

AZITHROMYCIN, ERYTHROMYCIN AND OTHER MACROLIDE ANTIBIOTICS



THE MOST RELIABLE LC-EC APPLICATIONS FOR PHARMACEUTICAL & BIOTECH ANALYSIS EVER FORMULATED

Aminoglycosides

- Amikacin
- Framycetin Sulphate
- Gentamicin Sulphate
- Kanamycin Sulphate
- Lincomycin
- Neomycin
- Spectinomycin
- Tobramycin

PET imaging tracer

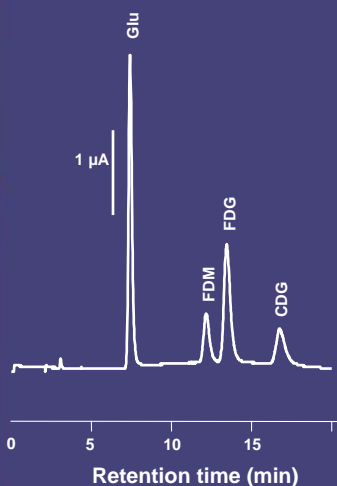
- FDG

Macrolide antibiotics

- Azithromycin
- Azaerythromycin
- Clarithromycin
- Erythromycin
- Roxithromycin

Bioanalysis of pharmaceuticals

- Artemisinin
- Dihydro-artemisinin
- Artemether
- Etoposide
- 8-OH-DPAT
- mesna BNP7787
- Vincristine



INTRODUCTION

The macrolides are a group of antibiotics whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars may be attached. Macrolide antibiotics are used to treat infections caused by Gram positive bacteria, *Streptococcus pneumoniae*, and *Haemophilus influenzae* infections such as respiratory tract and soft tissue infections. The antimicrobial spectrum of macrolides is slightly wider than that of penicillin, and, therefore, macrolides are a common substitute for patients with a penicillin allergy.

Azithromycin (Azi) is a semi-synthetic macrolide antibiotic chemically related to erythromycin and clarithromycin [1-4]. It is effective against a wide variety of bacteria organisms, such as *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and many others. Azithromycin is used to treat bacterial infections such as bronchitis; pneumonia; sexually transmitted diseases (STD); and infections of the ears, lungs, skin, and throat.

- Method based on US monograph USP 30-NF25, May 2007
- Analysis of main substituent and impurities
- Column USP L49 stationary phase

Summary

In this note the USP monograph USP 30-NF25 issued in May 2007 has been used as a basis for the development of a method for the analysis of macrolide antibiotics, using the ALEXYS Macrolide Antibiotics analyzer with a dual flow cell configuration and a column with USP L49 stationary phase.



Fig. 1. ALEXYS Macrolide Antibiotics Analyzer.

With this LC system macrolide antibiotics such as Clarithromycin, Erythromycin, Roxithromycin, and Azithromycin were analyzed.

Method

The USP method for Azi uses two flow cells in series, cell 1 for screening and cell 2 for detection of the analytes. The first flow cell is a Reactor cell, the second is the VT-03 cell.

Stock solutions of 220 μM Azaerythromycin (Aza) and 440 μM Azi were prepared in 100% acetonitrile. The final standards were obtained by diluting the stock solutions with mobile phase.

Table 1

Conditions	
HPLC	ALEXYS Azithromycin Analyzer
Temperature	35 °C for separation and detection
Flow rate	1.0 mL/min
Flow cell 1	Flexcell™, GC with Hy-REF™
Flow cell 2	VT-03, 2 mm GC sb REF, 50 μm spacer
ADF	0.5 Hz
Range	100 nA/V

Some conditions differ slightly from the USP method.

Hydrodynamic voltammogram

The USP method requires a working potential of 0.70 ± 0.05 V for cell 1 and 0.82 ± 0.05 V for cell 2. As can be seen in the voltammogram this working potential is not optimal for cell 2. Therefore a working potential of 0.87 V for cell 2 has been used in all experiments, which is the best possible setting within the USP specifications. The working potential of cell 1 was set to 0.65 V.

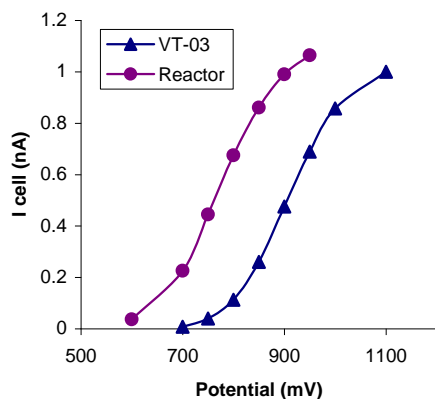


Fig. 2. Hydrodynamic voltammogram for Azi on cell 1 (dots) and 2 (triangles).

USP criteria

The USP method has a number of method specifications for selectivity, reproducibility, peak asymmetry, plate number and relative retention time. In Table 2 the criteria of the USP are compared with the LC performance obtained with the system.

