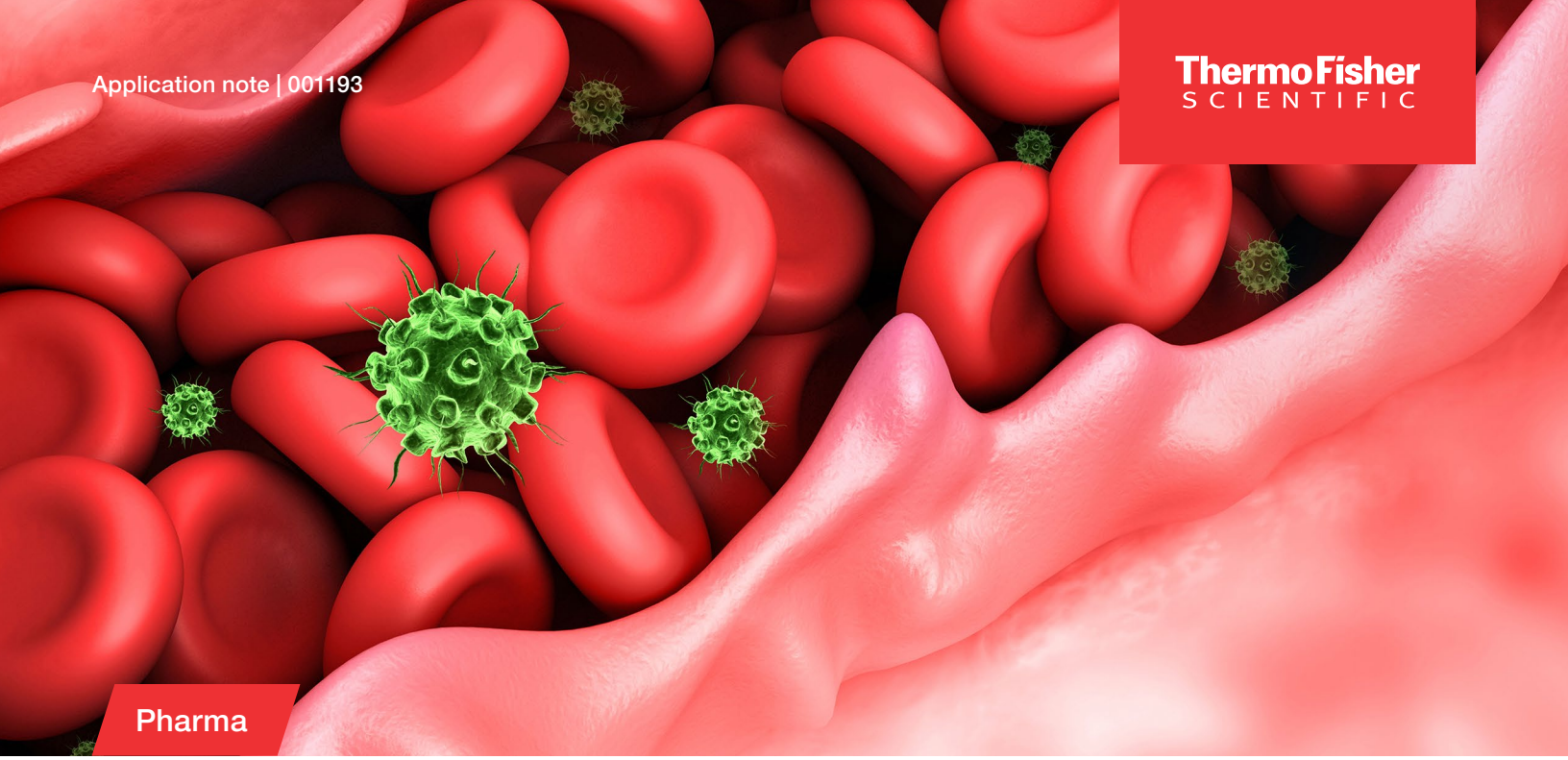

Application note | 001193

Pharma

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

Authors

Xuepu Li[1], Min Du[2]

