Azithromycin Analysis According Latest USP Monographs Using HPLC-ECD

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Azithromycin — USP Monographs

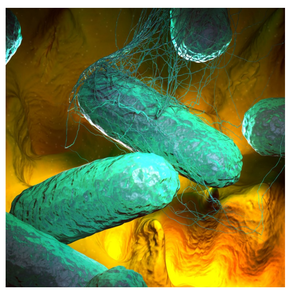


Figure 1: *Salmonella typhi, Gram-negative bacterium that causes systemic infections and typhoid fever in humans, which can be treated with the macrolide antibiotic Azithromycin.*

Azithromycin is a semi-synthetic macrolide antibiotic, that is effective against a wide variety of bacteria.
Several USP monographs [1] describe the analysis method for Azithromycin in various formulations. Some of these methods are based on HPLC with electrochemical detection (ECD). Unfortunately, the measurement criteria for background current and cell potential in the USP method appeared to be quite counter-productive. In spite of the narrow window of allowable cell current, we managed to develop an LC-ECD system that produces results fully in compliance with USP criteria and requirements for all tested USP monographs.

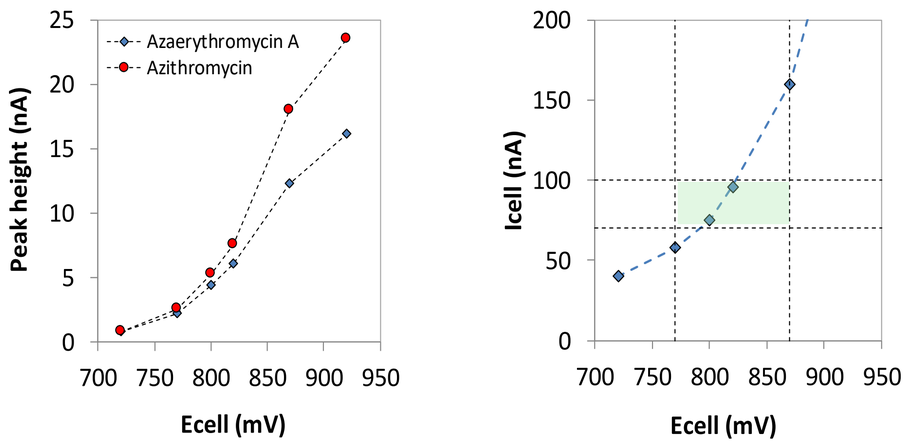


Figure 2: *Hydrodynamic voltammogram of Azithromycin and Azaerythromycin A measured with SenCell equipped with GC working electrode vs. salt bridge (left panel) and the response of the background current (Ic) to the various working potentials (right panel). The green area in the right plot indicates the allowed settings as given in the USP monographs for Azithromycin in capsules or oral suspension ('Assay'). Results based on the analysis of the 'System Suitability Solution'.*

ALEXYS Azithromycin Analyzer

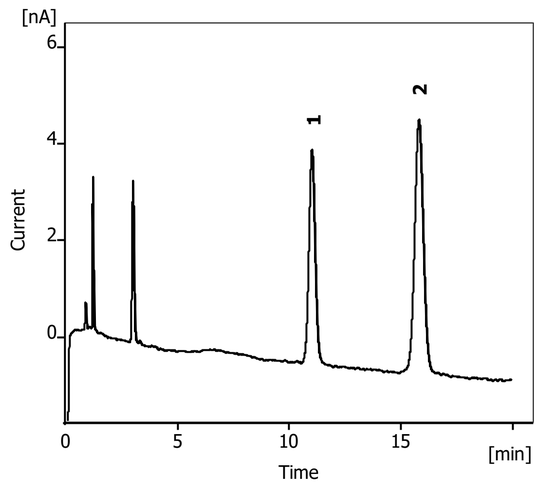
The ALEXYS Azithromycin Analyzer offers a turn key solution for the analysis of Azithromycin according to the USP methods. It consists of an AS110 autosampler, a P6.1L HPLC pump and the DECADE Elite ECD equipped with two flow cells.

