iFunnel Technology for Enhanced Sensitivity in Tandem LC/MS

Now available on the New Agilent 6490 Triple Quadrupole LC/MS System

Technical Overview

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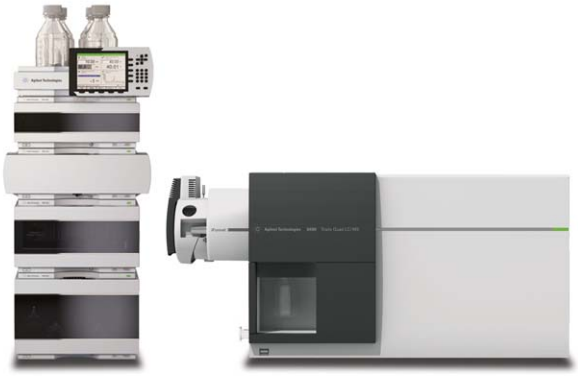

Agilent 1290 Infinity LC System and the new Agilent 6490 Triple Quadrupole LC/MS with iFunnel Technology

Abstract

Electrospray ionization (ESI) has proven to be robust and widely applicable for the analysis of many types of chemical compounds. Researchers have documented that typically far less than 1% of analyte ions produced by ESI enter the mass spectrometer and only one in 10^3 to 10^5 trigger a signal response at the MS detector[1]. This fundamentally limits the sensitivity of LC/MS systems. Agilent's new iFunnel Technology combines the high ESI ion generation and focusing of Agilent Jet Stream technology with a hexabore capillary sampling array, which enables a much larger fraction of the ESI spray plume to enter the mass spectrometer ion optics. A unique dual-stage ion funnel allows increased ion transmission, while evacuating a much higher gas load. This innovative design reduces contamination and neutrals to greatly improve overall system signal and reduce system noise. This technical overview demonstrates the iFunnel Technology on the new 6490 Triple Quadrupole LC/MS System enabling zeptomole (10^{-21} mole) sensitivity for the first time in a quadrupole-based tandem mass spectrometer at conventional flow rates.

Agilent Technologies

Agilent Jet Stream Technology

In the design of the new 6490 Triple Quadrupole LC/MS System, Agilent has taken a holistic approach to improving sensitivity, paying particular attention to gas phase ion formation and their subsequent transfer into the mass spectrometer, while also driving gains in ion transfer efficiency and noise reduction throughout the system.

Photographs of the electrospray plume region reveal at least two reasons why many analyte ions are missed during the process of atmospheric pressure ESI: a) analyte is retained in nebulized spray droplets because of insufficient desolvation and b) many droplets and desolvated ions are moving away from the entrance MS inlet orifice – given that the ions are entrained in atmospheric gas, it is not possible to capture them all using only an electrostatic field.

Agilent Jet Stream (AJS) thermal gradient focusing technology helps to overcome both of these issues and improve sampling by atmospheric ESI. AJS technology uses heat to effectively desolvate droplets created during nebulization in the ion source. A precisely micro-machined sprayer surrounds the droplets in a sheath of superheated gas and creates flow dynamics which concentrate ions in a well-characterized thermal confinement zone for effective sampling by the MS system. Using fast-flowing gas heated to temperatures up to 400 °C confines the ion rich zone to a region about one fifth the size of the spray plume produced by a standard ESI source. Spray confinement is depicted in **Figure 1** which compares the standard ESI plume with the smaller AJS plume. The high gas temperature focuses the spray and produces substantially smaller droplet sizes.

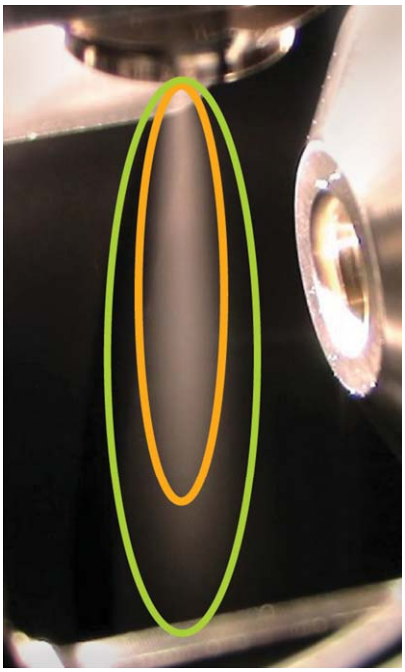

Figure 1. Agilent Jet Stream Spray Confinement
Green= Standard ESI
Orange = Agilent Jet Stream

Comparing signals produced by ESI or AJS demonstrate that signal intensities are several times higher with thermal focusing, as shown in **Figure 2**.

