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应用纪要

Azithromycin, Azaerythromycin A, and Clarithromycin: UPLC/ELS Baseline Gradient Separation

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Abstract

In this application note, we present an UltraPerformance LC (UPLC) method combined with evaporative light scattering (ELS) detection for the high-quality, rapid analysis of Azithromycin, Azaerythromycin A, and Clarithromycin, three structurally-similar compounds.

Benefits

Provides simplified workload and increased productivity

Introduction

Azithromycin, a commonly prescribed antibiotic worldwide, and its degradation products have been traditionally analyzed by thin layer chromatography and isocratic liquid chromatography under low wavelength UV[1,2] for quality control and final product formulation.

Analytical methods with high resolving power become necessary as the volume of these and other drugs increases. In this application note, we present an UltraPerformance LC (UPLC) method combined with evaporative light scattering (ELS) detection for the high-quality, rapid analysis of Azithromycin, Azaerythromycin A, and Clarithromycin, three structurally-similar compounds.

Experimental

For these experiments, an ACQUITY 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

Azithromycin, azaerythromycin A, and clarithromycin are drugs that are used for prescription against selective microbes, as small changes in structure can provide benefits in the fight against given microorganisms.[3] The work shown here consists of the development of a generic linear gradient using ELS detection for the effective separation of these three antibiotics. Figure 1 shows the chromatographic results of the analysis using the UPLC/ELS platform.

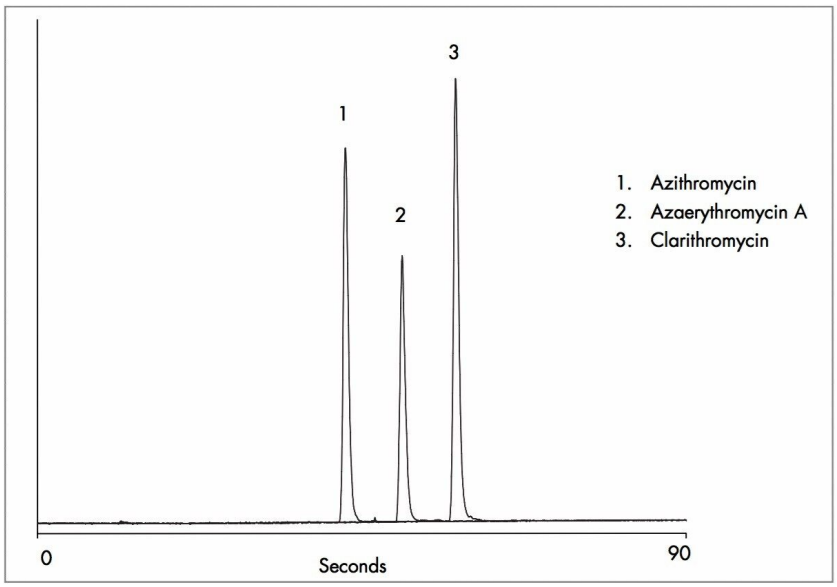

Figure 1. Evaporative light scattering detector trace of azithromycin, Azaerythromycin A, and clarithromycin, using a UPLC method with ELS detection.

As we can see, the chromatogram indicates that while these three compounds have very similar chemical compositions, baseline resolution is easily achieved for each when employing the ACQUITY UPLC System technology coupled with an ACQUITY UPLC ELS Detector, carrying out a very rapid (90-second), generic linear gradient.

These results are significant as resolution and fast chromatography are key for the effective, high-quality analyses of batches of compounds, as well as the determination of potential impurities when analyzing compounds that lack chromophores.

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 for high-quality antibiotic analysis performed in just 90 seconds using linear gradients. For the detection of erythromycins and its closely-related compounds, this new technique provides a simplification of the workload and increased productivity for busy pharmaceutical laboratories.

References

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