Pharmaceutical analysis

Automated HPLC method development and robustness tests for abacavir, lamivudine, dolutegravir and their related compounds in Triumeq drug product

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Abstract

Purpose: To demonstrate an automated method development and robustness tests workflow for abacavir, lamivudine, dolutegravir, and their related compounds in drug Triumeq.

Methods: By using the ChromSwordAuto Chromeleon Connect in combination with the Thermo Scientific ™ Vanquish ™ Core HPLC system and automated method scouting kit, the method scouting, method optimization, robustness tests and data processing were completed automatically and intelligently.

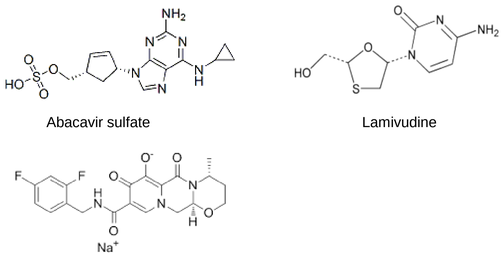
Results: This workflow reduced manual instrument operations and accelerated method development significantly. The total time required for method development and robustness testing was about one-fifth of the time needed by the manual development process described previously.[1]

Introduction

Triumeq is a “once-daily” tablet for the treatment of HIV-1 infection, which contains abacavir 600mg, dolutegravir 50mg, and lamivudine 300mg in each tablet (Figure 1).[2] It was approved by the US Food and Drug Administration in 2014, and now is the best-selling and most competitive anti-HIV drug on the market.[3]

Using the traditional trial-and-error process to develop an HPLC method for this drug is challenging and time-consuming due to the presence of more than 20 components.[4] It requires testing many different columns, mobile phases, and gradients to find an acceptable condition to achieve separation in a reasonable time, and this process is highly dependent on the skills and knowledge of the chromatographer. Automated method development platform provides an alternative solution. In this poster, ChromSwordAuto Chromeleon Connect and the Vanquish Core HPLC system were used to develop an automatic HPLC method development and robustness testing workflow for abacavir, lamivudine, dolutegravir, and their related compounds in drug Triumeq.

Figure 1: Chemical Structures of abacavir sulfate, lamivudine and dolutegravir sodium.



Abacavir sulfate

Lamivudine

Dolutegravir sodium

Materials and methods

Sample Preparation

10 Triumeq tablets were ground into fine powder, weigh the powder equivalent to 2 tablets, add 100mL 50% methanol to dissolve it. The solution was sonicated for 30 min and centrifuged for 10 min at 8000 rpm. After centrifugation, the supernatant was transferred to tubes for the next step.

Then the related compounds were spiked into the supernatant at a 1.0% concentration level relative to APIs for method scouting, 0.1% concentration level relative to APIs for method optimization and robustness tests.

Instrumentation

Thermo Scientific™ Vanquish™ Core Quaternary HPLC System with the Thermo Scientific™ Vanquish™ Diode Array Detector CG.

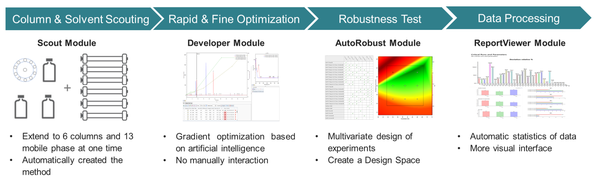
Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Version 7.3.

ChromSwordAuto Chromeleon connect.

Results

Figure 2. The workflow and benefits of automated method development and robustness testing by using ChromSwordAuto Chromeleon connect and Thermo Scientific™ Vanquish™ automated method scouting kit.