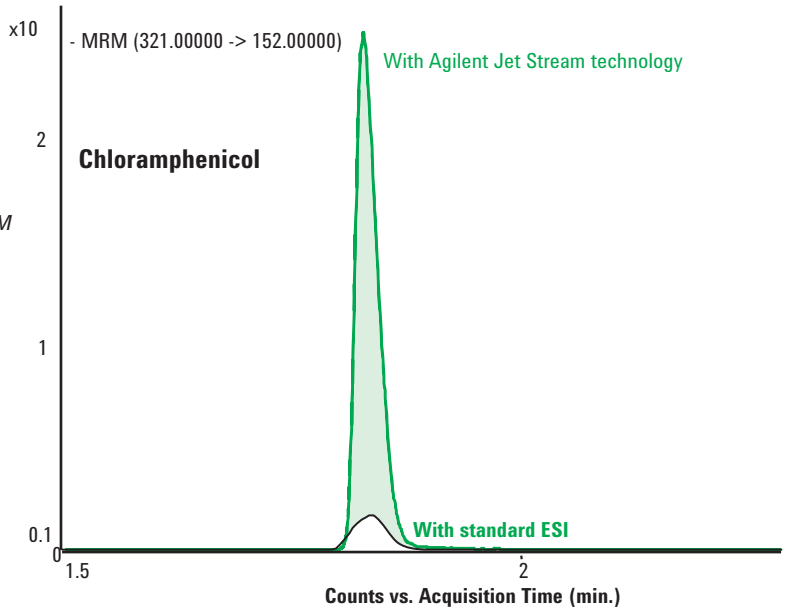

Figure 2. Comparison of the MRM intensity for chloramphenicol spiked into potable water analyzed in negative ion mode. Injected volume was 5 µL of a 50 ppb solution.

Hexabore Sampling Capillary

In spite of these sensitivity gains, further experimentation has revealed that many ions formed in the collimated AJS plume were not captured by the mass spectrometer. Experiments were undertaken to vary the lateral position of the AJS plume in front of the entrance capillary for the mass spectrometer. The data in **Figure 3** show that the area of maximum ion generation occurs across a 3-5 mm horizontal region at the center of the plume. Ions are generally sampled by a single inlet capillary for the MS system. Standard Agilent capillaries have a 600 µm internal diameter.

Greater ion sampling should be possible by increasing the interface area of the capillary inlet within the thermal ion confinement zone. Previous MS systems have tried to increase sensitivity by simply increasing the diameter of a single entrance capillary. It is not surprising that these approaches have showed limited gains because they do not significantly improve ion sampling efficiency – most ions are not spatially accessible and are not transmitted into the mass spectrometer. For large diameter capillaries, gas dynamics change from benign laminar flow to turbulent flow leading to ion losses. (Furthermore, the gas load on the mass spectrometer increases proportional to the capillary diameter to the 4th power, meaning that even nominal increases in diameter create increased atmospheric gas load and a need for extensive new vacuum systems).

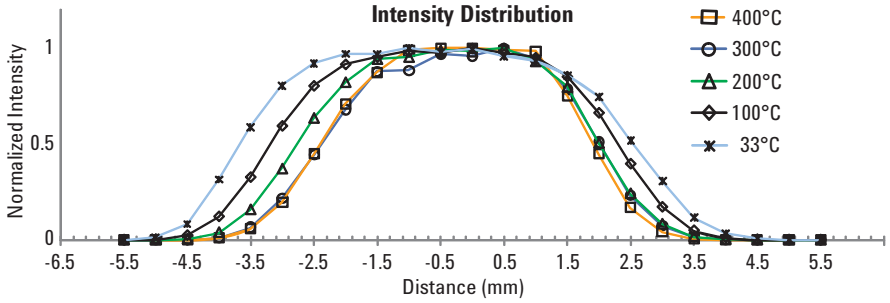

Figure 3. Normalized ion intensities as the spray plume from AJS is moved laterally off-center. The highest ion intensity occurs in a 3-5 mm wide band in front of the sampling capillary.

