# Breaking the Sensitivity Barrier in Triple Quad LC/MS Performance

**Tim Schlabach**

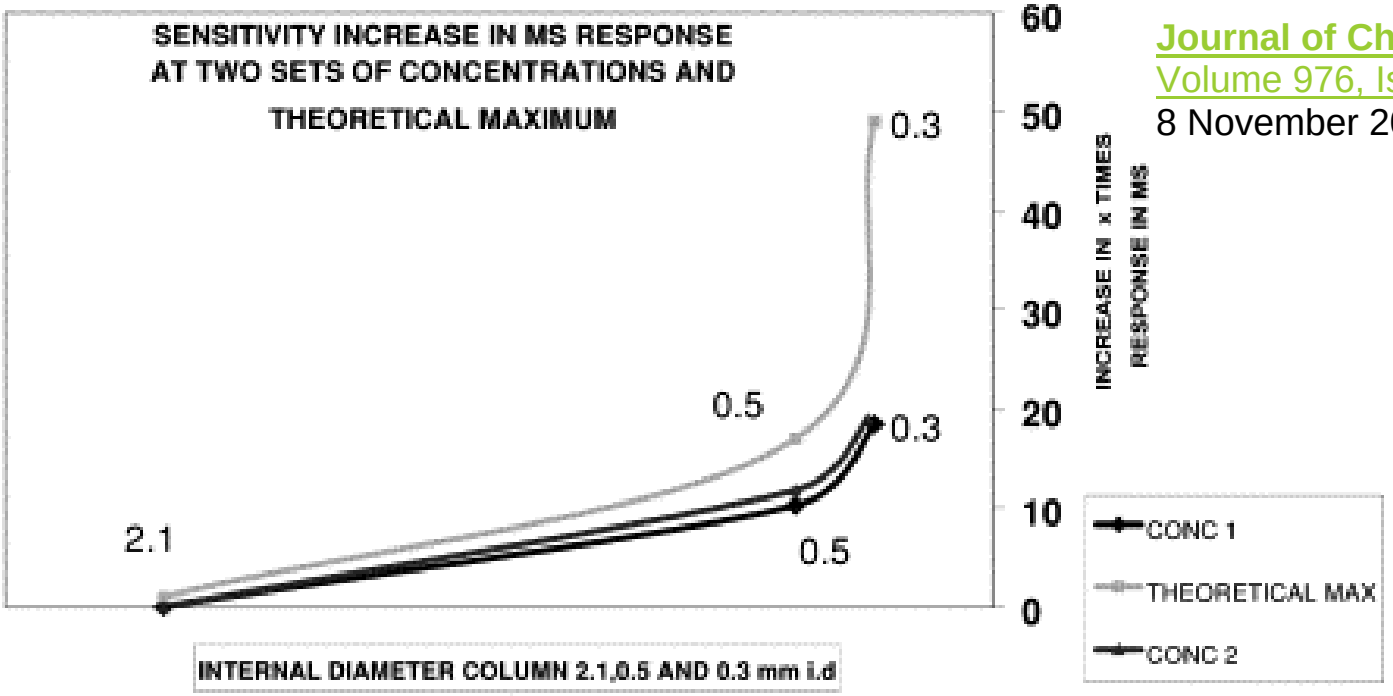
**Alex Mordehai**

**Michael Zumwalt**

# Sensitivity enhancements for ESI-MS

## Reduce the column diameter!

## 30 fold gains in sensitivity achievable



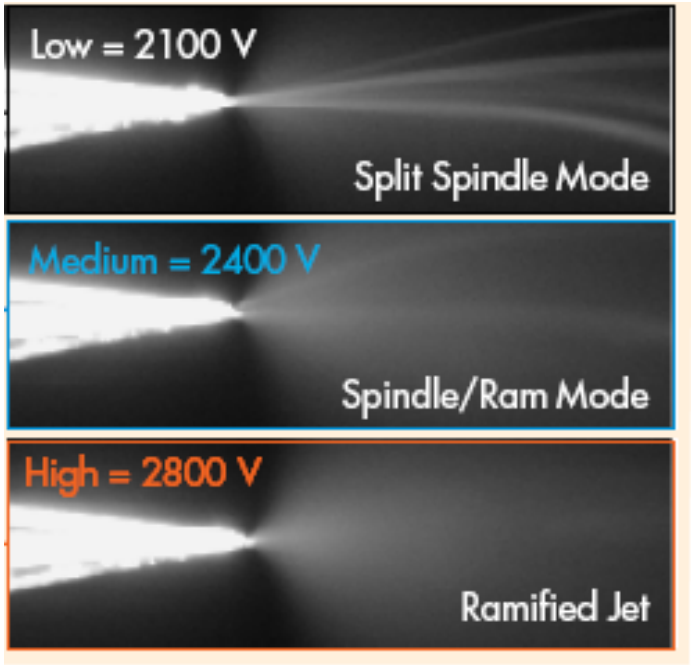

Journal of Chromatography A
Volume 976, Issues 1-2,
8 November 2002, Pages 1 1-1

**Quantification of the sensitivity increase of a micro-high-performance