Waters™

RenataDX System – Tips and Tricks for Flow-Injection Tandem Mass Spectrometry (FIA-MS/MS)

Heather Brown, Jonathan Jones
Waters Corporation, Wilmslow, UK

INTRODUCTION

To ensure high-quality and consistent data, appropriate system setup, optimization, and maintenance should be considered allowing for minimal need of ongoing user supervision or intervention.

This white paper describes a number of key steps that can support the generation of high quality analytical data by flow injection analysis tandem mass spectrometry (FIA-MS/MS), using the Waters RenataDX™ System, as well as how to recognize common system occurrences that can negatively affect data quality.

MOBILE-PHASE AND WASH SOLVENT PREPARATION

Always use LC-MS grade solvents for sample preparation and analysis, and ensure all glassware is clean and free from contaminants. The purity and cleanliness of solvents can vary between commercial lot and batch numbers. Similarly, the performance of laboratory water purification systems can vary. Recording lot, batch, and/or "prepared on" dates when preparing solvents can be helpful when troubleshooting instrument performance issues, which can be attributable to solvent quality.

SETTING UP A VARIABLE FLOW PROFILE

A typical flow profile for FIA-MS/MS can be achieved using a relatively simple method, as illustrated in Table 1. The type 11 curve applies the conditions in the table row at the time-point specified. A linear change in flow rate between two time-points with a type 6 curve can also be used.

Step	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.13	100	0	Initial
2	0.2	0.01	100	0	11
3	1.2	0.50	100	0	11
4	1.7	0.13	100	0	11

Table 1. Example pump parameters for a variable FIA-MS/MS analysis.

This is an example of a typical variable flow profile, with the horizontal axis representing time.

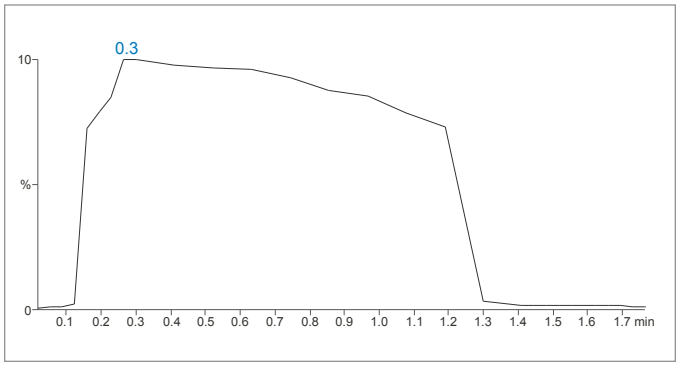

Figure 1. Typical variable flow profile for a FIA-MS/MS analysis.

This desired shape is achieved by starting with a pump flow rate between 0.1 and 0.2 mL/min, and decreasing it to between 0.01 and 0.02 mL/min as the sample enters the tandem mass spectrometer. This flow rate is held constant for approximately one minute while data collection occurs. After the data collection step, the flow rate is sharply increased to 0.5 mL/min to clear any remaining sample from the system flow path. The final step is to return the flow rate back to the same rate as the initial step.

OPTIMIZING THE FLOW PROFILE

When performing FIA-MS/MS it is critical that the flow profile has sufficient MS scans for data processing. This will allow for the most reproducible results, because low intensity signals from chemical noise will be averaged out, improving the signal to noise ratio. The target to aim for is typically a minimum of 8–10 MS data points in the central region of the flow profile. The analyte and internal standard response ratio should be consistent across the peak, including the data points at the extremities. Beware, that if the data points are processed there is a chance of averaging baseline noise if the flow profile peak shifts in either direction.

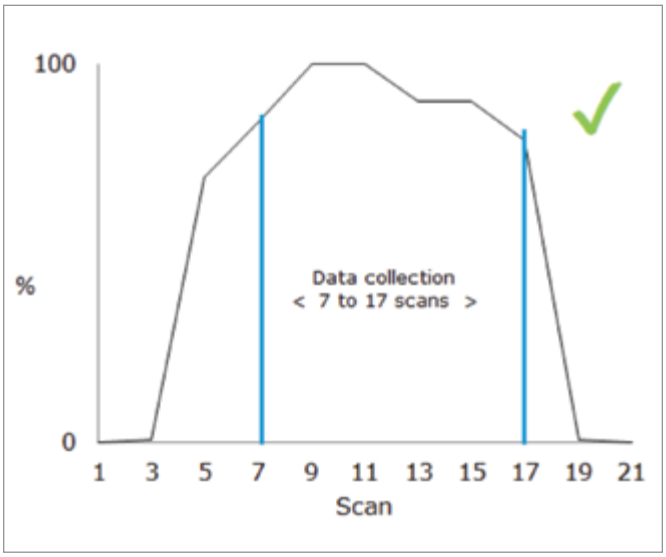

Figure 2. An example of an acceptable flow profile showing region of data averaging.

In this example, the flow profile has an acceptable shape and 8–10 data points. This spectrum is being processed by IonLynx™ Application Manager Software.

RECOGNIZING ISSUES

Below are multiple examples of issues that are commonly encountered where performing FIA-MS/MS and their potential causes.

In Figure 3, half of the flow profile is missing. This can occur as a result of a flow issue caused by a blockage somewhere in the sample path from the injector to the mass spectrometer. As a result of not achieving the minimum acceptable number of scans, this sample should be rejected and re-acquired.

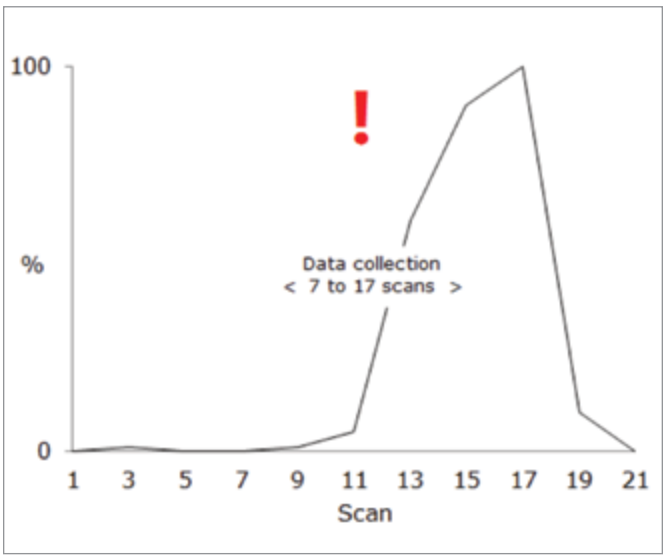

Figure 3. Typical non-acceptable flow profile with an inadequate number of data points.

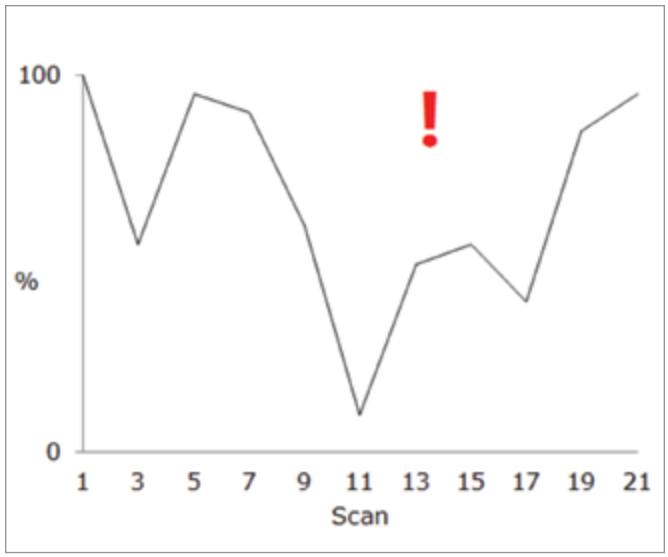

Figure 4. Typical flow profile indicating an issue with the injector module, or sample preparation.

In Figure 4, there is an extreme example where inadequate sample has reached the mass spectrometer. This scenario can be due to a major sample preparation issue, such as low concentration, missing internal standards or, if samples are derivatized, inadequate derivatization being achieved.

This result can also be related to insufficient sample being delivered to the mass spectrometer due to an injector blockage or a lack of detection by the mass spectrometer, which could result from inefficient ionization, desolvation, or transmission of ions through the ion-focussing regions, quadrupoles or collision cell. An entirely flat baseline might indicate an issue within the tandem mass spectrometer, such as improper installation of the sample cone, forgetting to re-open the sample cone isolation valve after performing maintenance, or a loss of communication between the tandem mass spectrometer and the operating software.

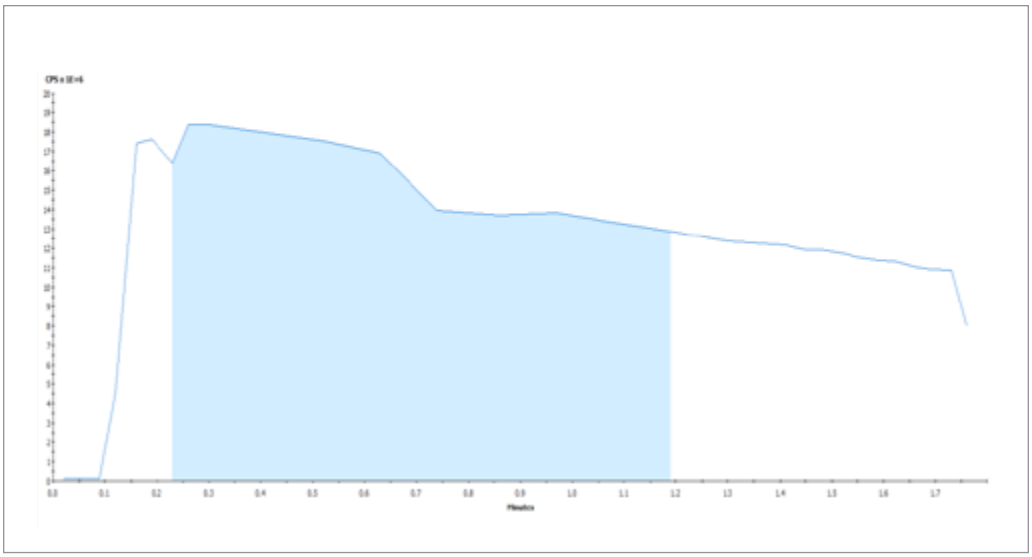

Figure 5. Typical flow profile indicating an issue in the LC pump module.

The final example in Figure 5 demonstrates ten MS scans have been achieved for data collection, which is represented by the area highlighted in blue, however, the step in the variable flow injection responsible for pushing any remaining sample out of the injector and in to the tandem mass spectrometer has failed. In this scenario, the injection itself is likely acceptable, but the result could present a risk to carry over that may affect the subsequent injection.

The most common cause of this type of observation is within the pump, and it can indicate that periodic maintenance is needed.

CONSIDERATIONS FOR MS METHOD

FIA-MS/MS utilizing variable flow rate method may be chosen when scan-based MS data are required. If the highest *m/z* of interest is just less than 500 Da, then the upper setting of the *m/z* acquisition range should be set to 500 Da.

If the instrument is set to scan an unnecessarily high (or low) mass range, then time is wasted by scanning outside of the relevant mass range. This means it will take longer to acquire the minimum number of scans for processing, and this diminishes the efficiency of the system.

It can be useful to take advantage of the advanced scanning modes of the mass spectrometer. In the example below, the MS method is set to acquire specified MRMs (Multiple Reaction Monitoring), precursor ions (parent ions) of a specified *m/z*, and constant neutral loss data within a single FIA-MS/MS run. Using scanning modes of data acquisition is helpful when a qualitative evaluation of the spectrum is needed. An example of this is when a scanning acquisition identifies an interference, the natural isotope of that interference could affect the intensity of an analyte or internal standard of interest.

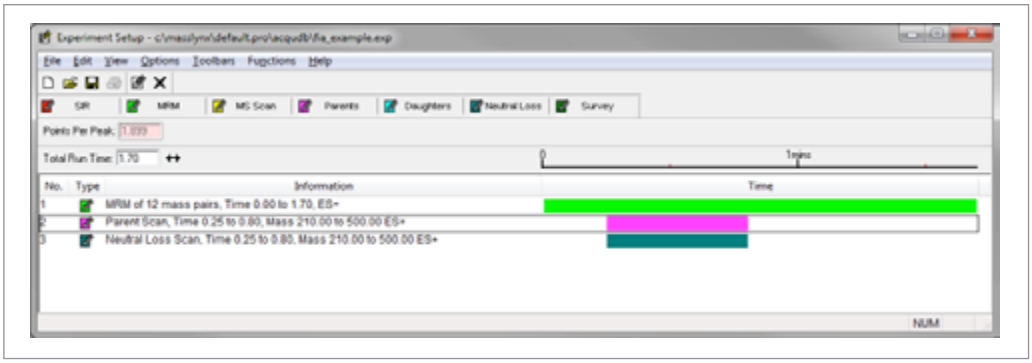

Figure 6. An example of an MS acquisition method combining precursor ion and constant neutral loss scanning with multiple reaction monitoring (MRM) experiments.

Monitoring ion intensities and response ratios of analytes and internal standards in a system suitability test mixture can allow you to identify potential instrument issues before batches of precious test samples are analyzed. Infusing the test mixture through the MS fluidics allows you to monitor detector performance in isolation from the pump, sample manager and system tubing. Injecting the test mixture via the sample manager can further support troubleshooting by helping you identify the source of artifacts, such as environmental contamination.

CONCLUSIONS

- Using these tips and tricks, system issues can be quickly identified and corrected ensuring a return to optimal operation with the minimum of user intervention.
- Monitoring the flow profile in FIA-MS/MS can give an indication of system health and provide insight as to a potential cause or results variation.
- Optimizing the MS data acquisition and pump flow methods can maximize the quality of data collected from a single sample.

References

1. Waters RenataDX System, *Customer Familiarization Guide*, p/n 715005697EN.
2. *Maintaining Accuracy and Consistency of Results in Flow Injection Analysis Mass Spectrometry in the Clinical Laboratory – Lessons from Newborn Screening Ontario* – On-demand webinar via SelectScience.net.

For *in vitro* diagnostic use. Not available in all countries.

Waters™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com