[1]Thermo Fisher Scientific, Shanghai, China

[2]Thermo Fisher Scientific, Boston, MA, US

Keywords

Automated method development, ChromSwordAuto Chromeleon Connect, Vanquish Core HPLC, abacavir, lamivudine, dolutegravir, Triumeq, aQBD

Application benefits

- The seamless integration between ChromSword and Thermo Scientific™ Chromeleon™ Chromatography Data System fully automates the method development and robustness testing process.
- HPLC method development is accelerated significantly by using ChromSwordAuto Chromeleon Connect and Thermo Scientific™ Vanquish™ switching valves.
- The statistical design of experiments (DoE) and the design space in the robustness test are in accordance with analytical quality by design (aQbD) principles.
- A robust HPLC method for abacavir, lamivudine, dolutegravir, and their related compounds in the drug Triumeq™ is provided.

Goal

Demonstrate an automated method development and robustness test workflow for abacavir, lamivudine, dolutegravir, and their related compounds in the drug Triumeq by using ChromSwordAuto Chromeleon Connect and a Thermo Scientific™ Vanquish™ Core HPLC

Introduction

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

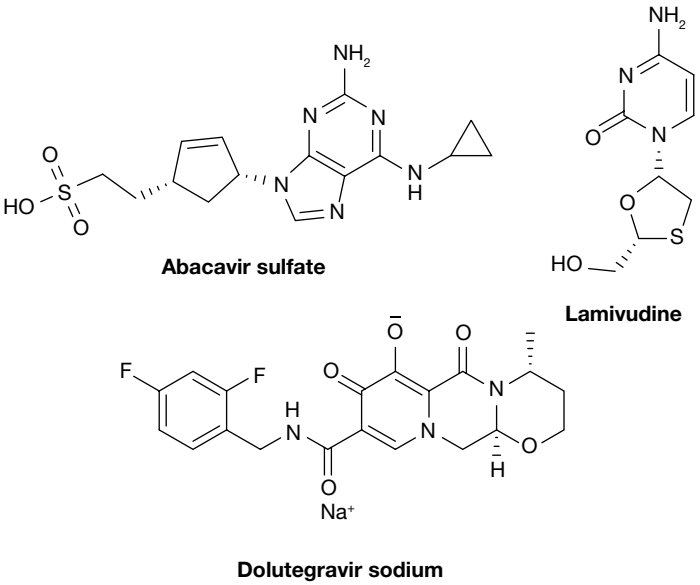

Figure 1. Chemical structures of abacavir sulfate, lamivudine, and dolutegravir sodium

Using the traditional trial-and-error process to develop an HPLC method for this drug is challenging and time-consuming in the analytical lab due to the presence of more than 20 components with different polarity, including three active pharmaceutical ingredients (APIs), impurities, and excipients.[3] It requires testing many different columns, mobile phases, and gradient programs to find acceptable conditions, to achieve separation in a reasonable time, and it’s highly dependent on the skills and knowledge of the chromatographer. Even for a highly skilled analyst, it can take weeks to months to develop a suitable chromatographic method. The latest automated HPLC method development techniques offer a solution to these problems. By using an artificial intelligence algorithm, automated method development software can run method scouting, method optimization, robustness testing, and data processing automatically, intelligently, and rapidly,[4] which can accelerate the method development process significantly.

In this application note, ChromSwordAuto Chromeleon Connect and a Thermo Scientific Vanquish Core HPLC system were used for the automated method development. The Vanquish Core HPLC was fitted with two six-position, seven-port switching valves, which can allow for up to six columns at once for method scouting. After the method was developed, the robustness was also studied. ChromSwordAuto provides a statistical design of experiments to study the effects of multiple variables on the separation, then generates a two-dimensional resolution map with a design space for the method, which are in accordance with aQbD principles. The results demonstrated that the method developed by the ChromSwordAuto was robust, with good separation and peak shape. Compared with the manual trial-and-error method development process, this workflow reduces manual instrument operations and accelerates method development significantly. The total time required for method development and robustness testing is about 5.5 days, around one-fifth of the time needed by the manual development process described previously.[5]

