

SPion System™: Introduction and Performance Evaluation

SPion System™ is a revolutionary mass spectrometry technology that defines a new architecture for the front end of a mass spectrometer. Empowered by the world's first and only flexible ion guide technology for lossless ion transfer, SPion System™ seamlessly bridges the gap between sample and mass spectrometer. By overcoming a major instrumentation bottleneck, SPion System™ offers endless opportunities for scientists and researchers to develop and introduce new generations of advanced front end peripheral technologies for mass spectrometry that were not possible to develop before, including probes, multimodal imaging stations, and sample preparation product suites. In this technical note, SPion System™ is introduced, some application areas are proposed, and the results of a proof-of-principle demonstration of SPion System™ in conjunction with Thermo Fisher's TSQ Altis triple quadrupole mass spectrometer are presented. The proof-of-principle results demonstrate that SPion System™ offers excellent analytical performance while maintaining easy and robust operation.

Keywords: SPion System™, sample-to-mass spectrometer interface, remote analysis, atmospheric pressure sampling

Introduction

SPion System™ is a novel high-performance and easy-to-use atmospheric pressure sampling (APS) interface for mass spectrometry. SPion System™ bridges the gap between sample and mass spectrometer, and delivers unprecedented sensitivity in mass spectrometry analysis for samples located remotely to a mass spectrometer. Designed with simplicity in mind, SPion System™ can be easily and quickly installed and operated as a stand-alone sample-to-mass spectrometer interface. It can be integrated into a variety of probes, imaging stations, and sample preparation product suites to provide a streamlined and seamless mass spectrometry workflow.

At the core of SPion System™ lays a ground-breaking ion guide technology —SPion Tube™. SPion Tube™ extends the APS inlet of the instrument while maintaining excellent analytical performance. In a sense, SPion Tube™ is a flexible

extension to a mass spectrometer's internal ion funnel or ion guide and is engineered to provide the same highly-efficient ion transfer mechanism for transferring ions from sample to mass spectrometer. The flexibility offered by SPion Tube™ allows for the decoupling of the ion source from the inlet of a mass spectrometer to freely move in three-dimensional space.

SPion System™ has a compact, lightweight design and can be easily and quickly installed on advanced state-of-the-art mass spectrometry instruments. It provides the most versatile and modular frontend technology for APS on the market and is priced competitively to allow more scientists and researchers to access the technology. The enhanced sensitivity resulting from lossless ion transfer and the option for an integrated internal ion source as well as compatibility with external custom ion sources makes SPion System™ a highly-desirable mass spectrometry technology.

SPion System™ Improves Upon Commercial APS Interfaces

State-of-the-art mass spectrometers are equipped with an APS interface that includes a heated capillary inlet to transfer ions from atmospheric pressure to fore vacuum region, then to an ion funnel downstream from the capillary inlet, which then receives and focuses the ions. SPion System™ employs the same components and offers the same advantages while simultaneously improving upon the commercial interfaces. Instead of a short and rigid ion funnel fixed inside the instrument, SPion Tube™ employs a patented long and flexible stacked-ring ion guide that extends out of the instrument. Similar to the APS interfaces that are installed by manufacturers on commercial instruments, SPion System™ is compatible with any atmospheric pressure ionization source and employs a heated capillary inlet for transferring ions from atmospheric pressure to the fore vacuum region of a mass spectrometer.

SPion System™ Solves the Dilemma of Remote Sample Analysis

SPion Tube™ solves a significant challenge that has remained unsolved for many decades: when ions are produced far from a mass spectrometer, it is difficult, if not impossible, to efficiently transfer the ions to the mass analyzer. This problem has been a significant ion transfer bottleneck until now. SPion Tube™, an award winner from the NASA's Science Mission Directorate, was originally conceived to solve this ion transfer problem in the context of planetary exploration by enabling *in-situ* mass spectrometry analysis directly from the robotic arm of a rover.

When a sample is located at a distance from a mass spectrometer, generally two approaches are available for mass spectrometry analysis. In the first approach, called *in-situ sampling*, neutral molecules from the sample are transferred to the mass spectrometer and ionized after reaching the mass spectrometer. In the second approach, called *in-situ ionization*, the sample is ionized *in situ*, and the ions are transferred to the mass spectrometer for analysis.

In both approaches, insulator or metal tubes are commonly used to transfer molecules or ions from the sample to the mass spectrometer. However, there are limitations associated with both approaches. For example, techniques employing

in-situ sampling suffer from carry-over issues, which can be difficult to overcome, whereas techniques utilizing *in-situ ionization* suffer from loss of sensitivity due to significant ion loss along the insulator or metal tubes. Moreover, these issues scale exponentially as tube length increases.

SPion System™ was conceived by analyzing the problem from a different perspective: instead of using an insulator or a metal tube to bridge the gap between a remote sample and a mass spectrometer, what if, instead, the front end of the mass spectrometer extends to reach the sample? This reconceptualization of the problem set the foundation to re-engineer the front end of a mass spectrometer into an "elongated" form to solve the remote sample analysis dilemma. SPion System™ is a highly engineered solution that has been designed and constructed with the same level of precision that goes into the design of mass spectrometers.

Components of SPion System™

SPion System™ consists of four components: SPion Inlet™, SPion Tube™, SPion Interface™, and SPion Controller™ shown in Figure 1 and described below.

SPion Inlet™ is an advanced microprocessor-controlled, handheld atmospheric pressure sampling inlet for receiving and transporting ions from an ionization source to vacuum. SPion Inlet™ incorporates a capillary inlet, a heater block with a PID temperature control providing up to 150°C, an absolute orientation sensor for accurate positioning, a status indicator for intuitive operation. It also includes electronics to supply voltages to the capillary inlet and SPion Tube™ and to monitor and report the health of the SPion Inlet™ and SPion Tube™ back to SPion Controller™.

SPion Tube™ is a flexible ion guide that allows lossless ion transfer in a flexible path. SPion Tube™ is maintained at 1-3 Torr and employs two radio frequency (RF) voltages for lossless ion transfer from SPion Inlet™ to SPion Interface™ and eventually to the mass spectrometer. SPion Tube™ operates with RF voltages (0.1 to 1MHz and 0 to 80Vpp supplied by SPion Controller™). The frequency and voltage adjustment allows for tuning the system for optimal transfer of low and high mass range ions. A resistive network along SPion Tube™ provides a DC gradient along SPion Tube™ between the first ring electrode (on the SPion Inlet™ side) and the last ring electrode (on the SPion Interface™ side). The end of SPion Tube™ can incorporate an optional ion

funnel at no cost to focus ions for transferring into the mass spectrometer.

