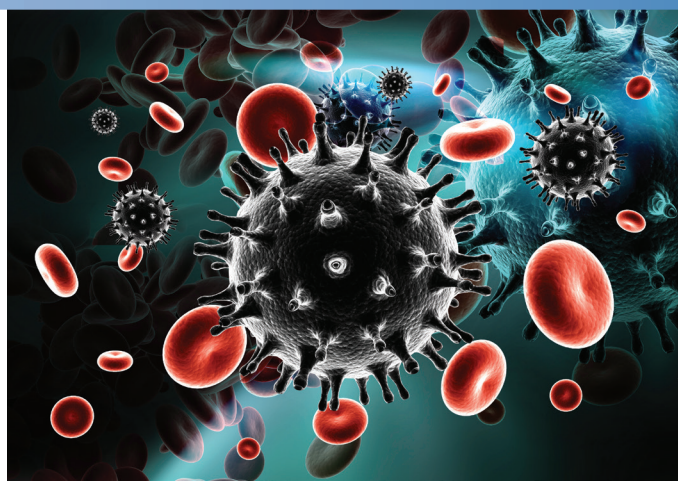
Application Note 1129

Ternary Gradient for Tenofovir Disoproxil Fumarate Impurity Profiling

Susanne Fabel and Markus Martin
Thermo Fisher Scientific, Germering, Germany

Key Words
USP, Aqueous Gradient, Active Pharmaceutical Ingredient, Equilibration, Accucore aQ Column

Goal
Demonstrate the robust performance of the Thermo Scientific™ Vanquish™ Flex UHPLC System in the challenging ternary gradient application starting at zero percent organic solvent.



Introduction
Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, an enzyme crucial to viral production. Tenofovir is in formulation given as the prodrug tenofovir disoproxil fumarate (TDF) in combination with the nucleoside reverse-transcriptase inhibitor emtricitabine. The combination drug is marketed under the tradename Truvada® by Gilead.

The organic impurities of TDF are analyzed following the instructions noted in a monograph posted on the USP website as USP Pending monograph.[1] The eluents of the original method of the procedure to analyze the organic impurities is modified to gain a mass spectrometry (MS) compatible method with easier eluent preparation and shortened run time. The system suitability testing of the original method requires the challenging separation of the polar compounds adenine and tenofovir. In this work, the simultaneous separation of early-eluting polar compounds and later-eluting nonpolar compounds is achieved by applying a ternary gradient with the Vanquish Flex UHPLC System[2] using a Thermo Scientific™ Accucore™ aQ column.[3]

Experimental
Equipment
Vanquish Flex UHPLC system consisting of:

- System Base (P/N VH-S01-A)
- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- LightPipe Flow Cell, Standard (10 mm; P/N 6083.0100)

Thermo SCIENTIFIC

Chromatographic Conditions	
Column:	Accucore aQ, 2.6 µm, 2.1 × 100 mm (P/N 17326-102130)
Mobile Phase:	A - 25 mM ammonium acetate buffer, pH 3.8 with acetic acid
	B - Methanol
	C - Acetonitrile
Gradient:	0–4.0 min 0% B–70% B, 0% C–15% C
	4.0–4.5 min 70% B, 15% C
	4.5–5 min 70% B–25% B, 15% C–70% C
	5–6 min 25% B, 70% C
	6.0–6.1 min 25% B–0% B, 70% C–0% C
	6.1–15 min 0% B, 0% C
Flow Rate:	0.6 mL/min
Temperature:	40 °C, Still air
	Active pre-heater: 40 °C
Injection Volume:	1 µL
Detection:	260 nm
	Data Collection Rate: 100 Hz
	Response time: 0.04 s
Analytes:	Test solution: Adenine (50 µg/mL), tenofovir (150 µg/mL), emtricitabine (100 µg/mL), tenofovir disoproxil fumarate (100 µg/mL) in mobile phase A Sample solution: Tenofovir disoproxil fumarate (100 µg/mL) in mobile phase A

Data Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2, SR 3

Results and Discussion

The USP referenced mobile phases for *Organic Impurities, Procedure 1* requests dibasic sodium phosphate and tertiary butyl alcohol mixed with methanol. The reproducible premixing of eluents consisting of three different components is generally challenging. The consistent composition of the mobile phases from batch to batch might be questionable. In addition, these eluents are not MS-compatible, are prone to salt precipitation, and are inconvenient for the preparation. Specifically, the tertiary alcohol has a melting point of 25 °C, which makes it difficult to handle at room temperature. For these reasons, the described eluents might not be ideal for running routine methods with high eluent consumption. Here, the mobile phase was changed to an MS-compatible 25 mM ammonium acetate buffer, pH 3.8, as eluent, mixed with methanol and acetonitrile by the quaternary pump. The literature method uses a 250 mm long column with 5 µm fully porous particles and applies gradients between 60 and 70 minutes for organic impurity profiling. To decrease the method run time, a 100 mm, 2.6 µm fused core column is used.

