

Screening Method for Fluoroquinolones Using an Advanced UHPLC Column and System Combination

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Key Words

Hypersil GOLD VANQUISH, fluoroquinolones, UHPLC, non-linear gradient

Goal

To provide an application for the analysis of fluoroquinolones using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ 1.9 µm UHPLC column the Vanquish UHPLC system.

Introduction

The Hypersil GOLD VANQUISH UHPLC column and the Vanquish UHPLC system were designed to achieve the best possible chromatographic performance. The Vanquish UHPLC system features intelligent SmartInject technology prior to injection and extremely low pump pulsation that delivers exceptional flow stability and improves column life time. The system is optimized to reduce extra column band dispersion, and allow users to significantly improve the separation power of their analytical assays and the binary high pressure-mixing pump is capable of delivering non-linear gradient for separation of complex mixtures.

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



Fluoroquinolones are popular broad spectrum antibiotics used in animal husbandry, some of which have been banned by US FDA and EU regulations. They are poly-aromatic compounds with fluorine atoms and carboxylic acid functionality. As such, they are sensitive to silanol interactions with the stationary phase that can lead to compromised peak shape and reduce effective peak resolution.

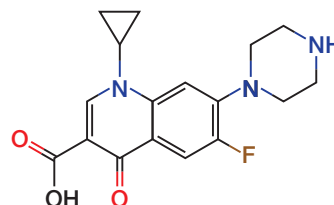


Figure 1. Structure of ciprofloxacin.

Experimental

Consumables

- Hypersil GOLD VANQUISH, 1.9 μm UHPLC column, 200 \times 2.1 mm (P/N 25002-202130-V)
- LC-MS grade 18 M Ω ·cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ Optima™ UHPLC-MS grade acetonitrile (P/N A956-1)
- Fisher Scientific Optima LC-MS grade formic acid (P/N A117-50)
- 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)
- Active Pre-heater (P/N 6732.0110)
- Column Compartment H (P/N VH-C10-A)
- 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 twenty-five compounds shown in Table 2 were prepared by dissolving 10 mg amounts in 10 mL of water/methanol (1:1, v/v) to produce 1 mg/mL primary solutions. Full dissolution of a number of the compounds was not achieved but this was overcome by addition of a few drops of formic acid. Further dilutions of the primary solutions were then made with water/methanol (1:1, v/v) to produce nominal 100 $\mu\text{g}/\text{mL}$ working solutions.

Vial labeling was supported by the Virtuoso Vial Identification System.

UHPLC Conditions

UHPLC Column	Hypersil GOLD VANQUISH, 1.9 μm , 200 \times 2.1 mm
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
Flow Rate:	400 $\mu\text{L}/\text{min}$
Column Temperature	40 $^{\circ}\text{C}$, still air with eluent pre-heating
Injection Volume	1 μL
UV Detection	254 nm

Table 1. Gradient table.

Time	%B	Curve
0.00	10	5
1.00	10	5
17.00	60	8
18.00	60	5
20.00	10	5
24.00	10	5

Results and Discussion

By exploiting the non-linear capabilities of the Vanquish UHPLC system, in conjunction with the Hypersil GOLD VANQUISH UHPLC column, it was found that a non-linear curve with a value of 8 and a full method

time of 24 minutes produced excellent results. Some compounds are not baseline resolved but do give sufficient separation for consistent peak identification between all the main analytes of this complex mixture, as shown in Figure 2.