SPion Interface™ replaces a mass spectrometer's original vacuum interface and is engineered to mechanically match the mass spectrometer's interface while maintaining high-efficiency ion transfer from SPion Tube™ to the mass spectrometer. The RF voltages and the last ring electrode DC voltage are supplied to SPion Tube™ via connectors on SPion Interface™. The ion funnel at the end of SPion Tube™ is precisely aligned inside SPion Interface™ to maximize ion transfer from SPion Tube™ to the mass spectrometer. Exchanging instruments' original APS interface to install SPion Interface™ is accomplished quickly and easily, and requires breaking instrument's vacuum.

SPion Controller™ is a stand-alone unit that incorporates a microprocessor and a touchscreen display with a user-friendly menu to modify system

parameters. System parameters, such as temperature, voltages, and frequencies, can be set and monitored from the touchscreen display. Adjusting system parameters is typically not required during normal operation and is provided to allow for technical flexibility. SPion Controller™ includes an advanced power amplifier that allows for the operation of SPion Tube™ over a wide range of frequencies (0.1 to 1MHz) and provides up to 80Vpp. The upper limit for the DC voltages supplied by SPion Controller™ can be set to 50V or 100V via the touchscreen display. Current and voltage readbacks are easily accessible via SPion Controller™. The current readback (~1nA resolution) provides an easy mechanism to monitor ion loss on the capillary inlet. For reliable operation, SPion Controller™ continuously monitors the health of SPion Tube™ by monitoring the resistance of SPion Tube™ and the level of RF voltages at SPion Inlet™. When enabled, a motion-activated timer idles the system when it is not in use.

SPion System™

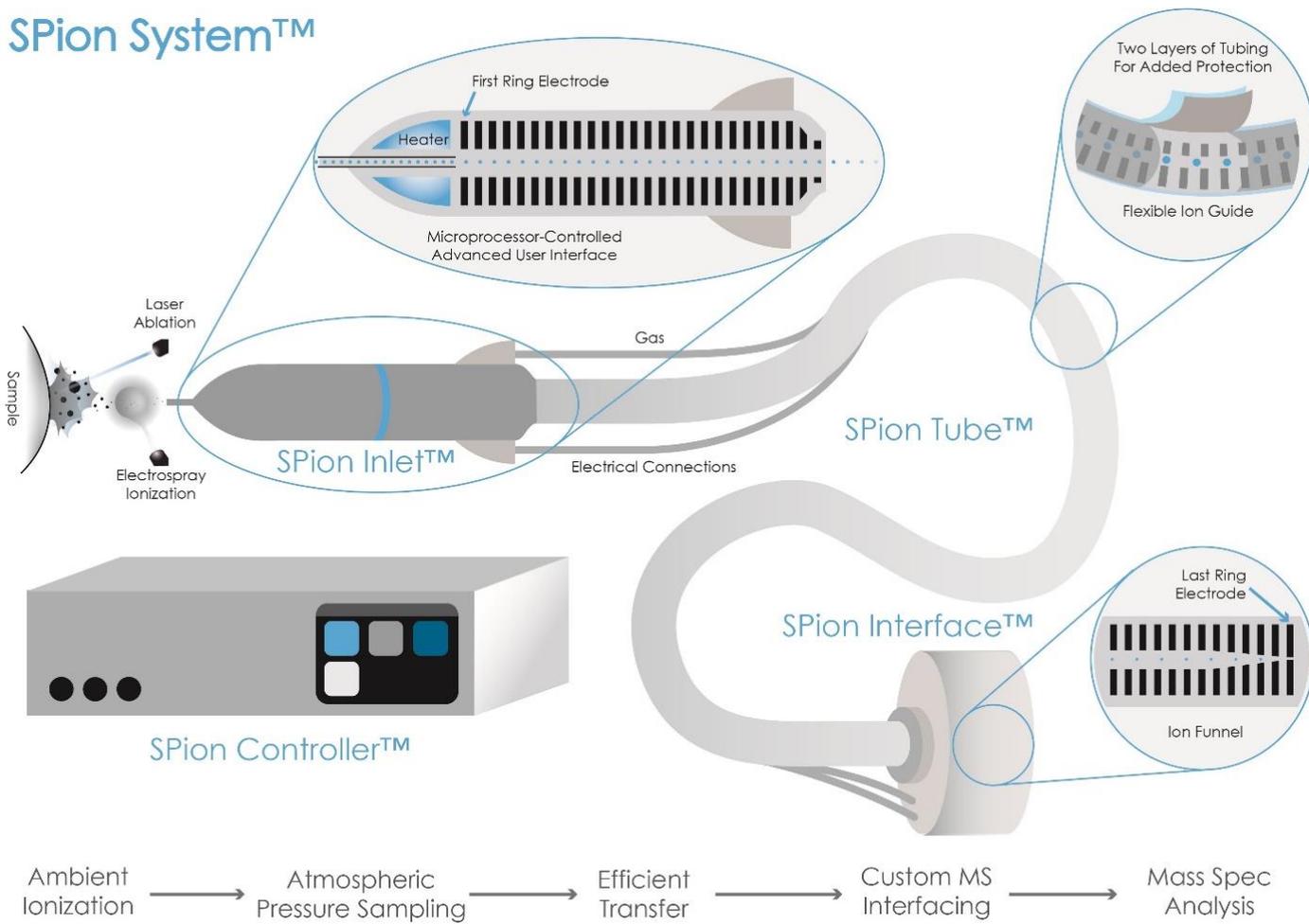


Figure 1. The block diagram of SPion System™. Ions are sampled via SPion Inlet™, confined and transferred in SPion Tube™, and delivered to the mass spectrometer via SPion Interface™. The flexibility of SPion Tube™ allows movement of SPion Inlet™ in three-dimensional space.

Configuration and Application Ideas for SPion System™

Some configuration and application ideas for SPion System™ are presented below. SPion System™ configurations can be easily shifted between these different configurations, thus enabling its use in multiple applications.

Handheld Mass Spectrometry Probes

The most common configuration for using SPion System™ is shown in Figure 2 (left). In this configuration, SPion Inlet™ is used as a handheld probe that can be freely moved in three-dimensional space to reach samples of interest for analysis. In this figure, SPion Tube™'s length is 120cm (4 feet), but SPion Tube™ can be made longer than pictured. Due to variety and diversity of available ion sources, an ion source is not provided with SPion Inlet™, but can be integrated internally or externally upon request. Alternatively, a modified nosecone for SPion Inlet™ (with tapped mounting holes) can be supplied upon request to allow externally mounting custom ion sources by the user.