The separation challenge of the here-described analytical problem is to achieve a resolution of at least 1.5 between the early eluting peaks adenine and tenofovir in the test solution. The Accucore aQ columns are compatible with 100% aqueous mobile phases and offer special selectivity for polar analytes. Starting at 100% aqueous mobile phase and the increase of acetonitrile content with a lower slope than methanol allowed the separation of adenine and tenofovir with a resolution of more than two (Figure 1). This is not achievable with a binary water/acetonitrile gradient and fast method. The increase of the acetonitrile content during the progression of the gradient allowed an earlier elution of the more hydrophobic emtricitabine, tenofovir disoproxil fumarate, and the impurities. An adequate equilibration time is beneficial for the retention time precision.

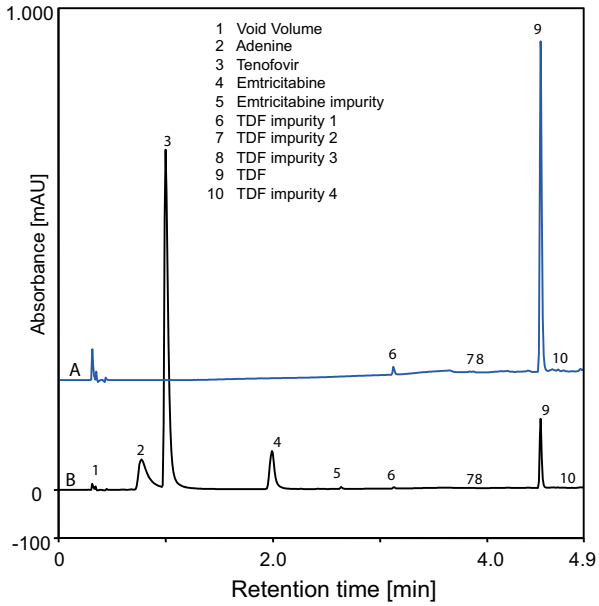


Figure 1. The sample solution (A) shows the active pharmaceutical ingredient and impurities. Test solution (B) shows sufficient resolution for the critical substances 2 and 3 running a ternary gradient starting with 100% aqueous conditions.

To investigate the long-term robustness of the ternary gradient method the sample solution was injected repeatedly over a 15 hour time period. For the tenofovir disoproxil fumarate, a retention time RSD of 0.03% and a peak area RSD of 0.3% was achieved. The robustness demonstrating results are visualized in the trendplot of Figure 2. These results are by far better than the requested limits in the pending monograph to be not more than 5–10% relative standard deviation.

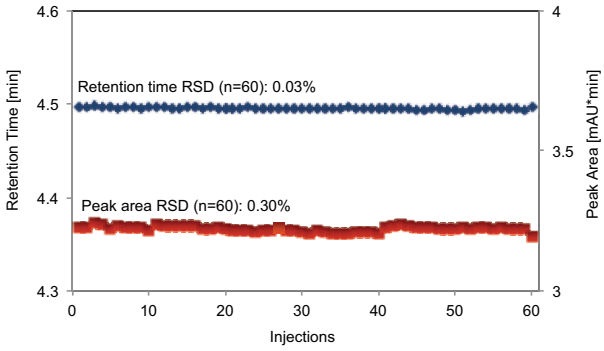


Figure 2. Trendplot showing the good retention time RSD of 0.03% and peak area RSD of 0.3% for the tenofovir disoproxil fumarate main peak analyzing 60 replicates over a run time period of more than 15 h.

Conclusion

This work combines an innovative column material with a versatile UHPLC instrument to solve a challenging separation problem. The capabilities of the Vanquish Flex System with quaternary pump allowed a selective increasing of the elution strength to create a fine-tuned gradient on the Accucore aQ column material. The modified method employs mass spectrometry compatible solvents. The column and instrument robustness allows the analysis of the active pharmaceutical ingredient with stable retention times and peak areas to give maximum confidence in the results.

References

1. Authorized USP Pending Monograph for Tenofovir Disoproxil Fumarate, Version 1. [Online] http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/pendingStandards/m3429_authorized.pdf (accessed May 21, 2015)

2. Thermo Fisher Scientific. Vanquish UHPLC System. [Online] http://info1.thermoscientific.com/content/Vanquish-Overview/ (accessed May 21, 2015)

3. Thermo Fisher Scientific. Accucore aQ C18 Polar Endcapped LC Column. [Online] http://www.thermoscientific.com/en/product/accucore-aq-2-6-m-particle-size-hplc-columns.html (accessed May 21, 2015)

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

AN71676-EN 0615S

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand