UHPLC Method Development for Analyzing a Once-Daily Tablet Formulation for HIV-1 Infection Treatment

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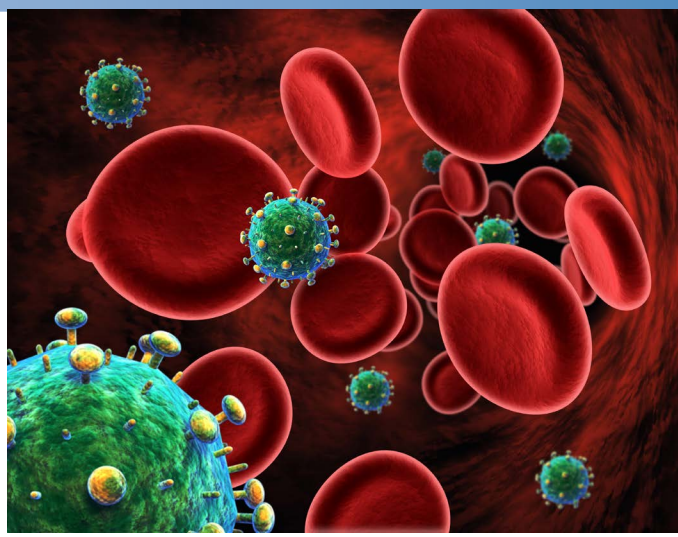
Application Note 1115

Key Words
UHPLC Instrumentation, Stribild® Drug, Autosampler Linearity, Pharmaceuticals, HIV, Generic Method

Goal
To develop an easy to use method for the fast separation of four main compounds of the Stribild pill utilizing the latest UHPLC equipment and technology. The method was further optimized to consider the simultaneous analysis of a variety of possible impurities in this rather complex drug formulation.



Introduction
The HIV-1 specific medication has progressed from monotherapy in the early 1990s towards combination therapies of different antiretroviral agents (ARVs), resulting in numerous pills that a patient must consume.[1] A new formulation for the therapy of HIV-1 is marketed by Gilead Science under the trade name Stribild.[2] This novel "once-daily" tablet was approved by the Food and Drug Administration (FDA) in 2012.[3] Instead of consuming several pills a day, a patient only has to take a single tablet. This tablet formulation is comprised of different types and varying amounts of active pharmaceutical ingredients (API). Emtricitabine (200 mg) is a nucleoside reverse-transcriptase inhibitor (NRTI), tenofovir disoproxil fumarate (300 mg) a nucleotide reverse-transcriptase inhibitor (NtRTI), elvitegravir (150 mg) an integrase strand transfer inhibitor (INSTI), and cobicistat (150 mg) a pharmacokinetic boosting agent.[4]

Several High Performance Liquid Chromatography (HPLC) methods that are used to separate some of these APIs have already been published.[5–7] However, an HPLC method that enables the separation of all those four APIs in a single run has not yet been published within the literature. In this application note, we describe the development of an Ultra High Performance Liquid Chromatography (UHPLC) method for the simultaneous determination of the four components of a simulated Stribald formulation by applying the latest-generation of UHPLC-instrumentation.[8]

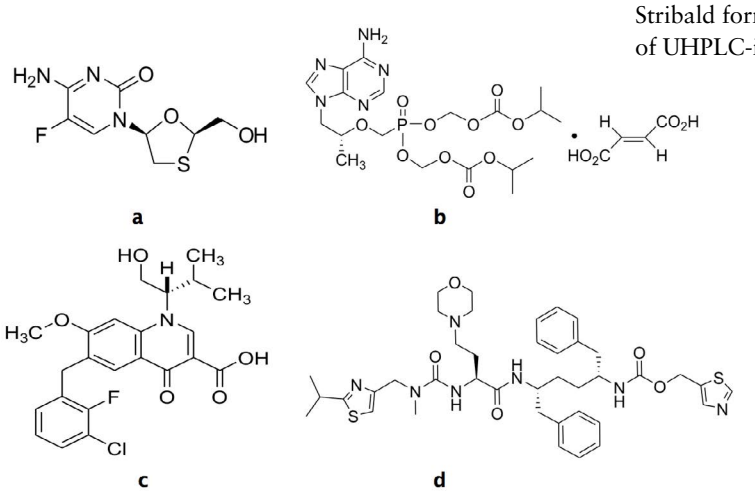


Figure 1. a–d: APIs in Stribild tablets are emtricitabine (a), tenofovir disoproxil fumarate (b), elvitegravir (c), and cobicistat (d).