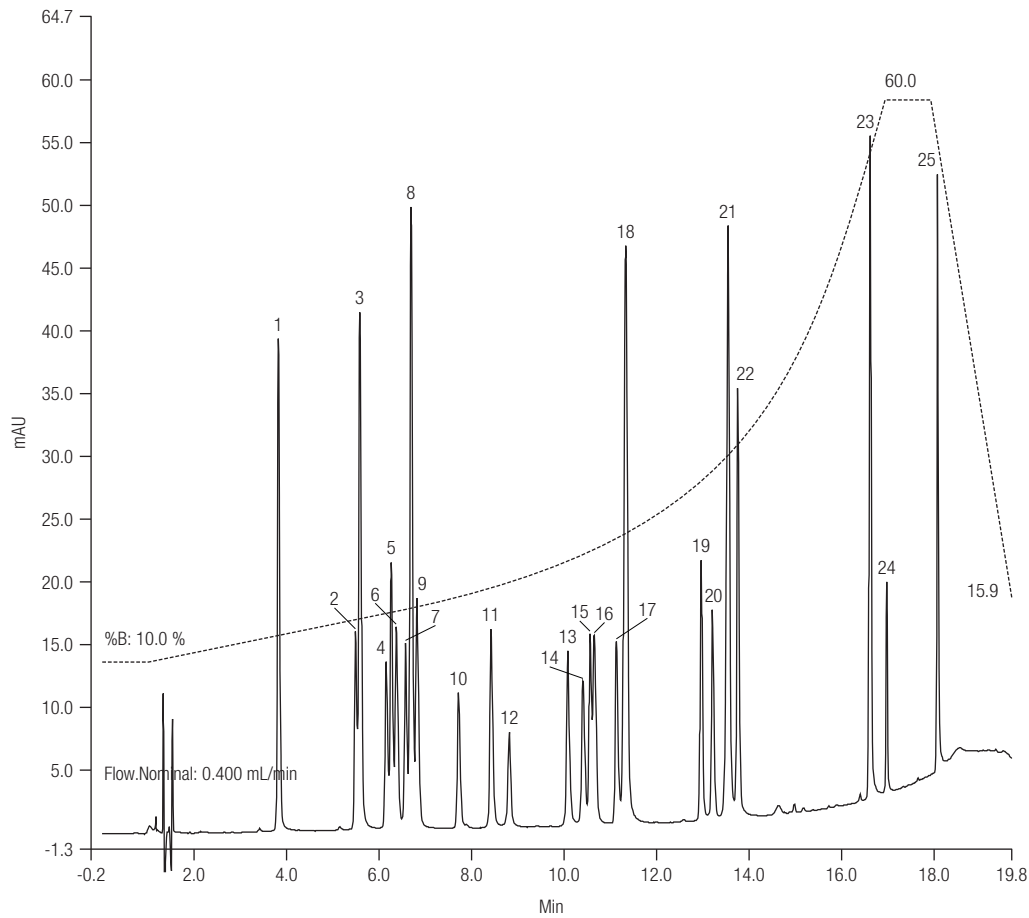


Figure 2. Fluoroquinolone sample mixture, non-linear gradient.

Table 2. Peak identification based on chromatogram in Figure 2.

Peak	Analyte	Peak	Analyte
1	Piromidic acid	14	Sarafloxacin
2	Marbofloxacin	15	Clinafloxacin
3	Enoxacin	16	Difloxacin
4	Fleroxacin	17	Sparfloxacin
5	Norfloxacin	18	Moxifloxacin
6	Ofloxacin	19	Balofloxacin
7	Pazufloxacin	20	Cinoxacin
8	Pefloxacin	21	Oxolinic acid
9	Ciprofloxacin	22	Trovafloxacin
10	Danofloxacin	23	Nalidixic acid
11	Enrofloxacin	24	Nadifloxacin
12	Prulifloxacin	25	Pipemidic acid
13	Gatifloxacin		

Replicate injections of the sample mixture showed that the Vanquish UHPLC system and Hypersil GOLD VANQUISH UHPLC column produced stable and reproducible results (Table 3 and Figure 3).

One reason for this is that the new pumping technology results in extremely low pump and outstanding gradient

precision, even for challenging non-linear gradient profiles. Furthermore, the autosampler features the unique intelligent SmartInject technology which compresses the sample to system pressure prior to injection. This results in a very stable flow delivery and consequently, unmatched retention time precision.

Table 3. Peak identification, retention time variability, and resolution for six replicate injections using a non-linear curve gradient method.

Peak	Analyte	Average Retention Time [min]	% RSD of Retention Time	Average Resolution (USP)
1	Piromidic acid	3.824	0.09%	
2	Marbofloxacin	5.500	0.06%	17.36
3	Enoxacin	5.596	0.06%	0.92
4	Fleroxacin	6.167	0.05%	5.42
5	Norfloxacin	6.277	0.05%	1.04
6	Ofloxacin	6.388	0.05%	1.02
7	Pazufloxacin	6.589	0.06%	1.82
8	Pefloxacin	6.707	0.06%	1.14
9	Ciprofloxacin	6.836	0.05%	1.14
10	Danofloxacin	7.741	0.05%	7.74
11	Enrofloxacin	8.446	0.04%	6.28
12	Prulifloxacin	8.845	0.05%	3.52
13	Gatifloxacin	10.112	0.04%	11.08
14	Sarafloxacin	10.443	0.03%	2.72
15	Cinafloxacin	10.596	0.04%	1.21
16	Difloxacin	10.688	0.03%	0.68
17	Sparfloxacin	11.168	0.04%	3.71
18	Moxifloxacin	11.373	0.03%	1.60
19	Balofloxacin	13.012	0.02%	13.97
20	Cinoxacin	13.255	0.03%	2.56
21	Oxolinic acid	13.598	0.03%	3.06
22	Trovafloxacin	13.805	0.03%	1.93
23	Nalidixic acid	16.686	0.01%	34.70
24	Nadifloxacin	17.045	0.01%	5.52
25	Pipemidic acid	18.150	0.01%	21.46

The use of advanced peak detection algorithms and data wizards provide for rapid development of data processing methods for complex samples, which lead to consistent peak assignment even when peaks are not fully resolved.

