# Avantor® ACE® Pharmacopoeia LC Translator

## Contents



## Abbreviations

| | |
|---|---|
| D | Dwell volume (mL) |
| dc | Column internal diameter (mm) |
| dp | particle diameter (µm) |
| EuPh | European Pharmacopoeia |
| F | Flow rate (mL/min) |
| JP | Japanese Pharmacopoeia |
| L | Column length |
| N | Plate number |
| PDG | Pharmacopoeial Discussion Group |
| SPP | Superficially porous particles |
| t | Time point (min) |
| $t_G$ | Gradient time (min) |
| $t_R$ | Retention time (min) |
| TPP | Totally porous particles |
| USP | United States Pharmacopoeia |
| $V_{inj}$ | Injection volume (µL) |
| $W_h$ | Peak width at half-height |

## 1. Introduction

***IMPORTANT:** This translation tool is designed to be used to translate existing United States, European and Japanese Pharmacopoeia LC methods to different format columns in accordance with allowable changes specified in their harmonised general chapters, released in 2022/2023 (USP General Chapter <621> Chromatography,[1] EuPh 2.2.46. Chromatographic Separation Techniques[2] and JP 2.00 Chromatography[3]). As such, its functionality is partly restricted in line with changes that are permitted by the harmonised text. It is the users responsibility to refer to the relevant pharmacopoeia text to ensure compliance. For pharmacopoeias other than EuPh, JP and USP, please refer to their specific texts for guidance on allowable changes to monograph methods.*

***For translating methods that are not governed by pharmacopoeia guidelines, a more comprehensive and unrestricted method translation tool and its associated user guide is available from the following links:***

*https://uk.cmd.uwr.com/bin/public/idoccdownload/10156385/ace_lc_translator.xlsx*

*https://uk.cmd.uwr.com/bin/public/idoccdownload/10156179/ACE_LC_Translator.pdf*

Monograph methods contain all the method parameters required to run an analytical method. They have been validated and are ready to use by the analyst (although the analyst is required to verify that the method can be used for its intended purpose, for example, selected analytical performance characteristics of USP methods require verification according to general chapter <1226> when run for the first time, but do not require full validation according to chapter <1225>).[4,5]

Although replicating monograph methods is, in principle, relatively straightforward, a number of factors can lead to differences in the results obtained from different laboratories running the same monograph method. Pharmacopoeias therefore include a general chapter on chromatography which specifies allowable changes that the analyst can make to the method if necessary, for example USP chapter <621> and the European Pharmacopoeia general chapter 2.2.46.[1,2]

In 2021 the Pharmacopoeial Discussion Group (PDG) signed off a harmonised general chapter (G-20) on Chromatography, which harmonised the three regional general chapters from the USP, EuPh and JP.[6] The harmonised chapter allows the analyst more flexibility to translate both isocratic and gradient methods to more modern LC practices (i.e. narrower bore columns packed with smaller particles).

This method translator tool is designed to help simplify the process of translating monograph LC methods to different format LC columns. The tool uses the original method and column details as input, along with the new column format. Translated methods are then automatically generated according to pharmacopeia guidance, with no need for the user to manually carry out numerous calculations.

## 2. Disclaimer

The Avantor® ACE® Pharmacopoeia LC Translator Excel spreadsheet tool and this document are provided 'as is'. All users do so at their own risk and without any acceptance of liability by Avantor

Inc. for damages, incorrect information, or any regulatory body implications. Use of this spreadsheet tool indicates acceptance of these conditions.

***It is important to note that even when a method is correctly translated, many factors such as frictional heating from the use of elevated flow rates and pressure induced changes in selectivity may mean that the exact chromatography of the original method may not be fully reproduced.***

## 3. Index page

The Index page of the translator appears as shown in Figure 1. The tools listed can be accessed by clicking on the relevant link on the index page or can be accessed via the tabs at the bottom of the spreadsheet. Users can navigate back to the Index Page by clicking on the Main Menu link at the bottom right of each page.