Experimental

Instrumentation

- Vanquish Core Quaternary HPLC system consisting of:
  - System Base Vanquish Core (P/N VC-S01-A)
  - Vanquish Quaternary Pump CN (P/N VC-P21-A)
  - Vanquish Split Sampler CT (P/N VC-A12-A)
  - Vanquish Column Compartment C (P/N VC-C10-A) (×2)
  - Vanquish Diode Array Detector CG (P/N VC-D11-A)
  - Flow cell, SST, 10 mm, 13 µL, (P/N 6083.0510)
- Thermo Scientific™ Vanquish™ 6-position, 7-port Switching Valve (2x) (P/N 6036.2530)

Software

- Chromeleon Chromatography Data System (CDS) Version 7.3
- ChromSwordAuto Chromeleon Connect

Reagents and consumables

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Methanol, HPLC grade (P/N A452-4)
- Fisher Scientific™ Acetonitrile, HPLC grade (P/N A998-4)
- Fisher Scientific™ Formic acid (FA), Optima™ LC/MS grade (P/N A117-50)
- Ammonium formate, LC-MS grade, ≥99% purity
- Abacavir, lamivudine, dolutegravir, and their related compounds reference standards (Table 1)
- ViiV Healthcare UK Limited, Triumeq film coated tablets, purchased from Huifan pharmacy, Shanghai, China

Table 1. Abacavir, lamivudine, dolutegravir, and their related compounds reference standards

Standards	CAS	Vendor	P/N
Abacavir sulfate	188062-50-2	Macklin	A833205
Lamivudine	134678-17-4	Damas-beta	21280A
Dolutegravir sodium	1051375-19-9	Macklin	G872900
Cytosine	71-30-7	Macklin	C804556
Uracil	66-22-8	Macklin	U820313
Salicylic acid	69-72-7	Macklin	S817529
Cyclopropyl diaminopurine abacavir	120503-69-7	Macklin	N892752
Lamivudine impurity I	173829-09-9	China National Institutes for Food and Drug Control (NIFDC)-National Drug Reference Standards	101317
Lamivudine impurity III	145986-07-8	NIFDC	101318
Lamivudine impurity V	160552-54-5	NIFDC	101319
Abacavir related compound A	124752-25-6	Sigma-Aldrich	PHR2064
Abacavir related compound B	141271-12-7	Sigma-Aldrich	PHR2065
Abacavir related compound C	172015-79-1	Sigma-Aldrich	PHR2066
Abacavir related compound D	1443421-69-9	USP	R152H0

Sample preparation

Triumeq drug product solution

Ten Triumeq tablets were ground into fine powder, the powder equivalent of two tablets was weighed into a 100 mL flask, and 50% methanol in water (v/v) was added to the volume. The solution was sonicated for 30 min, and then centrifuged for 20 min at 8,000 rpm. After centrifugation, the supernatant was transferred to tubes for the next step.

Sample solution for method scouting

The related compounds were spiked into the Triumeq drug product solution at a 1.0% concentration level relative to APIs.

Sample solution for method optimization and robustness study

The related compounds were spiked into the Triumeq drug product solution at a 0.1% concentration level relative to APIs.

Results and discussion

The workflow of the automated method development and robustness test is shown in Figure 2, mainly including the following steps:

Method scouting study: The main task in this phase was to find the most powerful parameters that influence the separation. According to how influential these factors are in affecting selectivity, the key three parameters are column stationary phase, pH of aqueous phase, and organic modifier. The Scout module of ChromSwordAuto Chromeleon Connect was used for this phase.

Method optimization study: In this phase, other parameters were adjusted to get a better separation and peak shape, such as gradient breakpoint time, ratio of the organic phase, column temperature, and injection volume. The Developer module of ChromSwordAuto Chromeleon Connect was used for this phase.

Robustness test: After the method development, the robustness was studied to demonstrate the method is reliable even under some variations in the lab. The AutoRobust module of ChromSwordAuto Chromeleon Connect was used for this phase, which provides one factor, full factorial, and Plackett-Burman test types.

