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Applikationsbericht

# Automated Creation of Chromatographic Methods for Analytical Method Development Using an Empower™ Sample Set Generator

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Dies ist ein Applikationsbericht, der keinen detaillierten Abschnitt zu Versuchen enthält.

## Abstract

This technology brief illustrates the use of an Empower™ Sample Set Generator (SSG) Software for automated creation of chromatographic methods for method development performed using a systematic screening protocol. The experimental study is conducted on the Arc™ Premier System equipped with PDA and an ACQUITY™ QDa™ Detectors. Data acquisition and analysis are performed using Empower™ Data Chromatography Software (CDS).

## Benefits

- Automated and quick creation of Empower instrument methods, method sets and sample set methods using the Empower Sample Set Generator (SSG) for analysis on theWaters ACQUITY™ LC instruments, optical detectors, and ACQUITY QDa Mass Detector
- Improved confidence that all chromatographic runs are completed with correctly created methods

## Introduction

Development of analytical methods is a complex process that involves screening a wide range of chromatographic parameters to generate desirable separations and robust methods. A systematic screening protocol is a three-phase approach to method development that incorporates scouting, screening, and optimization steps.[1–2] Impact of key factors affecting selectivity and resolution is systematically evaluated throughout the process and require careful creation of many chromatographic methods.

The Empower Sample Set Generator (SSG) Software automates the creation of chromatographic methods for a wide range of variables.[3] The Empower method sets and instrument methods are automatically created and structured in the sample set method, according to the experimental design, as a ready-to-run injection sequence. Using Empower SSG reduces the time and transcription errors, providing confidence that all chromatographic runs are completed with correctly created methods.

This technology brief demonstrates the use of the Empower Sample Set Generator (SSG) for automated and quick creation of chromatographic methods within a method development workflow. A systematic screening protocol is employed to develop a method for naphazoline hydrochloride (HCl) and pheniramine maleate active pharmaceutical ingredients (APIs) and their related substances. The chromatographic methods required to run screening and optimization studies are created using the Empower SSG.

## Experimental

### Sample Description

A standard solution mixture containing naphazoline HCl and pheniramine maleate APIs with their associated related substances was prepared as described previously.[2]

### MS Conditions

| | |
|---|---|
| LC system: | Arc Premier System with column manager (Active) with PDA and ACQUITY QDa Detectors |

| | |
|---|---|
| Vials: | LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV |
| Columns: | All with 4.6 x 100 mm, 2.5 µm, at 40 °C<br>XSelect™ Premier CSH™ $C_{18}$ (p/n: 186009873)<br>XSelect Premier CSH Phenyl Hexyl (p/n: 186009890)<br>XSelect Premier HSS T3 (p/n: 186009859)<br>Atlantis™ Premier BEH™ $C_{18}$ AX (p/n: 186009397) |
| Mobile phase: | A: 1% Formic acid in water<br>B: 1% Ammonium hydroxide in water<br>C: Water<br>D1: Acetonitrile<br>D2: Methanol |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 5.0 µL |
| Wash solvents: | Purge/Sample Wash: 80:20 water/methanol<br>Seal Wash: 90:10 water/acetonitrile |
| Detection: | UV at 260 nm |

## Gradient Table

| | Time (min) | Flow (mL/min) | %A | %B | %C | %D |
|---|---|---|---|---|---|---|
| 1 | Initial | 1.000 | 10.0 | 0.0 | 85.0 | 5.0 |
| 2 | 10.00 | 1.000 | 10.0 | 0.0 | 0.0 | 90.0 |
| 3 | 11.00 | 1.000 | 10.0 | 0.0 | 0.0 | 90.0 |
| 4 | 11.10 | 1.000 | 10.0 | 0.0 | 85.0 | 5.0 |
| 5 | 15.50 | 1.000 | 10.0 | 0.0 | 85.0 | 5.0 |

## Data Management

Chromatography software:

Empower™ 3 Feature Release 5 Service Release 5 (FR5 SR5) for data acquisition and analysis.

Empower Sample Set Generator is available as an optional add-on to the Empower Chromatography Data Software (CDS).[3]

# Results and Discussion

A method for naphazoline HCl, pheniramine maleate and their related substances was developed using a systematic screening protocol.[2] Integrating the Arc Premier System with column manager and solvent select valves enabled automated column and organic solvent switching.

## Screening

In the screening phase, columns with different chemistry were tested with acetonitrile and methanol solvents. Chromatographic methods (instrument, method sets, and sample set methods) required to run the screening study were created using the Empower SSG Software following the steps described below.

- An experimental design was imported to the Empower SSG by loading a comma separated value (CSV) file with solvents and columns (Figure 1)

- A base sample set method that included method set and instrument method with the system configuration for the analysis was loaded from the Empower project to Empower SSG and instrument components configured (Figure 2)
- Factors associated with the Empower settings for solvents (Figure 3) and columns (Figure 4) were mapped to pump and column manager modules
- Final generation settings included injection panel, equilibration time, and method names (Figure 5)

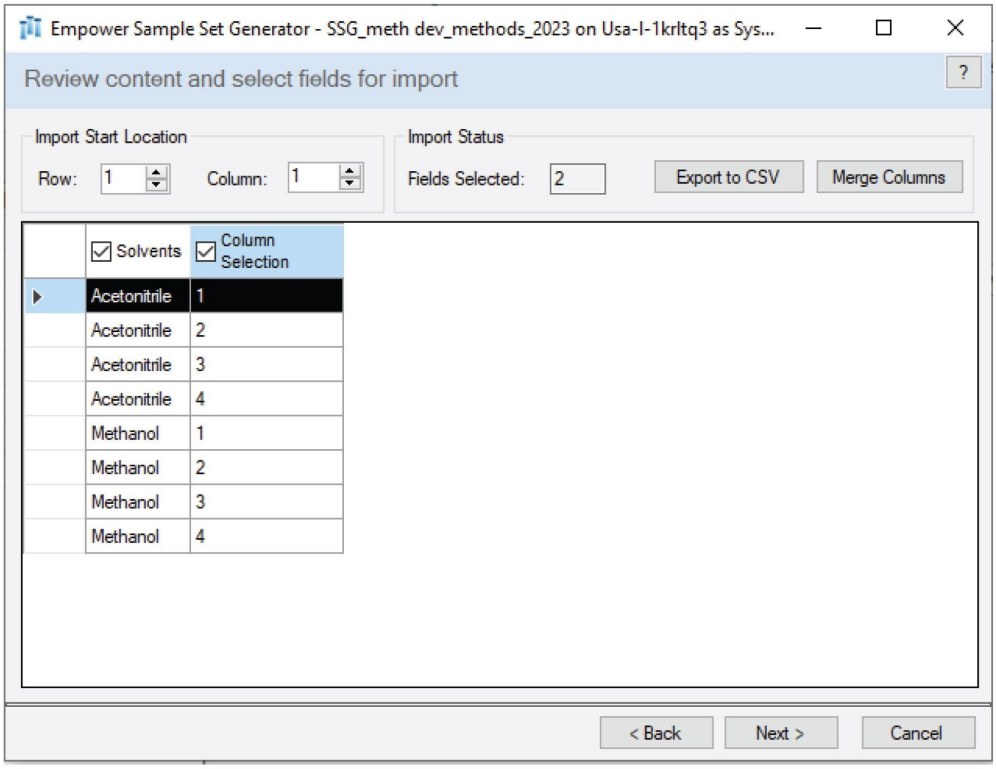


*Figure 1. Creation of chromatographic methods with Empower SSG. Imported experimental design for screening with solvents and columns.*

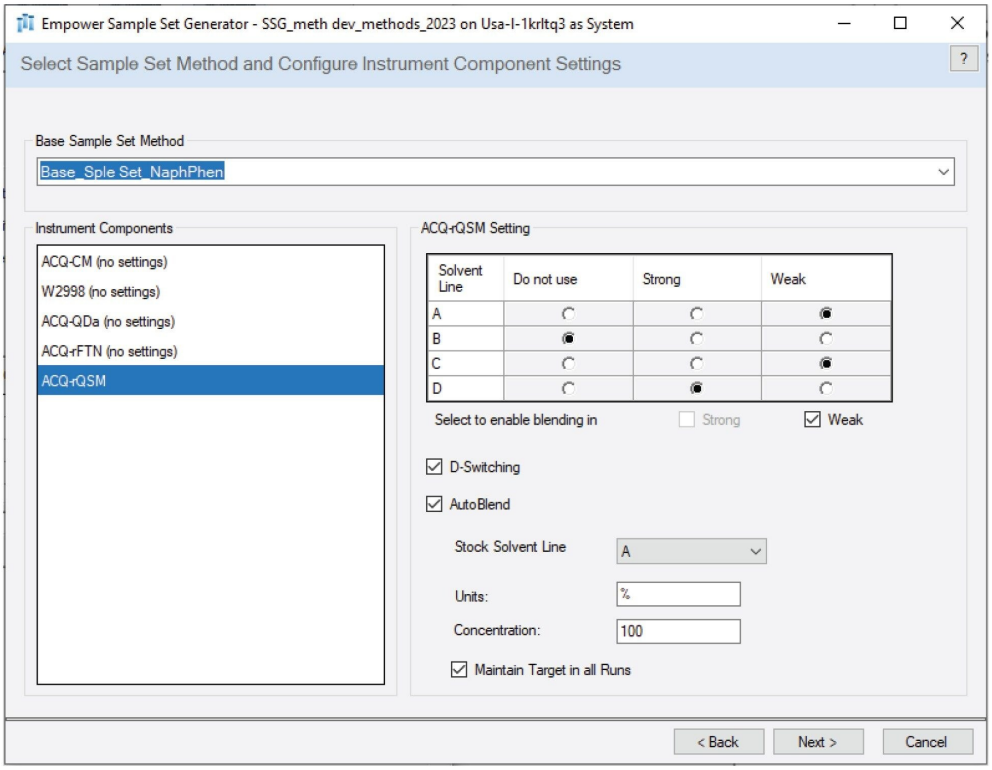


*Figure 2. Empower SSG. Load base sample set method and configure instrument components settings as required. ACQUITY rQSM pump setting: strong and weak solvent lines, D-switching for strong solvent, and AutoBlend with stock solvent line A.*

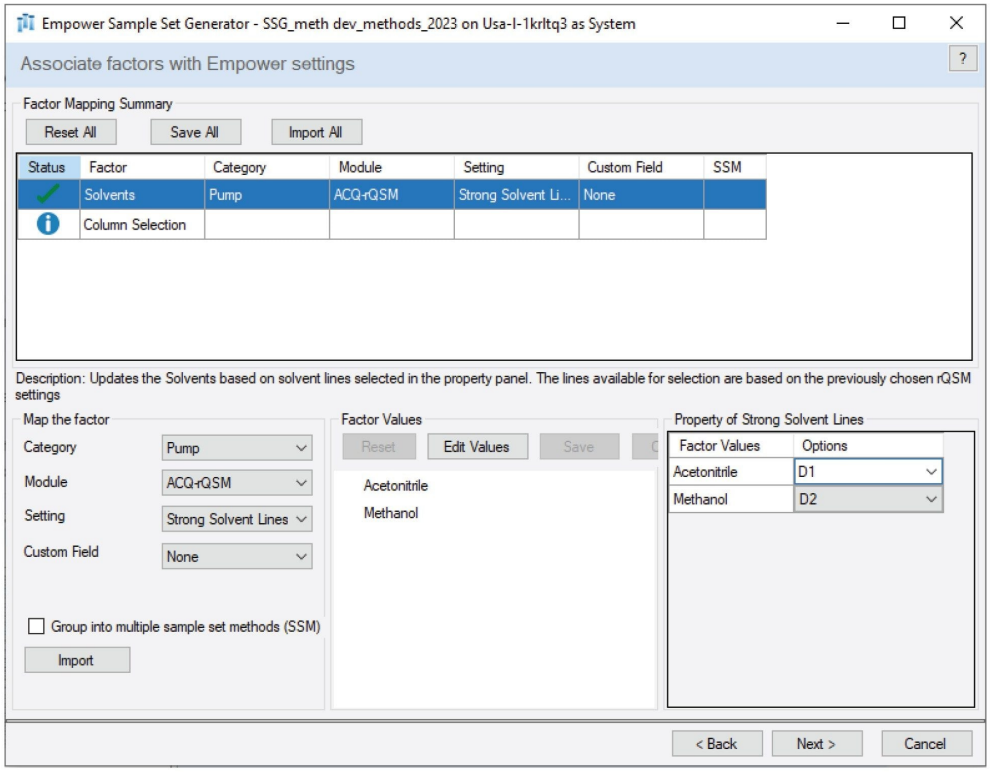


*Figure 3. Empower SSG. Settings for organic solvents: pump and strong solvent lines.*

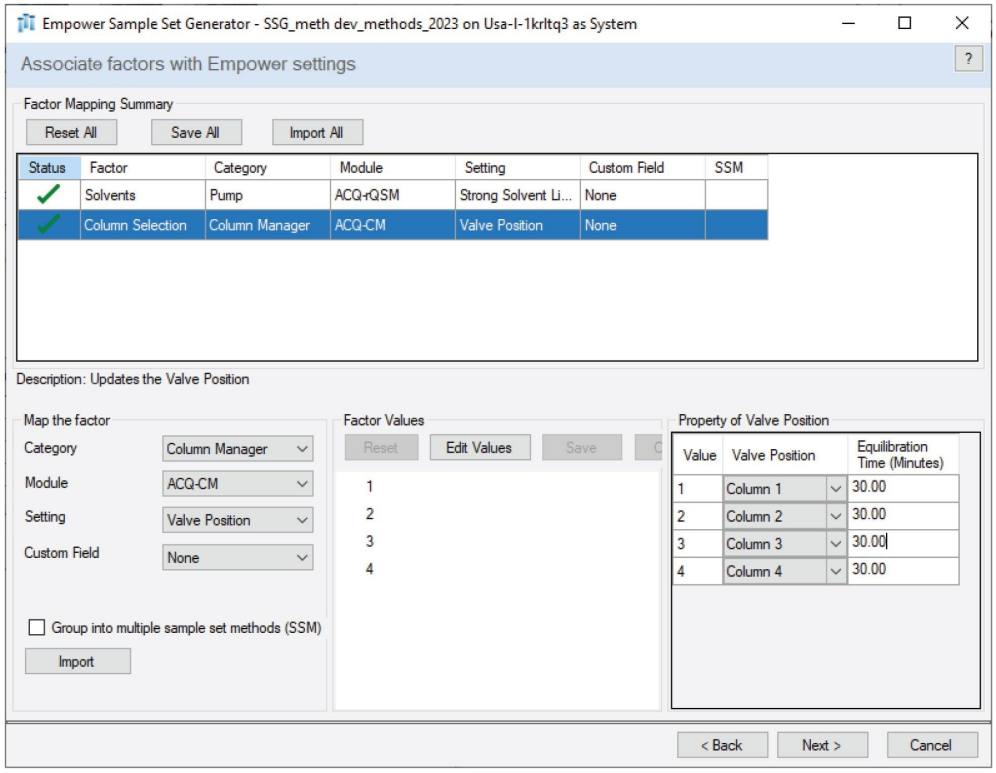


*Figure 4. Empower SSG. Settings for column selection: column manager and valve position.*

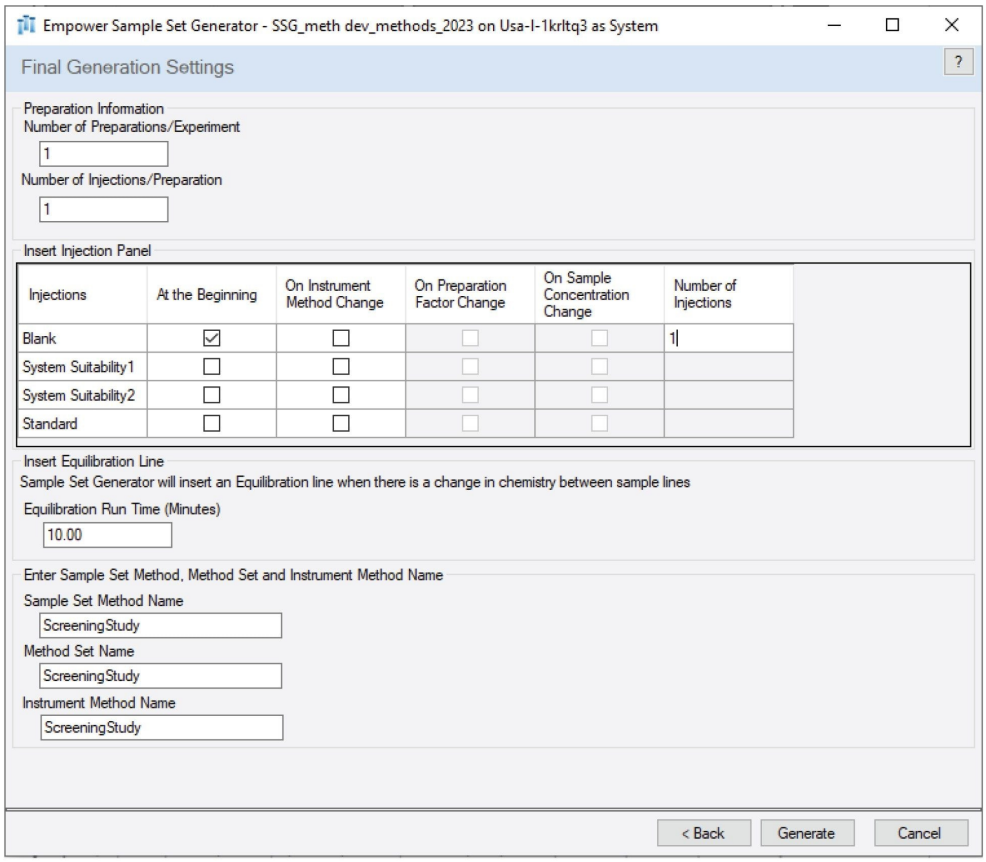


*Figure 5. Empower SSG. Final settings for generation of chromatographic method including sample preparation information, injection panel (blank, system suitability, standard), equilibration time, and names for the methods.*

After completing the final settings, the Empower SSG automatically created a sample set method according to the experimental design as a ready-to-run injection sequence (Figure 6). The Empower instrument methods and method sets with the associated column and solvent were automatically created and built into the sample set method. An equilibration step and blank injection were added at the beginning of the run as instructed by the Empower user. Using Empower SSG, enabled automated generation of the chromatographic methods, reducing the time and transcription errors associated with conducting this process manually.

| | Plate /Well | # of Injs | SampleName | Function | Method Set / Report or Export Method | Run Time (Minutes) | Column Position | Solvents |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | Equilibrate | ScreeningStudy 1 | 30.00 | | |
| 2 | 1:A,2 | 1 | Blank | Inject Samples | ScreeningStudy 1 | 15.50 | 1 | Acetonitrile |
| 3 | 1:A,3 | 1 | APIs/Imp mix 1 | Inject Samples | ScreeningStudy 1 | 15.50 | 1 | Acetonitrile |
| 4 | | | | Equilibrate | ScreeningStudy 2 | 30.00 | | |
| 5 | 1:A,3 | 1 | APIs/Imp mix 2 | Inject Samples | ScreeningStudy 2 | 15.50 | 2 | Acetonitrile |
| 6 | | | | Equilibrate | ScreeningStudy 3 | 30.00 | | |
| 7 | 1:A,3 | 1 | APIs/Imp mix 3 | Inject Samples | ScreeningStudy 3 | 15.50 | 3 | Acetonitrile |
| 8 | | | | Equilibrate | ScreeningStudy 4 | 30.00 | | |
| 9 | 1:A,3 | 1 | APIs/Imp mix 4 | Inject Samples | ScreeningStudy 4 | 15.50 | 4 | Acetonitrile |
| 10 | | | | Equilibrate | ScreeningStudy 5 | 30.00 | | |
| 11 | 1:A,3 | 1 | APIs/Imp mix 5 | Inject Samples | ScreeningStudy 5 | 15.50 | 1 | Methanol |
| 12 | | | | Equilibrate | ScreeningStudy 6 | 30.00 | | |
| 13 | 1:A,3 | 1 | APIs/Imp mix 6 | Inject Samples | ScreeningStudy 6 | 15.50 | 2 | Methanol |
| 14 | | | | Equilibrate | ScreeningStudy 7 | 30.00 | | |
| 15 | 1:A,3 | 1 | APIs/Imp mix 7 | Inject Samples | ScreeningStudy 7 | 15.50 | 3 | Methanol |
| 16 | | | | Equilibrate | ScreeningStudy 8 | 30.00 | | |
| 17 | 1:A,3 | 1 | APIs/Imp mix 8 | Inject Samples | ScreeningStudy 8 | 15.50 | 4 | Methanol |

*Figure 6. Empower sample set method for screening generated using Empower SSG software.*

## Optimization

In the optimization phase, chromatographic parameters are optimized systematically to achieve the desired resolution and chromatographic performance.

To illustrate the use of Empower SSG for creation of chromatographic methods in optimization experiments, impact of gradient slope was evaluated in the range of 90–60% of organic solvent over ten minutes. Methods were created following the steps described in the screening section. The gradient slope was mapped to the pump and strong solvent percentage, as well as to lines 2 and 3 (or steps) in the gradient separation (Figure 7).

# Conclusion

The Empower SSG Software automated the creation of chromatographic methods as part of a method development workflow performed using a systematic screening protocol. Instrument methods and the method

set were automatically created and built into a sample set method as a ready-to-run injection sequence for screening and optimization studies. Automating creation of chromatographic methods minimized the transcription errors and time associated with conducting these steps manually. Use of the SSG provided confidence that chromatographic runs were completed using correctly created methods.

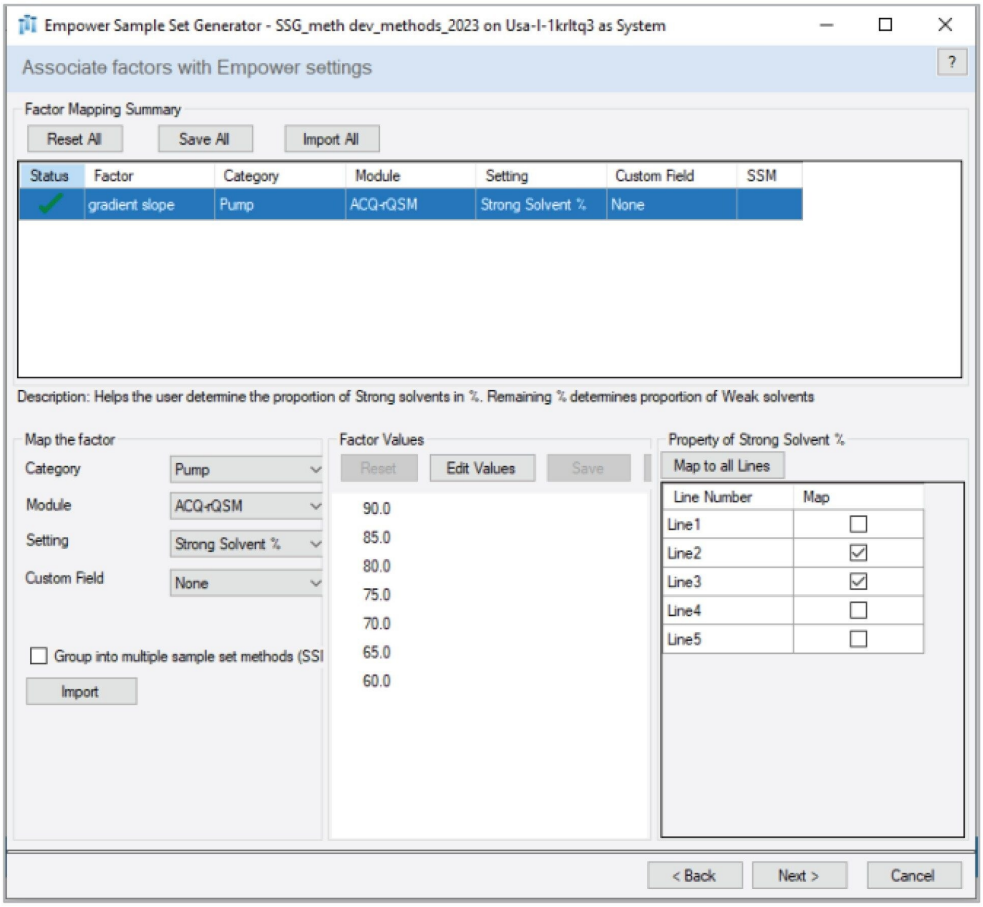


*Figure 7. Empower SSG for creation of chromatographic methods for optimization of gradient slope. Gradient slope mapped to pump and strong solvent percentage, applied to lines 2 and 3 in the gradient separation.*

## References

1. Hong P, McConville P. A Complete Solution to Perform a Systematic Screening Protocol for LC Method Development. Waters White Paper 720005268 < https://www.waters.com/webassets/cms/library/docs/720005268en.pdf> , 2018.

2. Maziarz M, Rainville PD. Efficient Method Development for the Analysis of Naphazoline Hydrochloride, Pheniramine Maleate and Associated Related Substances Using a Systematic Screening Protocol. Waters Application Note 720007850, 2023.

3. Waters Empower 3 Sample Set Generator Release Notes. Waters Corporation, 716004237 < https://www.waters.com/webassets/cms/support/docs/716004237ra.pdf> .

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Empower Chromatography Data System <https://www.waters.com/10190669>

720008224, February 2024