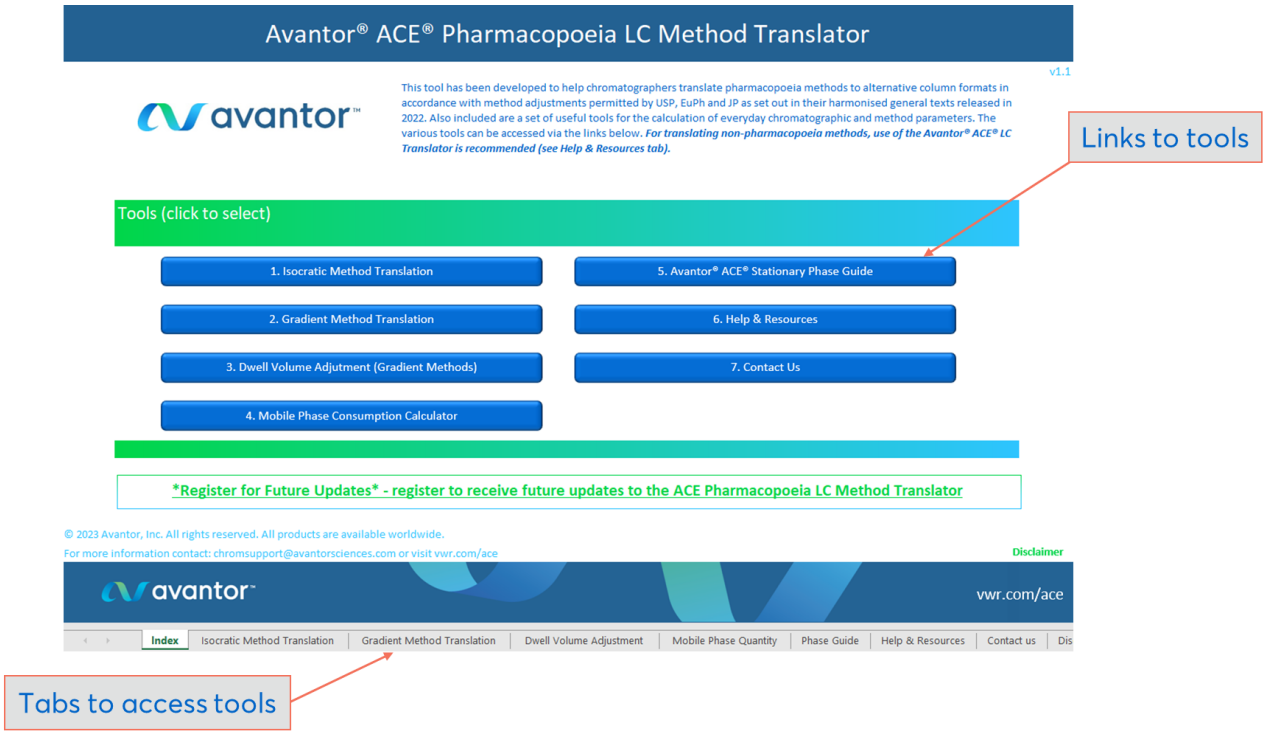


**Figure 1:** Avantor® ACE® Pharmacopoeia LC Translator – Index Page.

## 4. General guidance

All the tools follow a similar formatting style. To carry out a calculation, all the input boxes (displayed as ) must be completed. Once all input fields are completed, calculated values are displayed (Figure 2).

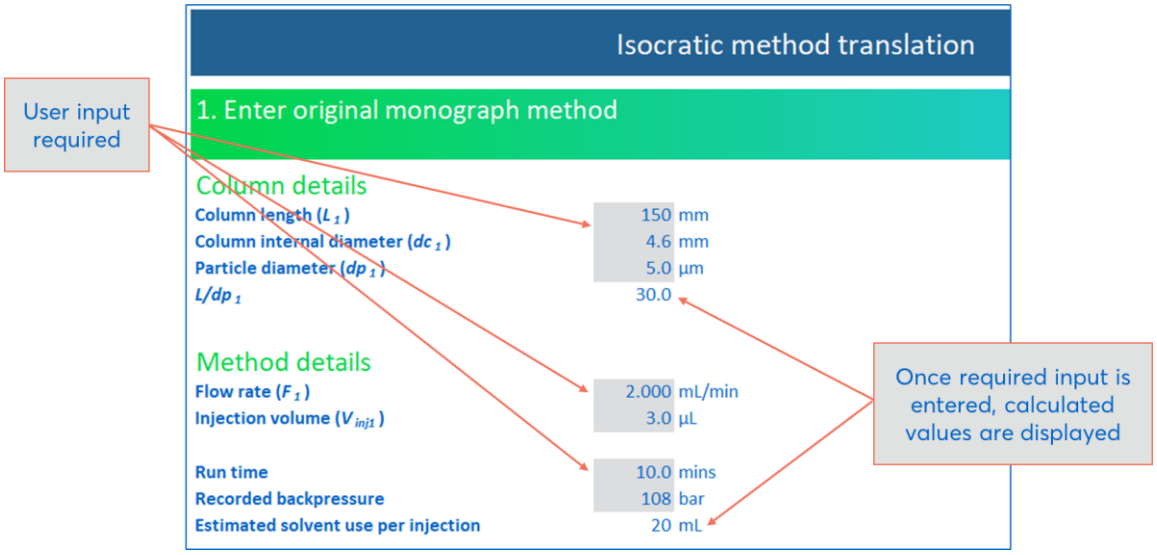


**Figure 2:** Data entry.

The Index page can be accessed by clicking on the **Main Menu** link which can be found in the lower left corner of each page in the spreadsheet.

## 5. Method translation calculators

### *5.1 General*

Two method translation tabs are provided, the first to translate isocratic monograph methods and the second to translate gradient monograph methods. Both are divided into five sections. Once all input fields ( ) have been completed, the translated method parameters are automatically displayed.

### *5.2 Section 1 – Enter original monograph method*

The user is required to enter the details of the original column specified in the monograph and the specified method conditions (flow rate, injection volume and run time) as shown in Figure 3. The back pressure generated by the original method can also be entered, although this is not mandatory. The required pressure units should be selected. If entered, this value is used to estimate the likely backpressure of the translated method. Note that for gradient methods, the backpressure will vary during the gradient program. The maximum backpressure observed during the gradient should be entered.