The alternative approach is the use of multiple capillaries spread across the central, ion rich part of the AJS thermal confinement zone. Multiple capillaries have been shown to increase ion sampling efficiency and maintain good desolvation performance. A circular arrangement of six capillaries, as shown in **Figure 4**, was chosen for the 6490 Triple Quad. The capillaries span a horizontal distance of 3 mm and provide a high efficiency sampling interface for the gas phase ions in the AJS thermal confinement zone. Benefits should also be expected with standard ESI, but the gains are most dramatic with the concentrated ion zone created by AJS.

In addition to increasing the number of capillaries, the capillary length was shortened by 50% to decrease capillary gas flow resistance, minimize high mobility ion loss, and thus further improve atmospheric sampling.

Increasing the number of capillaries increases the gas load on the mass spectrometer in linear proportion to the number of capillaries. The single bore capillary on the Agilent 6460 Triple Quad conducts about 2 L/min of atmospheric gas. The shortened hexabore capillary sampling array conducts about 23 L/min. Many more ions are sampled from the AJS thermal confinement zone, but there is now a requirement to separate them from a large volume of gas.

Figure 4. Hexabore capillary sampling array and orifice plate. The hexabore capillary spans the AJS thermal confinement zone to allow for much greater ion sampling.

Dual Ion Funnel

The solution to this challenge has been achieved with the addition of an ion funnel system – an ion optics element which can be used to efficiently capture and confine highly dispersed ions as well as direct the flow of ions through the initial stages of the MS system, which are under higher gas loads.

A dual ion funnel assembly was chosen. It removes gas in two stages, as shown in **Figure 5**. The initial funnel operates at high pressure and is pumped by a dedicated rough vacuum system, while the funnel voltages and RF propel ions forward, and focuses their trajectories to align with the entrance to the second, low pressure funnel. (Note that the first funnel is offset from the capillary and the inlet of the second funnel to prevent neutral species from entering the downstream ion optics.) The process is repeated with a second low pressure ion funnel. A reduced amount of gas emerges from the second funnel compared with a standard skimmer/Q0 optics assembly and reduces the load on the turbo pump, improving its service life.

The thermal focusing capability of AJS collimates the ESI spray plume so more ions can be captured by capillary assembly. The hexabore capillary sampling array captures a larger solid angle of the spray plume and works in concert with the dual ion funnel, which separates the gas and neutrals from the ions. The combined result of all three elements of iFunnel Technology is a dramatic gain in sensitivity.

Agilent's iFunnel Technology combines unique **Agilent Jet Stream technology** with a **hexabore capillary sampling array** and **dual-stage ion funnel** assembly to increase ion sampling and transmission from the AJS spray plume into the 6490 ion focusing optics.

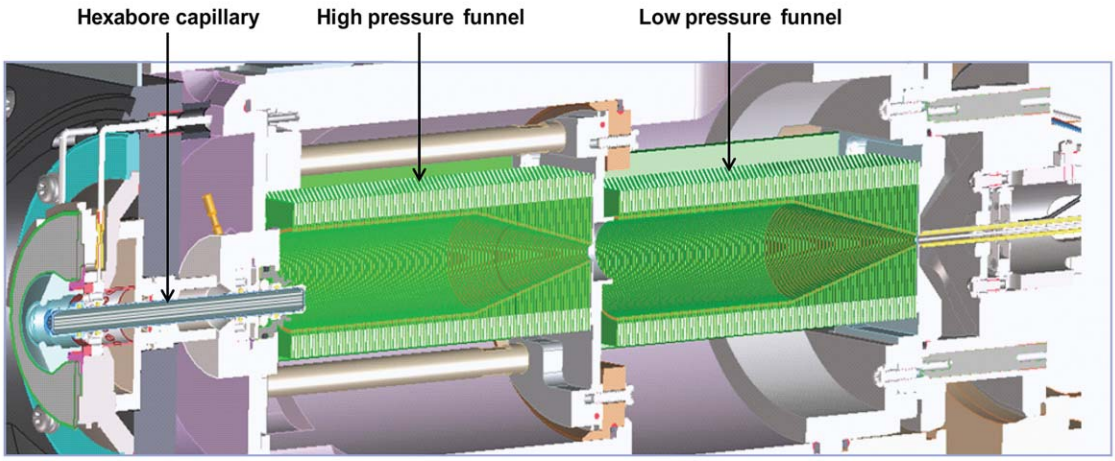

Figure 5. iFunnel dual ion funnel assembly removes atmospheric gas and neutral species, and focuses and directs ions into the low pressure optics of the Agilent 6490 Triple Quadrupole LC/MS.