liquid chromatography–electrospray ionization mass spectrometry system with decreasing column diameter**

**C. Legido-Quigley, , a, N. W. Smitha and D. Malletb**
**a Centre for Analytical Sciences, RSC1, Imperial College London, Exhibition Road, London SW7, UK**
**b CEDD DMPK, GlaxoSmithKline R&D, Park Road, Ware, Herts SG1**

# Why is NanoSpray only popular in Proteomics?

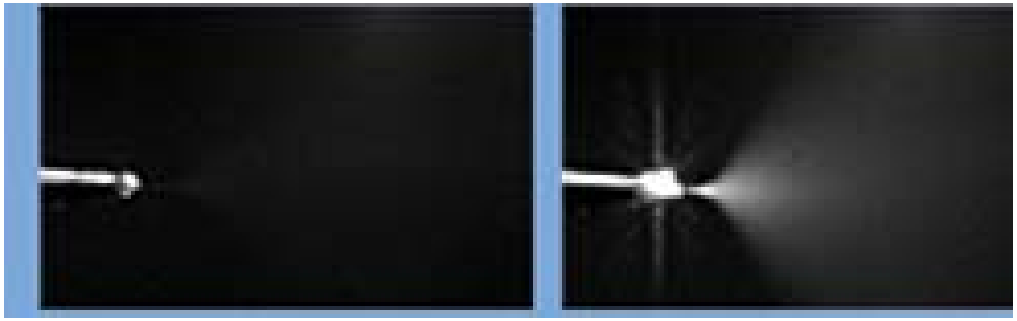
**ASMS Poster TPJ150, 2003**
**by Mike Lee and Gary Valaskovic**