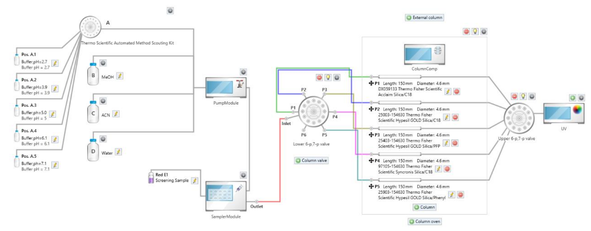
Method Scouting Study:

Table 1. Columns, eluents, and gradient used for method scouting, along with column temperature of 30°C, injection volume 10μL, and UV wavelength of 275nm and 300nm.

Columns (3 μm, 4.6 × 150 mm)			
Thermo Scientific Acclaim™ 120 C18 (P/N 059133)			
Thermo Scientific Syncronis™ C18 (P/N 97105-154630)			
Thermo Scientific Hypersil Gold™ C18 (P/N 25003-154630)			
Thermo Scientific Hypersil Gold™ Phenyl (P/N 25903-154630)			
Thermo Scientific Hypersil Gold™ PFP (P/N 25403-154630)			
Aqueous Eluent			
0.1% formic acid in water, pH 2.7			
0.02% formic acid and 10 mM ammonium formate in water, pH 3.9			
0.002% formic acid and 10 mM ammonium formate in water, pH 5.0			
10 mM ammonium formate in water, pH 6.1			
20 mM ammonium formate, pH 7.1			
Organic Eluent			
Methanol/ Acetonitrile			
Gradients			
Time (min)	Flow Rate(mL/min)	Aqueous Eluent (%)	Organic Eluent (%)
0	1.0	95	5
12	1.0	10	90
15	1.0	10	90

The column equilibration, column washing, system purging, and valve switch can be completed automatically by ChromSwordAuto Chromeleon Connect 5 according to your settings.

Figure 3：Schematic for method scouting system using ChromSwordAuto Chromeleon Connect, two 6-p, 7-p column switching valves and method scouting kit for automated method scouting.



After method scouting study, the Acclaim 120 C18 column, aqueous phase with pH 5.0, and methanol were selected for the further optimization, due to the best separation performance.

Method Optimization Study:

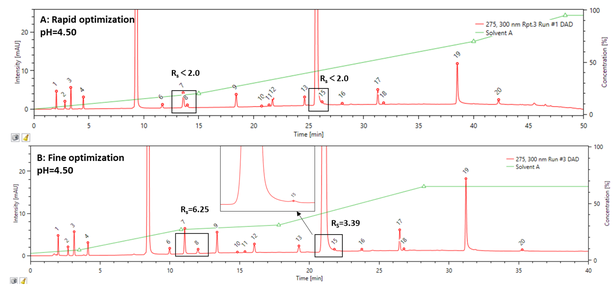
Table 2: Chromatography conditions used for method rapid optimization.

Conditions for Rapid Optimization:	
Columns	Thermo Scientific Acclaim™ 120 C18 (P/N 059133)
Aqueous Eluent	10 mM ammonium formate in water, pH 4.5
	10 mM ammonium formate in water, pH 5.0
	10mM ammonium formate in water, pH 5.6
Organic Eluent	Methanol
Injection volume:	10μL, 5μL, 2μL
Column Temperature:	25°C, 30°C, 40°C
Flow Rate	1.0 mL/min
Detector	UV wavelength: 275nm and 300nm

Figure 4: The best chromatogram selected from rapid and fine optimization results on Acclaim 120 C18, with column temperature 30°C, injection volume 2μL. After the rapid optimization, the resolution of peak 7 and peak 8, peak 14 and peak 15 was still less than 2.0. After fine optimization, the resolution between peak 7 and 8, peak 14 and 15 were improved and the run time also reduced.

The green line represents the gradient. Signal standard solutions were injected to identify the retention time of the APIs and impurities.

