Application Note 21499

A High-Throughput Method for the Analysis of 13 Antiretroviral Compounds Using a UHPLC Column and System Combination

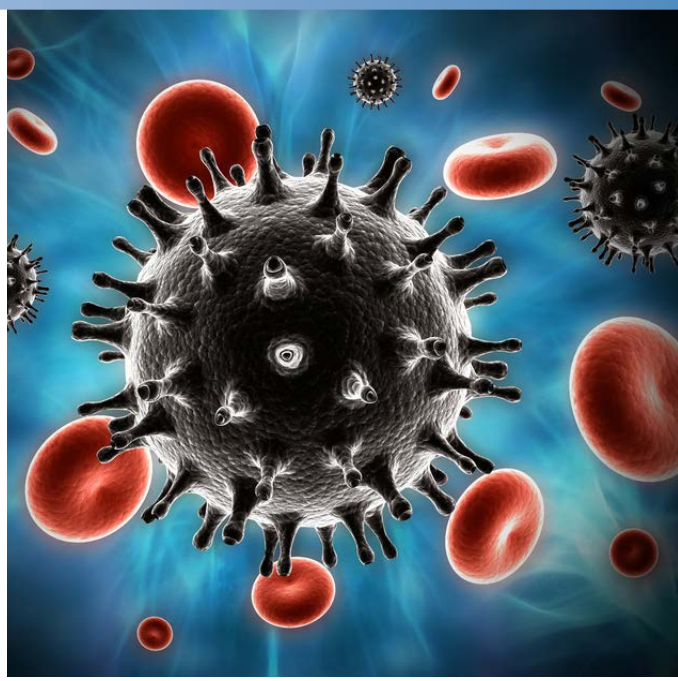
Joanne Jones, Thermo Fisher Scientific, Runcorn, UK

Key Words

Hypersil GOLD VANQUISH, antiretrovirals, UHPLC, tenofovir, lamivudine, emtricitabine, abacavir, zidovudine, rilpivirine, raltegravir, dolutegravir, darunavir, cobicistat, atazanavir, efavirenz, elivitegravir

Goal

To demonstrate the advantages of using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ UHPLC 1.9 µm column and the Vanquish UHPLC system for the fast analysis of 13 antiretroviral compounds.



Introduction

Screening methods for multiple analytes can be more cost-effective than dedicated methods for fewer analytes. However, dedicated methods can provide reduced analysis times for quicker release of data and greater sample throughput. The analysis of 13 antiretroviral compounds provides a good demonstration of a multiple analyte method. However, also demonstrated are the dedicated methods for the co-administered compounds.

The complementing technologies of the Hypersil GOLD VANQUISH UHPLC column and the Vanquish UHPLC system allow for the best possible chromatographic performance. The low system gradient delay volume is ideal for high-throughput gradient separations. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. Intelligent sample pre-compression prior to injection and extremely low pump pulsation help achieve outstanding flow stability and improved column lifetime.

The Hypersil GOLD VANQUISH range of UHPLC/HPLC columns was developed to give reproducible and reliable chromatography analysis with excellent peak shape. Based on highly pure silica, Hypersil GOLD VANQUISH UHPLC columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. The Hypersil GOLD VANQUISH medium provides a stationary phase with C18 selectivity and a predictable elution order but can yield new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis.

Thermo SCIENTIFIC

Experimental

Consumables

- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 50 × 2.1 mm (P/N 25002-052130-V)
- LC-MS grade 18 MΩ·cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade methanol (P/N M/4056/17)
- Fisher Scientific Analytical grade formic acid (P/N 10559570)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)

Instrumentation

Analyses were performed using a Vanquish UHPLC System consisting of:

- System Base (P/N VH-S01-A)
- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ LightPipe™ flow cell, 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT-100)

Software

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 SR2 MUa Chromatography Data System

Sample Preparation

Solutions of the 13 compounds shown in Table 5 were prepared by dissolving 10 mg amounts in 10 mL of methanol or water to produce 1 mg/mL primary solutions. Subsequent dilutions resulted in a final working solution in 75:25 methanol/water (v/v) with a concentration of 25 µg/mL. (Cobicisat and abacavir had a final concentration of 75 µg/mL.)

Vial labeling was supported by the Virtuoso Vial Identification System.

