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Application Article

Automated AI-Driven HPLC Method Development

By Gesa Schad, Shimadzu Europa GmbH, Duisburg, Germany;
Shinichi Fujisaki, Shimadzu Corporation, Kyoto, Japan

Abstract

This article introduces a software-supported, AI-assisted workflow for optimizing LC gradient methods. The method-development software automatically evaluates chromatograms and adjusts gradient parameters until user-specified targets for minimum resolution and maximum elution time are met, recording every iteration for complete documentation. This approach reduces manual work, improves consistency, and supports method development, transfer, and lifecycle updates in regulated environments.

Introduction

Pharmaceutical laboratories face tight timelines yet must deliver validation-ready liquid chromatography (LC) methods. Method development can be especially time consuming, as it often relies on manual trial-and-error and requires creating multiple analysis methods, running them, interpreting chromatograms, and deciding the next adjustment. While this iterative approach can be effective, it is labor-intensive, introduces variability, and depends heavily on the analyst's experience and judgment.
This application article describes the use of an AI-guided algorithm, implemented within dedicated method-development software, to automate the exploration and refinement of gradient conditions. The algorithm alternates between condition search and correction analysis, using the results from each run to modify the gradient profile until predefined criteria are met. In this case study, the objective was to meet explicit criteria for minimal resolution and maximum total analysis time, reflecting typical requirements for release testing and impurity assessment. The results demonstrate that a neutral, criteria-driven workflow can converge rapidly on robust separation conditions while reducing the need for empirical trial-and-error, thereby supporting development, transfer, and lifecycle management of LC methods.

Analytical conditions

The case study employed a compact integrated UHPLC system configured with a C18 column and PDA detector. The software generated several initial gradient curves and applied an AI-guided search for improved separation. Criteria were defined in terms of minimal resolution for critical pairs and the elution time of the last peak. The software executed the initial analyses under five starting gradients and then proceeded through

iterative correction analyses, each informed by the measured resolution and retention behaviour. Detailed analytical conditions and sample compounds are listed in table 1.

Table 1: Analytical conditions and target compounds

System:	LC-2080C 3D integrated UHPLC (Shimadzu, Japan)
Sample:	(1) Antipyrine (40 mg/L), (2) Benzoic acid (80 mg/L), (3) Salicylic acid (80 mg/L), (4) Hydrocortisone (80 mg/L), (5) Furosemide (80 mg/L), (6) Naproxen (40 mg/L), (7) Probenecid (80 mg/L) in Acetonitrile/Water (50:50)
Mobile phase:	Pump A: 0.1% formic acid in water
	Pump B: Acetonitrile
Column:	Shim-pack Scepter C18-120 (100 × 3.0 mm I.D., 1.9 µm)
Injection Volume:	5 µL
Gradient time program:	20%B (0 min) → X%B (3 min) → 95%B (3.01-4min) → 20%B (4.01-8 min)
	X = 90, 91, 92, 93, 94 (five initial gradients)
Column Temperature:	40 °C
Flow rate:	0.7 mL/min
Detection (PDA):	254 nm (PDA, standard cell)
Specified target criteria for gradient optimization	
Minimal resolution (Rs)	3.0
Time of last eluting peak	< 10 minutes

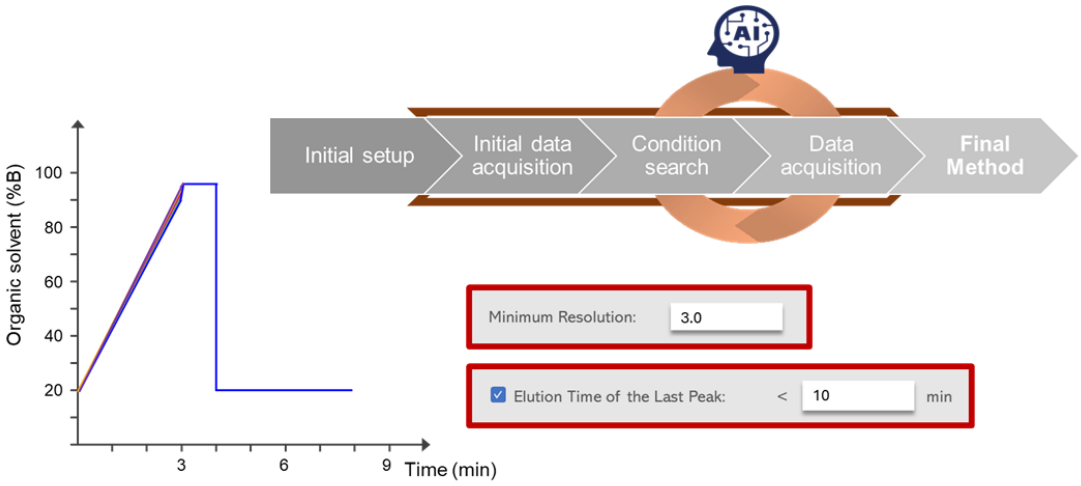
These conditions were selected to reflect routine pharmaceutical workflows in which robust resolution is required within a limited runtime.