Sensitivity Gains with the iFunnel Technology on 6490 Triple Quadrupole

The new 6490 Triple Quadrupole with iFunnel Technology shows a ten-fold gain in signal-to-noise for alprazolam relative to the 6460 Triple Quadrupole with AJS and a single sampling capillary (**Figure 6**). Significant gains in sensitivity for many compounds in both positive ion and negative ion modes have been observed with the new 6490 Triple Quad.

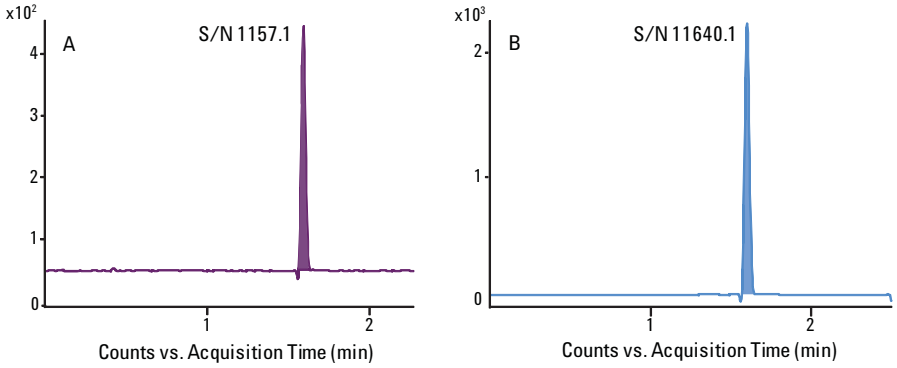

Figure 6. Alprazolam, 1 pg on-column A. Standard 6460 with Agilent Jet Stream: **S/N = 1,157:1** *B. New 6490 with iFunnel Technology:* **S/N = 11,640:1**

Sensitivity gains with iFunnel Technology occur across the mass spectrum as shown in **Figure 7.** The average gain in signal intensity is about 6-fold for positive ESI mode. These gains are typically consistent across the entire mass range of the 6490 Triple Quadrupole.

Increases in ion intensity are even more pronounced for negative ions as shown in **Figure 8**. The average gain in signal intensity was 10-fold across the mass range of the 6490 Triple Quadrupole.

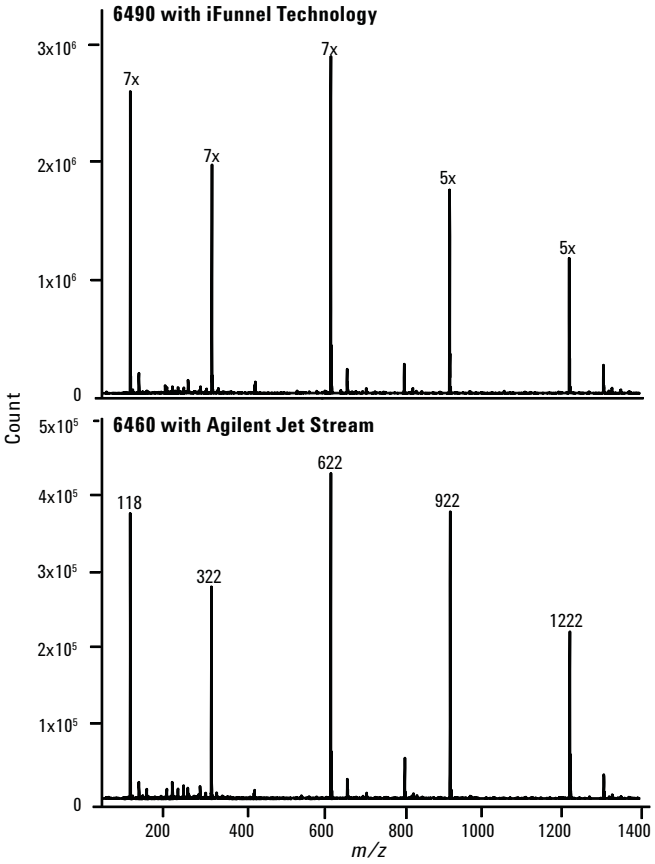

Figure 7. Signal intensities for calibration standards in positive ESI for 6490 Triple Quad (top) and 6460 Triple Quad (bottom) show increases across a wide mass range.

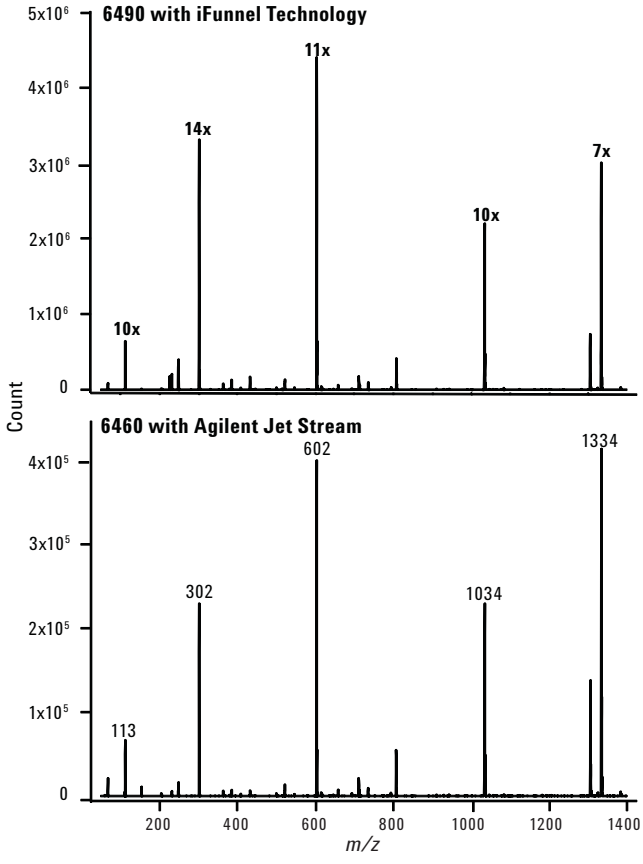

Figure 8. Signal intensities for calibration standards in negative ESI for 6490 Triple Quad (top) and 6460 Triple Quad (bottom) show increases across a wide mass range.

Dramatic gains in signal intensity achieved with **iFunnel Technology** translate into significantly improved limits of detection. Injecting just 100 attograms of verapamil on-column produces a response that is clearly distinguishable from noise (**Figure 9**). The limit of detection in this case is approximately **100 zeptomoles** of verapamil.

Samples in complex matrices such as plasma and urine represent a greater analytical challenge. One solution to increase specificity in bioanalysis is to decrease the isolation window of the precursor ion to exclude potential chemical interferences. This is shown in **Figure 10** for the analysis of 2.5 fg of fluticasone propionate in plasma. The limit of detection (LOD) with the narrow mass window (0.4 *m/z*) is two-fold higher than at lower resolution (0.7 *m/z*) because of a substantial decrease in chemical noise. The LOD at 0.4 *m/z* is about 1 fg.

