Fortis™ Technologies Ltd.

Application Note

Azithromycin

Introduction

Developed in the late 1970's Azithromycin is still one of the worlds most popular antibiotics[1]. Present on the WHO's (World Health Organisations) Essential Medicines Library, due in part to the clinical efficacy of single dose azithromycin[2]. Used to treat certain bacterial infections, concerned mainly with ear infection, troat, pneumonia, typhoid and sinusitis. It is derived from Erthromycin a macrolide antibacterial compound, differing in that a methyl substituted nitrogen atom is incorporated into the lactone ring, thus making the lactone ring 15 membered. Azithromycin interfer's with the protein synthesis of bacteria's growth mechanism[3]. Pliva is the original developer of Azithromycin in Crotia and in 1981 they patented the drug, and sell under the name Sumamed in Europe, whilst Pfizer sells the licensed product under the name Zithromax. In 2005 the patent ran out so now azithromycin is manufactured as a generic drug making it widely available at a competitive price.

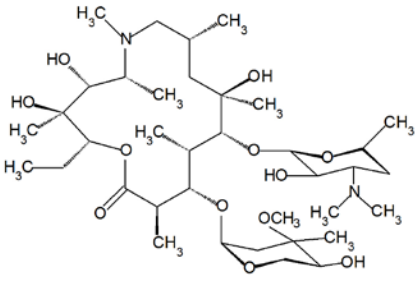
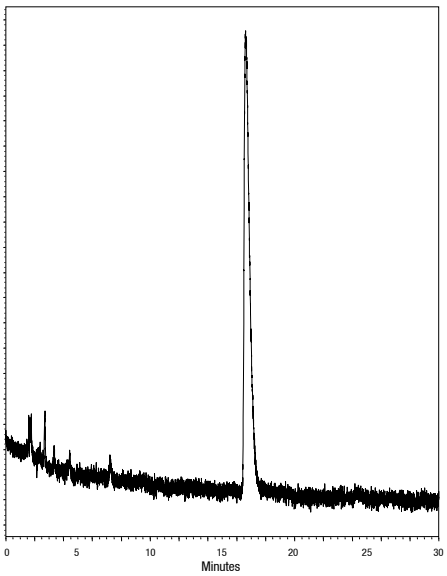


Figure 1. Azithromycin

Arithromycin is readily transported to the site of infection by the phagocytes where large doses can be realised and with a good half-life and a propensity for lipid layers the drug can remain at the infected tissue site for a significant length of time. The active substance azithromycin is the subject of a European Pharmacopeia (Ph.Eur.) and USP monograph.

Experimental

Azithromycin is best analysed by HPLC if a high pH mobile phase can be utilised. Solubility is suspect in water hence high concentrations of organic solvent are generally preferred. In the Ph.Eur. monograph potassium phosphate is used to provide this increased pH level. Unfortunately not many columns will handle these extended pH regions >8.



In this application note Fortis™ C18 was used since it can provide analysis at high pH upto pH 12. Typically C18 columns will not operate at these higher pH values. Fortis C18's optimised bonding allows for excellant stbility and lifetime at high pH. An ammonia buffer was used instead of the recommended phosphate buffer in order to make the methodology more compatible with LC-MS. The volatile nature of ammonia allowing for a good spray in MS without suppression of signal and therefore no loss of sensitivity. An isocratic mix of aqueous:organic provides a suitable mobile phase for solubility, retention and selectivity from any possible degradents.

Column: 5µm Fortis™ C18 250 x 4.6 mm
p/n F18-050905

Mobile phase
20 : 80 +0.1% NH_3 in H_2O : ACN
Flow Rate: 1.0ml/min
Temp: 20ºC
Detection: UV 200nm

Results

Azithromycin shows good retention in a simple isocratic mobile phase system, the high pH/high organic mobile phase system meaning that the compounds stays in solution well and also gives good sensitivity, meeting the requirements of the various monograph compendial methods. The variety of its structure leads to difficulty if other lower pH reversed-phase methods are employed.

Conclusion

Fortis™ C18 allows for good retention, sensitivity and selectivity of Azithromycin. The method developed allows for the simple use of mobile phase conditions which are compatible with todays most sensitive detection technologies, Mass Spectroscopy and ELSD (Light Scattering Detector).

Azithromycin is a popular drug with great proliferation, by having this simple analysis method for QC and a highly stable column then robustness and reproducibility can be assured.

References

1. http://www.wipo.int/sme/en/case_studies/pliva.htm

2. http://apps.who.int/emlib/MedicineDisplay.aspx?Language=EN&MedIDName=423%40azithromycin

3. Yamauchi K., et al., Azithromycin suppresses interleukin-12p40 expression in lipopolysaccharide and interferon-gamma stimulated macrophages. Int. J. Biochem. Cell Biol. 5, 667-678, (2009)