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Reagents and Chemicals

Compound	Supplier	P/N
Cobicistat	Selleckchem	S2900
Elvitegravir	Selleckchem	S2001
Emtricitabine	USP Standard	1235106
Tenofovir Disoproxil Fumarate	Selleckchem	S1400
Formic Acid	Fisher Scientific™	F/1900/PB15
Methanol Optima™ LC/MS	Fisher Scientific	A456-212
Acetonitrile Optima LC/MS	Fisher Scientific	A955-212
Ultra-Pure Lab Water, 18.2 MΩ·cm at 25 °C	NA	NA

Equipment

- Thermo Scientific™ Vanquish™ System consisting of:
  - Binary Pump H (P/N VH-P10-A)
  - Split Sampler HT (P/N VH-A10-A)
  - Column Compartment H (P/N VH-C10-A)
  - Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS)

Experimental Conditions

Column:	Thermo Scientific™ Accucore™ Vanquish™ C18+, 1.5 µm, 2.1 × 100 mm (P/N 27101-102130)
Mobile Phase:	A – water with 0.1% formic acid B – methanol with 0.1% formic acid
Gradient:	0–6.0 min 5–90% B, 6.0–9.0 min 90% B, 6.9–7.0 min 90–5% B, 7.0–10.0 min 5%B
Flow Rate:	0.500 mL/min
Pressure:	1320 bar (max.)
Temperature:	50 °C, still air, easy mode
Injection Volume:	0.5 µL (for calibration: 0.01 µL, 0.05 µL, 0.1 µL, 0.3 µL 0.5 µL, 0.8 µL, 1.0 µL)
Autosampler wash mode: after draw, wash solvent: 40% acetonitrile	
Detection:	214 nm, 260 nm, 50 Hz, 0.1 s response time, 4 nm slit width, 4 nm bandwidth
Flow Cell:	LightPipe™, 10 mm

Standard Preparation

Single stock solutions of 1 mg/mL were prepared for all APIs by dissolving the appropriate amount in methanol, except for emtricitabine which was dissolved in water. The single standard solutions were mixed and diluted with water to yield a standard mixture containing 100 µg/mL emtricitabine, 150 µg/mL tenofovir disoproxil fumarate, 75 µg/mL elvitegravir and 75 µg/mL cobicistat. The concentration ratios of the APIs reflect their dosage ratios in the Stribild formulation.

Results and Discussion

When developing a method, it is a well-tried procedure to begin this approach with a generic elution gradient. As rule of thumb, such a mobile phase gradient starts from 5% and goes up to 90% organic content within a run time that reflects 20 column volumes divided by an appropriate flow rate. Assuming the porosity of 0.6 for an Accucore Vanquish column, the generic mobile phase gradient was performed at a flow rate of 0.4 mL/min within a 10.35 minute gradient time. This generic method already allowed a separation of all APIs and their impurities (data not shown). Nevertheless, the mobile phase gradient was optimized by decreasing the gradient rise-time and increasing the flow rate for faster separation. The limiting factor for this method speed-up was the resolution of a critical peak pair at the detection wavelength of 260 nm (see Figure 2, peak 9 and 10). After final method optimization, a resolution factor of 2.1 was achieved between cobicistat and the elvitegravir impurity. With a flow rate of 0.5 mL/min and a generated system back pressure of 1320 bar, the method worked well within the operational optimum of the Vanquish UHPLC system and Accucore Vanquish column.

Simultaneous recording of a wide range of detection wavelengths (3D-field) with the Vanquish diode array detector revealed that the cobicistat peak is not interfered by the elvitegravir impurity at a detection wavelength of 214 nm (see Figure 2, blue line in zoom). The further analysis of cobicistat was, therefore, made at 214 nm detection wavelength.

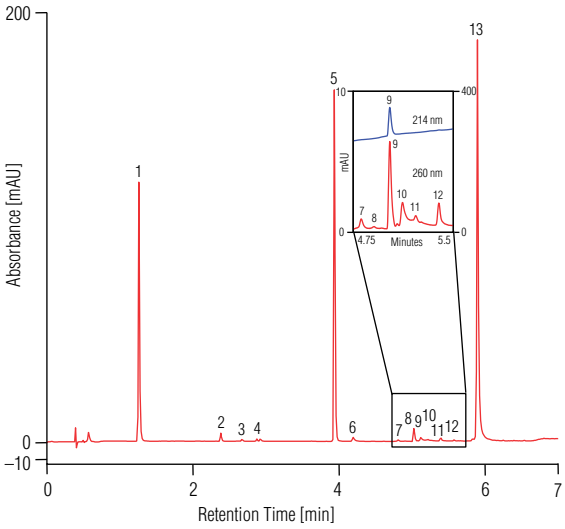

Figure 2. Separation of the four APIs and their impurities (for peak details refer to Table 1) detected at 260 nm. The zoom shows the separation of compound Cobicistat (peak 9) and impurities being compared at 214 nm and 260 nm.

The sources of impurities were identified by injections of single API standard solutions. This approach in method development gives a hint to the possible occurrence of secondary components in the formulation. The occurence of impurities in the commercial formulation has not been evaluated here.

The excellent retention time reproducibility allows for reliable peak assignments. Relative standard deviations of the retention times were <0.008% as shown in Table 1.