The chromatographic resolution is determined by injecting a standard solution of 4.5 μM Aza and 4.4 μM Azi. Resolution of Azi and Aza is better than 4 (Fig.3). The relative retention time of Aza vs. Azi is 0.7.

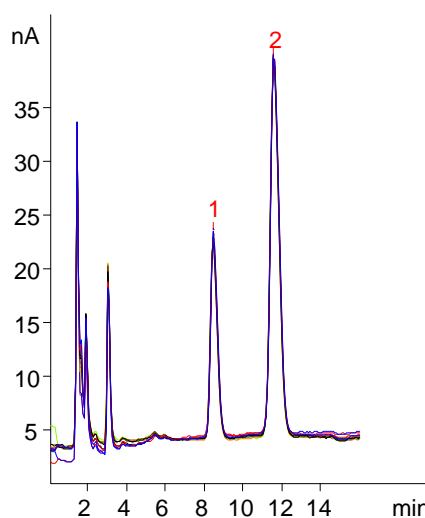


Fig. 3. Overlay of repeatability study of 4.4 μM Aza (1) and 4.4 μM Azi (2). Signal cell 2.

The repeatability has been studied for 10 replicate injections of 4.4 μM of Azi and Aza. The relative standard deviation in peak areas was < 1% (Fig.3).

Table 2

USP system suitability requirement			
Parameter	USP criteria	Aza	Azi
Retention time (min)	-	8.4	11.6
Rel. retention time	0.7 and 1	0.7	1
%RSD area (4.4 μM)	< 2.0%	0.9 %	0.9 %
Theoretical Plates	> 1000	2725	3020
Resolution	> 2.5		4.2
Tailing factor	0.9 to 1.5	1.4	1.3

A small shift in retention time was found when using the mobile phase for more than one day. In a period of 5 days this shift is about 30 seconds. This is probably due to a drop in pH of mobile phase. Over the same period of time the pH dropped from 11.0 to 10.8. It should be noted that a 15 mmole/L phosphate buffer at pH

11.0 is not optimal. It is therefore advised to prepare fresh mobile phase every day.

The asymmetry (A) of the Azi peak was calculated using $A = 0.5 \cdot [\text{width}] / [\text{width of left-half of peak}]$ measured at 5% of peak height. According to the USP the asymmetry (tailing factor) should be a value between 0.9 and 1.5. We found values between 1 and 1.4. The USP requires a column efficiency of more than 1000 plates for Azi and Aza. A column efficiency of more than 2500 plates was found for both substances.

Linearity is measured by constructing a calibration line in the concentration range 0.1 – 40 μM Azi (not shown). The regression line $Y = a + bX$ is given by $Y = 127 [\pm 59] + 212 [\pm 3] X$. Correlation coefficient r is 0.9992.

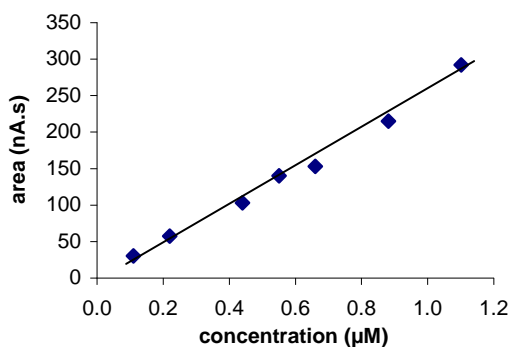


Fig. 2. Regression line for 0.1 – 1.1 μM Azi.

For calculation of detection limit a regression line in the concentration range 0.1 – 1.1 μM has been used (Fig. 2). The detection limit calculated as $3 S_{y/x}/b$ is 0.1 μM [5]. Calculation of detection limit as 3 times the peak-to-peak noise of the baseline, results in a detection limit of about 40 nmol/L (Fig. 3). Note that the detection limit is negatively affected by non-optimised working potential, required by USP.

Clarithromycin, Roxithromycin and Erythromycin

A mixture of several macrolides was analysed to illustrate the chromatographic performance of the method. All components are separated with at least a resolution of 2.5.

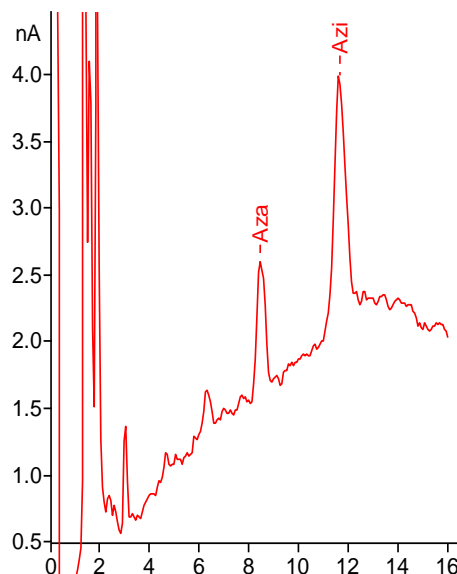


Fig. 3. Analysis of 200nM Aza and Azi.