Once the column details have been entered, the L/dp ratio is displayed (the ratio of column length to particle size). Once the method details have been entered, an estimate of the solvent consumption per injection is displayed, this is compared to that of the translated method in Section 3.

If the critical pair resolution obtained with the original monograph method is available, this can be entered and will be used to estimate the critical pair resolution that will be obtained for the translated method in Section 2.

Additionally, the data collection rate used for the original method can be entered.

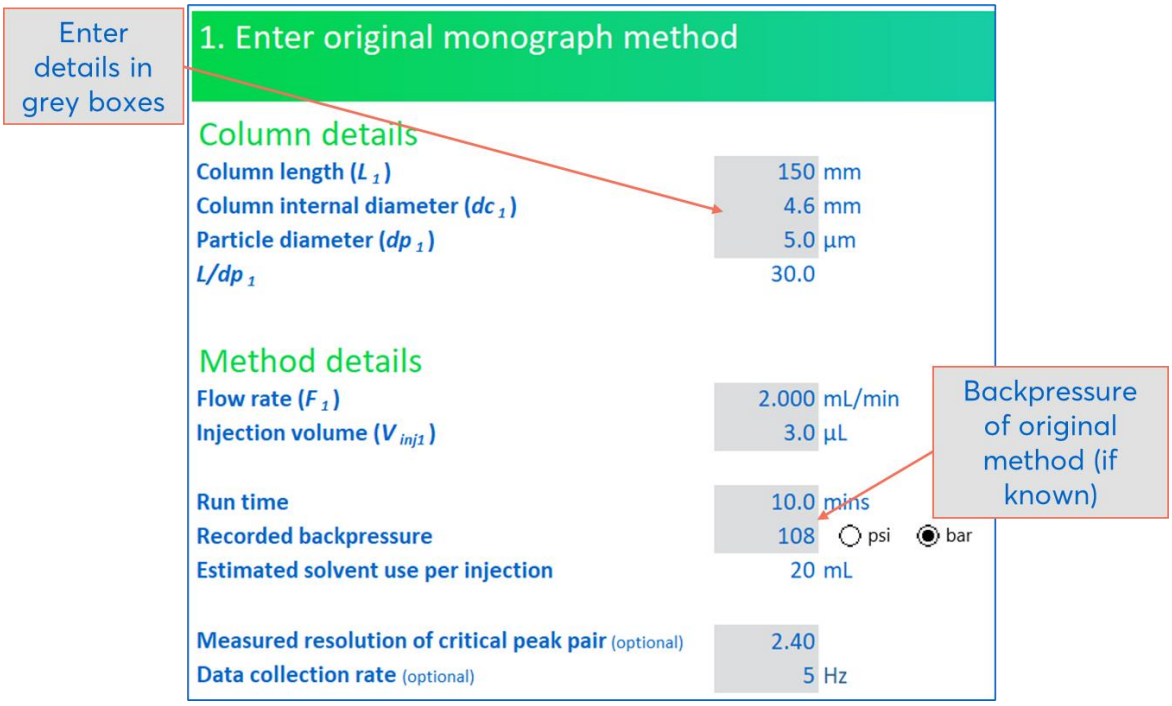


**Figure 3:** Entering the column details and original monograph method for an isocratic method.

For gradient monograph methods, the gradient table, as specified in the monograph, must also be entered (Figure 4). It is advisable to also include the post-gradient re-equilibration time in the gradient table, so that this time period is translated to the new column format. The tool can be used to translate linear and step gradients. Concave and convex gradients are not supported.

