Fast Nevirapine Impurity Profiling Using UHPLC-DAD

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Key Words
Active Pharmaceutical Ingredient (API), Impurity profiling, Ballistic gradient, HPLC to UHPLC transfer tool, ICH requirements

Goal
To develop a fast ballistic gradient UHPLC method optimized for simultaneous analysis of an API and impurities in a nevirapine tablet

Introduction
Nevirapine is a non-nucleoside reverse transcriptase inhibitor with activity against human immunodeficiency virus type 1 (HIV-1), currently marketed for the treatment of HIV-1 infected adults.[1] The United States Pharmacopeia (USP) uses a reversed-phase high-performance liquid chromatography (HPLC) separation with UV detection to determine nevirapine and its impurities. The related column is a 4.6 × 150 mm column packed with L60 (spherical, porous silica gel, 5 µm in diameter).[2] Due to the strong retention of impurity C, the USP monograph method requires about 30 minutes to separate this API and known impurities. A previous Dionex, now part of Thermo Scientific, application note demonstrated that an HPLC-UV separation can meet or exceed the chromatographic requirements of the USP monograph method for nevirapine while requiring about half the analysis time per sample.[3]

Here we report further optimization of this approach, using a state of the art gradient UHPLC-UV method. Applying ballistic gradients with latest-generation UHPLC equipment achieves significantly shorter analysis time while maintaining compliance with ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) requirements.

Equipment
- Thermo Scientific™ Vanquish™ UHPLC 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™ Chromatography Data System software 6.80 SR14

Table 1. Reagents and chemicals.

Compound	Supplier	P/N
Nevirapine Anhydrous	USP Standard	1460703
Related compound A	LGC Standards	MM1146.01
Related compound B	LGC Standards	MM1146.02
Impurity C	LGC Standards	MM1146.03
Ammonium acetate	Fisher Scientific	A114-50
Acetonitrile OPTIMA™ LC/MS	Fisher Scientific	A955-212
Ultra-pure lab water, 18.2 MΩ·cm at 25 °C	n.a.	n.a.

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Experimental Conditions	
Column:	Thermo Scientific™ Syncronis™ C18, 1.7 µm, 2.1 × 100 mm
Mobile Phase:	A) 10 mM NH_4OAc, pH 5.0 with acetic acid/ acetonitrile (85%/15% v/v) B) Acetonitrile
Gradient:	0–0.730 min: 30–70% B; 0.730–1.100 min: 70% B; 1.100–1.150 min: 70–30% B; 1.150–2.800 min: 30%
Flow Rate:	0.800 mL/min
Pressure:	950 bar (max.)
Temperature:	50 °C
Injection Volume:	1 µL
Detection:	240 nm, 100 Hz, 0.05 s response time, 4 nm slit width, 4 nm bandwidth
Flow cell:	LightPipe™, 10 mm

Results and Discussion

A number of rules should be followed for method transfer from HPLC to UHPLC, to adapt parameters such as flow rate, injection volume, or gradient profile (if applicable) to the new column characteristics. The Thermo Scientific Method Transfer Tool is a universal, multi-language tool that streamlines this process[4] and was used to transfer the USP method to a high-efficiency Syncronis UHPLC column. The application was accelerated by a factor of 1.3 by using the tool's boost factor functionality. The result was 130% of the initial linear mobile phase velocity through the column. With sub-2 µm particle columns this can easily be done while keeping the chromatographic efficiency almost constant.

The USP monograph uses 25 mM NH_4OAc, pH 5.0/ acetonitrile (80%/20% v/v) as mobile phase. In the method transfer, the buffer concentration was reduced to 10 mM and a gradient was applied with a maximum acetonitrile content of 80%. The buffer concentration was reduced to maintain compatibility of the buffer with the higher organic content. The detection wavelength was changed from 220 nm to 240 nm to eliminate baseline drift caused by varying absorption over the course of the gradient.