UHPLC Conditions	
UHPLC Column	Hypersil GOLD VANQUISH, 1.9 µm, 50 × 2.1 mm
Mobile Phase A	Water + 0.1% formic acid
Mobile Phase B	Methanol + 0.1% formic acid
Flow Rate	See Tables 1, 2, 3, and 4
Column Temperature	40 °C, still air with eluent pre-heating
Injection Details	1 µL
UV Detection	254 nm
Data Collection Rate	200 Hz
Response Time	0.02 s

Table 1. LC gradient conditions and flow rate for Method A.

Method A		
Gradient	Time (min)	% B
	0	2
	1.60	85
	1.61	2
	2.40	2
Flow rate (mL/min)	1.0	
Maximum backpressure (bar)	820	

Table 2. LC gradient conditions and flow rate for Method B.

Method B		
Gradient	Time (min)	% B
	0	2
	0.50	50
	0.51	2
	1.60	2
Flow rate (mL/min)	1.2	
Maximum backpressure (bar)	948	

Table 3. LC gradient conditions and flow rate for Method C.

Method C		
Gradient	Time (min)	% B
	0	2
	0.40	90
	0.50	90
	0.51	2
Flow rate (mL/min)	1.2	
Maximum backpressure (bar)	945	

Table 4. LC gradient conditions and flow rate for Method D.

Method D		
Gradient	Time (min)	% B
	0	50
	0.50	100
	0.51	50
	1.60	50
Flow rate (mL/min)	1.2	
Maximum backpressure (bar)	956	

Results and Discussion

Full resolution of the 13 compounds was achieved with excellent peak shape within a 2 minute detection window (Figure 1 and Table 5) using the conditions as described in Method A, Table 1. The maximum system pressure throughout the gradient was 820 bar, which is within the backpressure specification of both the Hypersil GOLD VANQUISH 50 mm column (1000 bar) and the Vanquish UHPLC system, which can routinely operate at back pressures up to 1500 bar.

To increase throughput further, the antiretroviral compounds were then analyzed in the fixed dose combinations. A fixed dose combination is a combination of drugs administered in a single tablet to increase effectiveness and reduce drug resistance (Table 6). By exploiting the maximum operating conditions of the column (by increasing the flow rate), the detection window has reduced from 2 minutes to approximately 0.7 minutes, therefore doubling throughput while maintaining resolution. Example chromatograms for three of the fixed-dose combinations are shown in Figure 2. The three chosen chromatograms have been selected to represent each of the three gradients used to analyze the combinations (Methods B, C, and D). Baseline resolution was achieved for each application with excellent retention time reproducibility (Table 7).

