

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

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

Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1krltq3 as Sys...

Review content and select fields for import

Import Start Location
 Row: 1 Column: 1

Import Status
 Fields Selected: 2 Export to CSV Merge Columns

	<input checked="" type="checkbox"/> Solvents	<input checked="" type="checkbox"/> Column Selection
▶ Acetonitrile	1	
Acetonitrile	2	
Acetonitrile	3	
Acetonitrile	4	
Methanol	1	
Methanol	2	
Methanol	3	
Methanol	4	

< Back Next > Cancel

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

Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1kritq3 as System

Select Sample Set Method and Configure Instrument Component Settings

Base Sample Set Method
Base_Sple Set_NaphPhen

Instrument Components

- ACQ-CM (no settings)
- W2998 (no settings)
- ACQ-QDa (no settings)
- ACQ-FTN (no settings)
- ACQ+QSM**

ACQ+QSM Setting

Solvent Line	Do not use	Strong	Weak
A	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
B	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
C	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
D	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>

Select to enable blending in ☐ Strong ☒ Weak

☒ D-Switching

☒ AutoBlend

Stock Solvent Line

Units:

Concentration:

☒ Maintain Target in all Runs

< Back Next > Cancel

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.

Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1krtq3 as System

Associate factors with Empower settings

Factor Mapping Summary

Reset All Save All Import All

Status	Factor	Category	Module	Setting	Custom Field	SSM
✓	Solvents	Pump	ACQ-rQSM	Strong Solvent L...	None	
i	Column Selection					

Description: Updates the Solvents based on solvent lines selected in the property panel. The lines available for selection are based on the previously chosen rQSM settings

Map the factor

Category: Pump

Module: ACQ-rQSM

Setting: Strong Solvent Lines

Custom Field: None

☐ Group into multiple sample set methods (SSM)

Import

Factor Values

Reset Edit Values Save

Acetonitrile

Methanol

Property of Strong Solvent Lines

Factor Values	Options
Acetonitrile	D1
Methanol	D2

< Back Next > Cancel

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

Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1krtq3 as System

Associate factors with Empower settings

Factor Mapping Summary

Reset All Save All Import All

Status	Factor	Category	Module	Setting	Custom Field	SSM
✓	Solvents	Pump	ACQ+QSM	Strong Solvent Li...	None	
✓	Column Selection	Column Manager	ACQ-CM	Valve Position	None	

Description: Updates the Valve Position

Map the factor

Category: Column Manager

Module: ACQ-CM

Setting: Valve Position

Custom Field: None

☐ Group into multiple sample set methods (SSM)

Import

Factor Values

Reset Edit Values Save

1

2

3

4

Property of Valve Position

Value	Valve Position	Equilibration Time (Minutes)
1	Column 1	30.00
2	Column 2	30.00
3	Column 3	30.00
4	Column 4	30.00

< Back Next > Cancel

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

Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1krltq3 as System

Final Generation Settings

Preparation Information

Number of Preparations/Experiment
1

Number of Injections/Preparation
1

Insert Injection Panel

Injections	At the Beginning	On Instrument Method Change	On Preparation Factor Change	On Sample Concentration Change	Number of Injections
Blank	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
System Suitability1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
System Suitability2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Insert Equilibration Line
Sample Set Generator will insert an Equilibration line when there is a change in chemistry between sample lines

Equilibration Run Time (Minutes)
10.00

Enter Sample Set Method, Method Set and Instrument Method Name

Sample Set Method Name
ScreeningStudy

Method Set Name
ScreeningStudy

Instrument Method Name
ScreeningStudy

< Back Generate Cancel

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.

The screenshot shows the 'Empower Sample Set Generator - SSG_meth dev_methods_2023 on Usa-I-1krltq3 as System' window. The title bar includes standard window controls. The main area is titled 'Associate factors with Empower settings' and contains a 'Factor Mapping Summary' table with columns: Status, Factor, Category, Module, Setting, Custom Field, and SSM. A single row is shown with a green checkmark in the Status column, 'gradient slope' in the Factor column, 'Pump' in the Category column, 'ACQ+QSM' in the Module column, 'Strong Solvent %' in the Setting column, 'None' in the Custom Field column, and an empty SSM column. Below the table are 'Reset All', 'Save All', and 'Import All' buttons. A description states: 'Helps the user determine the proportion of Strong solvents in %. Remaining % determines proportion of Weak solvents'. The 'Map the factor' section has dropdowns for Category (Pump), Module (ACQ+QSM), Setting (Strong Solvent %), and Custom Field (None), with an unchecked checkbox for 'Group into multiple sample set methods (SSI)' and an 'Import' button. The 'Factor Values' section has 'Reset', 'Edit Values', and 'Save' buttons, followed by a list of values: 90.0, 85.0, 80.0, 75.0, 70.0, 65.0, and 60.0. The 'Property of Strong Solvent %' section has a 'Map to all Lines' button and a table with columns 'Line Number' and 'Map'. The table lists Line1 through Line5, with Line2 and Line3 having checked boxes in the 'Map' column. At the bottom are '< Back', 'Next >', and 'Cancel' buttons.

Status	Factor	Category	Module	Setting	Custom Field	SSM
✓	gradient slope	Pump	ACQ+QSM	Strong Solvent %	None	

Description: Helps the user determine the proportion of Strong solvents in %. Remaining % determines proportion of Weak solvents

Map the factor

Category: Pump
Module: ACQ+QSM
Setting: Strong Solvent %
Custom Field: None

☐ Group into multiple sample set methods (SSI)
Import

Factor Values

Reset Edit Values Save

90.0
85.0
80.0
75.0
70.0
65.0
60.0

Property of Strong Solvent %

Map to all Lines

Line Number	Map
Line1	<input type="checkbox"/>
Line2	<input checked="" type="checkbox"/>
Line3	<input checked="" type="checkbox"/>
Line4	<input type="checkbox"/>
Line5	<input type="checkbox"/>

< Back Next > Cancel

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](#) <
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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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