Application Brief 157

High Throughput Impurity Profiling of Nevirapine

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Key Words
Ballistic Gradient, Quality Control, United States Pharmacopeia, Reporting Threshold, Nevirapine Tablets

Introduction

Receiving a reaction yield of 100% in organic synthesis is rare and often not possible as there is always a chance for byproducts and residual reaction educts. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) provides recommendations on treatment of so-called impurities beside the active pharmaceutical ingredient (API).[1] The impurity profile describes the identified and unidentified impurities present in the new drug substance. A fast assessment of the product purity is important information for quality control but also during the manufacturing of drugs. End point and achieved yield can be quickly determined and allow better control of the overall process. Also during process and synthesis development fast or near-real time feedback on laboratory scale synthesis experiments will reduce development time and resources required.

This application brief demonstrates the separation of Nevirapine and its related compounds A, B and C on a Thermo Scientific™ Dionex™ UltiMate™ 3000 XRS LC System. Nevirapine (trade name: Viramune, Boehringer Ingelheim) is a non-nucleoside reverse transcriptase inhibitor with activity against human immunodeficiency virus type 1 (HIV-1).

The analysis was done on a UHPLC column with 1.7 µm particle size. The Thermo Scientific™ Syncronis™ column material ensures reliable separation column after column by rigorous quality control and testing procedures.[2] The detection of the API and its impurities is possible in one run. The Thermo Scientific Dionex UltiMate 3000 VWD-3400RS Rapid Separation Variable Wavelength Detector with the semi-micro flow cell allows the detection of threshold <0.01% of the API considering LOD criteria of S/N equal to three. Depending on the maximum daily dose the reporting threshold for impurities is set to 0.05% by ICH. This threshold amounts are visualized in Figure 1.

Equipment

- An UltiMate 3000 XRS LC system[3] designed for high throughput analysis consisting of following modules was used in this experiment:
  - LPG-3400XRS Quaternary Rapid Separation Pump with Extended Pressure Range (P/N 5043.0036)
  - OAS-3300TXRS Rapid Separation Thermostatted Open Autosampler with 2 µL sample loop (P/N 6845.8109)
  - TCC-3000RS Rapid Separation Thermostatted Column Compartment (P/N 5730.0000)
  - VWD-3400RS Rapid Separation Four Channel Variable Wavelength Detector (Without Flow Cell) (P/N 5071.0010), installed with semi-micro flow cell (stainless steel) (P/N 6074.0360)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software 7.1 SR 2

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Column:	Syncronis C18, 1.7µm, 2.1 × 100 mm
Eluent:	A: 10 mM NH_4Ac, Acetic acid (pH 5.0)/ACN (85/15 v/v); B: ACN
Flow rate:	918 µL/min
Gradient:	30–70% B in 0.65 min, 70% B for 0.33 min, 0.8 min equilibration before injection at 30% B
Column Temp.:	50 °C
Inj. Volume:	1 µL
Detection:	UV, 240 nm

Peaks:	1. Nevirapine Impurity B	0.15 µg/mL (0.05% of API)
	2. Nevirapine	0.3 mg/mL (API)
	3. Nevirapine Impurity A	0.15 µg/mL (0.05% of API)
	4. Nevirapine Impurity C	0.15 µg/mL (0.05% of API)

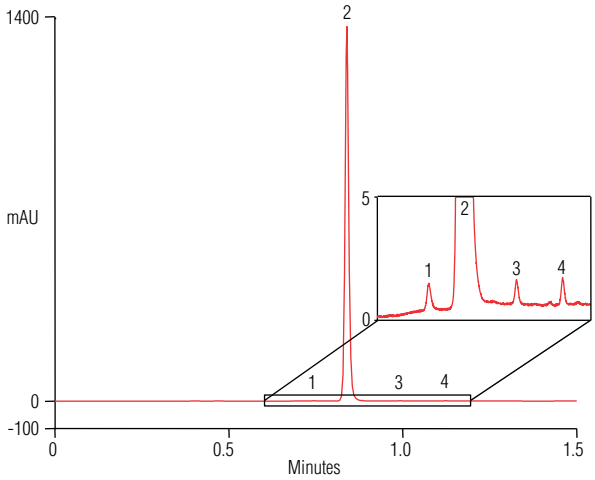

Figure 1. Separation of Nevirapine and its related compounds using a Syncronis C18 column.

Experimental Conditions

Experimental data are listed in Figure 1. The 1 mg/mL (0.1 mg/mL for impurity B) stock solutions of Nevirapine and related compounds are prepared by dissolving the solids in acetonitrile first, using 10% of the final flask volume. The solution is sonicated to dissolve and filled up with eluent A. The standard solutions are prepared by diluting with eluent A.

Results and Discussion

The UltiMate 3000 XRS LC system is designed for high-throughput analysis with extra low internal volume and extended pressure range up to 1250 bar. This allows a further acceleration of Nevirapine impurity analysis compared to USP methods or formerly published application notes.[4] Here, a ballistic gradient in less than 40 seconds runs on the gradient delay volume optimized system. This allows a tremendous short analysis time well below two minutes inclusive equilibration step. The fast cycle time and the high sample capacity make the OAS-3000XRS autosampler a real high throughput sample manager. The blank injections show no carryover using the two wash solvents water and acetonitrile. The wide linear range of the variable wavelength detector completes the high throughput impurity profiling system. The detector records signals of low impurity thresholds and of high APIs in one single run without the need for dilution.

References

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