Automated gradient optimization workflow

The automated workflow begins with an initial setting in the software (LabSolutions MD, Shimadzu, Japan), where input gradient curves, column temperature, flow rate, and separation goal are specified. The system performs the initial analyses under the starting conditions and computes resolution among adjacent peaks, along with the time of the last eluting component. Based on these outcomes, the AI algorithm proposes a modified gradient designed to improve separation while respecting the runtime constraint. This proposal is tested in a correction analysis, and the loop continues until the specified criteria are satisfied. Next to auto-integration and accurate tracking of peak movement, the distinctive feature of the algorithm is its capacity to autonomously decide on suitable changes in the gradient curve, including the introduction of isocratic segments at specific times where resolution deficits are localized. By temporarily holding the organic fraction,

diffusion and differential partitioning can unfold sufficiently to separate closely eluting pairs before the gradient resumes. The algorithm also adjusts slopes and turning points to fine tune selectivity across the chromatographic space. Together, these interventions form a closed loop optimization guided by measured resolution rather than manual trial-and-error strategies.
Figure 1 schematically illustrates the software's workflow, indicating the gradient curves of 5 initial analyses, as well as the optimization targets as specified in the software setting.

Figure 1: Workflow of automated gradient optimization in the LabSolutions MD method development software

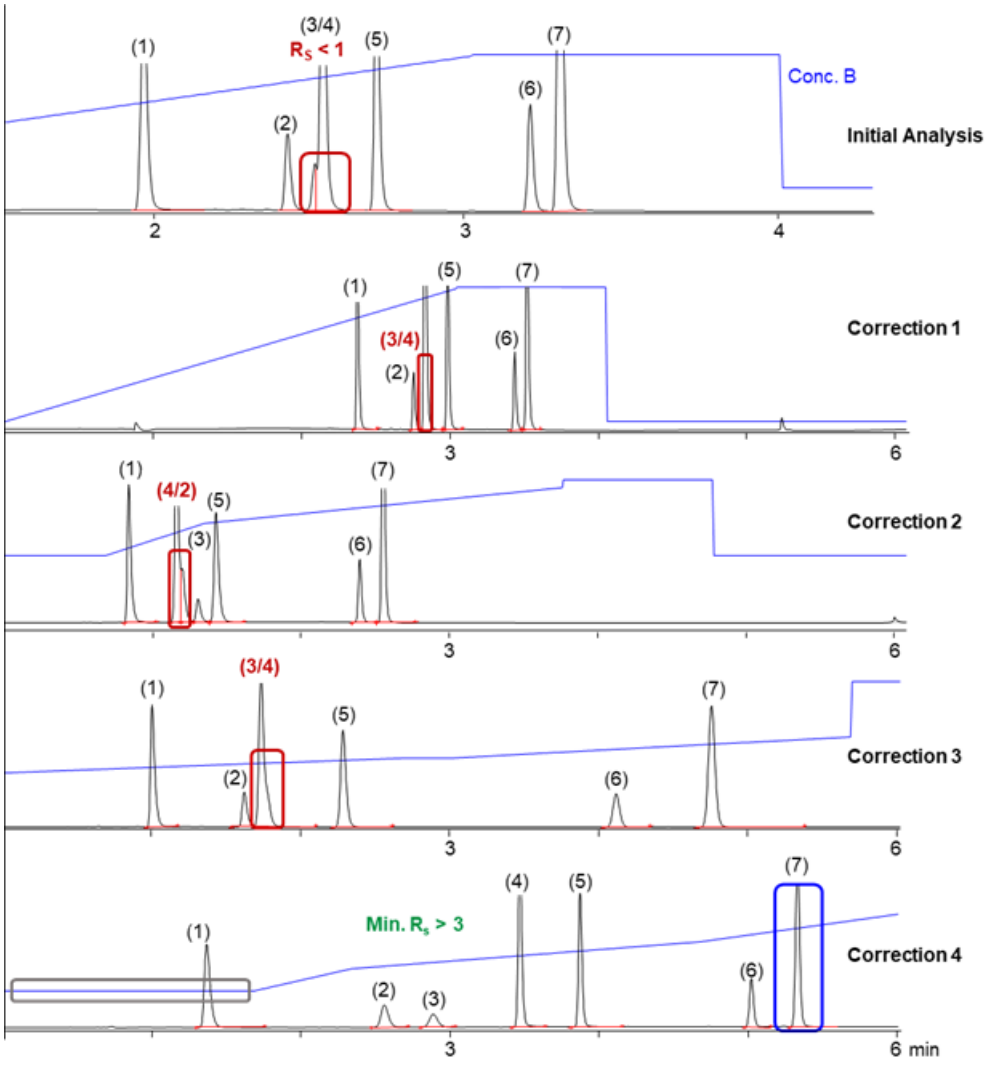


Results and discussion

In the example seven compound mixture, the initial analyses conducted across the five gradient patterns revealed insufficient separation for peaks (3) Salicylic acid and (4) Hydrocortisone. The poorest case showed a minimal resolution of approximately Rs ≈ 0.81 as can be seen in figure 2, indicating substantial overlap of this critical peak pair. Subsequent analyses modified the gradient slope, starting with a lower %B in the first correction analysis. However, peaks (3) and (4) remained unresolved. In the second correction analysis, introducing a step gradient improved separation among other components and resulted in overlap of the pair involving (2) Benzoic acid and (4) Hydrocortisone. The third correction analysis, a flat, linear gradient from 45 – 60%B preserved overall runtime but led to complete coelution of compounds (3) and (4). Resolution improved decisively in the fourth correction analysis, when the algorithm introduced a short isocratic hold and recalibrated the gradient thereafter. This intervention achieved a minimal resolution of at least Rs ≥ 3.3 for all peaks of interest, satisfying the predefined resolution criterion. Importantly, the retention time of the last eluting peak