Competitor solutions for this application use the additional capability of MS detection to resolve individual analytes. The advanced low-dispersion design of the Vanquish UHPLC system and the high performance of the Hypersil GOLD VANQUISH UHPLC column allow sufficient resolution for a robust screening method using only UV detection.

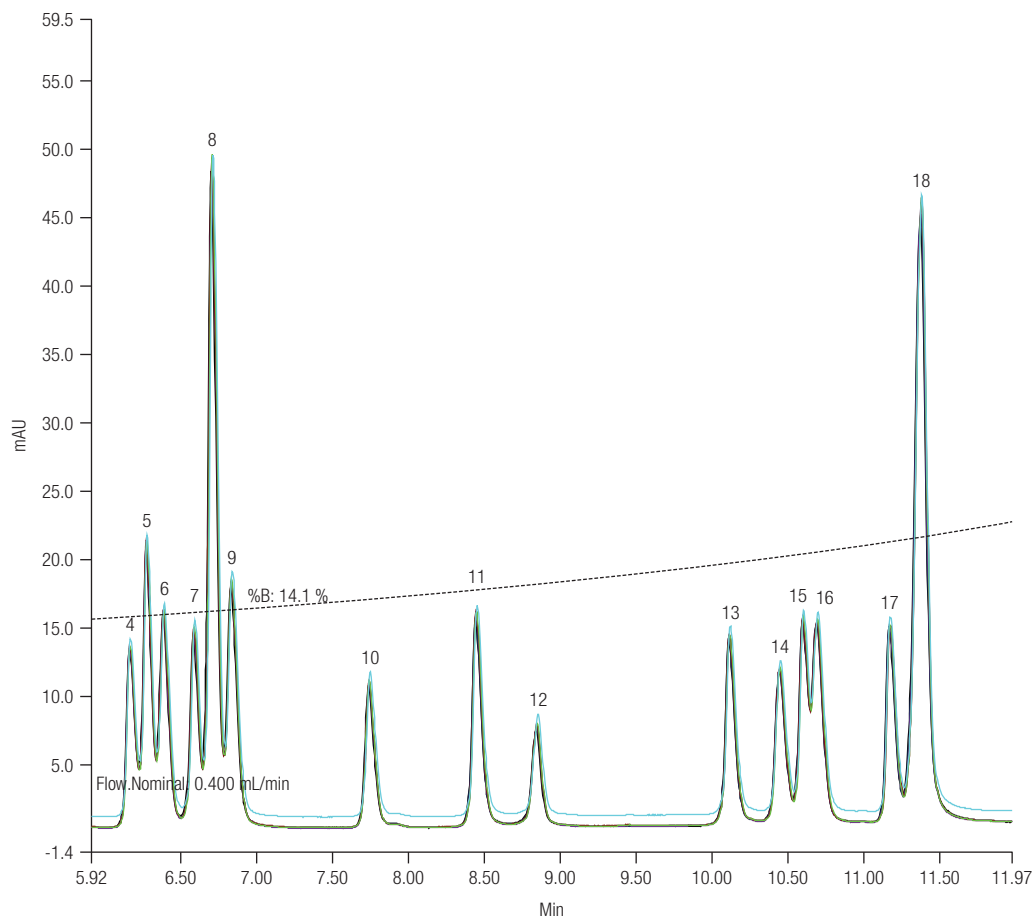


Figure 3. Chromatogram overlay of six consecutive injections, expanded to show excellent RT reproducibility, typically less than 0.05% RSD.

Conclusion

This application demonstrates the advantages of using the Hypersil GOLD VANQUISH 1.9 μ m UHPLC column in conjunction with the Vanquish UHPLC system and Chromeleon software. The performance of the Hypersil GOLD VANQUISH UHPLC column and the low internal extra-column volume and superior flow delivery capabilities of the Vanquish UHPLC, coupled with Chromeleon software deliver:

- Screening UHPLC method for fluoroquinolones
- Excellent retention time reproducibility
- Consistent data processing

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library:

www.thermofisher.com/appslab

www.thermofisher.com/LC-columns

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