Figure 3: *ALEXYS™ Azithromycin Analyzer*

[1] Official Monographs / Azithromycin, USP 41 (2018)

Results System Suitability Test

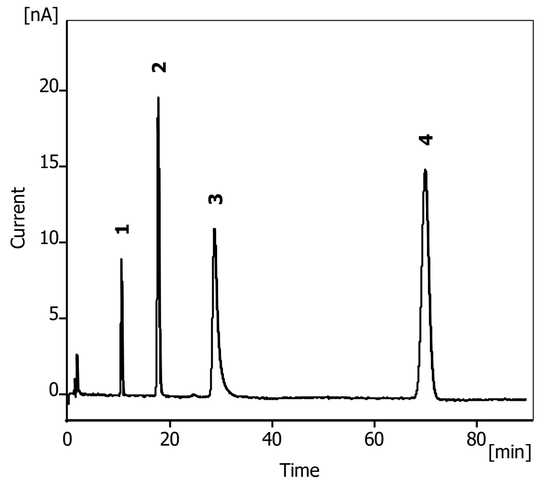
1. Azithromycin for Oral Suspension/Capsules - Assay



System suitability parameter	*USP criteria*	Result
Resolution Azaerythromycin A Azithromycin	> 2.5	7.4
Column efficiency Azithromycin	> 1000	7651
Tailing factor Azithromycin	0.9 - 1.5	1.0
RSD (n=5) Azithromycin	< 2.0%	1.4%

Figure 4: *Chromatogram from 3.2 ug/mL USP azaerythromycin A (1) RS and 3.3 ug/mL USP azithromycin (2) RS in mobile phase. System suitability solution and conditions as described in the USP monograph for 'Azithromycin in Capsules' and 'Azithromycin for Oral Suspension', section 'Assay' using a ZirChrom column with packing L49.*

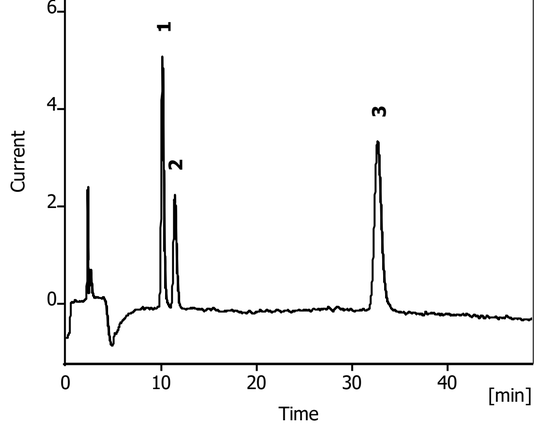
2. Azithromycin for Injection - Impurities 1



System suitability parameter	USP criteria	Result
Tailing factor		
Azithromycin	< 2.0	1.1
N-demethylazithromycin	< 2.6	2.0
RSD (n=6)		
Azithromycin N-oxide	< 10%	6%
Desosaminylazithromycin	< 10%	4%
N-demethylazithromycin	< 10%	6%
Azithromycin	< 10%	3%

Figure 5: *Chromatogram from 1 ug/mL USP azithromycin N-oxide RS (1), 0.9 ug/mL USP desosaminylazithromycin RS (2), 3.2 ug/mL USP N-demethylazithromycin RS (3) and 3.2 ug/mL USP azithromycin RS (4) in mobile phase. Standard solution' and conditions as described in the USP monograph for 'Azithromycin for injection', section 'Impurities: limit of azithromycin N-oxide, desosaminylazithromycin and N-demethylazithromycin'.*

3. Azithromycin for Injection - Impurities 2



System suitability parameter	*USP criteria*	Result
Resolution desosaminylazithromycin N-demethylazithromycin	> 1.5	2.3
Tailing factor Azithromycin	< 1.5	1.2
RSD (n=5) Azithromycin	< 5%	1.4%

Figure 6: *Chromatogram overlay from 1.8 ug/mL USP desosaminylazithromycin RS (1), 4.2 ug/mL USP N-demethylazithromycin RS (2) and 6 ug/mL USP azithromycin RS (3) in 46% acetonitrile. Standard solution and conditions as described in the USP monograph for 'Azithromycin for injection', section 'Impurities: limit of aminoazithromycin and others'.*

Conclusion

By using the ALEXYS Azithromycin Analyzer the obtained results are in full compliance with the latest USP 41 (2018) monograph for azithromycin analysis.