This configuration is particularly useful in applications where the sample under test is large and immobile, for example, a patient on a surgical bed or a museum object. This configuration is also useful when physical or operational constraints require physically locating the mass spectrometer at a distance from samples, for example, in stand-off detection, luggage and cargo screening, or real-time inline monitoring of manufacturing lines or reaction chambers.

Multimodal Imaging Stations

Another common configuration for using SPion System™ is shown in Figure 2 (right) and Figure 3 (middle and right). In this configuration, SPion Inlet™ is mounted on a 3D robotic stage for computer programmed mass spectrometry imaging at atmospheric pressure. SPion System™ provides ample space for integrating various ionization sources and accessories, such as cameras and light sources. The scan area can be easily scaled to support scanning of larger surface areas by increasing the scan size of the robotic stage. SPion Inlet™ can be modified upon request for reduced pressure mass spectrometry imaging (e.g., reduced pressure MALDI or MALDI-2) to provide increased sensitivity.

This configuration is particularly suitable in applications where custom multimodal imaging stations are being developed and additional space is required for integrating large modules into the imaging station. Generally, mass spectrometry imaging stations are complex systems and are constructed by integrating various optical, mechanical and ion optical components, thus quickly adding to the size and complexity of the system. With the extra space provided around the imaging station, size will not be a constraint in designing and constructing advanced imaging stations, and therefore, additional imaging modalities, such as fluorescence imaging, can be easily added for visualizing dynamic biological processes in real time.

This configuration is also useful in applications where a non-expert user regularly interacts with an imaging station, as in current use of 3D printers. State-of-the-art imaging stations are either embedded inside an instrument, or are tied closely to the front end of the instrument, making them complex and not user-friendly. However, SPion System™ allows the imaging station to be physically separated from the mass spectrometer, thus the mass spectrometer itself can be placed behind a wall with only the imaging station made available to a user. An important application of this configuration is tissue analysis performed in surgical theaters for real-time surgical decision making.

Sample Preparation Product Suites

Proper sample preparation for mass spectrometry analysis is a critical step in many -omics workflows, and many dedicated sample preparation product suites have been developed. SPion System™ can be integrated with a variety of mass spectrometry sample preparation product suites to provide seamless and streamlined mass spectrometry workflows.

The flexibility offered by SPion Tube™ permits SPion Inlet™ to be pointed in any arbitrary direction, including perpendicular to a horizontal surface, thus allowing vertical integration of SPion Inlet™ into autosamplers. When integrated into an autosampler, SPion System™ allows direct infusion mass spectrometry analysis straight from microplates (e.g., 96, 384 and 1536 well-plates), microcentrifuge tubes, or similar sample containers, which are always handled horizontally.

Direct infusion mass spectrometry analysis straight from sample containers reduces the amount of

sample consumption per analysis, and eliminates the need for slow microfluidic transfer of samples to mass spectrometer, thereby, preventing potential carryover issues and speeding up the analysis. Speed of analysis in this configuration is determined by the speed at which the sample and SPion Inlet™ can travel with respect to each other. This speed is much faster than that of microfluidic flow.

The increased analysis speed combined with reduced sample consumption make SPion System™ particularly appropriate for use in single-cell mass spectrometry. With SPion System™, it is also possible to store living-cell-containing microplates in an

incubator while conducting an analysis to continuously preserve cell culture conditions.

In addition to the advantages noted above, SPion System™ enables new configurations to be used that were previously impossible with state-of-the-art mass spectrometry instrumentation. For example, as shown in Figure 3, two SPion Inlets™ each connected to a different mass spectrometer can be coupled to a single ion source. This configuration can be helpful in cases where only a small amount of sample is available, or when the user aims to extract the maximum amount of data from the sample as quickly as possible by employing two different mass analyzer technologies.



Figure 2. Examples in which SPion System™ is being used with a mass spectrometer: (left) SPion Inlet™ as a hand-held probe that can be moved around (ion source not included), and (right) a robotic stage implementation of SPion Inlet™ for imaging applications with laser/ESI ion source. The length of SPion Tube™ shown in these figures is 120cm (4 feet). SPion Tube™ can be made longer than pictured.

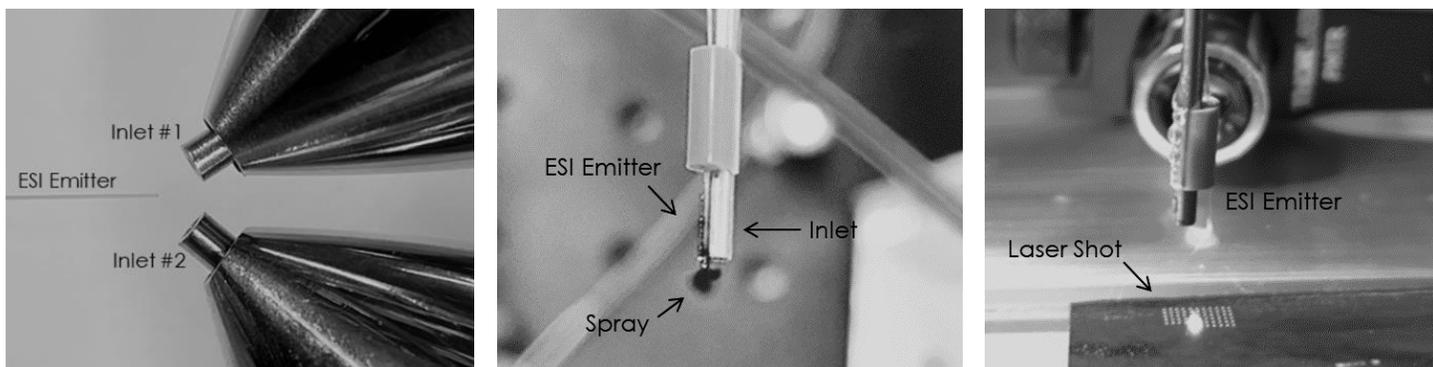


Figure 3. (Left) zoomed-in view of two SPion Inlets™ each connected to a different mass spectrometer coupled to a single ion source. This configuration was previously impossible with state-of-the-art mass spectrometry instrumentation, (middle) zoomed-in view of ESI emitter parallel to the inlet, and (right) zoomed-in view of ESI emitter and laser ablation for imaging applications.