Data processing: The ReportViewer module of ChromSwordAuto Chromeleon Connect was used for all data processing, including peak integration, data analysis and statistics, design space analysis, report creation, and export.

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

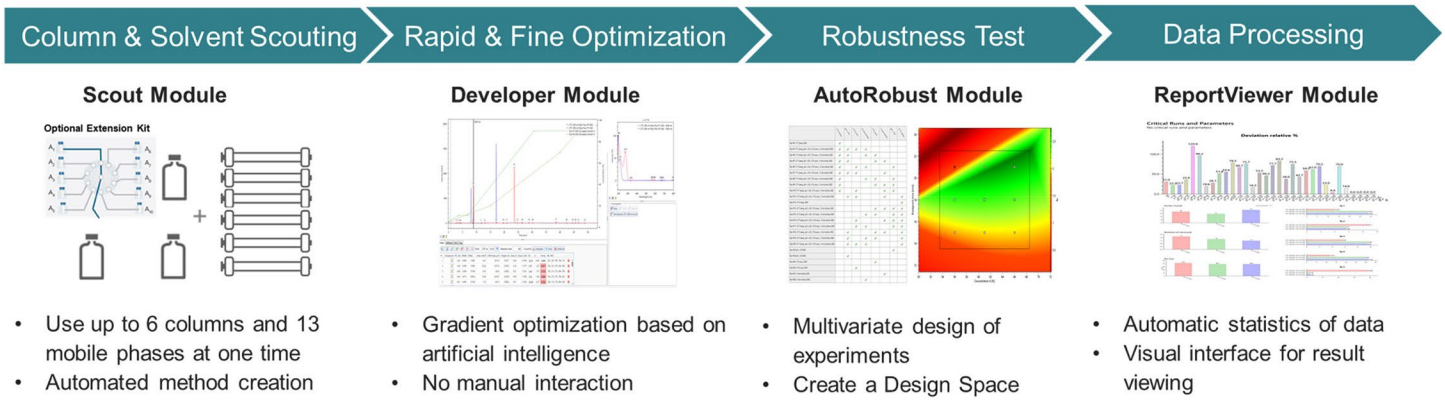

Figure 2. Workflow overview and benefits of automated method development and robustness testing based on ChromSwordAuto Chromeleon Connect and Thermo Scientific Vanquish automated method scouting kit

Table 2. Columns, eluents, and gradient used for method scouting

Columns			
Thermo Scientific™ Acclaim™ 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)			
Thermo Scientific™ Syncronis™ C18, 5 µm, 4.6 × 150 mm (P/N 97105-154630)			
Thermo Scientific™ Hypersil GOLD™ C18, 3 µm, 4.6 × 150 mm (P/N 25003-154630)			
Thermo Scientific™ Hypersil GOLD™ Phenyl, 3 µm, 4.6 × 150 mm (P/N 25903-154630)			
Thermo Scientific™ Hypersil GOLD™ PFP, 3 µm, 4.6 × 150 mm (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 7.0			
Organic eluent			
Methanol			
Acetonitrile			
Gradient			
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

Method scouting study

The detailed information for columns, eluents, and gradient used for method scouting is listed in Table 2, with the column temperature of 30 °C, injection volume 10 µL, and UV wavelength of 275 nm and 300 nm. The number of peaks, resolution, and peak asymmetry were used to evaluate the performance of different conditions. With pH 2.7 and 3.9, the peak number is less than 14, which means some compounds eluted at the same time or did not elute at all, and with pH 6.1 and 7.0, there is a peak tailing issue for dolutegravir. Compared with acetonitrile, methanol provides a better separation for all pH conditions. Finally, an Acclaim 120 C18 column, aqueous phase with pH 5.0, and methanol were selected for the further optimization, due to the best separation performance.