Peak ID: 1. Cytosine; 2. Uracil; 3. Lamivudine impurity I; 4. Lamivudine Impurity V; 5. Lamivudine; 6. Lamivudine Impurity III; 7. Cyclopropyl diaminopurine abacavir; 8. Salicylic acid; 9. Abacavir related compound A; 10. Unknown compound; 11. Unknown compound; 12. Abacavir related compound B; 13. Abacavir related compound C; 14. Abacavir; 15. Unknown compound; 16. Unknown compound; 17. Abacavir related compound D; 18. Unknown compound; 19. Dolutegravir; and 20. Unknown compound



Method Robustness Study:

Table 3. The properties in the method robustness study, full factorial was used for the test. The breakpoint time represents the time points where the gradient slope changed.

Property	± values
Concentration of organic solvent A, %	± 5%
Column temperature, °C	± 5°C
Breakpoint time, min	± 0.6 min
pH of the mobile phase buffer	±0.5 pH units

Figure 5：Two-dimensional resolution map for the effect of column temperature (X axes), pH (Y axes), and breakpoint time (Z axes, value=3.4 min). (Red: Resolution ＜2.0, Yellow and Green: Resolution≥2.0). The blue box depicts the robust region for temperature, pH, and breakpoint time (3.4±0.6min), produced by changing the value of Z axes to find an aera with the resolution ＞2.0.

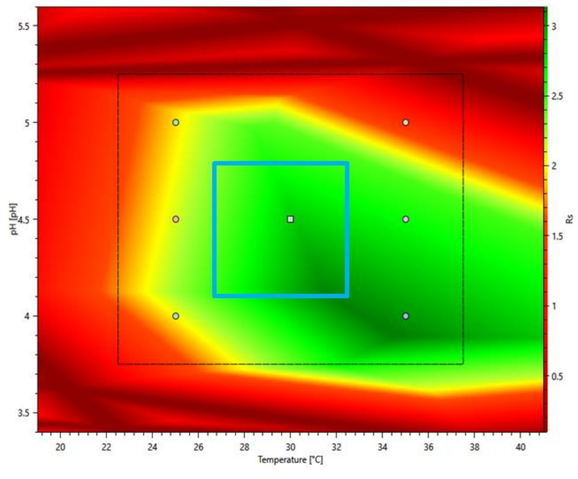


Table 4: Time required for automated method development and robustness tests for Triumeq and its related compounds. The total time required for method development and robustness tests is about 5.5 days, around one-fifth of the time needed by the manual development process described previously.[1]

Experiment	Instrument time (h)	Analyst time (h)	Total time (h)
Method scouting	33.5	1.5	35.0
Rapid optimization	10.5	0.5	11.0
Fine optimization	40.2	1.0	41.2
Robustness study	48.2	1.5	49.7
Total time	132.4	4.5	136.9

Conclusions

- Accelerated the HPLC method development and reduced the costs significantly by using ChromSwordAuto Chromeleon Connect and Thermo Scientific™ Vanquish™ switching valves.
- The final method provides an adequate separation for all analytes with a USP resolution ≥2.0, and peak asymmetry within 0.9 to 2.1.
- The statistical design of experiments and the design space in the robustness tests are in accordance with analytical quality by design principles.

References

1. Thermo Fisher Scientific, Application Note 000582, Development and validation of a HPLC-DAD method for simultaneous determination of abacavir, lamivudine, dolutegravir, and their related compounds in Triumeq. https://thermofishercmd.highspot.com/items/62680066ea1179e4c175824c?lfrm=shp.0
2. ViiV Healthcare Press Release. ViiV Healthcare receives FDA approval for Triumeq | ViiV Healthcare
3. Belk, D. "Pharma’s 50 Best Sellers." True Cost of Health-Care. https://truecostofhealthcare.org/pharmas-50-best-sellers/
4. Tol, T., Kadam, N., Raotole, N., Desai, A., & Samanta, G. (2016). A simultaneous determination of related substances by high performance liquid chromatography in a drug product using quality by design approach. Journal of Chromatography A, 1432, 26-38

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