Automated UHPLC method development for mebendazole and related impurities, from method scouting to robustness testing

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Introduction

Process automation and acceleration in (U)HPLC method development are areas of constant interest, leading to significant reduction in development time, cost, and labor. The systematic automated method development approach generally includes method scouting, optimization, and robustness testing. Key chromatographic parameters such as column, mobile phase composition and pH, and organic solvent type are first screened during method scouting. Method optimization is then followed where detailed chromatographic parameters (e.g. gradient profile, column temperature and flow rate) are iteratively adjusted. Robustness testing investigates the effects of method parameters on the responses, to ensure long-term method stability and facilitate method transfers between instruments and laboratories.

Mebendazole belongs to a class of anthelmintic drugs and is commonly used in the treatment of nematode infestations such as roundworm, hookworm, pinworm, whipworm, and threadworm. According to the International Council for Harmonization (ICH) guidelines, the active pharmaceutical ingredient (API) and related impurities must be well resolved for accurate quantification.

In this work, we used a software-assisted and automated UHPLC method development workflow to develop a method for determination of mebendazole impurities. A fast and robust UHPLC method was developed within 5 days, where mebendazole and related impurities were baseline separated with analysis time of 12 min. The method was found to be robust within ranges of ±1.5%B for organic solvent composition, ±2° C for column temperature, and ±0.1 pH unit.

Materials and methods

Sample Preparation

1 mg of the reference standard, consisting of the API mebendazole and related impurities A, B, C, D, E, F, and G, was dissolved in 1 mL dimethylformamide (DMF).

Instrumentation and Methods

Table 1. Instrumentation

Module	Part number
Thermo Scientific™ Vanquish™ Quaternary Flex system consisting of:	
System Base Vanquish Horizon/Flex	VF-S01-A
Quaternary Pump F	VF-P20-A
Split Sampler FT	VF-A10-A
Column Compartment H	VH-C10-A-02
Diode Array Detector FG	VF-D11-A
Thermo Scientific™ Automated Viper™ Method Scouting kit for Vanquish Systems	6036.2807
Vanquish Switching Valve with 6-position/7-port	6036.2530 (2x)
Extension Kit for Automated Method Scouting, Vanquish Systems	6036.0100

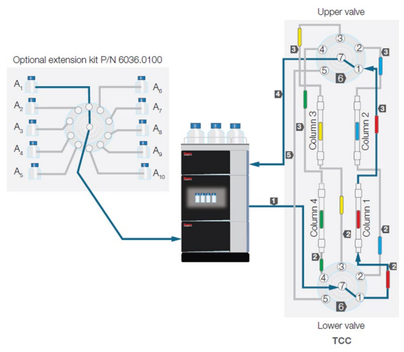

Figure 1. Flow scheme for the Vanquish Flex Quaternary UHPLC System with Automated Viper Method Scouting kit for Vanquish Systems.

Table 2. Columns, aqueous mobile phases, organic solvents, and other method parameters used for method scouting

Columns	Thermo Scientific™ Acclaim™ Polar Advantage II (PA2) (100 x 2.1 mm, 2.2 mm) P/N 068990	
	Thermo Scientific™ Accucore™ Phenyl-Hexyl (100 x 2.1 mm, 2.6 mm) P/N 17926-102130	
	Thermo Scientific™ Accucore™ Phenyl-X (100 x 2.1 mm, 2.6 mm) P/N 27926-102130	
	Thermo Scientific™Hypersil GOLD™ (100 x 2.1 mm, 1.9 mm) P/N 25002-102130	
Aqueous mobile phases	0.1% (v/v) formic acid in water	
	20 mM aqueous ammonium acetate buffer, pH 4.7	
	20 mM aqueous ammonium bicarbonate in water, pH 6.7	
	20 mM aqueous ammonium bicarbonate in water, pH 7.7	
Organic solvents	90/10 (v/v) acetonitrile/water, 90/10 (v/v) methanol/water	
Flow rate	0.3 mL/min	
Gradient	Time (min)	%B
	0	6
	25	100
Column temperature	40 °C (still air)	
Sampler temperature	10 °C	
Injection volume	2 µL	
UV detector parameters	Detection at 250 nm	
	3D scan: 190-350 nm	
	Data collection rate: 10 Hz	

Data Analysis

ChromSword Chromeleon Connect complete (P/N 7200.0165), integrated with Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS), was used for data acquisition during method scouting, rapid and fine optimization, and robustness testing. The ReportViewer module of ChromSword Chromeleon Connect was used for data analysis and evaluation.

