APPLICATION BRIEF



Liquid Chromatography

HPLC Analysis of the Semi-Synthetic Penicillin, Amoxicillin

Introduction

Antibiotics are natural substances released by bacteria and fungi which are capable of killing, or inhibiting, competing microbial species. This phenomenon has long been known; but it was not until 1928 that penicillin, the first true antibiotic,

was discovered by Alexander Fleming, Professor of Bacteriology at St. Mary's Hospital in London.1 However, it 12 years later that pen years later before penicillin was isolated and developed as a medicine by Howard Florey and Ernst Chain.2

Since this breakthrough in therapeutic medicine, derivates of penicillin were developed with increased efficacy levels. Amoxicillin is one of several such semisynthetic penicillin's. First discovered in the 1960's its patent has now expired. Consequently, amoxicillin and co-amoxiclav is now marketed under many different trade names worldwide. It is commonly used in the treatment of numerous infections including pneumonia, skin infections and Lyme disease.

This application brief illustrates the rapid analysis of amoxicillin, Figure 1, using the Quasar C18 column.





Experimental Conditions

Method Parameters

All LC method parameters are shown in Table 1.

Table 1. LC Method Parameters.

| Quasar C18 | 100 mm | 4.6 mm | 3 µm | N9308806 | | |
|----------------|--|--------|------|----------|--|--|
| Mobile Phase | A: H0 (+0.1% formic acid) B: AČN (+0.1% formic acid) Gradient: 5-50% B in 10 minutes | | | | | |
| Flow Rate | 1 mL/min | | | | | |
| Тетр | 20 °C | | | | | |
| Wavelength | 254 nm | | | | | |
| Injection Vol. | 5 µl | | | | | |
| Analyte | Amoxicillin | | | | | |

Column Used:

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a $0.45 \mu m$ nylon filter.

Results and Discussion

Amoxicillin, Figure 1, is successfully analysed in just over 5 minutes using the Quasar C18 column, 100 mm in length, Figure 2. Ideally suited to the analysis of small molecules, such as this antibiotic, the Quasar C18 phase provides enough retention via ligand/analyte interactions whilst also maintaining peak shape due to the ultra-high purity silica base and low residual silanol activity.

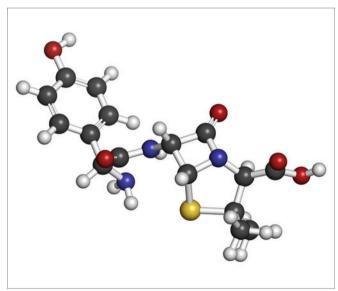
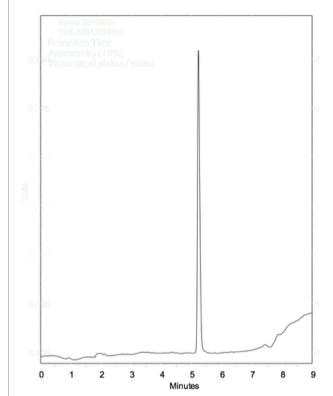


Figure 1. Chemical Structure of Amoxicillin.





Conclusion

• The Quasar C18 HPLC phase offers high efficiency

separation of this semi-synthetic antibiotic.

- The ultra-high purity silica base and low residual silanol activity yields excellent peak shape even for basic analytes, such as amoxicillin.
- Run time could be further reduced by switching to UHPLC and using a Quasar C18 1.7 μm column.

References

1. Haven KF (1994). Marvels of Science : 50 Fascinating

5-Minute Reads. Littleton, CO: Libraries Unlimited. p. 182. ISBN 978-1-56308-159-0.

 "Making Penicillin Possible: Norman Heatley Remembers". ScienceWatch. Thomson Scientific. 2007. Archived from the original on February 21, 2007. Retrieved 2007-02-13.



Consumables

| Nylon filters | Part Number 02542880 | | | | |
|---------------|-------------------------|--------------|-----|----------|--|
| Nyton Inters | | | | | |
| Phase | Length (mm) | I.D. (mm) | μm | Part | |
| Quasar C18 | 300 | 3.9 | 5 | N9308800 | |
| Quasar C18 | 250 | 4.6 | 5 | N9308801 | |
| Quasar C18 | 150 | 4.6 | 5 | N9308802 | |
| Quasar C18 | 100 | 4.6 | 5 | N9308803 | |
| Quasar C18 | 50 | 4.6 | 5 | N9308804 | |
| Quasar C18 | 150 | 4.6 | 3 | N9308805 | |
| Quasar C18 | 100 | 4.6 | 3 | N9308806 | |
| Quasar C18 | 50 | 4.6 | 3 | N9308807 | |
| Quasar C18 | 150 | 3.0 | 3 | N9308808 | |
| Quasar C18 | 100 | 3.0 | 3 | N9308809 | |
| Quasar C18 | 50 | 3.0 | 3 | N9308810 | |
| Quasar C18 | 150 | 2.1 | 3 | N9308811 | |
| Quasar C18 | 100 | 2.1 | 3 | N9308812 | |
| Quasar C18 | 50 | 2.1 | 3 | N9308813 | |
| Quasar C18 | 100 | 4.6 | 1.7 | N9308814 | |
| Quasar C18 | 50 | 4.6 | 1.7 | N9308815 | |
| Quasar C18 | 100 | 3.0 | 1.7 | N9308816 | |
| Quasar C18 | 50 | 3.0 | 1.7 | N9308817 | |
| Quasar C18 | 100 | 2.1 | 1.7 | N9308818 | |
| Quasar C18 | 50 | 2.1 | 1.7 | N9308819 | |

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