Application Note:
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Fast Analysis of Zidovudine by UHPLC Using a Syncronis C18 1.7 µm Column

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Key Words

- Zidovudine
- UHPLC
- USP
- Syncronis C18

Abstract

Thermo Scientific Syncronis C18 is a highly retentive stationary phase, with a surface area of 320 m^2/g.

This application note demonstrates the use of Syncronis™ C18, packed with 1.7 µm particles, for the fast HPLC analysis of zidovudine, allowing an increase in sample throughput. The method of analysis was adapted from the USP monograph, by using an in-house method transfer calculator [1].

Introduction

Zidovudine, or azidothymidine (tradename: Retrovir) is an antiretroviral drug used for the treatment of HIV/AIDS. It acts by stopping HIV from infecting healthy cells, but it can not help cells already infected with the virus [2]. It was the first HIV treatment to be marketed and it is now a widely used generic drug.

Zidovudine is included in the World Health Organisation's "Essential Drugs List" (list of minimum medical needs for a basic healthcare system) [3].

The United States Pharmacopeia (USP) provides worldwide guidance for the chromatographic analysis of zidovudine [4], which is based on High Performance Liquid Chromatography (HPLC) L1 columns. The implementation of an L1 type column, such as Syncronis C18, packed with 1.7 µm particles, allowed for the zidovudine USP method to be transferred to UHPLC (ultra High Performance Liquid Chromatography).

Experimental details

The analysis was run on an Thermo Scientific Accela UHPLC system. The data was acquired and processed using Thermo Scientific ChromQuest 5.0 Software.

The separation of zidovudine was achieved on a Syncronis C18 column, a recently developed, highly retentive reversed phase material, specifically designed to target consistent chromatography for a wide range of compounds.

Sample Preparation

A 1000 µg/mL of zidovudine standard solution was prepared in methanol; this solution was then diluted to 100 µg/mL in mobile phase (20:80 (v/v) methanol/water) and used for the analysis.

Thermo Scientific Column	Part Number
Syncronis C18 1.7 µm, 50 x 2.1 mm	97102-052130

Thermo Scientific Accela HPLC/UHPLC	
Column temperature	25 °C
Injection volume	0.5 µL
Flow rate	0.8 mL/min
UV detection	265 nm
Mobile phase	20:80 (v/v) methanol/water

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Thermo Scientific 2 mL clear vial and Si/PTFE seal	60180-600

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Results

The original USP analytical conditions, based on a 5 µm, 250 x 4.0 mm column were scaled down using our method transfer calculator to accommodate for the column geometry reduction (as shown in Figure 1). The final run time achieved on the 1.7 µm, 50 x 2.1 mm column was eight times faster than the original run, with zidovudine eluting at 2.42 min (retention time of 26 min on the 5 µm, 250 x 4.0 mm column). Figure 2 shows the chromatograms obtained employing Syncronis C18 1.7 µm, 50 x 2.1 mm column and Syncronis C18 5µm, 250 x 4.0 mm column.. The USP acceptance criteria were met, as demonstrated in Table 1. The statistical assessment is based upon data derived from six replicate injections (see Table 1 for mean values).

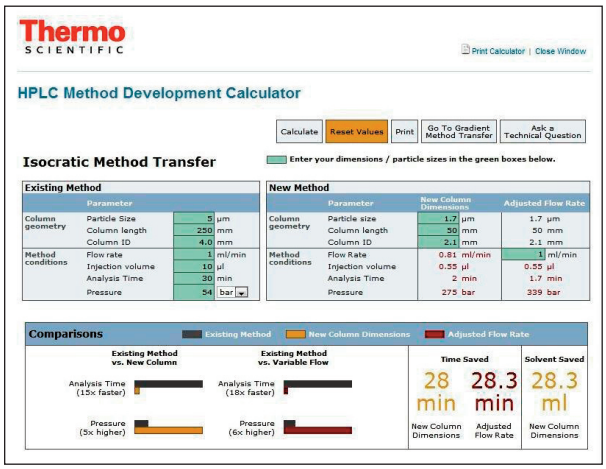

Figure 1: Isocratic method transfer calculator used in this application note to scale down the original USP chromatographic conditions [1]

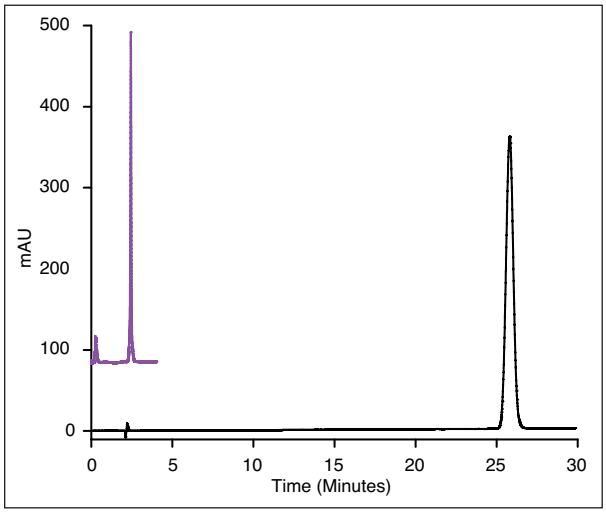

Figure 2: Chromatograms of 100 µg/mL of zidovudine separated on a Syncronis C18 1.7 µm, 50 x 2.1 mm column (top chromatogram), and on a Syncronis C18 5 µm, 250 x 4.0 mm column (bottom chromatogram)

Conclusions

The use of a Syncronis C18, packed with 1.7 µm particles column, allowed to successfully scale down the USP method for the analysis of zidovudine, in order to significantly increase sample throughput. The analytical results passed the specifications stated in the USP monograph. Syncronis C18 1.7 µm columns are therefore an excellent choice for the high speed, high efficiency analysis of zidovudine, allowing a high sample throughput.

References

[1] http://www.separatedbyexperience.com/products/IsocraticMethod.aspx

[2] W. V. Caufield; J. T. Stewart; Chromatographia (2001); Vol 54; pp 561-568.

[3]. http://whqlibdoc.who.int/hq/2005/a87017_eng.pdf. World Health Organization. March 2005 Retrieved 2006-03-12

[4] http://www.pharmacopeia.cn/v29240/usp29nf24s0_m89510.html

	T_r (min)	Tailing Factor	Efficiency	Peak Area
Average	2.416	0.970	118567	242096
% RSD	0.05	0.30	0.78	0.79
USP acceptance criteria	≤ 2.0 (% RSD)	≤ 2.0	≤ 2.0 (% RSD)	≤ 2.0 (% RSD)

Table 1: Average and Method Precision (%RSD) of chromatographic parameters, derived from the analysis of zidovudine on a Syncronis C18 1.7 µm, 50 x 2.1 mm column (data calculated from six replicate injections) USP acceptance criteria are also reported.

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