Performance Evaluation

In this section, the results for a proof-of-principle demonstration of SPion System™ used in conjunction with Thermo Fisher's TSQ Altis triple quadrupole instrument are presented. The goal of this study was to benchmark the instrument's performance with SPion System™ front end against that of the instrument's original atmospheric pressure ionization (API) interface with calibration solutions.

Initially, the instrument was calibrated and baselined according to the instrument's calibration procedure. Then, the instrument was vented, and the API interface was replaced with SPion System™ to conduct the experiments. Lastly, the instrument's interface was re-installed, and the instrument's performance was checked (and successfully confirmed) against its original calibration. The instrument was maintained for 24 hours under vacuum prior to acquiring results after venting.

SPion Interface™ used in this study was constructed by modifying the instrument's original API interface. For the modification, the Ion Sweep Cone, the Ion Transfer Tube, the Heater Block, and the RF Lens of the original API interface (refer to TSQ Altis manual for details) were removed. The end of SPion Tube™, made in form of an ion funnel, was extended to reach the Exit Lens of the API interface.

As noted in Table 1, commercial calibration solutions were used in this study in both positive and negative ion modes. Unless otherwise specified, the experimental conditions provided in Table 1 were used in the performance evaluation experiments. In all experiments, the electrospray emitter was placed facing SPion Inlet™.

Table 1. The experimental conditions in performance evaluation.

Electrospray Source

Voltage	3.2KV
Flow rate	1uL/min
Calibration mix.	Pierce™ Calibration Solution (cat. # 88340) Pierce™ FlexMix™ Calibration Solution (cat. # A39239) Pierce™ Triple Quadrupole Calibration Solution (cat. # 88325)
Emitter	Taper tip metal or fused silica emitter facing SPion Inlet™
ESI solution	H ₂ O:MeOH:ACN (50:25:25) mixture with 1% Formic Acid

SPion System™

Frequency	1MHz
RF amplitude	80Vpp
First ring voltage*	0
Last ring voltage*	0
Capillary voltage	0
Capillary	1/16" OD x .030 ID 20cm
Temperature	150°C
SPion Tube™	120cm (47") Length

TSQ Altis™ Mass Spectrometer

Scan type	Full Scan
Scan range	20-2000 amu
Resolution	0.7
Scan rate	1000 amu/second
CID gas	0
Source Pressure	1 Torr
Analyzer Pressure	5.80E-07 Torr
TSQ Altis™ interface	The Ion Sweep Cone, Ion Transfer Tube, Heater Block, and RF Lens (ion funnel) of the interface (refer to TSQ Altis manual for details) were removed and replaced by SPion Tube™.

* First and last rings are the two ring electrodes on two ends of SPion Tube™, the first ring positioned on capillary inlet side and the last ring on the mass spectrometer side.

Signal Stability with ESI Solution and the Corresponding Spectrum

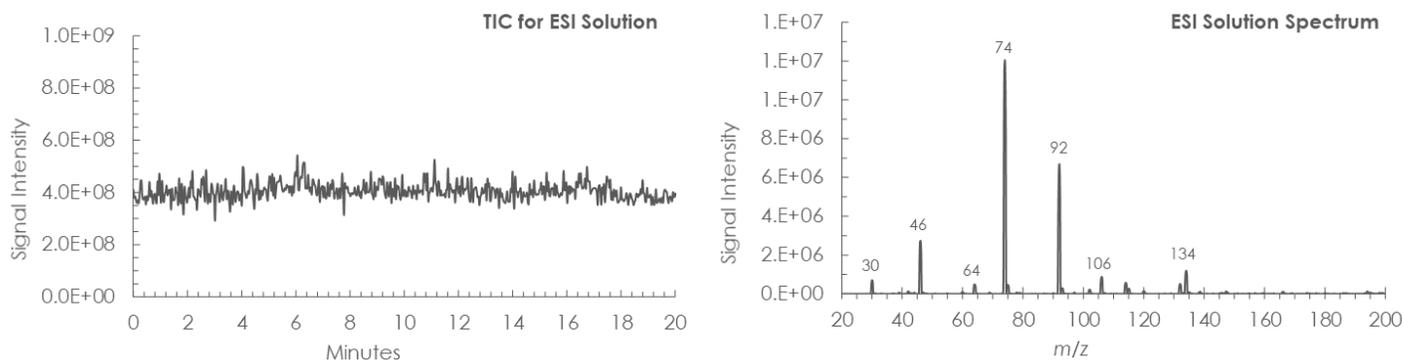


Figure 4. Measurement of total ion current from ESI solution during a 20-minute period (left) and the corresponding mass spectra (right). No major peaks were observed beyond m/z of 200. No solvent clusters were observed.

Signal Stability with Pierce™ Calibration Solution (cat. # 88340)

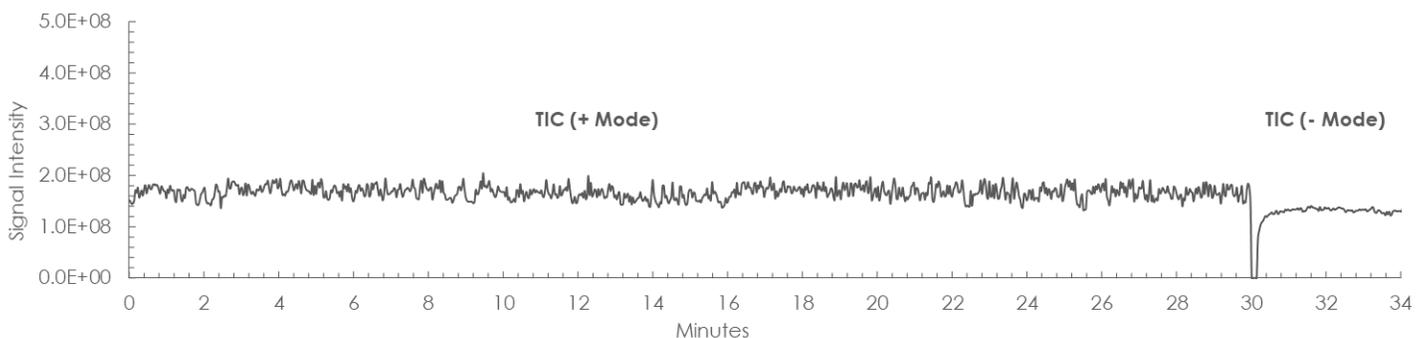


Figure 5. Measurement of total ion current from the Pierce™ Calibration Solution (cat. # 88340) in positive and negative mode. At the 30-minute time mark, the ESI and mass spectrometer were switched to negative ion mode. No changes were made to SPion System™ for polarity switching.