Figure 1 shows a calibration curve of the active pharmaceutical ingredient nevirapine with absorbances up to 3000 mAU. Even up to this high absorption, the calibration curve is almost perfectly linear with a correlation coefficient of $R^2 = 0.9998$.

Figure 2 shows a chromatogram of the 0.66 mg/mL standard. The zoom into the baseline reveals a number of impurities, including known compounds A, B, and C mentioned in the USP method as well as four additional impurities. Table 2 identifies the peaks of the chromatogram, the related signal-to-noise ratio and the relative area of the individual compound.

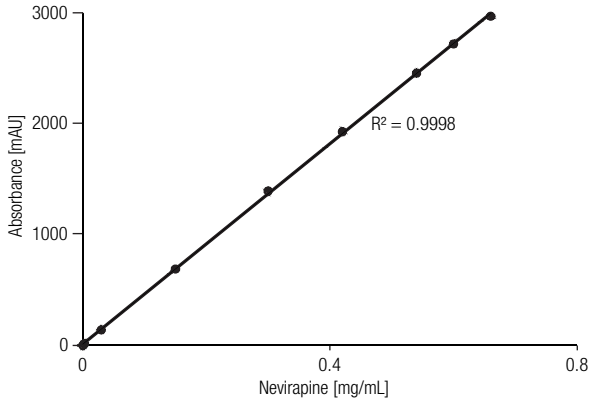

Figure 1. Calibration curve of nevirapine demonstrating excellent linearity up to 3000 mAU.

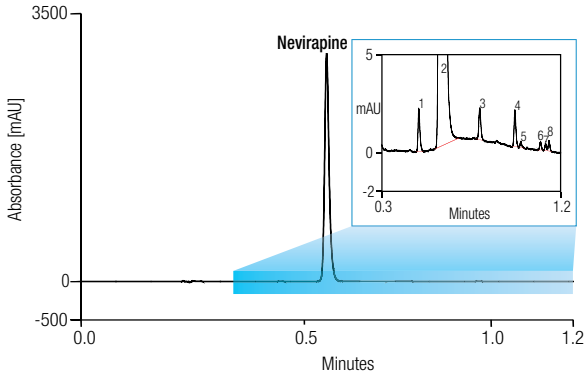

Figure 2. Impurity profiling of Nevirapine (0.66 mg/mL) with UHPLC gradient and zoom into the baseline to show related impurities.

Table 2. Peak identification for Figure 2 with related signal-to-noise ratio and area percentage of the individual compound.

Peak No.	Compound	Signal-to-Noise	Area [%]
1	Related compound B	28	0.065
2	Nevirapine	17602	99.790
3	Related compound A	16	0.050
4	Impurity C	53	0.053
5	Unknown 1	8	0.007
6	Unknown 2	17	0.011
7	Unknown 3	10	0.008
8	Unknown 4	11	0.013

The ICH defines the reporting threshold for impurities depending on the maximum daily dose. For nevirapine, a dosage of 400 mg/day translates into a reporting threshold of 0.05%.[5] According to the common definition of the Limit of Quantitation (LOQ)[6] defined as S/N ratio of at least 10, the nevirapine assay here described allows quantitation down to 0.008% relative area.

The compliance with the ICH guidelines was achieved despite challenging chromatographic conditions. We applied a ballistic 44 s linear gradient, achieving the elution of all relevant impurities within 1.2 min. The total run time was 2.8 min, using default detection parameters and 100 Hz data collection rate. This application is therefore a good example that the Vanquish system performance easily supports even ambitious analysis goals without the need for time-consuming instrument optimization.

Conclusion

This application describes an optimized method for the impurity profiling of nevirapine using a ballistic gradient method. The separation is completed in 2.8 min, compared to 80 min of the isocratic USP method. Even under challenging chromatographic conditions, the Vanquish UHPLC system easily enables the simultaneous detection of the API and related impurities while achieving compliance with the ICH guidelines on impurity monitoring.

References

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