Application Note: ANCCSSYNCARTDIH

Analysis of Artesunate and Dihydroartemisinin Using a Syncronis C18 HPLC Column

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Key Words

- Syncronis C18
- Artesunate
- Dihydroartemisinin
- Malaria

Abstract

This application note demonstrates the use of the Thermo Scientific Syncronis C18 HPLC column for the analysis of artesunate and its active metabolite dihydroartemisinin.

Introduction

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. The Syncronis column range has been engineered to provide exceptional reproducibility due to its highly pure, high surface area silica, dense bonding and double endcapping, all controlled and characterized through the use of rigorous testing.

Artesunate is obtained as an extract from the plant *Artemisia annua* and is commonly used for the treatment of malaria. Artesunate (active metabolite dihydro-artemisinin) and artesunate-based combination therapy (ACT) is recommended by the World Health Organization (WHO) for the treatment of severe and multidrug resistant malaria. The analytical method for artesunate described in the WHO monograph, uses a mobile phase which includes potassium phosphate, which is incompatible with mass spectrometry [1]. Considering the C_{MAX} of artesunate is 0.09+/-0.04 µg/mL [2] and that artesunate lacks an intensive chromophore for UV absorption, MS detection is required to give the required sensitivity. Within this application note we will demonstrate an alternative method using an MS-compatible mobile phase.

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17

Sample Handling Equipment	
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Preparation

Working standard contained 1 mg/mL of artesunate and 2 mg/mL of α and β isomers of dihydroartemisinin in acetonitrile.

Separation Conditions		Part Number
Instrumentation:	Accela 600 HPLC System	
Column:	Syncronis™ C18 5 µm, 100 x 2.1 mm	97105-102130
Measured pressure:	50 bar	
Column temperature:	30 °C	
Injection volume:	1 µL	
Flow rate:	0.2 mL/min	
UV detection:	210 nm	
Mobile phase:	40:60 (v/v) water + 0.1% formic acid/ acetonitrile + 0.1% formic acid	

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Results

The analysis was carried out on a Syncronis C18 5 µm, 100 x 2.1 mm column. As shown on Figure 1, artesunate and its active metabolites, α and β dihydroartemisinin are eluted in less than 5 minutes with outstanding peak symmetry. The low peak intensity (Figure 1) is due to the lack of a UV chromophore indicating that the method would be suited to MS detection.

Replicate injections of the test mix showed that Syncronis C18 produced highly reproducible retention and peak shape (Table 1).

	α-Dihydroartemisinin	β-Dihydroartemisinin	Artesunate
Peak position	1a	1b	2
Average As	1.05	1.05	1.05
Average Rs	0.00	4.49	2.52
%RSD t_R	0.06	0.11	0.10

Table 1: Method precision (%RSD) for artesunate and α and β dihydroartemisinin (data calculated from six replicate injections)

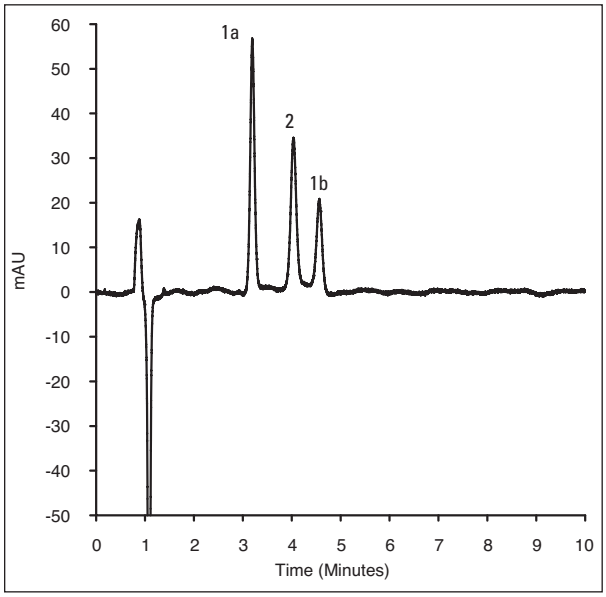

Figure 1: Chromatogram for α and β dihydroartemisinin (1a), (1b) and artesunate (2) separated on a Syncronis C18 5 µm, 100 x 2.1 mm column

Conclusions

Syncronis C18 successfully separated artesunate and dihydroartemisinin in less than 5 minutes. Syncronis C18 columns are an excellent choice for fast highly reproducible analysis with a MS compatible mobile phase.

References

[1] Artesunate: Final text for revision of The International Pharmacopoeia (December 2009) World Health Organisation (2009). Working document QAS/09.340/Final December 2009.
[2] Am. J. Trop. Med. Hyg., 58(3), 1998.pp.365-368

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