Spectra from Pierce™ Calibration Solution (cat. # 88340)

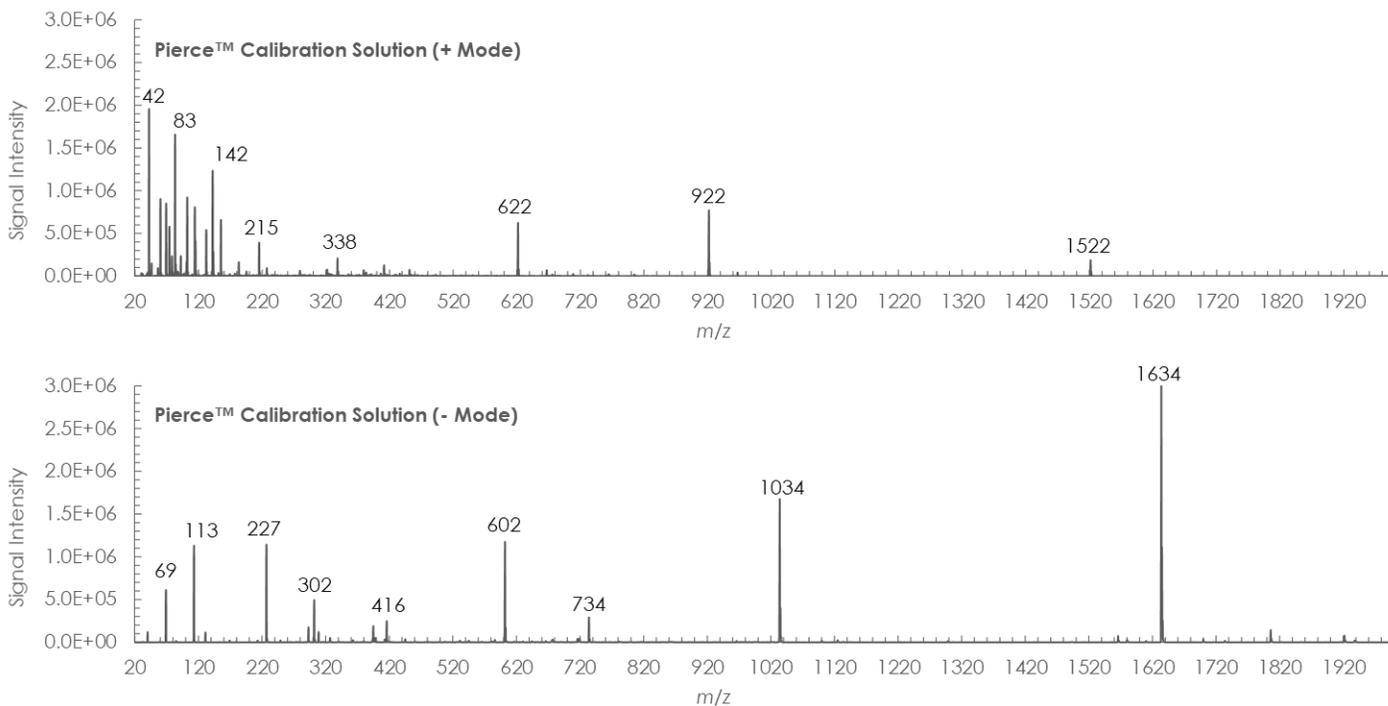


Figure 6. Measurement of mass spectra, as expected, from the Pierce™ Calibration Solution (cat. # 88340) in positive mode and negative mode.

Effect of RF Amplitude on TIC of Pierce™ Calibration Solution (cat. # 88340)

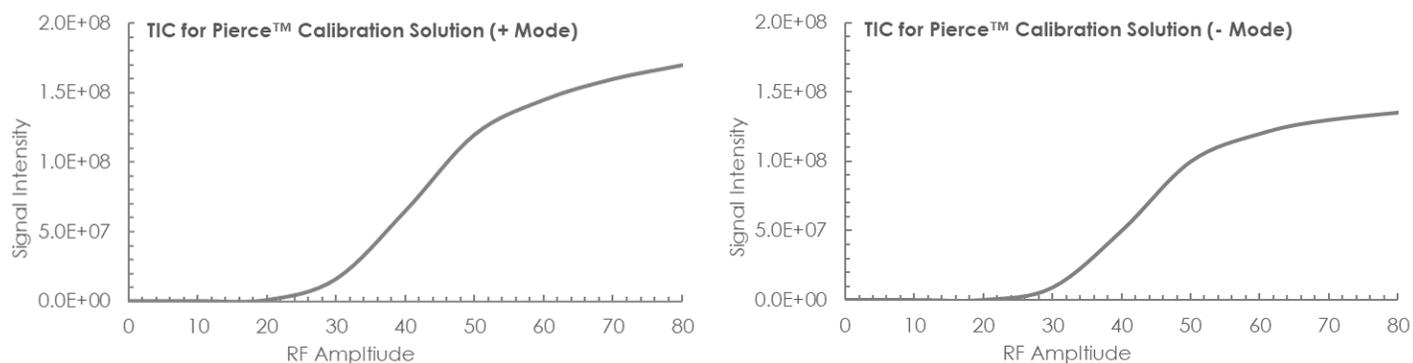


Figure 7. Measurement of total ion current from Pierce™ Calibration Solution (cat. # 88340) in the positive ion mode (left) and the negative ion mode (right) while RF Amplitude is being increased from 0 to 80Vpp.