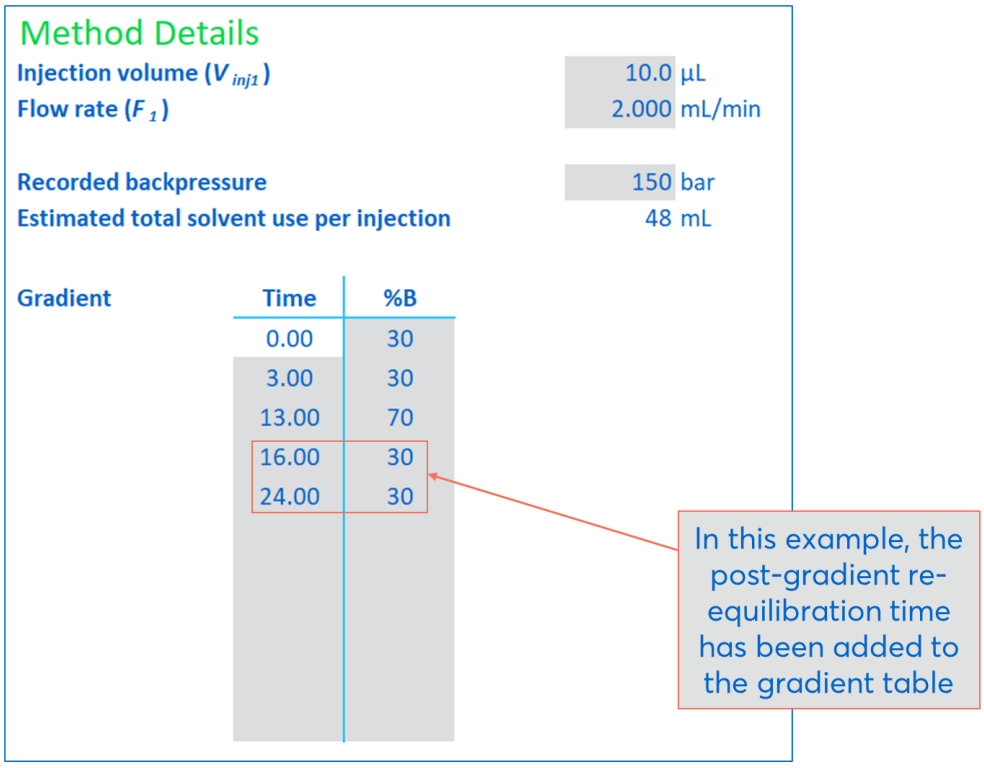


**Figure 4:** Entering the gradient table for a gradient method.

### *5.3 Section 2 – Select new column format*

For both isocratic and gradient methods, the harmonised pharmacopoeia guidelines state that:

> *"The particle size and/or length of the column may be modified, provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range between -25% to +50% of the prescribed L/dp ratio"*

The Avantor® ACE® Pharmacopoeia LC Translator takes the column length and particle size from section 1 and displays in table form common column L/dp combinations that are permitted according to this statement (Figure 5). The L/dp ratio for the original method, along with the corresponding allowed L/dp range (-25% to + 50%) are displayed below the table.

Permitted column length to particle size combinations are highlighted and colour coded in the table and their corresponding L/dp values displayed for readability. L/dp values equivalent to that of the original column can be expected to provide similar efficiency and resolution. Columns with a lower L/dp ratio will provide lower efficiency and resolution, whilst those with a higher L/dp value should provide greater resolution and efficiency.

The user should select their desired column from the table and enter the details in the grey input boxes (Figure 5). If the user wishes to select a column internal diameter that is different to the original method, this can also be specified here. If the desired column length or particle size is not included in the table, the details can still be entered into the input boxes and the L/dp value will be displayed. If an L/dp value outside the permitted L/dp range for the methods (-25% to + 50%) is entered, an error message is displayed (Figure 6).

If a value was entered in Section 1 for the critical pair resolution that is obtained with the original monograph method, an estimate of the resolution that can be expected to be obtained with the new column format will be displayed. Note that the actual value obtained may vary due to factors such as extra column band broadening (particularly for narrow bore columns), mobile phase flow rate and selectivity changes that may occur due to frictional heating or increased pressure under UHPLC conditions.

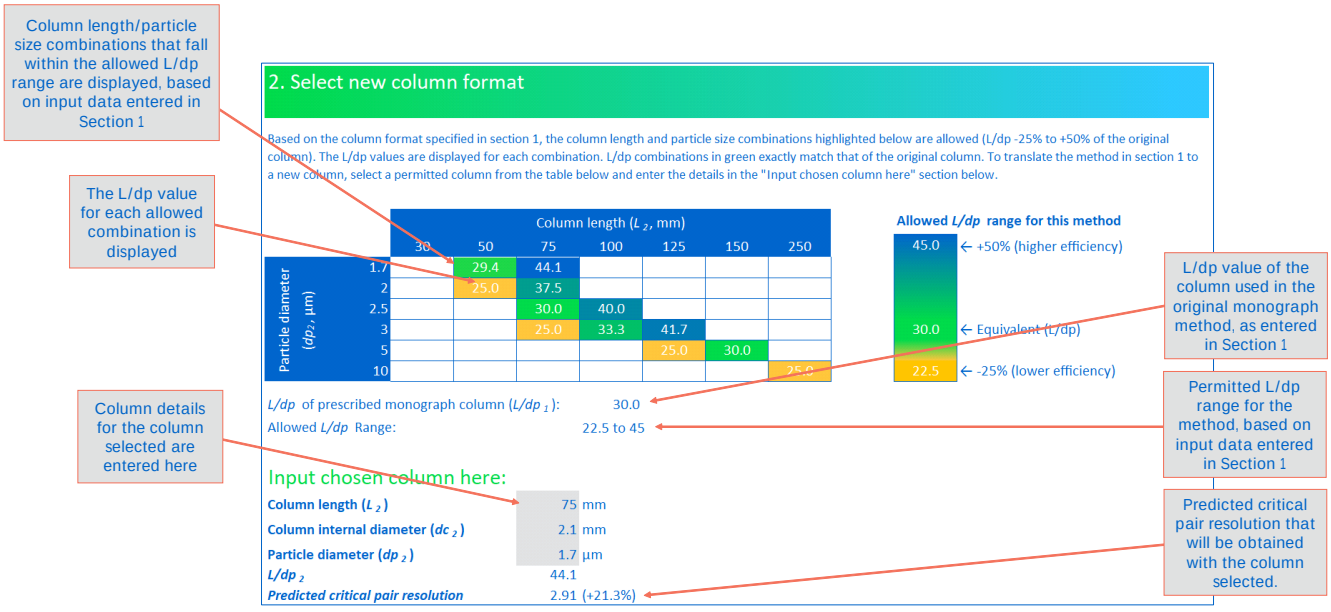


**Figure 5:** New column format selection.

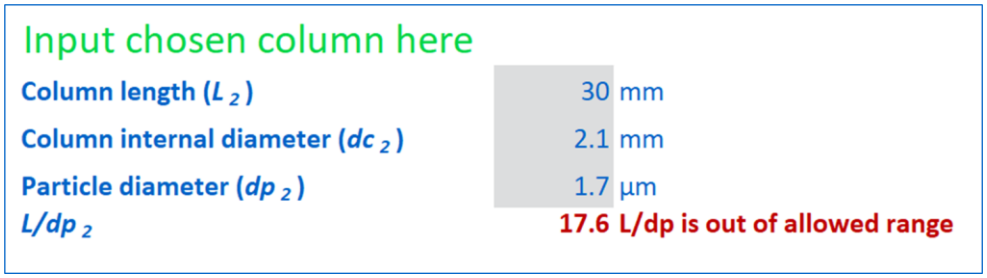


**Figure 6:** If column dimensions entered fall outside the permitted L/dp range, an error message will be displayed.

It is worth noting that the guidance also provides additional flexibility in the case of changing from totally porous particles (TPP) to superficially porous particles (SPP). In this case, other L/dp combinations are permitted, providing that the plate number (N) is within -25% to +50% relative to the prescribed column for isocratic methods and that the ratio $(t_R/W_h)^2$ is within -25% to +50% relative to the prescribed column, for all peaks used to determine the system suitability in gradient methods. Please refer to the relevant pharmacopoeia chapter for further details.[1,2,3]

### *5.3 Section 3 – Select new flow rate*

When the particle size is changed, the flow rate will require adjustment, as smaller particles require higher mobile phase linear velocities to achieve the same performance. The flow rate will also require scaling if the column internal diameter is changed. The adjusted flow rate is calculated according to equation 1 and is displayed in **bold underlined** text in section 3 (Figure 7).

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)] \quad (1)$$

$F_1$ = flow rate indicated in the monograph method (mL/min)

$F_2$ = adjusted flow rate (mL/min)

$dc_1$ = internal diameter of the column indicated in the monograph (mm)

$dc_2$ = internal diameter of the column used (mm)

$dp_1$ = particle size indicated in the monograph (µm)

$dp_2$ = particle size of the column used (µm)

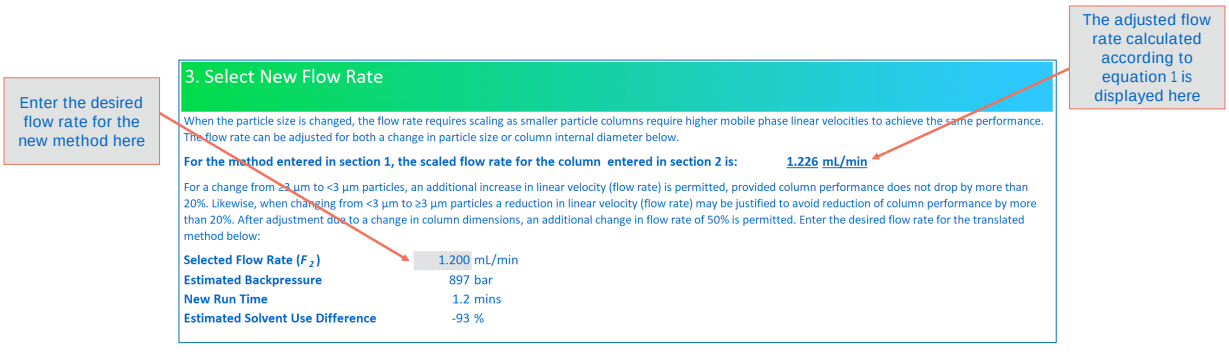


**Figure 7:** Flow rate selection.

For isocratic separations, the guidance provides additional allowances for varying flow rate:

> *"When a change is made from ≥3-µm to <3-µm particles in isocratic separations, an additional increase in linear velocity (by adjusting the flow rate) may be justified, provided that the column performance does not drop by more than 20%. Similarly, when a change is made from <3-µm to ≥3-µm particles, an additional reduction of linear velocity (flow rate) may be justified to avoid reduction in column performance by more than 20%.*
>
> *After an adjustment due to a change in column dimensions, an additional change in flow rate of ±50% is permitted."*

For maximum flexibility, the Avantor® ACE® Pharmacopoeia LC Translator requires the user to enter the desired flow rate for the new method in the grey input box provided (Figure 7). This allows the scaled flow rate to be rounded, or for the user to apply additional adjustments as per the guidance above if required. If the backpressure generated by the original monograph method is known and entered in section 1, then an estimate of the backpressure of the new method is given. The accuracy of this value will depend on factors such as the LC system used (HPLC or UHPLC), tubing internal diameter, detector flow cell and the absolute particle size and distribution. The value serves as a useful indicator that the flow rate and backpressure of the new method are compatible with the HPLC column and LC system specifications, however, the experimental back pressure may vary from this predicted value, particularly if the new method is run on a UHPLC instrument at a high flow rate.

For isocratic methods, a scaled run time and an estimate of the solvent usage relative to the original method are provided.

### *5.4 Section 4 – Injection volume*

When the column dimensions are changed, the injection volume requires scaling. The adjusted injection volume is automatically displayed in Section 4 (Figure 8) and is calculated according to equation 2.

$$V_{inj2} = V_{inj1}(L_2 dc_2^{\,2})/(L_1 dc_1^{\,2}) \qquad (2)$$

$V_{inj1}$ = injection volume indicated in the monograph (µL)

$V_{inj2}$ = adjusted injection volume (µL)

$L_1$ = column length indicated in the monograph (mm)

$L_2$ = new column length (mm)

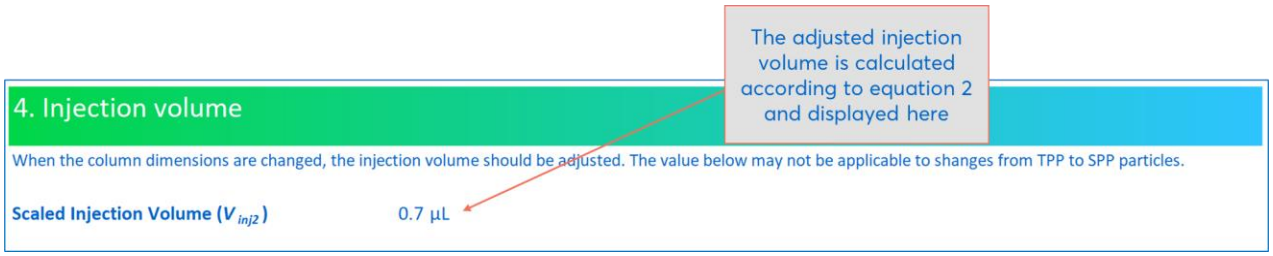


**Figure 8:** Injection volume scaling.

For guidance on using an alternative injection volume and for moving to SPP columns, please refer to the relevant pharmacopoeia text. [1,2,3]

### *5.4 Section 5 – New method summary*

This section provides a summary of the new method, adjusted to the column dimensions and flow rate specified. For gradient methods, the new gradient table is displayed, along with an estimate of the total solvent usage compared to the original monograph method (Figure 9). Note that solvent used during post gradient equilibration will only be included in this solvent use calculation if the post gradient re-equilibration step is specified in the original monograph table in Section 1. The new gradient times displayed in the table are calculated according to equation 3.

$$t_{G2} = t_{G1} \times (F_1/F_2)[(L_2 \times dc_2{}^2)/(L_1 \times dc_1{}^2)] \qquad (3)$$

$t_{G1}$ = original gradient time indicated in the monograph (min)

$t_{G2}$ = new gradient time (min)

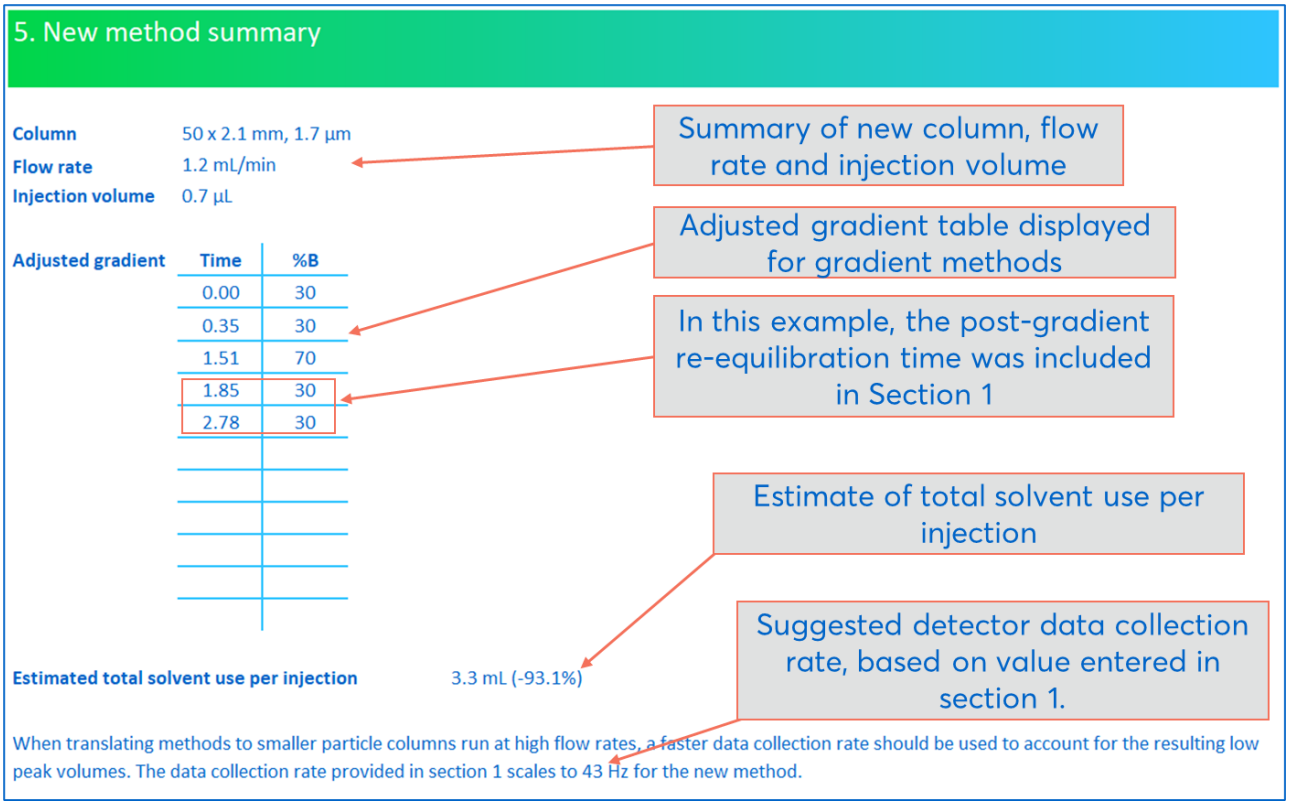


**Figure 9:** New method summary for a gradient method translation.

When translating a method to small format columns packed with small particles, it is typically necessary to increase the detector data collection rate to accurately record the lower volume, narrow chromatographic peaks that are generated. If a data collection rate was specified in section 1, a recommended data rate for the new method is stated for guidance, as in Figure 9. Note however, that if the data collection rate used for the original method was either excessively fast or slow, this recommended value may not be optimal. It is therefore advisable to experimentally assess a range of data collection rates when running the new method for the first time to optimise the value used.

## 6. Dwell volume adjustment

The guidance states that if a gradient monograph method includes an isocratic step before the start of the gradient program and that the dwell volume used during the elaboration of the monograph method is given, then the user can replace the time points stated in the gradient table with adapted gradient time points according to equation 4.

$$t_c = t - \frac{(D - D_0)}{F} \qquad (4)$$

$t$ = original gradient time point indicated in the monograph gradient table (min)

$t_c$ = adapted gradient time point (min)

$D$ = dwell volume (mL)

$D_0$ = dwell volume used for development of the method

The Dwell volume adjustment tab contains a tool which allows the user to adapt gradient time points according to equation 4 (Figure 10). The original gradient time points are entered, along with the dwell volume specified in the monograph method (if stated) and the dwell volume of the system to be used. Adapted gradient time points are then automatically generated.

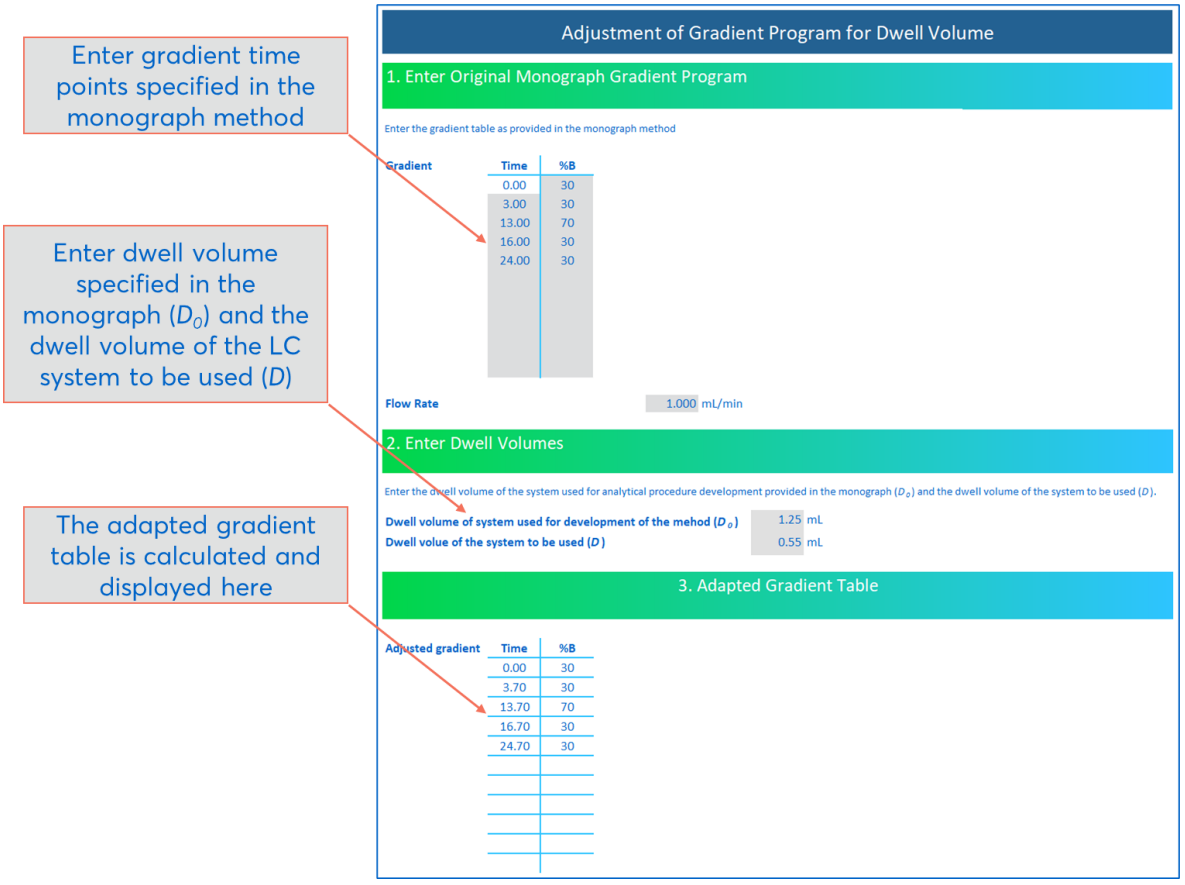


**Figure 10:** Dwell volume adjustment tool.

### 7. Mobile Phase Consumption Calculator

This tool allows the amount of mobile phase required to run *x* injections using a given LC method to be estimated (Figure 11). The calculated values should be rounded up to convenient amounts, ensuring at least 10% overage. It is important that sufficient mobile phase is prepared to allow for any flushing of solvent lines, columns and column for equilibration.

The calculator allows up to 4 solvent lines to be specified (denoted A, B, C and D). The total % across the 4 lines should always add up to 100% (green) as shown in Figure 11. If this is not entered correctly, this is highlighted in red.

***Tip: For gradient methods, the column re-equilibration time should be included as an additional step in the gradient table, as in Figure 11. For isocratic methods, include extra runs to factor in column equilibration.***

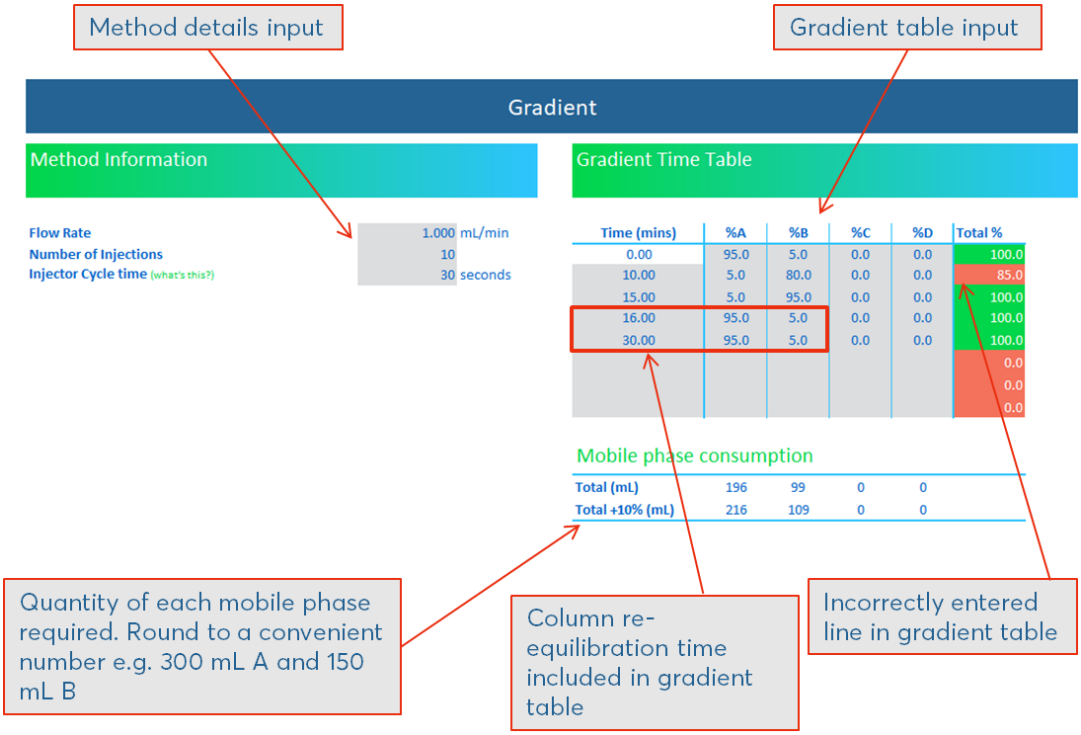


**Figure 11:** Mobile phase quantity calculation for a 10 minute gradient (5-95% in 10 minutes, hold for 5 minutes, ramp down to 5% in 1 minute and re-equilibrate for 14 minutes). Note that the second line of the gradient table is entered incorrectly.

### 8. Avantor® ACE® Stationary Phase Guide

This tab provides details of all stationary phases available in the Avantor® ACE® range and their corresponding USP listing (L-number). Avantor® ACE® LC phases have been specifically designed to help solve chromatographic challenges and include unique phases such as the C18-AR and C18-PFP, along with traditional phases, wide pore phases for the analysis of biomolecules and Method Development Kits.

### 9. Help and Resources

This tab contains links to useful articles and white papers related to LC method translation. In addition, the Avantor® ACE® Knowledge Note library contains a series of documents focussed on various aspects of chromatographic theory.

### 10. Contact Us

Use the links on this page for contact details and technical support for all Avantor® ACE® products and services.

### 11. References

1. United States Pharmacopoeia, Chapter <621> Chromatography (Dec 2022 onwards)

2. European Pharmacopoeia 11th Edition, Chapter 2.2.46 Chromatographic separation techniques

3. Japanese Pharmacopoeia 18th Edition Supplement I, Chapter 2.00 Chromatography

4. United States Pharmacopoeia, Chapter <1226> "Verification of compendial procedures"

5. United States Pharmacopoeia, Chapter <1225> "Validation of compendial procedures"

6. https://www.usp.org/harmonization-standards/pdg/excipients/chromatography