remained below 10 minutes, meeting the runtime constraint. Figure 2 summarizes the progression from initial analyses to the optimized chromatogram, with unresolved pairs highlighted in red and the gradient profiles shown in blue.

Figure 2: Automated LC gradient optimization for a seven compound sample mix. Progression from initial analyses to optimized chromatogram



The optimized method demonstrates the practical value of allowing the algorithm to insert isocratic segments. In conventional manual development, such interventions depend on recognizing local retention behavior and projecting the effect of holds on subsequent separation and runtime. Here, the algorithm identified and validated the hold duration and position empirically, using measured resolution data to guide the choice. This approach reduces the effort of recreating analytical conditions and manually interpreting

chromatograms between runs, while providing a transparent record of how the final conditions were reached. Visualizing the gradient curve alongside resolution and runtime clarifies how the method reaches its final conditions. Analysts can observe where the algorithm focuses its adjustments and correlate those edits with changes in peak spacing. Such transparency is useful when documenting method development.

Conclusion

Automated, AI guided gradient optimization offers a neutral, data driven approach to LC method development. In this example using a seven component mixture, the algorithm identified unresolved pairs in initial analyses and introduced a short isocratic hold to achieve the specified minimal resolution while maintaining a sub 10 minute total runtime. The optimization proceeded through alternating condition search and correction analysis, refining the gradient profile based on observed chromatographic behavior rather than manual trial-and-error.
Beyond the specific compounds and conditions examined here, the workflow generalizes to pharmaceutical tasks where robust separation and efficient runtime are required. By articulating clear criteria and allowing the algorithm to manipulate gradient shape, method developers can reduce manual iterations, document a transparent path to the final conditions, and improve the consistency of outcomes across analysts and sites.

Shimadzu Europa GmbH
Albert-Hahn-Str. 6-10
47269 Duisburg, Germany
Tel.: + 49 203 76 87 0
Fax: + 49 203 76 66 25
shimadzu@shimadzu.eu
www.shimadzu.eu