Low Flow Direct Infusion Mass Spectrometry Using AutoFocus™

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Abstract

Direct infusion mass spectrometry is a common technique for screening for both qualitative and quantitative analysis. In many direct infusion setups, samples are manually loaded into a syringe before infusion, limiting automation of the technique. The Axcend AutoFocus™, a compact and portable autosampler capable of direct infusion down to nL/min flow rates was used. In this application note, the AutoFocus was coupled to a small footprint MassTech MTE 30 mass spectrometer. This allows for automated MS analysis at nL/min flow rates with a low benchtop footprint. In this work, flow rates between 60 and 500 nL/min were analyzed to determine the sensitivity, stability, and viability of direct infusion MS at each flow rate. The AutoFocus demonstrated stable signal between 250 and 500 nL/min. Additional modifications were needed to operate at 60 nL/min.

Introduction

Detection of analytes using direct infusion into a mass spectrometer (MS) is a common technique for screening of both large and small molecules for structural elucidation and analyte quantification. When using electrospray ionization (ESI), ionization efficiency and sensitivity improve as flow rate decreases, a fact that has made low flow ESI-MS detection a staple in the omics fields. Traditional direct infusion applications often require manual loading of samples into a syringe, reducing throughput and limiting the ability to automate workflows. Additionally, mass spectrometers often require large benchtop footprints to accommodate not only the mass spectrometer but also its external vacuum pump system and gas supply. In this application note, the Axcend AutoFocus

autosampler was connected to the MassTech MTE30 for direct infusion of reserpine at various flow rates. The stability and intensity of analyte signal was used to quantify the system's ability to produce reliable, stable data at nL/min flow rates.

The Axcend AutoFocus is an autosampler specifically designed for integration into the Axcend instrument portfolio of compact and capillary scale LC instrumentation. With a form factor of 9.0 x 13.8 x 8.2 inches, the AutoFocus is one of the smallest commercially available autosamplers. It features the ability to sample from conventional 2 mL HPLC vials or from a 96 well plate. Sample volumes as low as 0.01 µL to as high as 50 µL can be withdrawn. Traditionally, these aliquots would be used to fill the sample loop of the Focus LC; however, the system can also be used to infuse sample into a mass spectrometer at nL/min flow rates.

The MassTech MTE30 is a compact mass spectrometer featuring internal vacuum pumping systems and gas supplies making it ideal for portable applications. The MTE30 uses a 3D ion trap with a mass range of 30-2000 Da. With a form factor of 8 x 12 x 13 inches, the MTE30 has a similarly compact form factor making it ideal for both low flow and portable analysis. In this study, the AutoFocus was connected to the MTE30 in order to monitor the performance of a direct infusion experiment.

Materials and Methods

For this experiment, a 0.5 ng/µL sample of reserpine in 50:50 (% by volume) H_2O/ACN with 0.1% formic acid was infused at various flow rates, with its expected mass (M+H= 609.28 m/z) being used to monitor stability of both flow and electrospray. Infusion rates of 500 nL/min, 250 nL/min, and 60 nL/min were all tested. The settings for the MassTech MTE 30 were as follows:

Table 1. MassTech MTE30 Acquisition Settings

ESI Voltage	+1.8 kV
Mass Range	500-700 m/z
Number of Microscans	3
MS Injection Time	5 ms

Results and Discussion

A series of three infusions of the reserpine standard solution were run at 500 nL/min, 250 nL/min and 60 nL/min in order to determine the stability and sensitivity of the setup at low flow rates. The extracted ion traces and mass spectra of these infusions are shown in Figures 1, 2, and 3, respectively.

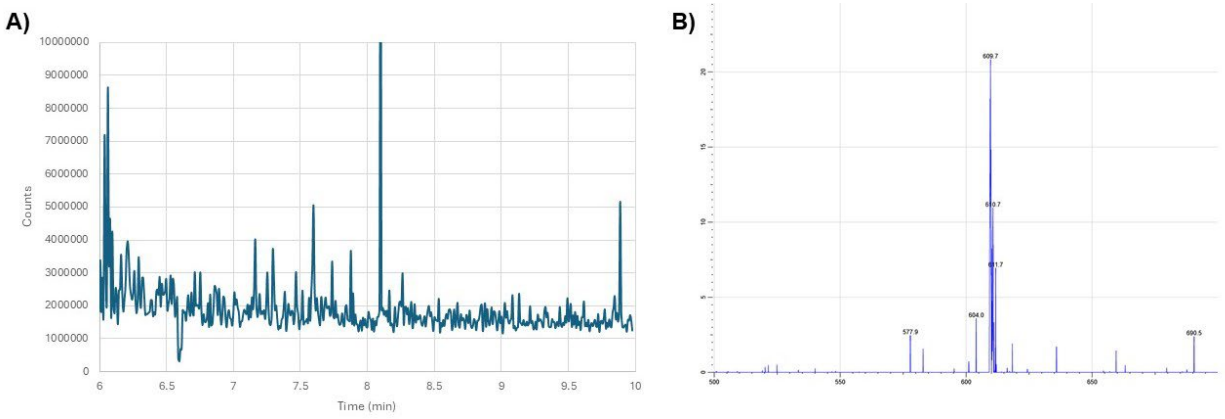

Figure 1. Direct infusion of reserpine at 500 nL/min showing A) the extracted ion trace of reserpine at 609 m/z and B) a mass spectrum collected during the infusion.

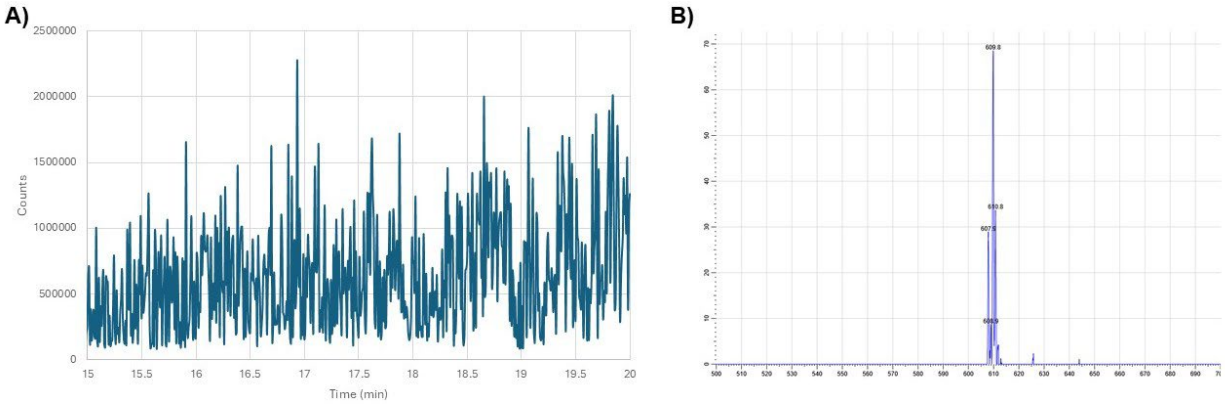

Figure 2. Direct infusion of reserpine at 250 nL/min showing A) the extracted ion trace of reserpine at 609 m/z and B) a mass spectrum collected during the infusion.

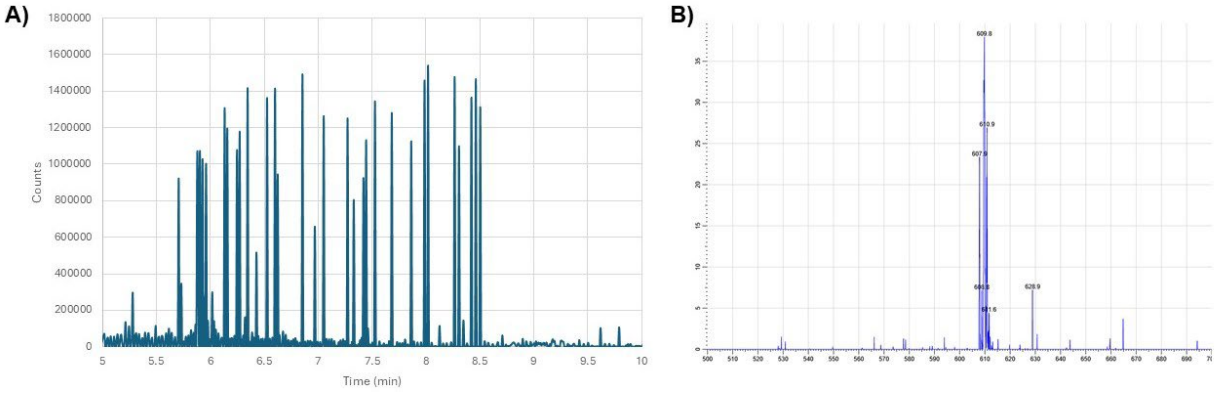

Figure 3. Direct infusion of reserpine at 60 nL/min showing A) the extracted ion trace of reserpine at 609 m/z and B) a mass spectrum collected during the infusion.

A comparison of results obtained from the three flow rates show several key differences in both spectra and intensity. As the flow decreases, the intensity of reserpine also decreases; however, when comparing the mass spectra, the background ions are decreased as well. This is clearly shown in Figures 1B and 2B where the background ions present at 577.9, 604.0, and 690.5 m/z become undetectable when changed from 500 nL/min to 250 nL/min. Another key difference is the stability of the spray. In each of the three cases, time was required to achieve stability, with lower flow rates requiring longer equilibration times. Using the AutoFocus, flows between 250 and 500 nL/min allowed direct infusion into a MassTech MTE30.

At the lowest flow rate of 60 nL/min, a stable spray could not be established; however, reserpine was still detectable. The inability to produce stable spray may be due to several factors including: the inner diameter of the ESI source being too large, the system lacking sufficient equilibration time, and pulsing resulting from the stepper motor causing fluctuations in flow. In each of these cases further studies and modifications can be made including reducing the i.d. of the ESI source, increasing equilibration time, and reducing the volume of the syringe used for infusion. With these modifications, it is reasonable to view the AutoFocus as capable of direct infusion flow rates down as low as 60 nL/min.

Conclusions

Direct infusion MS is a useful technique for many analytical workflows. The Axcend AutoFocus was used for low flow infusion of reserpine onto a compact and portable mass spectrometer. At flow rates of 500, 250, and 60 nL/min, the system was capable of detecting reserpine, with stable spray being achievable as low as 250 nL/min. This shows the viability of the AutoFocus for direct infusion experiments at nL/min flow rates.