應用手冊

# Automated Creation of Chromatographic Methods for Analysis with an ACQUITY™ QDa Detector Using Empower™ Sample Set Generator

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This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

Optimizing ion source-mass spectrometry (MS) parameters to enhance signal requires creation of multiple chromatographic methods over a range of ionization variables. This technology brief illustrates the use of an Empower™ Sample Set Generator Software to automatically create chromatographic methods for analysis with an ACQUITY QDa Mass Detector, to study the impact of probe temperature and cone voltage on the sensitivity of memantine hydrochloride.

## Benefits

- Automated and quick creation of Empower instrument methods, methods sets, and sample set methods using Empower Sample Set Generator, while varying chromatographic parameters
- Generation of chromatographic methods for Waters ACQUITY LC instruments, optical detectors, and ACQUITY QDa Mass Detector

## Introduction

Optimization of MS source ionization parameters plays an important role in obtaining high sensitivity analysis in liquid chromatography-mass spectrometry (LC-MS). The ionization parameters (*e.g.,* ionization mode, cone voltage, or probe temperature) are often investigated to enhance the MS signal and to achieve low detection limits for target analytes. To study the impact of different ionization parameters and their ranges on the sensitivity requires careful creation of multiple chromatographic methods over set of variables. Manually creating and verifying these methods can be time-consuming and prone to errors process.

The Empower Sample Set Generator (SSG) capabilities automates the creation of instrument methods, method sets, and sample set methods, while varying the chromatographic parameters. The Empower method sets and instruments methods are automatically created and structured in the sample set method according to the experiment design as a ready-to-run injection sequence. Automating creation of chromatographic methods minimizes transcription errors that may arise during the manual process and time spent generating methods.

This technology brief illustrates the use of Empower SSG to automatically create chromatographic methods for analysis with an ACQUITY QDa Mass Detector coupled to an Arc™ Premier System. The ACQUITY QDa is used for the detection of non-chromophoric memantine hydrochloride, a drug commonly used to treat dementia often associated with Alzheimer's disease. [1] The impact of QDa ionization parameters (probe temperature and cone voltage) on the method sensitivity for analysis of memantine is investigated.

## Results and Discussion

Memantine hydrochloride lacks a chromophore required for UV detection but produces a robust MS signal using an ACQUITY QDa Mass Detector. [2] Method conditions used in this work are summarized in Table 1. A standard solution at 10 ng/mL in 90:10 water/methanol was prepared from a concentrated stock solution containing 1 mg/mL in methanol and used to carry-out the sensitivity study with MS detection.

### Method conditions for analysis of memantine hydrochloride

| | |
|---|---|
| System: | Arc Premier System, 2998 PDA and ACQUITY QDa |
| Column: | CORTECS™ $C_{18+}$, 2.7 µm, 3 mm x 75 mm (p/n: 186007401) |
| Column temperature: | 45 °C |
| Mobile phase: | A: 0.1% Formic acid in water<br>B: 0.1% Formic acid in acetonitrile |
| Injection volume: | 3.0 µL |
| MS detection: | ACQUITY QDa Mass Detector (Extended Performance)<br>▪Ionization mode: ESI+<br>▪Single ion recording (SIR): 180.2 Da<br>▪Probe temperature: 600 °C<br>▪Cone voltage: 15 V<br>▪Capillary voltage: 0.8 kV<br>▪Data: centroid |
| Wash solvents: | Purge/sample wash: 50:50 water/methanol<br>Seal wash: 90:10 water/acetonitrile |

## Gradient Table

| Step | Time | Flow rate (mL/min) | %A | %B | Curve |
|---|---|---|---|---|---|
| 1 | Initial | 1.00 | 95.0 | 5.0 | 6 |
| 2 | 2.5 | 1.00 | 10.0 | 90.0 | 6 |
| 3 | 3.5 | 1.00 | 10.0 | 90.0 | 6 |
| 4 | 3.6 | 1.00 | 95.0 | 5.0 | 6 |
| 5 | 6.0 | 1.00 | 95.0 | 5.0 | 6 |

Chromatographic methods were automatically created using the Empower SSG to study the impact of probe temperature (600, 500, and 400 °C) and cone voltage (5, 10, 15, and 20 Volts) on the sensitivity of memantine HCl following the steps described below.