Effects of Inlet Temperature on Signal Intensity

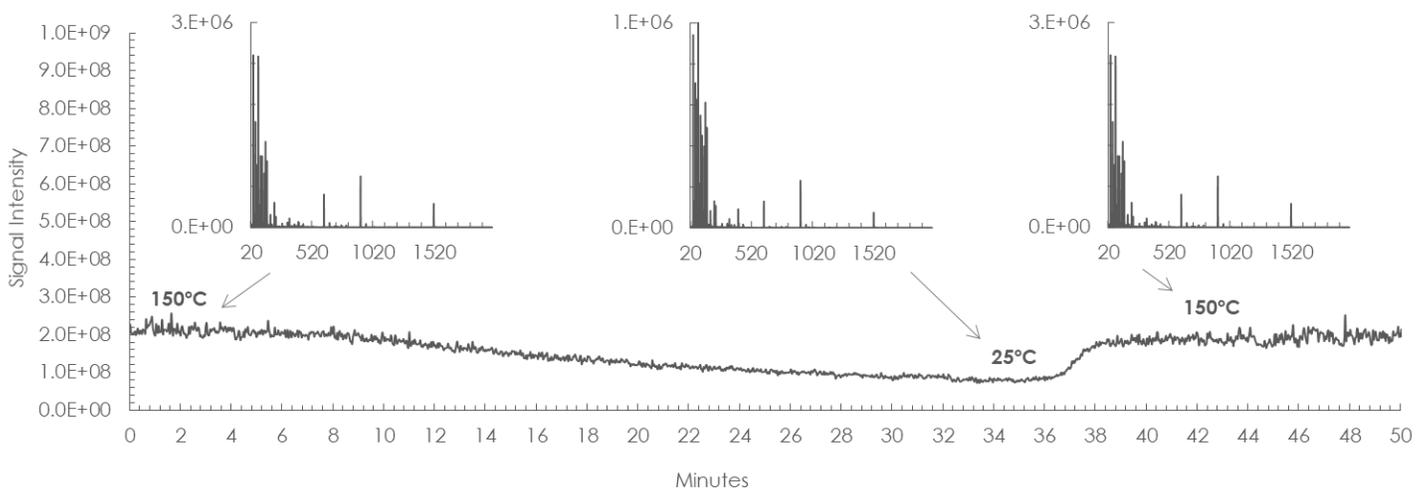


Figure 8. Measurement of total ion current from ESI solution during a 50-minute period. The heater of the capillary inlet was turned on at the 4-minute time mark to allow the capillary inlet to cool to room temperature. At the 36-minute time mark, the heater was turned on again. As expected, the measured mass spectra indicated nearly identical peaks, and no solvent clusters were observed at room temperature.

Effect of RF Frequency on Peak Intensity of Pierce™ Calibration Solution (cat. # 88340)

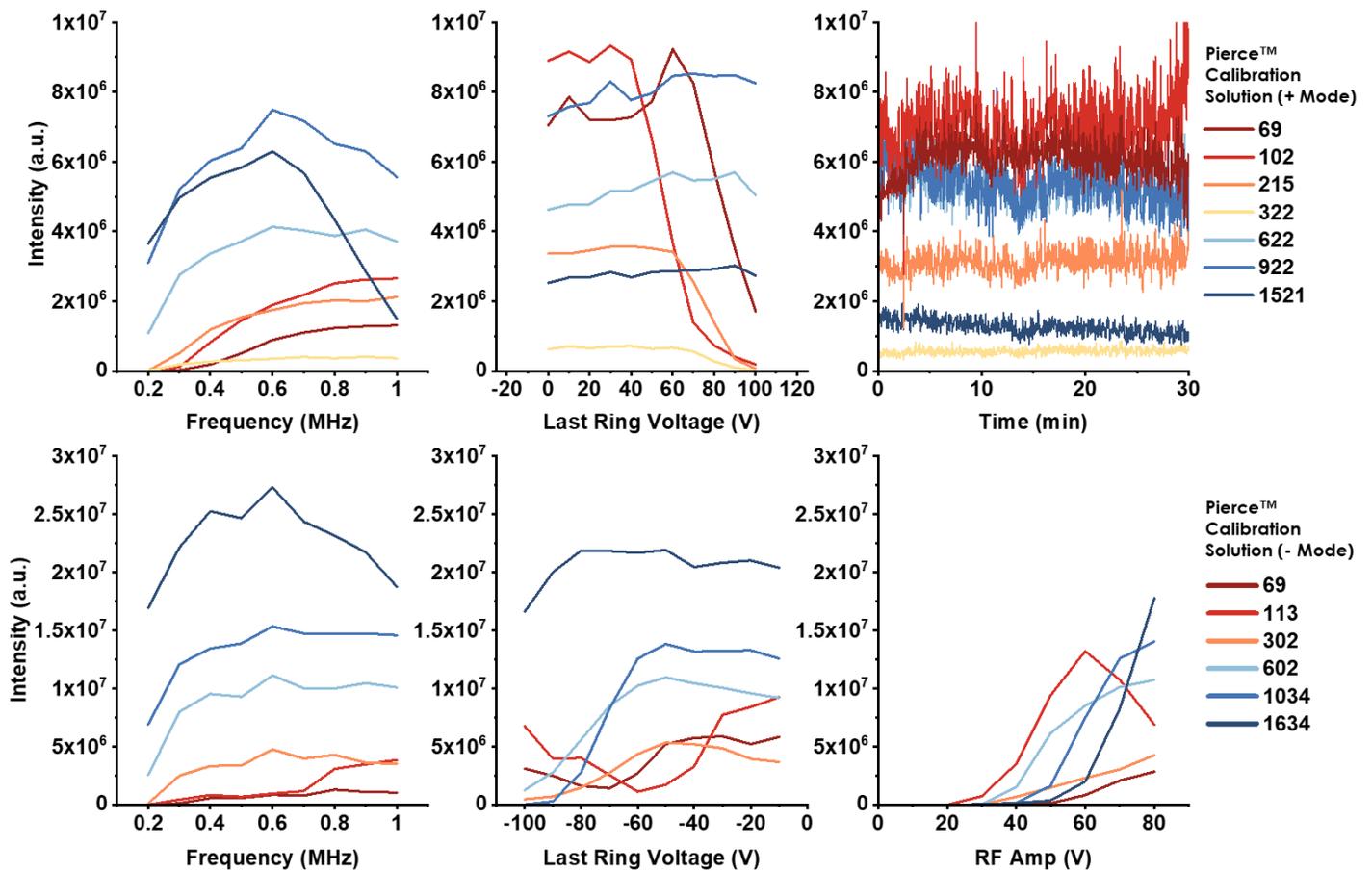


Figure 9. Measurement of individual ion intensities from Pierce™ Calibration Solution in the positive ion mode (top row) and negative ion mode (bottom row). The last ring electrode voltage affected the signal intensities in the low mass range in both positive and negative ion modes.