The total time spent on column and eluent scouting was 35 hours by using the column switching valves and ChromSwordAuto Chromeleon Connect, while the manual method scouting process described previously needs about 5 days.[5] The switching valves enable screening of up to six columns at one time. In this experiment, we selected five different columns for the method screening, and the five columns were screening at one time. ChromSwordAuto ReportViewer presents simple histograms of the statistical results on peak number, resolution, and run time for each injection, which greatly reduces data processing time. For a streamlined approach to column screening, an Extension Kit for Automated Method Scouting for Vanquish LC Systems can be employed to further improve the laboratory workflow.[6]

Method optimization study

In the method optimization study, adjustment of the gradient is usually the most time-consuming step. The analyst needs to change the gradient time, steepness, and the ratio of organic phase recurrently to get a better separation based on previous results. The Developer module in ChromSwordAuto calculates and analyzes the resolution and peak purity automatically after each injection. The resolution and peak purity that are lower than the value you set will be marked, and then the gradient is adjusted intelligently to resolve these issues.

The Developer module includes rapid method optimization, sample profiling (fine optimization)-isocratic optimization, and sample profiling (fine optimization)-isocratic and gradient optimization types. For rapid optimization, 3 to 4 gradient runs are generated to try to separate all the compounds. For sample profiling-isocratic and gradient optimization, ChromSwordAuto runs isocratic methods first to get the retention behavior for each compound and then generates gradient methods to get the optimum separation. In most cases, after the rapid optimization, it can provide a method with an acceptable separation. However, for some complex mixtures, sample profiling-isocratic and gradient optimization is required for better separation.

The optimization conditions are listed in Table 3. As pH has a significant effect on the resolution and retention time, we further tried pH 4.5, 5.0, and 5.6. After the pH optimization, the column temperature and injection volume were also optimized.

The best result from rapid optimization is shown in Figure 3A, with the chromatography condition aqueous phase pH = 4.5, column temperature = 30 °C, and injection volume = 2.0 µL. The results show that even after the rapid optimization, the resolutions of peak 7 and peak 8, peak 14 and peak 15 were still less than 2.0. Then, the sample profiling-isocratic and gradient optimization was conducted to improve resolution. The best result after fine optimization is shown in Figure 3B. It shows that after fine optimization, all the compounds can be separated well (with resolution > 2.0), and resolutions of peak 7 and 8, peak 14 and 15 have a significant improvement with values of 6.25 and 3.39, respectively. The run time was also reduced from 50 min to 40 min. The final HPLC conditions are listed in Table 4.

Peaks:

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
20. Unknown compound

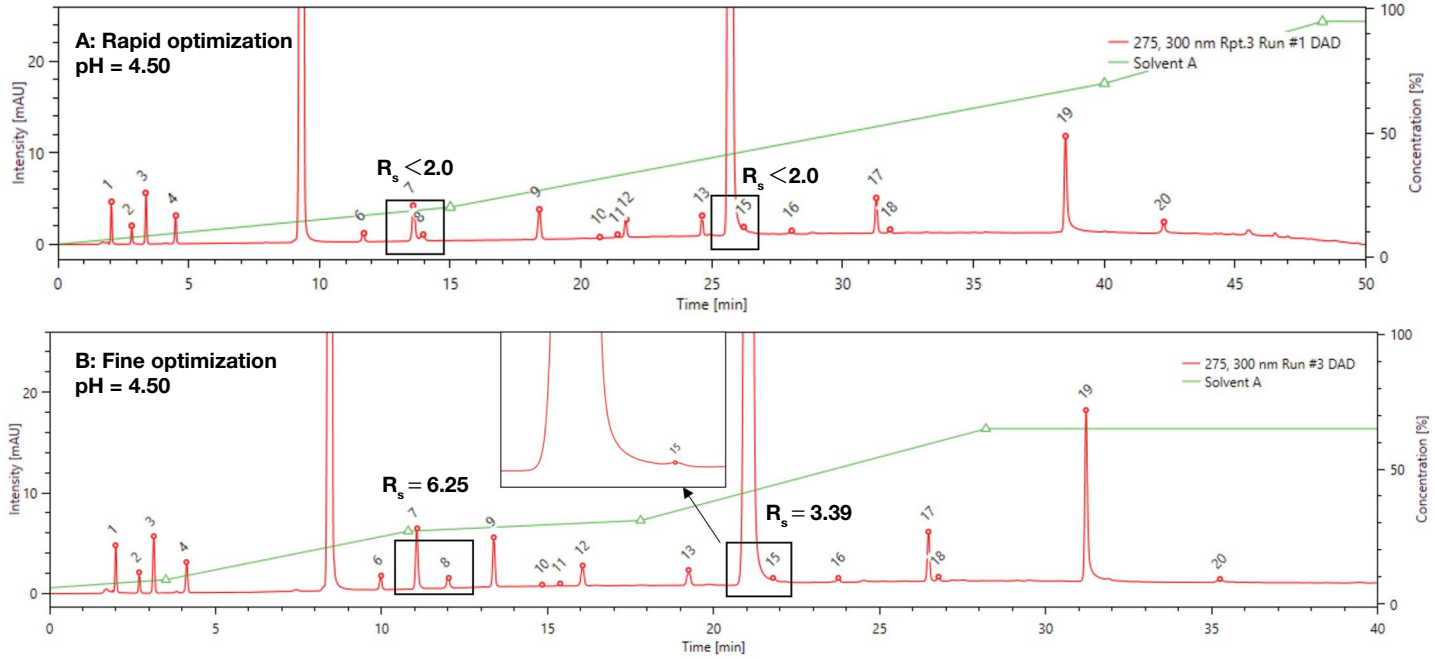

Figure 3. The best chromatogram selected from rapid and fine optimization results on an Acclaim 120 C18 column, with column temperature 30 °C, injection volume 2 µL. After fine optimization, the resolution between peak 7 and 8, peak 14 and 15 was improved and the run time reduced. The green line represents the gradient.

Table 3. Chromatography conditions used for method rapid optimization

Parameter	Setting
Column	Acclaim 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)
Organic eluent	MeOH
Aqueous eluent	10 mM ammonium formate in water, pH 4.5 (Add formic acid to pH =4.5) 10 mM ammonium formate in water, pH 5.0 (Add formic acid to pH =5.0) 10 mM ammonium formate in water, pH 5.6 (Add formic acid to pH =5.6)
Injection volumes	10 µL, 5 µL, 2 µL
Column temperatures	25 °C, 30 °C, 40 °C
Detection wavelength	275 nm, 300 nm
Flow rate	1.0 mL/min

Table 4. Final HPLC method conditions

Parameter	Setting
Column	Acclaim 120 C18, 3 µm, 4.6 × 150 mm
Mobile phase	A: Methanol B: 10 mM ammonium formate, pH = 4.5
Gradient	Time (min) %A %B 0 6 94 3.4 9 91 10.8 27 73 17.8 31 69 28.1 65 35 36.0 65 35 36.1 6 94 41.0 6 94
Flow rate	1.0 mL/min
Column temperature	30 °C
Sample compartment temperature	4 °C
Injection volume	2.0 µL
Needle wash solvent	50% methanol
Detector	275 nm, 300 nm, 20 Hz, 0.2 response time, 4 nm bandwidth 3D Field: 190–600 nm, bunchwidth, 4 nm

The total time spent on method optimization was about 51 hours. For rapid optimization, a total of 17 runs were performed (4 runs for each pH, 5 runs for injection volume and column temperature optimization), which required about 11 hours. For fine optimization, a total of 26 runs, including 14 isocratic and 12 gradient methods, were performed, requiring about 41 hours.

Method robustness study

The robustness of the method can be tested using the AutoRobust module by either varying one parameter at a time or by a multi-parameter design of experiments. It includes two types of multi-parameter design of experiments: the full factorial design and the Plackett-Burman statistical design. Plackett-Burman designs enable users to learn as much as possible from the smallest amount of data. However, there are some limitations to Plackett-Burman designs; users should know that the Plackett-Burman design does not tell if the effect of one factor depends on another factor. The full factorial design contains all possible combinations of a set of factors, the number of experiment runs will be very large when the factors and steps are higher than 3. Here, we used the full factorial design to study the method robustness; the properties used are listed in Table 5.