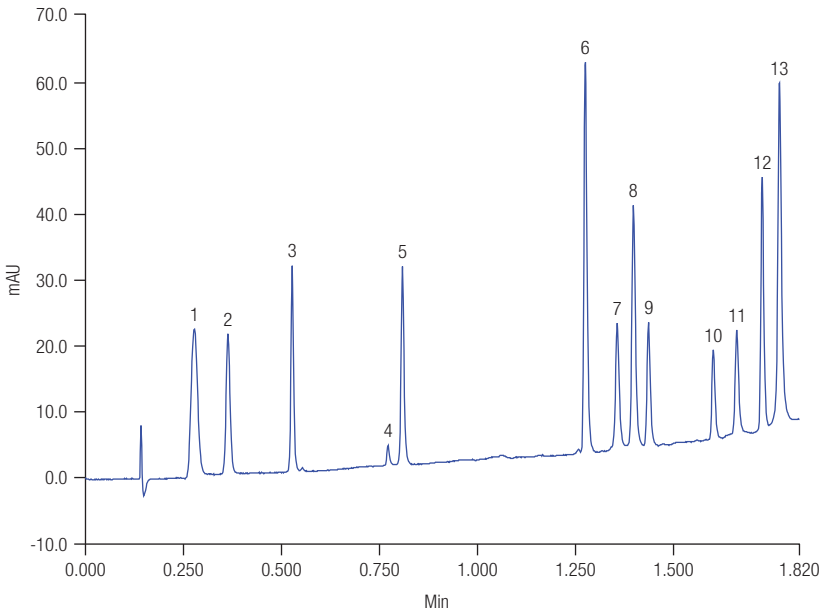


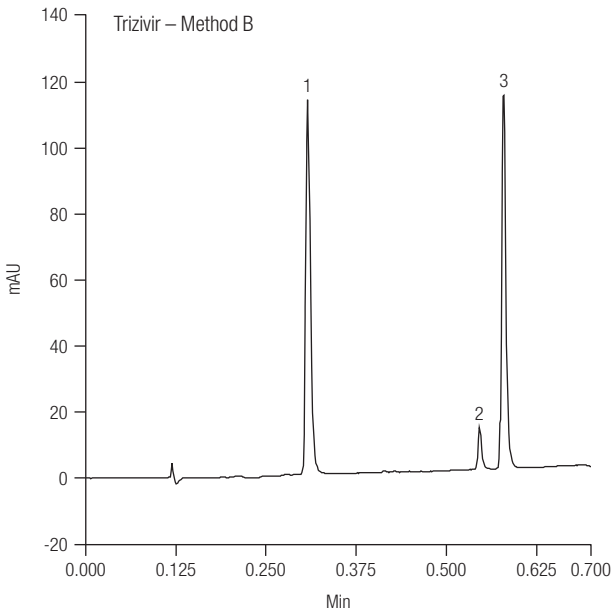
Figure 1. Chromatogram showing the separation of 13 antiretroviral compounds.

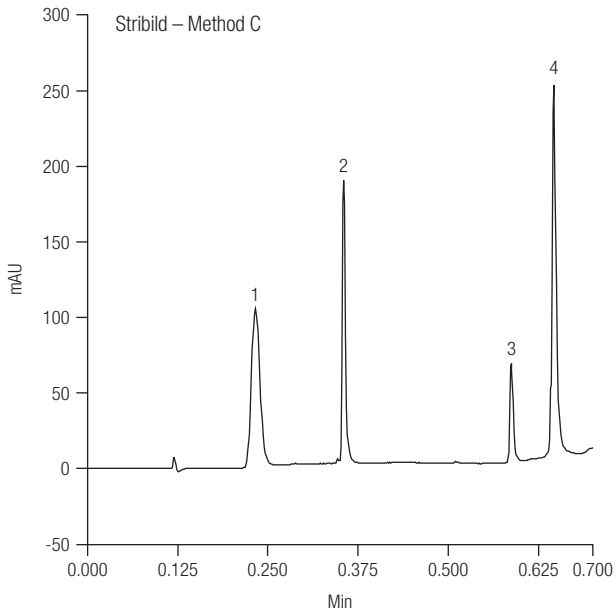
Table 5. Peak identification, resolution (Rs), and asymmetry (As) of 13 antiretroviral compounds.

Compound	Peak Position	Average Rt [min] (n=6)	Average Rs (n=6)	Average As (n=6)
Tenofovir	1	0.284	3.37	1.08
Lamivudine	2	0.369	10.70	1.14
Emtricitabine	3	0.531	2.24	1.15
Abacavir	4	0.774	2.91	1.22
Zidovudine	5	0.811	31.24	1.18
Rilpivirine	6	1.274	5.25	1.34
Raltegravir	7	1.355	2.50	1.20
Dolutegravir	8	1.396	2.43	1.24
Darunavir	9	1.434	11.12	1.22
Cobicistat	10	1.600	3.94	1.36
Atazanavir	11	1.660	4.44	1.27
Efavirenz	12	1.724	2.99	1.25
Elivitegravir	13	1.768	NA	1.22

Table 6. Antiretroviral drugs, the associated combinations, and analytical method used.

Antiretroviral Drug	Combivir	Trizivir	Epzicom	Truvada	Atripla	Complera	Stribild	Triumeq	Evotaz	Prezcobix	Dutrebis
Zidovudine	x	x									
Lamivudine	x	x	x					x			x
Abacavir		x	x					x			
Tenofovir				x	x	x	x				
Emtricitabine				x	x	x	x				
Efavirenz					x						
Rilpivirine						x					
Elivitegravir							x				
Cobicistat							x		x	x	
Dolutegravir								x			
Atazanavir									x		
Darunavir										x	
Raltegravir											x
Analytical Method	**B**	**B**	**B**	**B**	**C**	**C**	**C**	**C**	**D**	**D**	**C**





