Application Note: ANCCSCETARTDIHY

Analysis of Artesunate and Dihydroartemisinin Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

- Accucore RP-MS
- Fused core
- Superficially porous
- Core Enhanced Technology
- Artesunate
- Dihydroartemisinin
- Malaria

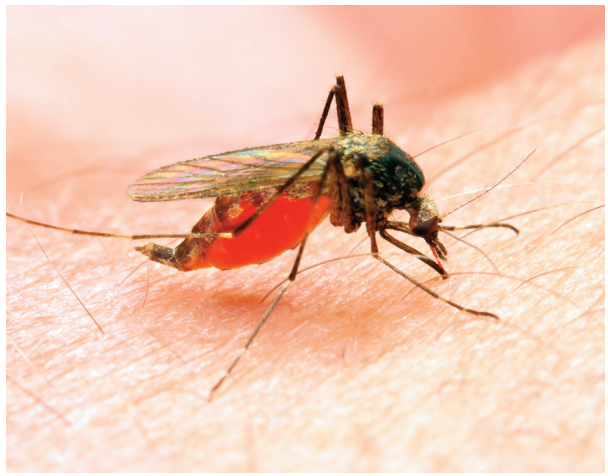
Abstract

This application note demonstrates the use of the Thermo Scientific Accucore RP-MS HPLC column for the fast analysis of artesunate and its active metabolite dihydroartemisinin.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

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, in addition to this the speed of analysis that can be obtained using Accucore RP-MS will also be demonstrated.



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		
Instrumentation:	Accela 600 HPLC System	
Column:	Accucore RP-MS 2.6 µm, 50 x 2.1 mm	17626-052130
Measured pressure:	185 bar	
Column temperature:	30 °C	
Injection volume:	1 µL	
Flow rate:	0.6 mL/min	
UV detection:	210 nm	
Mobile phase:	60:40 (v/v) water + 0.1% formic acid/ acetonitrile + 0.1% formic acid	

Results

The analysis was carried out on an Accucore RP-MS 2.6 µm, 50 x 2.1 mm column. Artesunate and its active metabolites, α and β dihydroartemisinin are eluted in under 2 minutes. The low peak intensity (Figure 1) is due to the lack of UV chromophore indicating that the method would be suited to MS detection.

Replicate injections of the test mix showed that Accucore RP-MS produced reproducible retention and peak shape (Table 1).

	α-Dihydroartemisinin	β-Dihydroartemisinin	Artesunate
Peak position	1a	1b	2
Average As	1.26	1.16	1.25
Average Rs	0.00	7.38	3.15
%RSD t_R	0.43	0.46	0.44

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

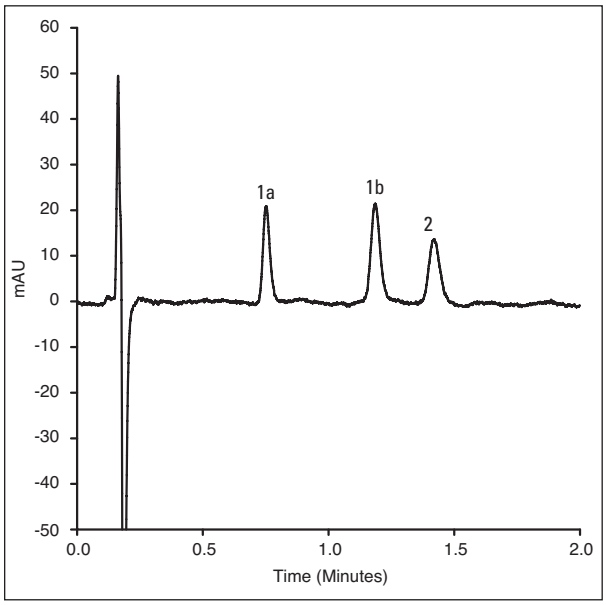


Figure 1: Chromatogram for α and β dihydroartemisinin (1a), (1b) and artesunate (2) separated on an Accucore RP-MS 2.6 µm, 50 x 2.1 mm column

Conclusions

Accucore RP-MS successfully separated artesunate and dihydroartemisinin in less than 2 minutes. Accucore RP-MS columns are an excellent choice for fast 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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