Spectrum from Pierce™ Calibration Solution (cat. # 88325)

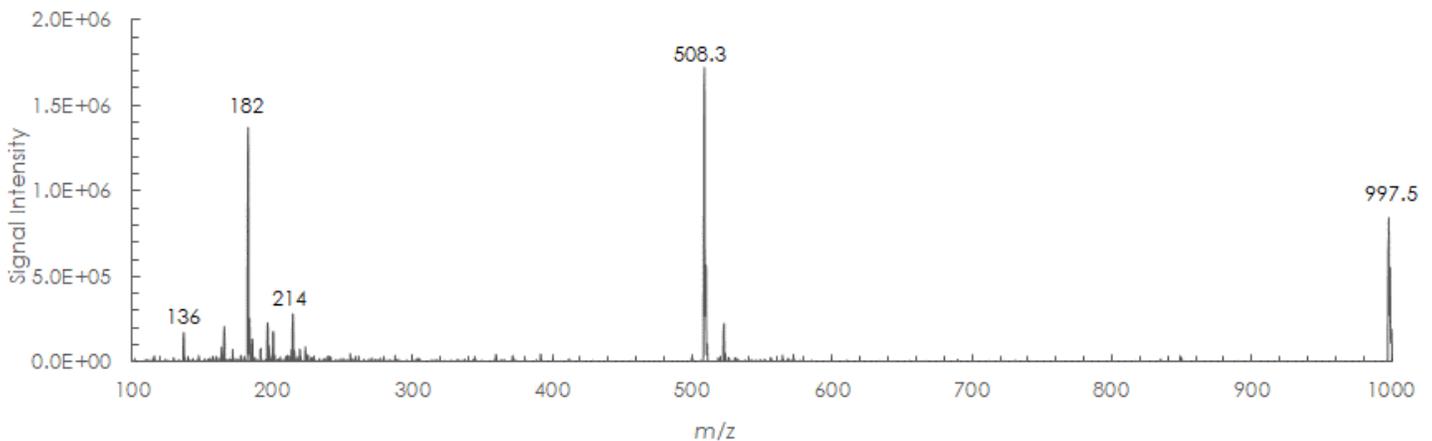


Figure 10. Measurement of mass spectra, as expected, from the Pierce™ Calibration Solution (cat. # 88325) in positive mode. The inlet temperature was maintained at 150C and the RF frequency and amplitude were 1MHz and 80Vpp, respectively. The ESI source flow rate and voltage were 2uL/min and 3.5KV, respectively.

Spectrum from Pierce™ FlexMix™ Calibration Solution (cat. # A39239)

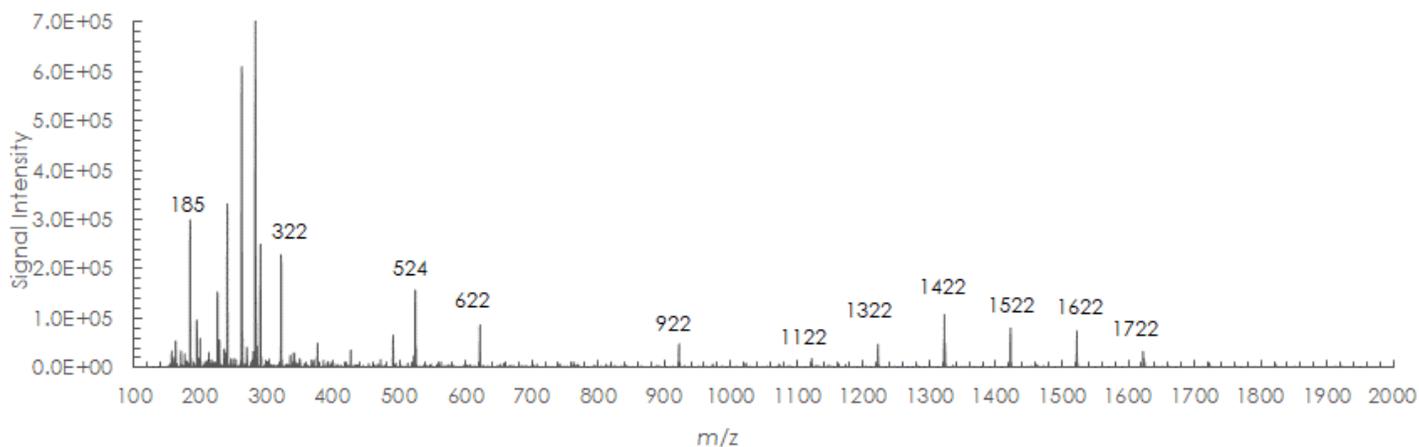


Figure 11. Measurement of mass spectra, as expected, from the Pierce™ FlexMix™ Calibration Solution (cat. # A39239) in positive mode. No fragmentation was observed in the SPion Tube™. ESI source flow rate and voltage were 2 μ L/min and 3.5KV, respectively.

Spectrum from Ubiquitin (8.5KDa) Denatured Charge State Envelope

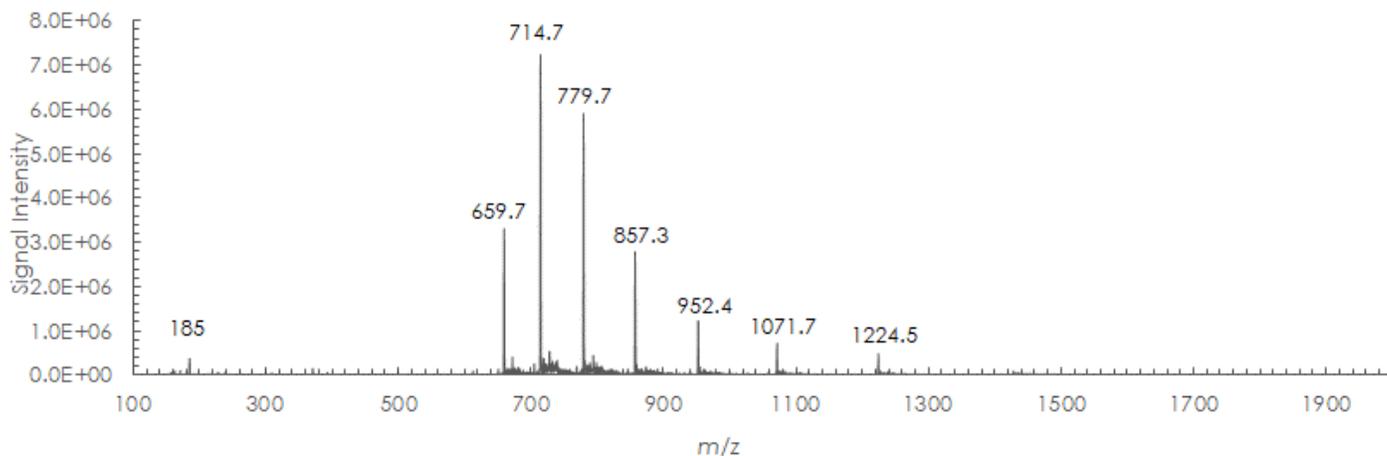


Figure 12. Measurement of mass spectra, as expected, from the ubiquitin solution in positive mode. ESI source flow rate and voltage were 2 μ L/min and 3.5KV, respectively.