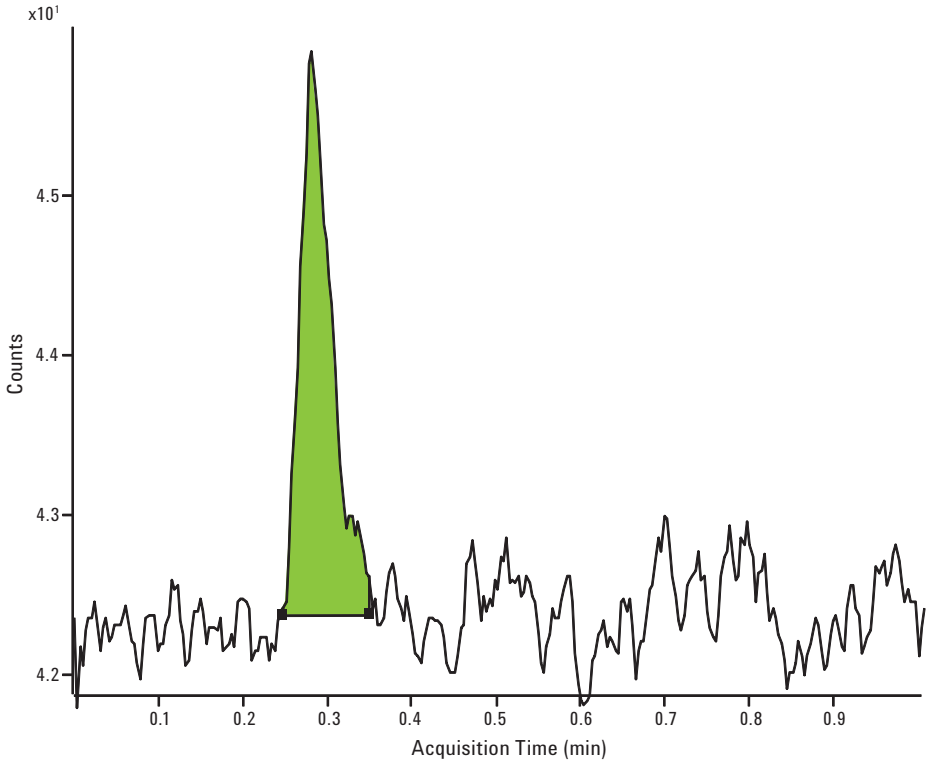

Figure 9. 100 attograms of verapamil, on-column, analyzed with the new 6490 Triple Quadrupole.

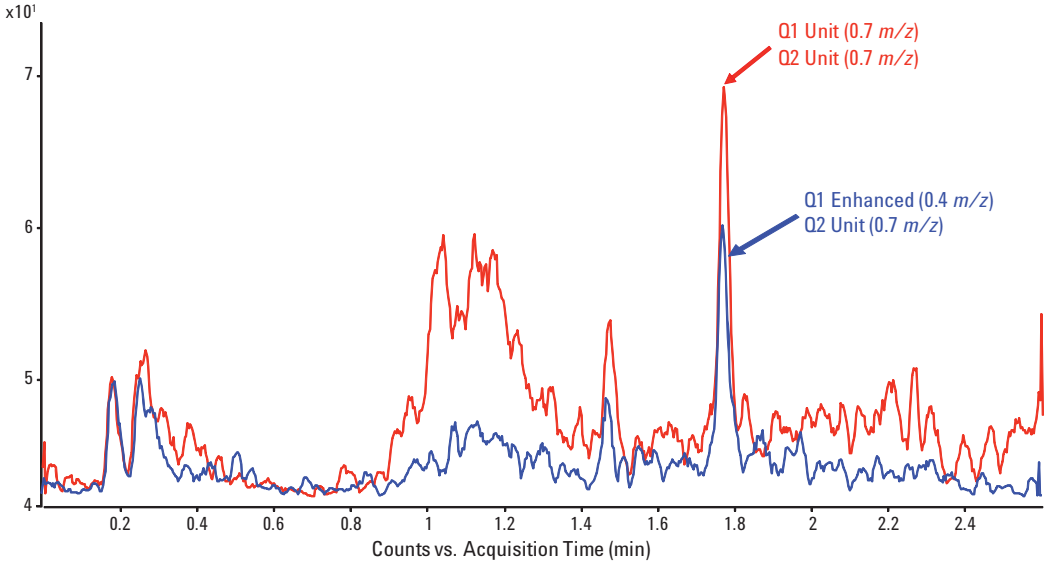

Figure 10. 2.5 fg of fluticasone propionate from a plasma sample. MRM data using a precursor isolation resolution of 0.7 m/z (red) and 0.4 m/z (blue). Narrow isolation reduces non-specific signal and improves detection limits.

Summary

New 6490 Triple Quadrupole with iFunnel Technology

- Significant, stable gains in tandem LC/MS sensitivity
- Dramatic improvements in both positive ion and negative ion sensitivity
- Robust design is resistant to contamination
- Linear dynamic range of up to 6 orders of magnitude
- 0.4 *m/z* Q1 resolution for enhanced precursor isolation
- Zeptomole detection limits for some compounds
- Attogram detection limits for compounds in matrix

References

1. J. S. Page, R. T. Kelly, K. Tang, R. D. Smith, "Ionization and transmission efficiency in an electrospray ionization-mass spectrometry interface," *J. Am. Soc. Mass Spectrom.*, 18:1582-1590, **2007**.

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