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LC Method Adjustment Made Simple: A Practical Guide

Are you looking to update a validated liquid chromatography (LC) method but not sure where to start? Whether your goal is to optimize your method's performance or address changing analytical needs, this guide offers a step-by-step approach to adjusting your methods that can help you achieve your goals and save time in the process.

Step 1 – Define goals and acceptance criteria

Defining your **goals** for method adjustment is crucial. Whether you aim to modernize column dimensions, reduce solvent usage and run time, cut costs, or scale to a new LC system, having clear objectives helps tailor your method adjustments for the desired outcome.

It is equally important to define the **acceptance criteria** to ensure that your adjusted method remains fit for its intended purpose. This involves evaluating system suitability parameters and setting appropriate limits for each parameter.

Step 2 – Select matching LC system and column

Matching the LC system and column that align with your goals is important to achieve optimum chromatographic performance.

System dispersion, dwell volume, operating pressure, and usability are the key LC characteristics to consider when selecting an LC system. It is also essential to ensure that the column dimensions match your system dispersion and that a similar length to particle size (L/dp) ratio is maintained.

Pharmacopoeial guidance requires the L/dp to remain between -25% to 50%, relative to the column specified in a given monograph.

Alliance iS HPLC System

ACQUITY Arc System

ACQUITY UPLC H-Class PLUS System

	Alliance iS HPLC System	ACQUITY Arc System	ACQUITY UPLC H-Class PLUS System
System Dispersion	HPLC Systems > 25 µL	HPLC/UHPLC Systems 12–25 µL	UHPLC/UPLC Systems < 12 µL
Columns			
Internal Diameter (ID)	≥ 4.6 mm	2.1–4.6 mm	1–3 mm
Particle Size	≥ 3.5 µm	2.5–5 µm	< 2 µm

Find an alternative to your existing column using the Column Coach.

For monograph methods, search for an appropriate column in the pharmacopoeial chromatographic database.

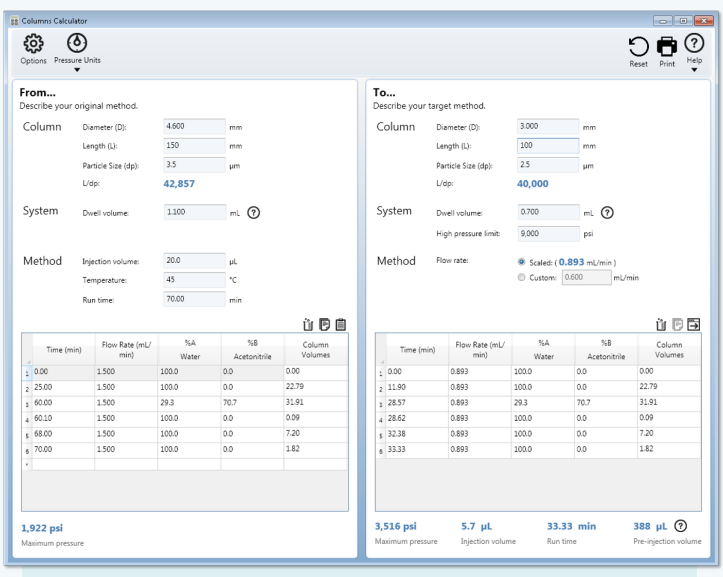
Step 3 – Adjust experimental conditions

Access Waters Column Calculator through Empower Software or Waters.com

The Column Calculator allows you to make method adjustments by calculating operating parameters that result in equivalent chromatographic performance.

Following the User Guide, enter the required system and column information, along with the original experimental conditions, to generate the adjusted method.

Note: The Column Calculator within Empower Software allows the adjusted method to be automatically transferred to Empower without manual transcription.

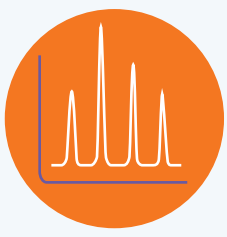


- ✓ **Customizable flow rates**
- ✓ **Calculation of pre- and post-injection volume for gradient start time adjustment**
- ✓ **Automatically flags if pressure exceeds system limit**

Step 4 – Perform analyses and method verification for adjusted method

Perform analyses with the adjusted method and verify that the results align with your expected outcomes.

Finally, conduct method verification tests according to internal or regulatory guidelines and make sure the system suitability results pass the monograph or your in-house requirements.



Read this application note to find out how to achieve method adjustment in accordance with the gradient allowances in USP General Chapter <621>.

Waters is here to guide you every step of the way.

For more support, please contact your local representative.

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