Table 5. 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
Percentage of organic solvent A, %	± 1.5
Column temperature, °C	± 5
Breakpoint time, min	± 0.6
pH of the mobile phase buffer, pH units	± 0.5

Figure 4 shows the design of robustness tests (top) and two-dimensional resolution map (bottom) for the effect of column temperature, pH of the aqueous phase, and the breakpoint time (value = 3.4 min) created by ChromSwordAuto Chromeleon Connect. The red and yellow area represents the condition with a resolution lower than 2.0, and the green area with a resolution higher than 2.0. The points on the map represent the actual test conditions completed by ChromSwordAuto. By changing the value of the Z axis (breakpoint time), the resolution map was also redesigned. Hence, we can get a robust region with a resolution >2.0 for temperature, pH, and breakpoint time (3.4 ±0.6 min), which is shown in the blue box in Figure 4.

The time spent on the robustness test is about 40 hours. A total of 29 runs were performed, requiring about 38.5 hours for instrument time and 1.5 hours for analyst time.

Design of robustness tests

	-5 °C temp.	pH = 4	+1.5 % conc.	+0.6 min b.time.	-1.5 % conc.	-0.6 min b.time.	pH = 5	+5 °C temp.
Run #2 -5 °C temp. DAD	✓							
Run #3 -5 °C temp.; pH = 4; +1.5 % conc.; +0.6 min b.time. DAD	✓	✓	✓	✓				
Run #4 -5 °C temp.; pH = 4; -1.5 % conc.; +0.6 min b.time. DAD	✓	✓		✓	✓			
Run #5 -5 °C temp.; pH = 4; -1.5 % conc.; -0.6 min b.time. DAD	✓	✓			✓	✓		
Run #6 -5 °C temp.; pH = 5; +1.5 % conc.; +0.6 min b.time. DAD	✓		✓	✓			✓	
Run #7 -5 °C temp.; pH = 4; +1.5 % conc.; -0.6 min b.time. DAD	✓	✓	✓			✓		
Run #8 -5 °C temp.; pH = 5; -1.5 % conc.; +0.6 min b.time. DAD	✓			✓	✓		✓	
Run #9 -5 °C temp.; pH = 5; -1.5 % conc.; -0.6 min b.time. DAD	✓				✓	✓	✓	
Run #10 -5 °C temp.; pH = 5; +1.5 % conc.; -0.6 min b.time. DAD	✓		✓			✓	✓	
Run #12 +5 °C temp.; pH = 4; +1.5 % conc.; -0.6 min b.time. DAD		✓	✓			✓		✓
Run #13 +5 °C temp. DAD								✓
Run #14 +5 °C temp.; pH = 5; -1.5 % conc.; -0.6 min b.time. DAD					✓	✓	✓	✓
Run #15 +5 °C temp.; pH = 5; -1.5 % conc.; +0.6 min b.time. DAD				✓	✓		✓	✓
Run #16 +5 °C temp.; pH = 5; +1.5 % conc.; -0.6 min b.time. DAD			✓			✓	✓	✓
Run #17 +5 °C temp.; pH = 5; +1.5 % conc.; +0.6 min b.time. DAD			✓	✓			✓	✓
Run #18 +5 °C temp.; pH = 4; -1.5 % conc.; -0.6 min b.time. DAD		✓			✓	✓		✓
Run #19 +5 °C temp.; pH = 4; -1.5 % conc.; +0.6 min b.time. DAD		✓		✓	✓			✓
Run #20 +5 °C temp.; pH = 4; +1.5 % conc.; +0.6 min b.time. DAD		✓	✓	✓				✓
Run #22 pH = 5 DAD							✓	
Run #23 pH = 4 DAD		✓						
Run #24 -1.5 % conc. DAD					✓			
Run #25 +1.5 % conc. DAD			✓					
Run #27 -0.6 min b.time. DAD						✓		
Run #28 +0.6 min b.time. DAD				✓				