Results

Vanquish Method Development systems with ChromSword Chromeleon Connect support the automation of method scouting, optimization, and robustness testing, resulting in significantly reduced development time and costs. An overall workflow for automated method development using ChromSword Chromeleon Connect is shown in Figure 2.

Step1: Method scouting
- ChromSword Chromeleon Connect software module: Scout
- Screen for a promising combination of column, solvent and mobile phase buffer pH

Step2: Rapid and fine optimization
- ChromSword Chromeleon Connect software module: Developer
- Optimize the gradient profile, with the column, mobile phase buffer and organic solvent chosen in method scouting

Step3: Method robustness test
- ChromSword Chromeleon Connect software module: AutoRobust
- Create design space (or robust region) by multivariate study

Figure 2. Workflow for automated method development using ChromSword Chromeleon Connect.

Step 1: Method scouting

Method scouting was done by evaluating performance criteria related to column selectivity and peak shape, namely total number of peaks, minimum peak resolution, peak asymmetry, and peak width. Figure 3 compares two separations of mebendazole and related impurities, which were filtered by applying two criteria: the number of peaks (≥8) and a minimum resolution of 1.5. The method using the Hypersil GOLD column was selected for method optimization, which yielded the largest number of peaks with Rs greater than 1.5.

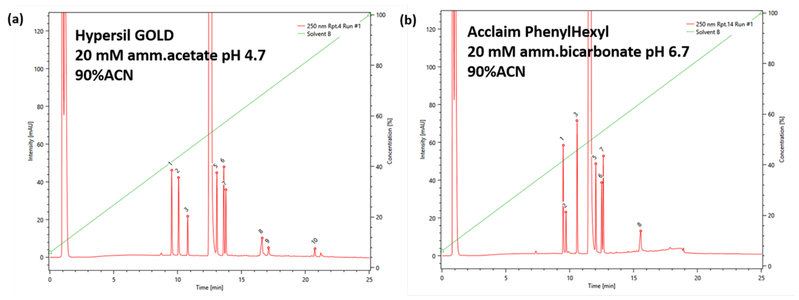

Figure 3. Chromatograms selected from method scouting as candidate for further optimization.

Step 2: Method optimization

Table 3 shows the chromatographic conditions used for method optimization including the above selected column, aqueous mobile phase pH, and organic solvent. The flow rate was increased to 0.8 mL/min both to reduce overall development time and improve final method throughput.

Table 3. Chromatographic conditions for method optimization

Column	Hypersil GOLD (100 x 2.1 mm; 1.9 µm)
Aqueous mobile phase	20 mM aqueous ammonium acetate buffer, pH 4.7
Organic solvent	90/10 (v/v) acetonitrile/water
Flow rate	0.8 mL/min
Other parameters were kept as those listed in Table 2	

Figure 4 shows the result of rapid optimization, where all related impurities were fully resolved from mebendazole within 13 minutes using a multi-step gradient profile. The resolution of the critical peak pair 6 and 7 was observed to be 3.15, and EP tailing factors for all mebendazole-related impurities were less than 1.2 (apart from peaks due to solvent matrix and API). The initial peaks eluting before 0.5 min, peaks 8 and 10, were seen as a result of sample matrix.

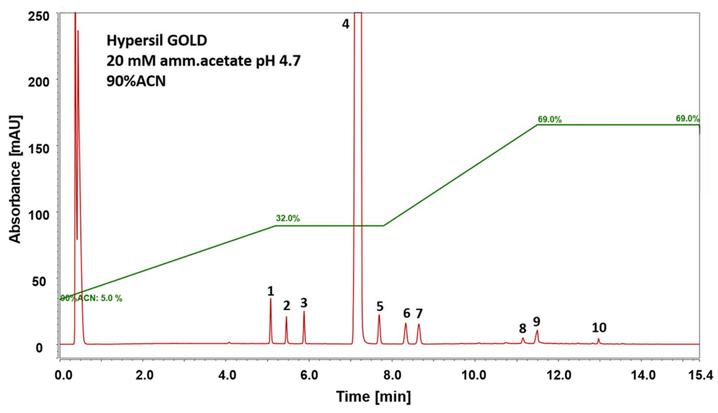

Figure 4. Chromatogram selected during rapid optimization.