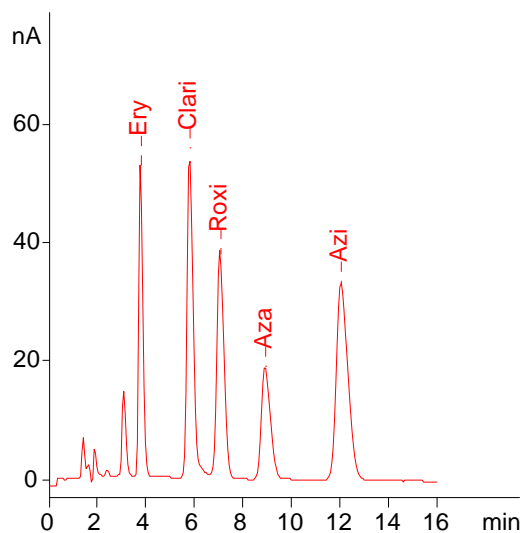


Fig. 4. Analysis of 4.5 μM Clarithromycin, Erythromycin, Roxithromycin, Aza and Azi. Signal cell 2.

Examples of Pharmaceutical formulations

Below a few examples are shown of the analysis of pharmaceutical formulations.

Zithromax tablet (Azithromycin)

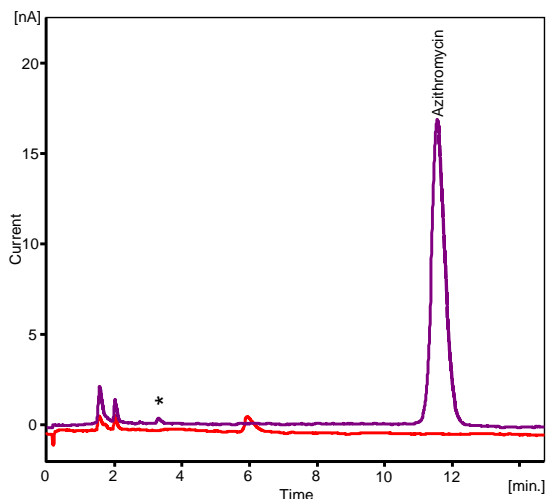


Fig. 5. Overlay of chromatograms from blank and 10 μ M Azithromycin in mobile phase, based on extraction of a Zithromax tablet. The peak marked with a * is an additional peaks to the main peak that is not present in the blank.

Inderm solution (Erythromycin)

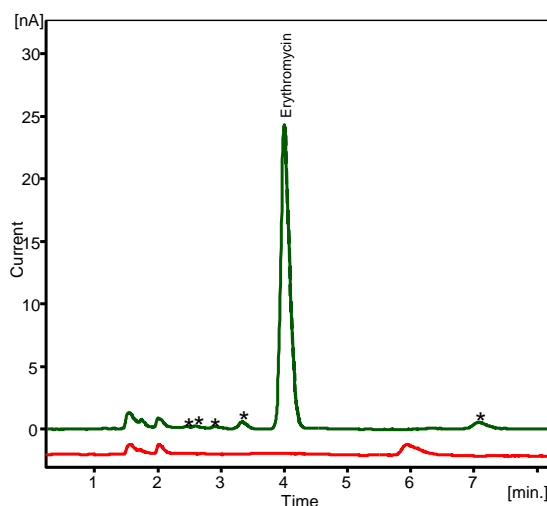


Fig. 6. Overlay of chromatograms from blank and 10 μ M Erythromycin in mobile phase, based on dilution of 'Inderm' solution. The peaks marked with a * are additional peaks to the main peak that are not present in the blank.

Conclusion

An USP method has been used as a basis for the development of an analysis solution for Macrolide Antibiotics. The ALEXYS Macrolide Antibiotics analyzer is configured with a dual flow cell setup and an L49 USP phase column.

References

1. USP-NF: page No. 185. Pharmacopeia form: Volume No.23 page No. 3407
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3. Chieko Taninaka, Hisakazu Ohtani, Erika Hanada, Hajime Kotaki, Hitoshi Sato, Tatsuji Iga, Journal of Chromatography B, 738 (2000) 405-411
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5. JN Miller and JC Miller; Statistics and Chemometrics for Analytical Chemistry, 5th ed, 2005, Pearson Education, Harlow, England. (ISBN: 0130228885).

PART NUMBERS

180.0086C	ALEXYS Macrolide Antibiotics analyzer
250.1126	ALG-515, 150 X 4.6 mm, 3 μ m
250.1128	ALG guard column inserts, 3pk
250.1130	ALG guard column holder

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. Conditions differ slightly from the USP method USP30-NF25 page No. 185 (2007). The actual performance may be affected by factors beyond Antec Leyden's control. Specifications mentioned in this application note are subject to change without further notice.