- A comma separated value (CSV) file with the QDa variables was imported to the Empower SSG (Figure 1). The experimental design included a combination of MS conditions with different probe temperatures and cone voltages.
- 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.
- Study factors probe temperature and cone voltage were associated with the Empower settings (Figure 2).
- Final settings (injection panel, equilibration time, and method names) were defined before completing generation of the sample set method (Figure 3).

After completing the above steps, the Empower SSG automatically created a sample set method according to the experiment design as a ready-to-run injection sequence (Figure 4). The Empower instruments methods and method sets were automatically created and built into the sample set method. The sample set method included the experiment name and 12 method sets with different probe temperatures and cone voltages. An equilibration step and injections of blank were added at the beginning of the run as instructed by the user. Without the Empower SSG, a user would need to manually create 12 instrument methods and method sets, which is time consuming and prone to errors process. Using automated generation with Empower SSG reduced the time and transcription errors, providing confidence that all chromatographic runs were completed with correctly created methods. The Empower data acquired using ACQUITY QDa Detector is shown in Figure 5. The peak data across the experimental runs indicated that the best sensitivity for memantine HCl was achieved with a probe temperature of 600°C and cone voltage of 15 Volts, resulting in highest signal-to-noise (s/n) value (Figure 5A).

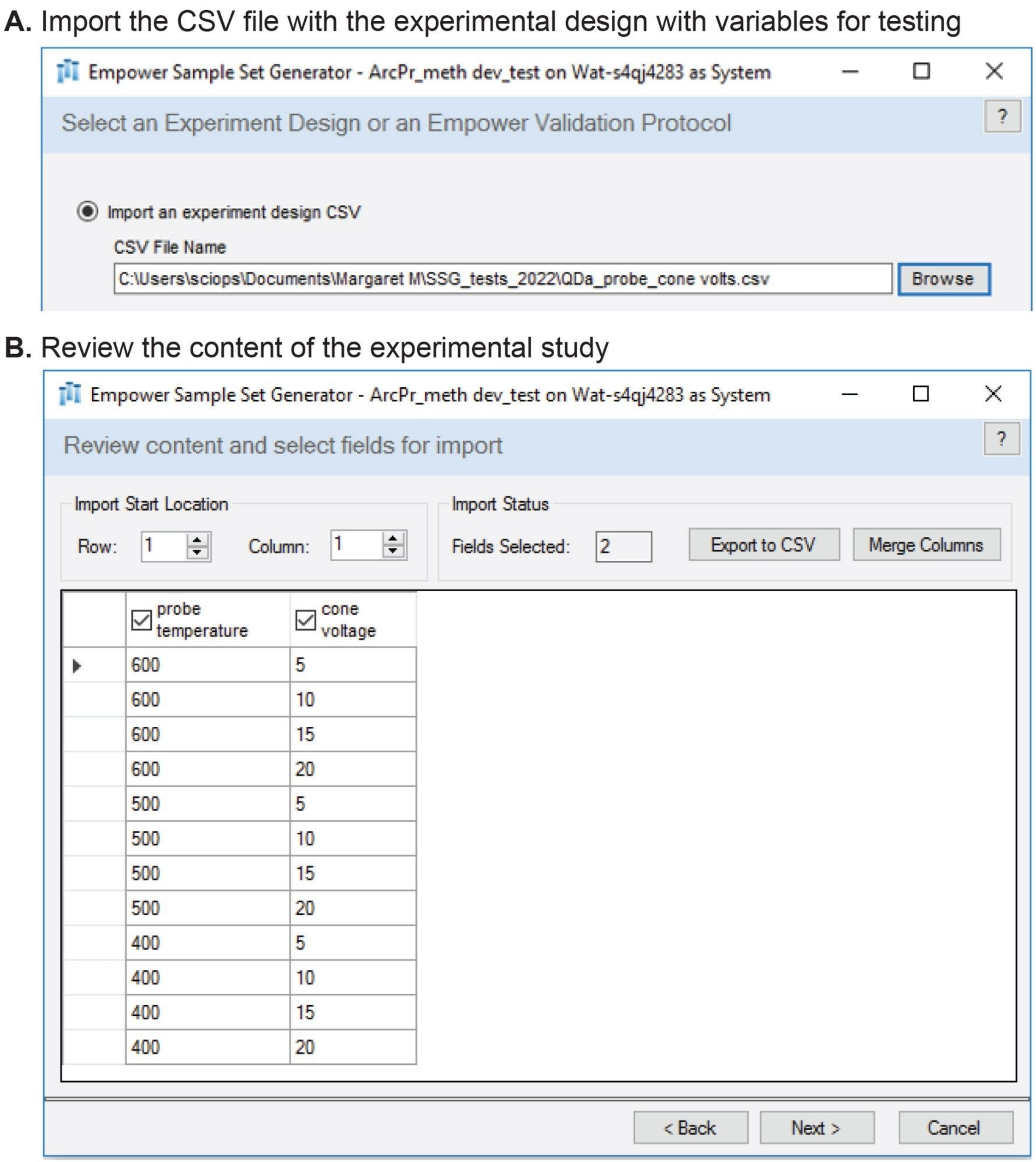


*Figure 1. Creating chromatographic methods with Empower SSG. Import CSV file (A) and review experiment design (B).*

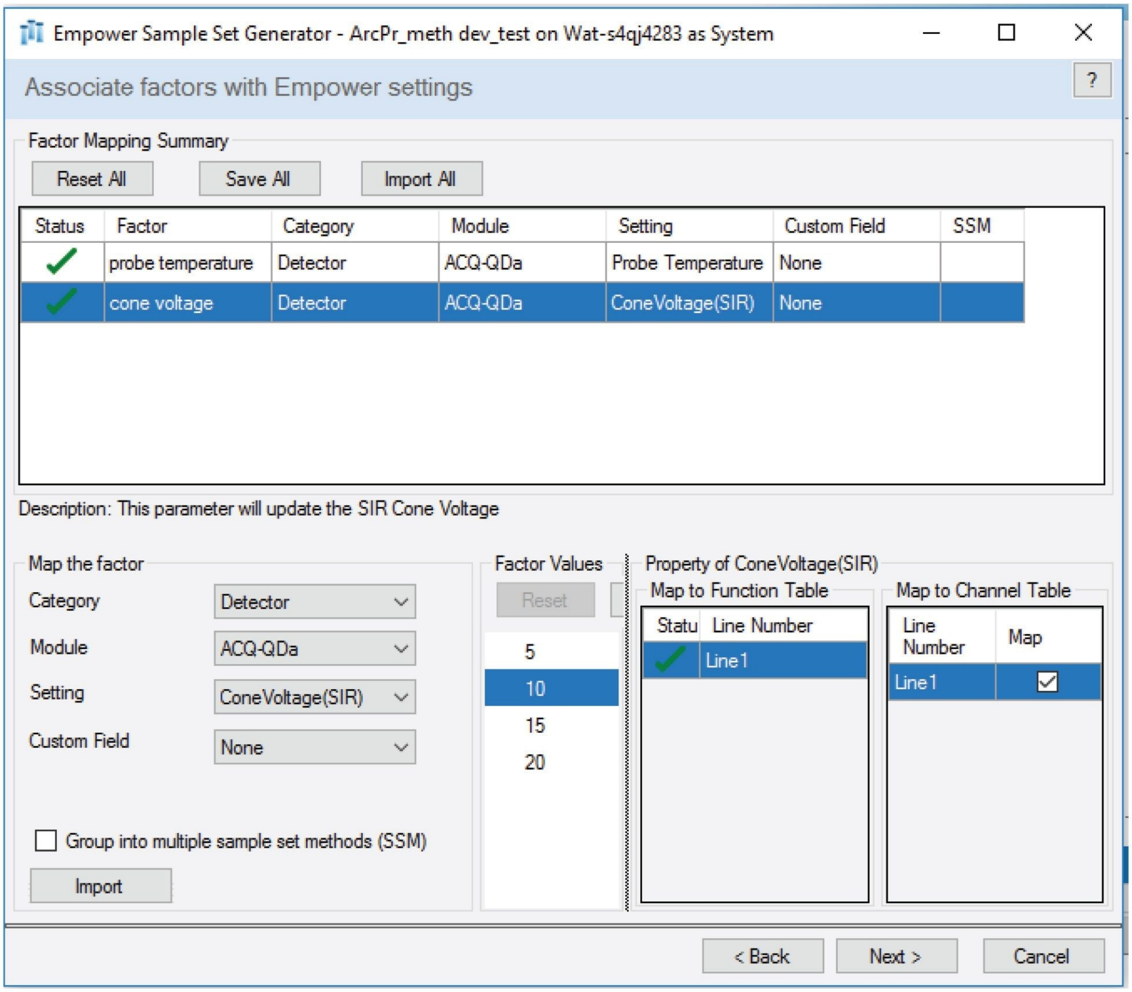


*Figure 2. Empower SSG. Associate factors or instrument variables with Empower settings.*

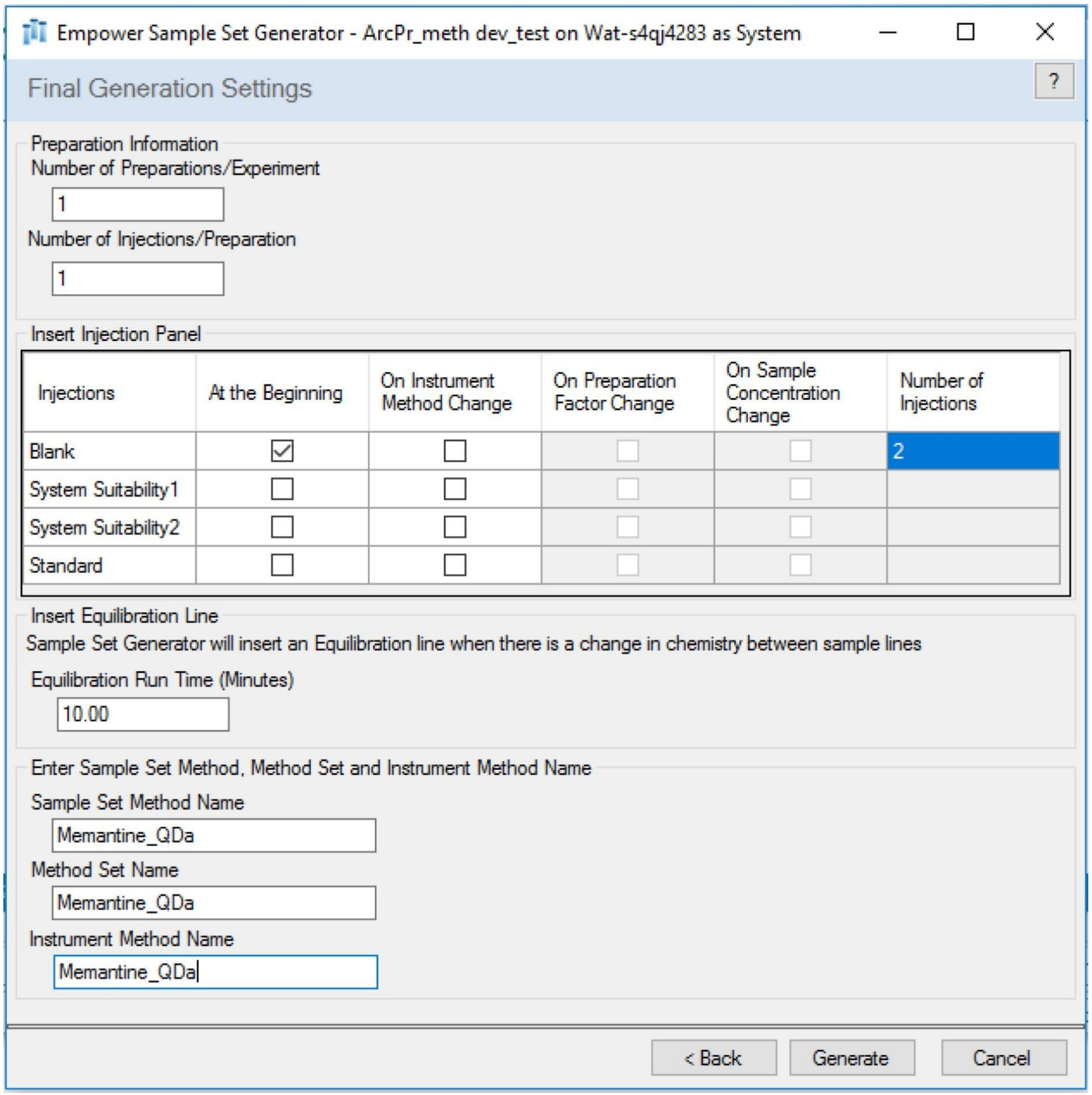


*Figure 3. Empower SSG final settings for generation of methods.*

| | Plate /Well | # of Injs | SampleName | Function | Method Set / Report or Export Method | Probe Temp | Cone Voltage | Run Time (Minutes) |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | Equilibrate | Memantine_QDa_1 | | | 10.00 |
| 2 | 1:A,1 | 2 | Memantine_1 | Inject Samples | Memantine_QDa_1 | 600 | 5 | 6.50 |
| 3 | 1:A,2 | 1 | Memantine_1 | Inject Samples | Memantine_QDa_1 | 600 | 5 | 6.50 |
| 4 | 1:A,2 | 1 | Memantine_2 | Inject Samples | Memantine_QDa_2 | 600 | 10 | 6.50 |
| 5 | 1:A,2 | 1 | Memantine_3 | Inject Samples | Memantine_QDa_3 | 600 | 15 | 6.50 |
| 6 | 1:A,2 | 1 | Memantine_4 | Inject Samples | Memantine_QDa_4 | 600 | 20 | 6.50 |
| 7 | 1:A,2 | 1 | Memantine_5 | Inject Samples | Memantine_QDa_5 | 500 | 5 | 6.50 |
| 8 | 1:A,2 | 1 | Memantine_6 | Inject Samples | Memantine_QDa_6 | 500 | 10 | 6.50 |
| 9 | 1:A,2 | 1 | Memantine_7 | Inject Samples | Memantine_QDa_7 | 500 | 15 | 6.50 |
| 10 | 1:A,2 | 1 | Memantine_8 | Inject Samples | Memantine_QDa_8 | 500 | 20 | 6.50 |
| 11 | 1:A,2 | 1 | Memantine_9 | Inject Samples | Memantine_QDa_9 | 400 | 5 | 6.50 |
| 12 | 1:A,2 | 1 | Memantine_10 | Inject Samples | Memantine_QDa_10 | 400 | 10 | 6.50 |
| 13 | 1:A,2 | 1 | Memantine_11 | Inject Samples | Memantine_QDa_11 | 400 | 15 | 6.50 |
| 14 | 1:A,2 | 1 | Memantine_12 | Inject Samples | Memantine_QDa_12 | 400 | 20 | 6.50 |

*Figure 4. Sample set method generated using Empower SSG.*

**A.** Peak data across experimental runs

Empower 3 Peak_summary report

Channel Name: QDa 1: SIR Ch1
Proc. Chnl. Descr.: 1: QDa Positive(+) SIR Ch1 180.20

| | SampleName | RT | Probe Temp (C) | Cone Voltage (V) | Height | USP s/n |
|---|---|---|---|---|---|---|
| 1 | Memantine_3 | 1.775 | 600 | 15 | 36023 | 72 |
| 2 | Memantine_2 | 1.797 | 600 | 10 | 35715 | 68 |
| 3 | Memantine_4 | 1.832 | 600 | 20 | 32793 | 40 |
| 4 | Memantine_7 | 1.792 | 500 | 15 | 31467 | 60 |
| 5 | Memantine_6 | 1.818 | 500 | 10 | 29286 | 36 |
| 6 | Memantine_8 | 1.771 | 500 | 20 | 28327 | 48 |
| 7 | Memantine_11 | 1.782 | 400 | 15 | 25978 | 50 |
| 8 | Memantine_1 | 1.761 | 600 | 5 | 24986 | 40 |
| 9 | Memantine_10 | 1.803 | 400 | 10 | 24413 | 39 |
| 10 | Memantine_12 | 1.822 | 400 | 20 | 23552 | 28 |
| 11 | Memantine_5 | 1.775 | 500 | 5 | 20856 | 39 |
| 12 | Memantine_9 | 1.779 | 400 | 5 | 17530 | 33 |

**B.** Chromatogram of 10 ng/mL memantine standard solution in 90:10 water/methanol

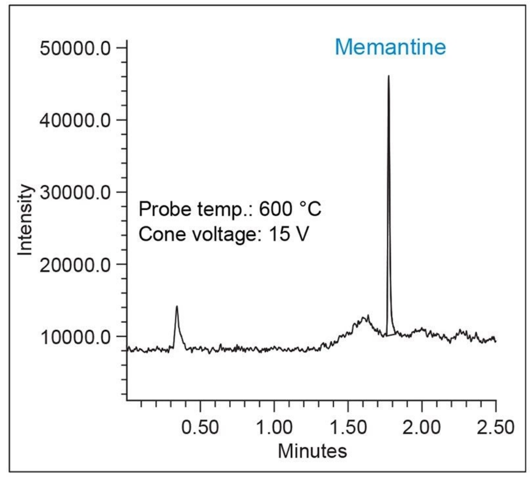


*Figure 5. Empower data. Peak data across the experimental runs (A) and representative chromatogram of the memantine standard solution (B).*

## Conclusion

Using the Empower Sample Set Generator, chromatographic methods were automatically created for analysis with an ACQUITY QDa Mass Detector, to investigate the effect of ionization parameters (probe temperature and cone voltage) on the sensitivity of memantine hydrochloride. The Empower instrument methods and method sets were automatically created and built into the a sample set method as a ready-to-run injection sequence. Automating methods generation reduced transcriptions errors and time associated with manual process.

The Empower Sample Set Generator automates creation of chromatographic methods for wide range of applications including method development and validation, performed on Waters ACQUITY LC Systems and detectors.

## References


1. https://www.webmd.com/drugs/2/drug-77932-377/memantine-oral/memantine-oral/details < https://www.webmd.com/drugs/2/drug-77932-377/memantine-oral/memantine-oral/details> .
2. Maziarz M, Wrona M, McCarthy SM. Benefits of Using QDa Mass Detection for Quantitative Analysis of Non-Chromophoric Memantine HCl in Tablet Formulation. Waters Application Note, 720005179, 2014.


## Featured Products

Arc Premier System <https://www.waters.com/waters/nav.htm?cid=135083359>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

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


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