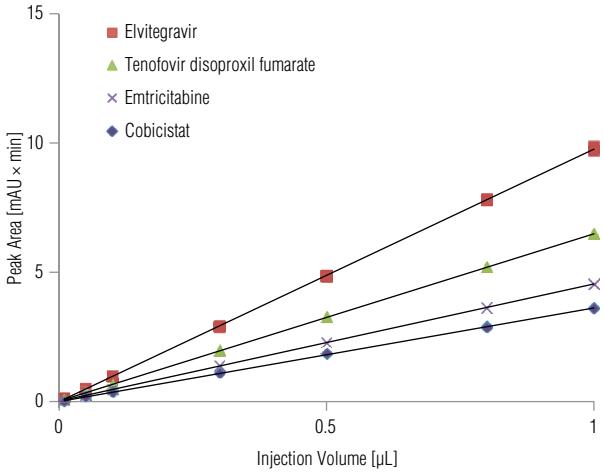

Figure 3. Calibration curves generated for the four APIs of the Stribild formulation by applying injection volumes from 0.01 µL up to 1 µL.

Table 1. Peak identification for Figure 2 with chromatographic performance indicators for three replicates.

Peak No.	Compound	Retention Factor k	Peak Width (min)	Asymmetry (EP)	Resolution (EP)	Retention Time (min)	Retention Time RSD (%)
1	Emtricitabine	2.23	0.02	1.5	41.5	1.26	0.008
2	Tenofovir disoproxil fumarate impurity	5.11	0.02	1.5	10.6	2.38	0.004
3	Elvitegravir impurity 1	5.86	0.02	1.3	9.2	2.67	0.003
4	Unknown 1	6.50	0.02	1.4	35.8	2.92	0.001
5	Tenofovir disoproxil fumarate	9.09	0.02	1.4	6.6	3.94	0.000
6	Elvitegravir impurity 2	9.75	0.03	2.1	13.5	4.19	0.000
7	Elvitegravir impurity 3	11.33	0.03	1	5.3	4.81	0.000
8	Elvitegravir impurity 4	11.58	NA	NA	NA	4.9	0.000
9	Cobicistat	11.88	0.02	1.5	2.1 (24.0 at 214 nm)	5.02	0.000
10	Elvitegravir impurity 5	12.13	0.03	5.3	6.1	5.12	0.000
11	Unknown 2	12.38	NA	NA	NA	5.22	0.000
12	Unknown 3	12.83	0.02	1.9	13.8	5.39	0.000
13	Elvitegravir	14.12	0.02	1.7	NA	5.9	0.000

A calibration curve was recorded for each API by utilizing the wide injection volume range of the Vanquish autosampler. Injection volumes from 0.01 µL up to 1 µL were injected with three replicates. The excellent injection linearity resulted in superior calibration coefficients (see Table 2).

The wide range of the sampler injection volumes allowed the determination of the limit of detection (LOD) and limit of quantitation (LOQ) without any standard dilution. The recorded 3D-field helped to discover the best detection wavelength for every single API to ensure best signal-to-noise ratios. The calculated values for the four APIs are listed in Table 2.

Table 2. Calibration coefficient and LOD/LOQ determination for the four APIs.

Compound	Detection Wavelength	Calibration Coefficent	Calibration Points	S/N (Injection Volume)	LOD (ng on Column)	LOQ (ng on Column)
Emtricitabine	260 nm	0.99998	21	159 (0.01 µL)	0.02	0.06
Tenofovir Disoproxil Fumarate	260 nm	0.99998	21	124 (0.01 µL)	0.04	0.12
Cobicistat	214 nm	0.99980	18	11 (0.05 µL)	1.02	3.41
Elvitegravir	260 nm	0.99987	21	23 (0.01 µL)	0.10	0.33

Conclusion

This application note demonstrates fast and easy method development with the latest generation of UHPLC equipment. The highly efficient Accucore Vanquish C18+ column with 1.5 µm particles provides a fast separation of the four main compounds and several impurities of a simulated Stribild pill. Excellent retention time reproducibility from run to run is utilized for reliable peak identification. Superior calibration curves were generated by applying the wide injection volume range of the Vanquish autosampler. The optimum detection wavelength was determined by acquiring a 3D-field and identifying the best signal-to-noise ratio for LOD and LOQ calculations.

References

1. E.J. Arts, D.J. Hazuda, *Cold Spring Harb Perspect Med 2(4) (2012):a007161.*
2. Package insert. Stribild (elvitegravir. cobicistat. emtricitabine. tenofovir). Foster City/CA: Gilead Sciences, August 2012 accessed via http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203100s000lbl.pdf
3. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm317004.htm
4. J. L. Olin, L. M. Spooner, O. M. Klibanov, *Ann Pharmacother.* 46 (2012) 1671–1677
5. N. L. Rezk, R. D. Crutchley, A. D. M. Kashuba, *J Chromatogr B* 822 (2005) 201–208
6. T. Le Saux, S. Chhun, E. Rey, O. Launay, L. Weiss, J.-P. Viard, G. Pons, V. Jullien, *J Chromatogr B* 865 (2008) 81–90
7. P. Swetha, V. V. S. R. Prasad, M. B. Raju, N. S. Kumar, *Indo American Journal of Pharm Research* 3(6) (2013) 4697–4705
8. Thermo Scientific Vanquish UHPLC System. http://www.thermoscientific.com/vanquish (accessed Nov 19, 2014).

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