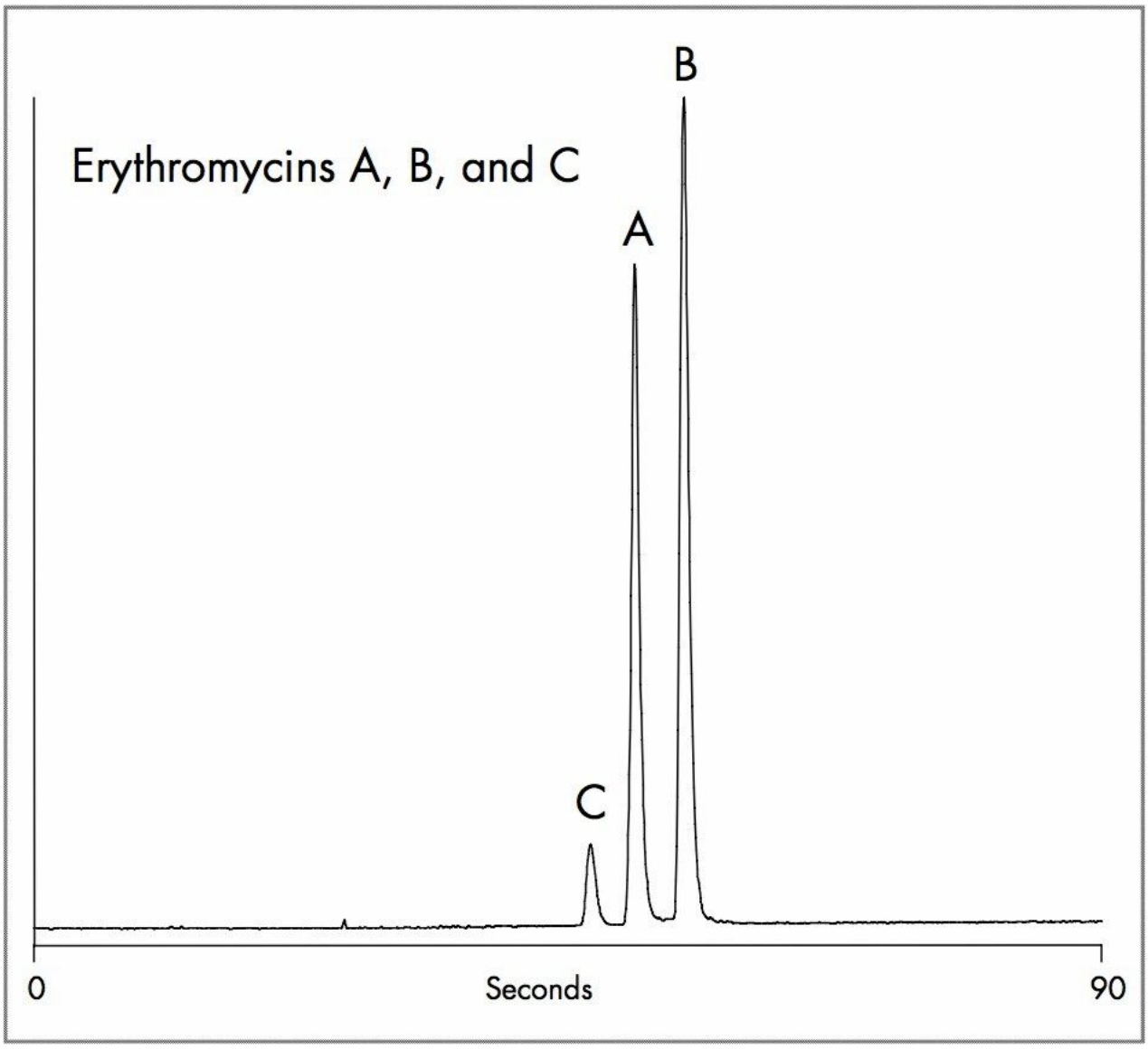

Figure 2. ELS detector trace of the structurally-related erythromycin A, B, and C standards using an ACQUITY UPLC System with an ACQUITY UPLC ELS Detector.

The resolving power and speed of the UPLC/ELS system is clearly demonstrated. These results indicate how this groundbreaking technology can deliver quality analyses in less time, providing a straightforward

technique for those facilities that may be contemplating the manufacture of closely-related products.

Conclusion

Antibiotics such as the erythromycins traditionally require complex analyses using low UV wavelengths, or by employing electrochemical detection involving outmoded column chemistries. This application note demonstrates a new UPLC/ELS approach to antibiotic analysis performed in just 90 seconds using linear gradients. This new technique provides a simplification of the workload in busy pharmaceutical laboratories for the detection of erythromycins and its closely-related compounds.

References

1. Orange Book: FDA Approved Drug Products with Therapeutic Equivalence Evaluations.

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ACQUITY UPLC System <https://www.waters.com/514207>

ACQUITY UPLC ELS Detector <https://www.waters.com/514219>

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