Polarity Switching for Potential Charging with Pierce™ Calibration Solution (cat. # 88325)

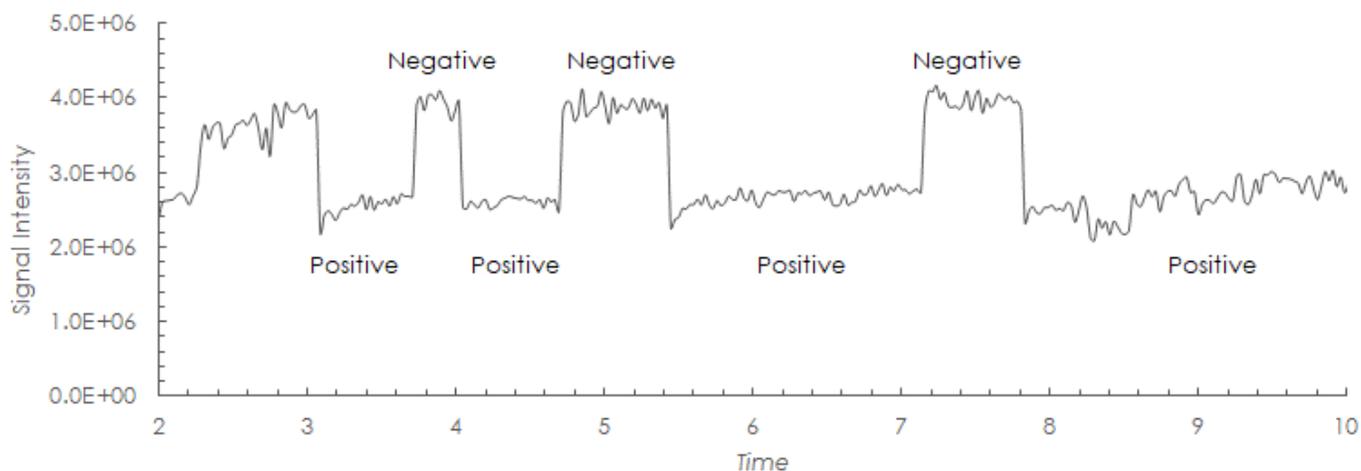


Figure 13. Polarity Switching was performed to study potential charging effects with the Pierce™ Calibration Solution (cat. # 88325). No charging was observed. ESI source flow rate and voltage were 2 μ L/min and 3.5KV, respectively.

Sample Calibration Curves from Serial Dilution of Polytyrosine Solution

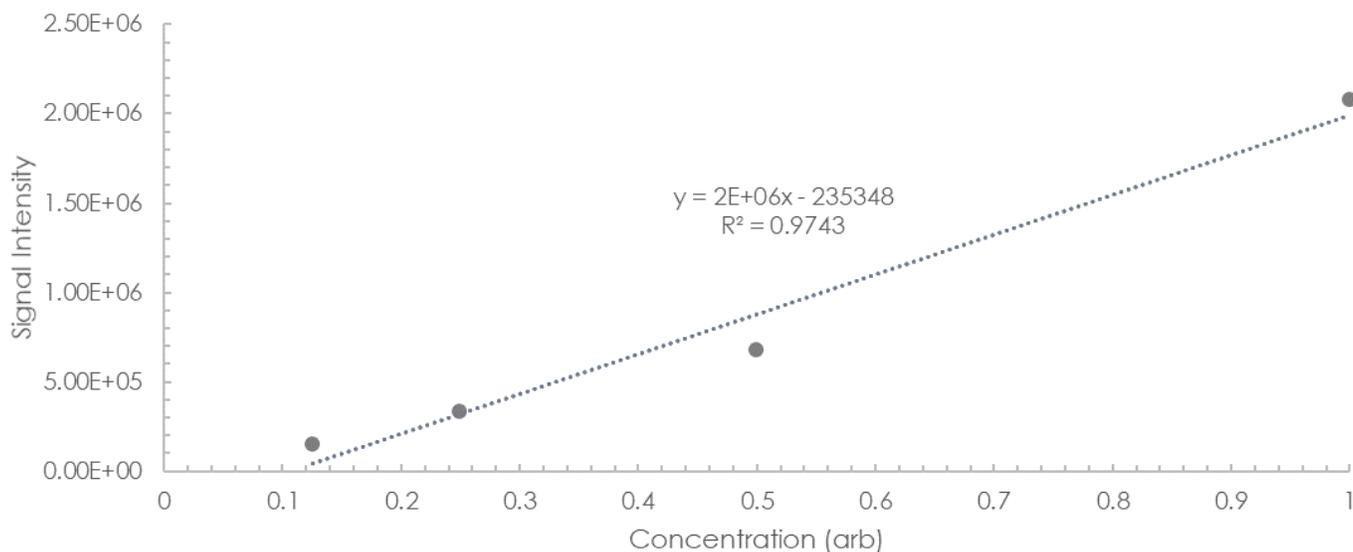


Figure 14. Calibration curve obtained from serial dilution of polytyrosine solution. Polytyrosine solution concentrations were 2.5, 5, 10, and 20 μ M. ESI source flow rate and voltage were 2 μ L/min and 3.5KV, respectively.

Conclusions

SPion System™ defines a new architecture for the front end of a mass spectrometer. By overcoming a major instrumentation bottleneck, SPion System™ offers endless opportunities for scientists and researchers to develop and introduce new generations of advanced front end peripheral technologies for mass spectrometry that were not possible to develop before.

With SPion System™, it is now possible to develop a variety of advanced sample-to-mass spectrometer interfaces, including probes, multimodal imaging stations, and sample preparation product suites. The development of these advanced technologies, which is now made possible by SPion System™, will shape the way mass spectrometers are used in the coming years.

SPion System™ offers excellent analytical performance. For proof-of-principle demonstration, SPion System™ was benchmarked against a commercial API interface, and mass spectrometry

results were acquired from standard calibration solutions. The experimental results confirm that:

- SPion System™ efficiently transfers ions in both positive and negative ion modes;
- SPion System™ can efficiently transfer ions from 20 to 8.5KDa;
- no adduct or cluster ions are present in the spectra;
- transfer of ions with SPion System™ does not cause fragmentation;
- compared to the standard TSQ Altis™ API interface, a slight drop in signal intensity was observed that is potentially related to the longer capillary inlet having a narrower inner diameter; and
- moving or bending SPion Tube™ does not affect ion transfer.

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