Figure 5 shows the best separation of mebendazole and related impurities, with the analysis time less than 11 min. The gradient provided by the fine optimization was linear. Linear gradients are preferred compared to a step-gradient in terms of method robustness and method transfer between different instruments and laboratories. Thus, the linear gradient method was chosen as the final method for further robustness testing.

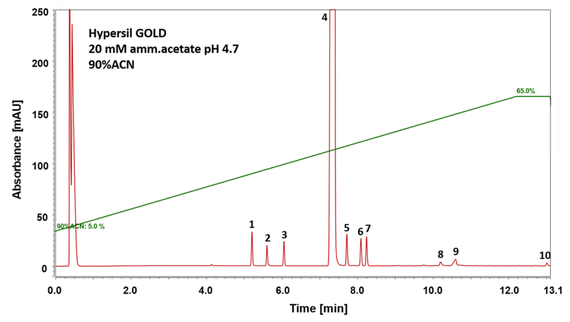

Figure 5. Chromatogram with best method from fine optimization.

Step 3: Method robustness test

Method robustness testing was performed to determine robust operating ranges of method parameters. The Plackett-Burman design was used. Table 4 lists method parameters and test ranges varied for robustness testing. Figure 6 shows the 2D resolution map for the effect of column temperature and concentration of organic solvent at pH 4.7. The blue box represents the robust region with resolution >1.8 for temperature, %B, and ±0.1 pH units.

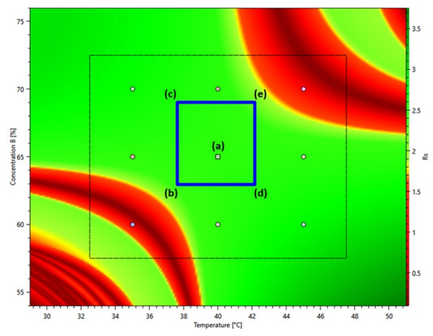

Table 4. List of variables.

Parameters	Test ranges
Column temperature	± 5° C
Concentration of organic solvent B (%)	± 5%
Break point time	± 0.25 min
Mobile phase buffer pH	± 0.2

Figure 6. A 2D resolution map illustrating the effect of temperature and concentration of organic solvent B (%), with the robust region indicated by a blue box. (a) Final method, and (b) to (e) – four corners of the blue box.

To demonstrate robust method operation throughout the design space (i.e. inside the blue box), separations were performed at four corners of the region, as shown in Figure 7.

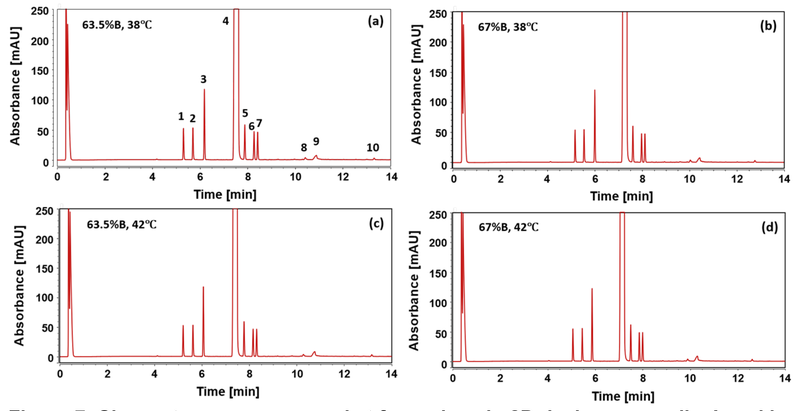

Figure 7. Chromatograms measured at four edges in 2D design space displayed in Figure 6. The critical peak pair resolution within the robust region remained above 2.4. The Hypersil GOLD column with 20 mM ammonium acetate pH 4.7 and 90% (v/v) acetonitrile in water was used.

Table 5. Summary of the time required during automated method development for mebendazole and related impurities.

	Step	Instrument time [h]	Analyst time [h]
1	Method scouting	51	1.5
2	Rapid optimization	1.5	0.25
3	Fine optimization	12.5	1
4	Robustness testing	5.6	1

Conclusions

- The Vanquish Method Development System, consisting of a combination of ChromSword Chromeleon Connect and the Vanquish Flex UHPLC system, enabled rapid, unattended development of a fast and robust method for the analysis of mebendazole and related impurities.
- The proposed workflow substantially reduced both development time and user intervention.
- By a systematic approach using method development software, the Thermo Scientific Hypersil GOLD column was rapidly selected as the most promising column for separating mebendazole and related impurities.

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