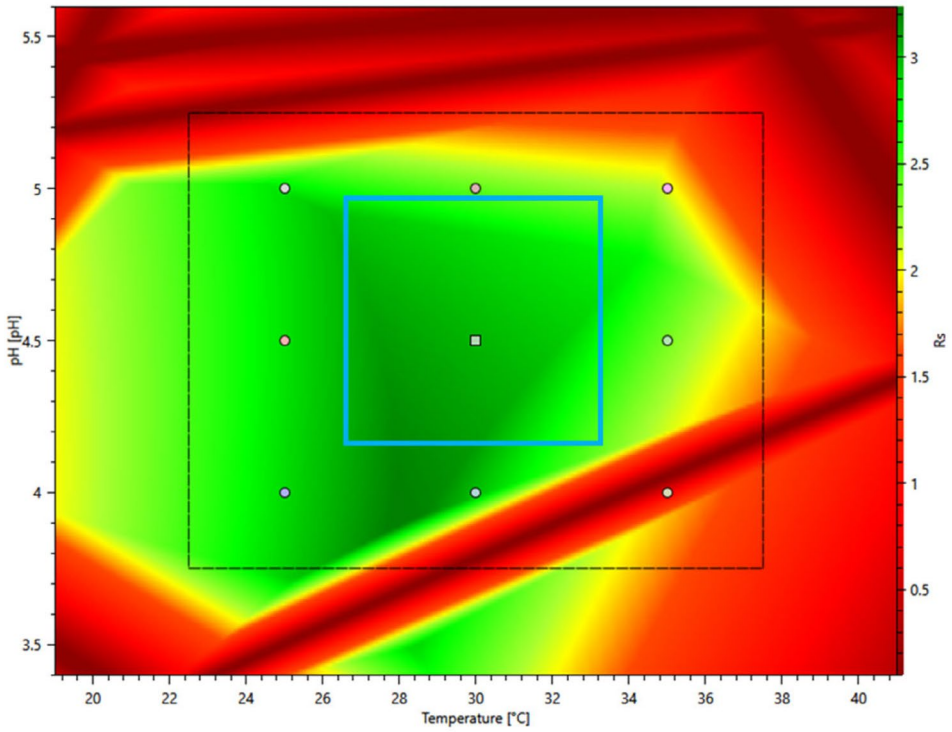

Figure 4. Robustness testing. Top: The design of robustness tests created by ChromSwordAuto Chromeleon Connect. Twenty-nine runs were performed, including five basic method runs in the sequence. Bottom: Two-dimensional resolution map for the effect of column temperature (X axes), pH of aqueous phase (Y axes), and the 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.

Table 6. Time required for automated and manual method development and robustness test for Triumeq and its impurities by using ChromSwordAuto Chromeleon Connect

Experiment	Automated method development and robustness test workflow			Manual method development and robustness test workflow
	Instrument time (hours)	Analyst time (hours)	Total time (hours)	Total time (days)
Method scouting	33.5	1.5	35.0	5.0
Rapid optimization	10.5	0.5	11.0	19.0
Fine optimization	40.2	1.0	41.2	
Robustness study	38.5	1.5	40.0	4.0
Total time	**122.7**	**4.5**	**127.2**	**28.0**

Table 6 shows the total time spent on this workflow, which was about 5.5 days. The analyst time for each step is less than 2.0 hours and can be completed in the daytime. As this automated workflow does not require any manual intervention, the instrument can run fully unattended and continuously even during night time and over the weekend, which supports increased efficiency in an analytical lab significantly.

Conclusion

- In this study, we have demonstrated an automated workflow for HPLC method development and a robustness test for abacavir, lamivudine, dolutegravir, and their related compounds in the drug Triumeq.
- The workflow reduced both the development time and manual operation, enabling the analytical laboratory to be more efficient and cost-effective in HPLC method development.
- The final method provides an adequate separation for all analytes with a USP resolution ≥ 2.0, and USP peak asymmetry within 0.9 to 2.1.
- The full factorial robustness test provided a robust region for the method.

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