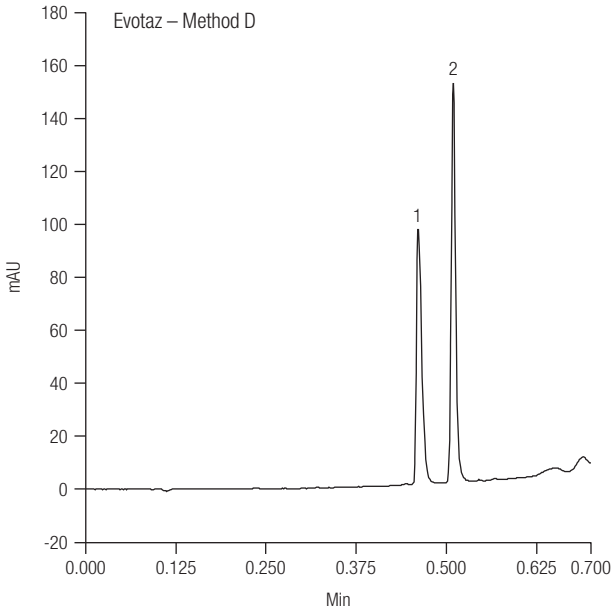


Figure 2. Representative chromatograms of the fixed-dose combinations, one combination from each of the three UHPLC methods.

Table 7. Peak identification, resolution, and retention time variability of the fixed-dose combinations.

	Compound	Peak Position	Average Rt [min] (n=6)	% RSD Rt (n=6)	Average Rs (n=6)
Combivir	Lamivudine	1	0.308	0.04%	25.08
	Zidovudine	2	0.580	0.02%	NA
Trizivir	Lamivudine	1	0.308	0.09%	23.15
	Abacavir	2	0.546	0.04%	3.49
	Zidovudine	3	0.579	0.01%	NA
Epzicom	Lamivudine	1	0.307	0.03%	23.31
	Abacavir	2	0.546	0.01%	NA
Truvada	Tenofovir	1	0.250	0.03%	13.47
	Emtricitabine	2	0.415	0.04%	NA
Atripla	Tenofovir	1	0.234	0.10%	10.45
	Emtricitabine	2	0.355	0.03%	35.24
	Efavirenz	3	0.639	0.05%	NA
Complera	Tenofovir	1	0.231	0.07%	8.35
	Emtricitabine	2	0.354	0.03%	21.48
	Rilpivirine	3	0.532	0.03%	NA
Stribild	Tenofovir	1	0.232	0.06%	8.26
	Emtricitabine	2	0.355	0.04%	29.88
	Cobicistat	3	0.587	0.03%	6.31
	Elivitegravir	4	0.646	0.02%	NA
Triumeq	Lamivudine	1	0.286	0.05%	15.04
	Abacavir	2	0.413	0.05%	18.74
	Dolutegravir	3	0.572	0.04%	NA
Evotaz	Cobicistat	1	0.461	0.10%	3.99
	Atazanavir	2	0.509	0.07%	NA
Prezcobix	Darunavir	1	0.373	0.12%	8.36
	Cobicistat	2	0.466	0.04%	NA
Dutrebis	Lamivudine	1	0.287	0.08%	29.64
	Raltegravir	2	0.562	0.06%	NA

Conclusion

By exploiting the high-pressure capabilities of the Vanquish UHPLC system, in conjunction with the Hypersil GOLD VANQUISH UHPLC column and a simple binary gradient, it was demonstrated that a method for 13 compounds within a 2 minute detection window (and a full method cycle time of 2.4 minutes) can be achieved.

For analysis of the fixed dose combinations, the analytical method can be modified and the detection windows decreased to 0.7 minutes, therefore increasing throughput.

The performance of the Hypersil GOLD VANQUISH UHPLC column, coupled with the low gradient delay volume and excellent flow stability of the Vanquish UHPLC system, deliver the following:

- Full-resolution UHPLC method for 13 antiretroviral drugs in a method time < 2.4 minutes
- Rapid, full-resolution UHPLC methods for 11 fixed-dose combinations in method times < 1.6 minutes
- Excellent retention time reproducibility and peak shape

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library: www.thermofisher.com/appslab

For Research Use Only. Not for use in diagnostic procedures

www.thermofisher.com/LC-columns

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand