Triple Quadrupole Optics: Enhanced Robustness for Challenging Applications

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ABSTRACT

Purpose: To demonstrate utility of an accelerated test to determine the long-term robustness of newly designed ion inlets.

Methods: Direct infusion of concentrated matrix was utilized with periodic monitoring of analyte response to detect attenuation of signal that could indicate contamination of ion optic elements. Matrix was added to LC stream at constant flow rate through a tee and introduced directly into the mass spectrometer ion source.

Results: Analyte response was plotted verses matrix volume. The matrix volume can be converted into number of injected samples allowing assessment of the ion inlet design on system robustness. Under the accelerated testing conditions, the Thermo Scientific™ TSQ Fortis™ mass spectrometer demonstrated excellent robustness.

INTRODUCTION

Robust performance is critical for most routine applications of triple quadrupole mass spectrometers. Those applications may involve samples with heavy matrices, such as food extracts and physiological fluids. A key to fast results is to minimize sample preparation and pre-treatment time, which may come at the expense of compromised system robustness. Electrospray ionization of these samples creates a high population of low volatility ionic and neutral species that tend to contaminate both source region and internal optics of mass spectrometers, leading to early degradation of performance.

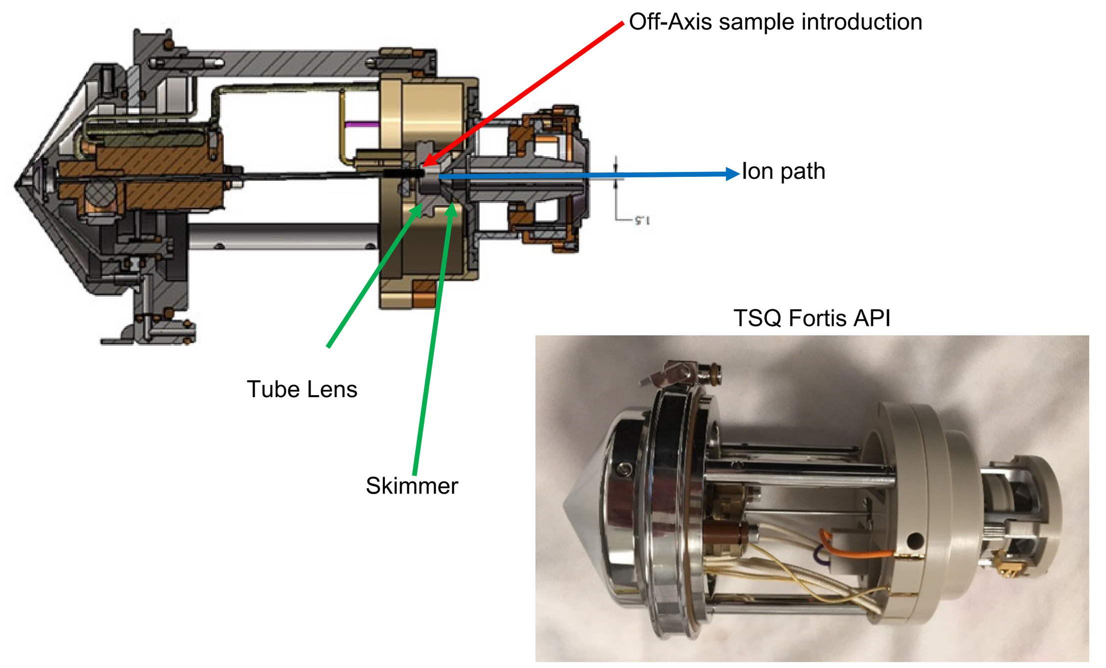
In the design of the new entry level TSQ Fortis™ instrument, the atmospheric pressure interface (API) was constructed to ensure that the internal optics stay clear of contaminants that could lead to transmission degradation. The API is designed such that the parts that were most prone to spray contamination have been replaced with a tube lens and skimmer configuration. This configuration reduces the need to perform maintenance tasks that requires breaking vacuum, resulting in increased instrument up-time.

To assess robustness performance of the API, a concentrated synthetic serum substitute matrix was teed into the LC stream and the combined eluent/matrix stream was introduced directly into the inlet of the mass spectrometer. The response of Alprazolam was monitored to assess performance. The test was designed to track robustness trends providing key information in diagnosing the API performance. Final volume of infused matrix can be correlated to a total number of injections.

MATERIALS AND METHODS

New Source Inlet Design

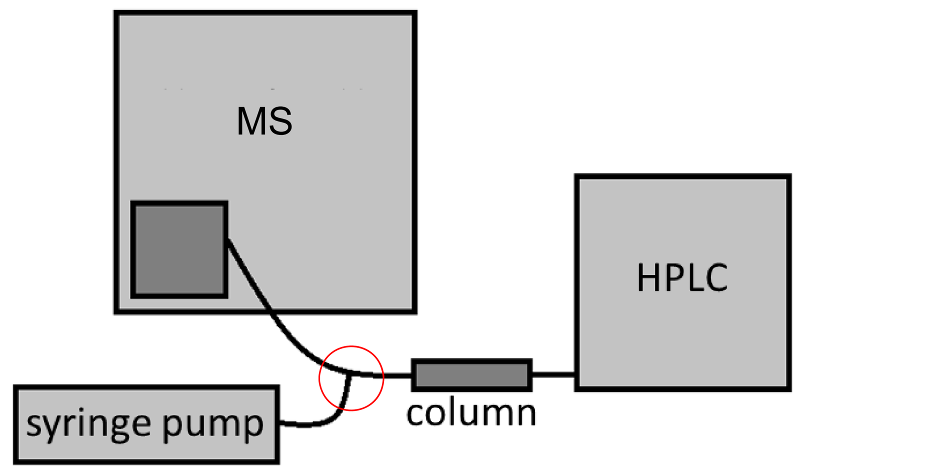
A prototype triple quadrupole mass spectrometer has been created by modification of an existing commercially available mass spectrometer by using an API with off-axis beam path geometry. This new configuration results in neutral species and any surviving droplets post desolvation being directed away from the critical surfaces on the ion beam path. Each source component and its arrangement has been optimized to maximize sensitivity and to maintain ion optics cleanliness, limiting required maintenance to removable parts such as ion transfer tube.



Test Configuration

The test system consisted of: mass spectrometer, HPLC with autosampler, syringe pump, LC column, LC Tee, and tubing (see Figure 1). The accelerated robustness test was performed by infusing SeraSub™ (a synthetic serum substitute) teed at 5 uL/min into 250 uL/min LC flow. Source conditions optimized for Alprazolam acquisition (see Figure 1).

Figure 1. System arrangement for robustness test. Red circle represents a Tee connector for HPLC and syringe pump to mass spectrometer. HPLC contains autosampler and column compartment. Syringe pump is controlled by MS instrument control software or by acquisition method.



Test Method: MS Method for Alprazolam

MS source conditions and MSMS parameters:

Test Method: HPLC Method

LC Solvent A: water (with 0.05% formic acid), Solvent B: methanol (with 0.05% formic acid)

LC flow: 0.25 mL/min isocratic 30% A + 70% B

LC column: Thermo Scientific™ Hypersil GOLD™ 50×2.11 (1.9 um)

Test Method: Test Sequence

1. Initial sensitivity was measured by making 5 injections of 5uL of 1 pg/uL concentration of Alprazolam.
2. Matrix infusion was teed into the LC flow continuously.
3. After 12 hr of matrix infusion (3.6mL of matrix), infusion was stopped and 5 replicate injections of alprazolam were run to asses signal intensity.

Steps 1–3 were repeated until significant signal attenuation was observed. When greater than 50% signal attenuation was observed, "routine user maintenance" was performed. "Routine user maintenance" consists of replacement of ion transfer tube and wiping the sweep cone and drain exhaust with methanol. The removed ion transfer tubes were then cleaned extensively by sonicating in water/methanol with formic acid (10%) and a follow up rinse of methanol and prepared for later use.

Software/Data Analysis

TSQ instrument control software, Thermo Scientific™ Xcalibur™ software, SII.

Sample Preparation

Test sample: Alprazolam 1 pg/uL (30% methanol in water)

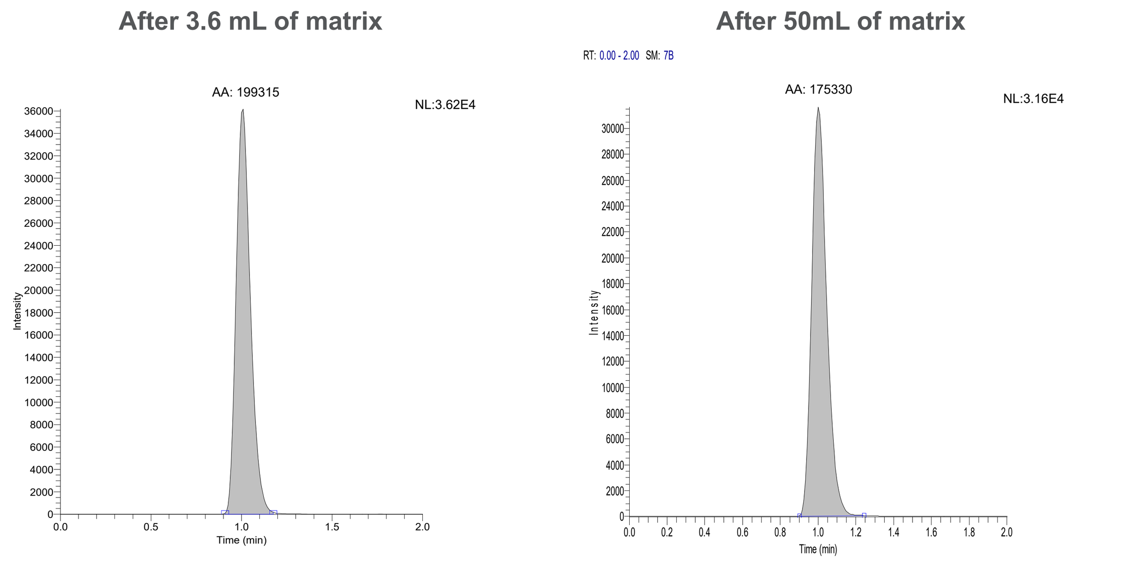
Matrix: SeraSub, a synthetic serum replacement containing salts and polymers.

RESULTS

Alprazolam Response

System performance was assessed by monitoring the average response for 5 replicate injections of 1 pg/uL Alprazolam made at 12 hour intervals. A depiction of Alprazolam chromatograms before and after infusion of 50 mL of SeraSub matrix is shown in Figure 2.

Figure 2. Chromatograms for 5uL injections of alprazolam at 4mL and after infusion of 50 mL of SeraSub.

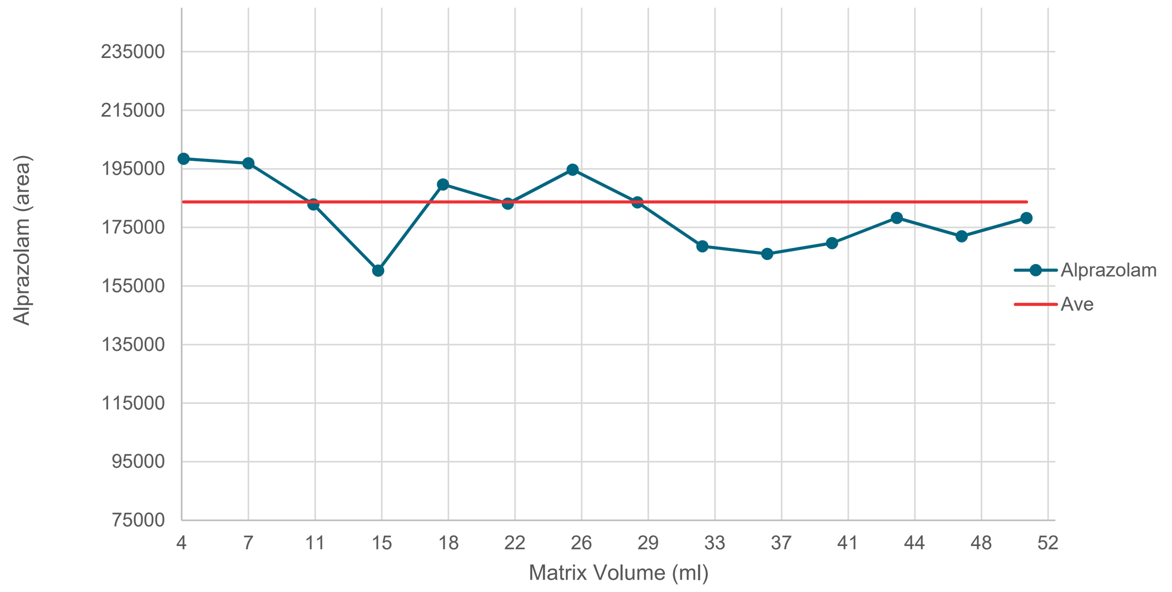


Robustness Trend

Alprazolam response from measurements were taken at 12 hr intervals during the matrix infusion into LC stream. To perform the alprazolam injections, the matrix infusion was stopped, the tee assembly was removed and replaced with a single line directly from the HPLC column to the grounding union of the source. The 5 replicate injections of alprazolam were made under the previously described conditions. The average of the 5 replicate injections was calculated and plotted versus the corresponding infusion volume of matrix (see Figure 3).

During the experimental period, if the alprazolam response dropped more than 50%, "routine user maintenance" was performed. This routine maintenance only requires the replacement of the ion transfer tube and quick wipe down of the sweep cone and exhaust tube with methanol. The entire operation takes less than 30 minutes, maximizing the instrument uptime.

Figure 3. Alprazolam peak area monitored during 50 mL of infused matrix. Alprazolam measurements were made every 12 hrs. Total experiment time was 170 hours.



A more detailed analysis of the data from the 170 hours of matrix infusion was performed to assess trends. In Table 1 the average of all the 12 hour alprazolam measurements was calculated. From the average alprazolam measurements the % RSD, % Loss (Alprazolam Area for Matrix Volume X-Alprazolam Area for Matrix Volume 4mL/ Alprazolam Area for Matix Volume 4mL*100), and the % Deviation (deviation=Alprazolam Area for Matrix Volume X-Average Alprazolam Area)/ Average Alprazolam Area*100) were determined. The % Loss and % Deviation calculations demonstrate that there was no consistent decrease in performance. The overall % RSD of the peak area for the entire 170 hour infusions was 7%, demonstrating excellent consistency for the equivalent of 10,000 injections of matrix (assuming 5uL injection volume).

Table 1. Signal vs. matrix volume. Signal average was 180k counts. % Loss was calculated from the first point after 4 mL of SeraSub matrix was infused, % Deviation was calculated from the average of alprazolam areas.

Matrix Volume (mL)	Alprazolam Area	%Loss	% Deviation
4	198436	0%	10%
7	196896	1%	9%
11	182879	8%	2%
14	160265	19%	-11%
18	189678	4%	5%
22	183109	8%	2%
25	194725	2%	8%
29	183549	8%	2%
32	168574	15%	-6%
36	165953	16%	-8%
40	169637	15%	-6%
43	178270	10%	-1%
47	171982	13%	-5%
50	178233	10%	-1%
Average	180156		
Standard Dev.	11965		
%RSD	7		

CONCLUSIONS

Robustness Testing

The experiment described here is a method of rapidly determining the robustness of internal ion optic designs without the need for complicated HPLC methods and lengthy experiments. The results demonstrate excellent robustness of the off-axis API design incorporated into the TSQ Fortis platform. The robustness demonstrated in this study enables users to run dirty samples with minimal clean up for extended periods of time, improving productivity by reducing sample preparation time and maximizing instrument up time.

- Rapid method of assessing internal ion optics robustness.
- Off-axis API design demonstrated consistent performance (RSD=7%) over the course of infusing 50mL of SeraSub matrix directly into the mass spectrometer (equivalent of 10,000 injections of plasma).
- Off-axis API design provides superior robustness enabling the analysis of "dirty" samples with minimal clean up and instrument maintenance.

TRADEMARKS/LICENSING

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