## NanoSpray Issues

- changing spray dynamics
- sputtering spray
- no spray at low organic

**Low Organic** **Higher Organic**



**Practical High Sensitivity LC-MS**
**by Gary Valaskovic**

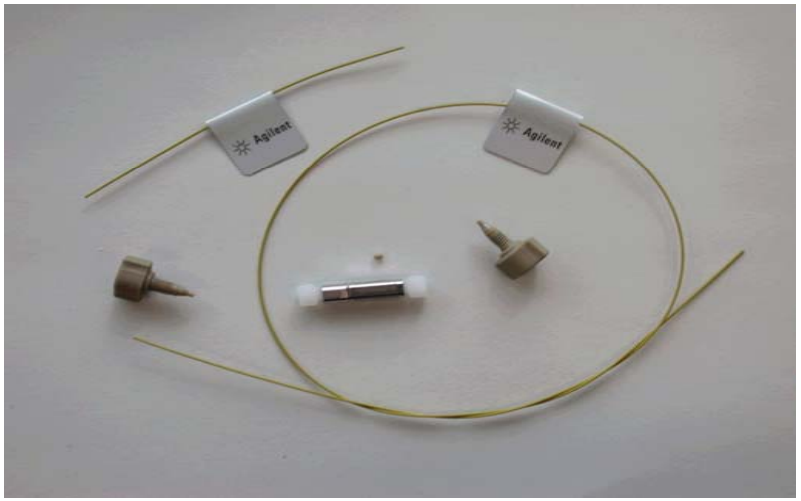
# Physical challenges of nanospray

**Nanoelectrospray LC/MS is challenging to implement :**

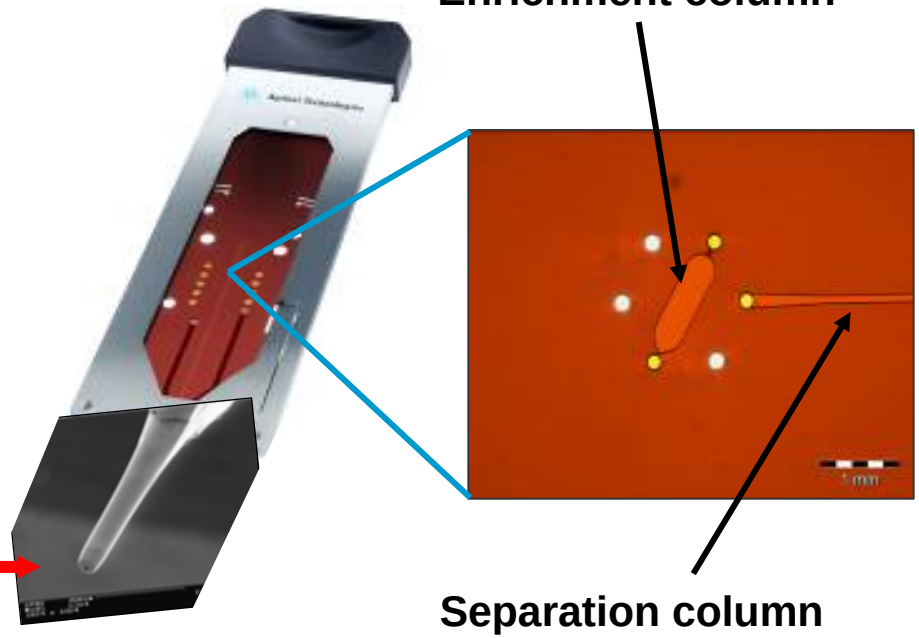
- frequent clogging
- leaks at the column and connections
- chromatographic degradation caused by tubing dead volume
- Not easy to trouble shoot problems
- Not ROBUST.

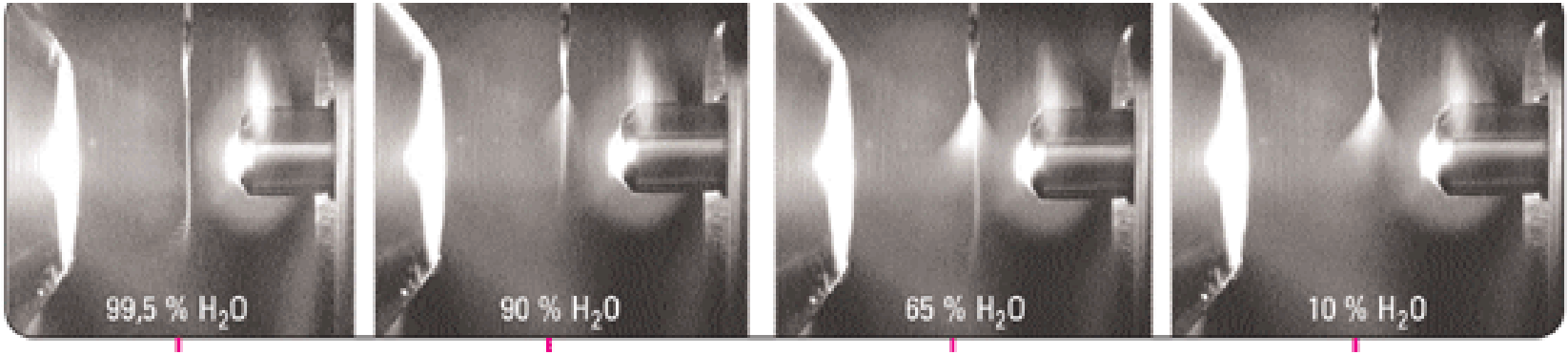
**Nano LC Column**

**Nano Fittings/Connectors**



# HPLC-Chip Triple Quad LC/MS



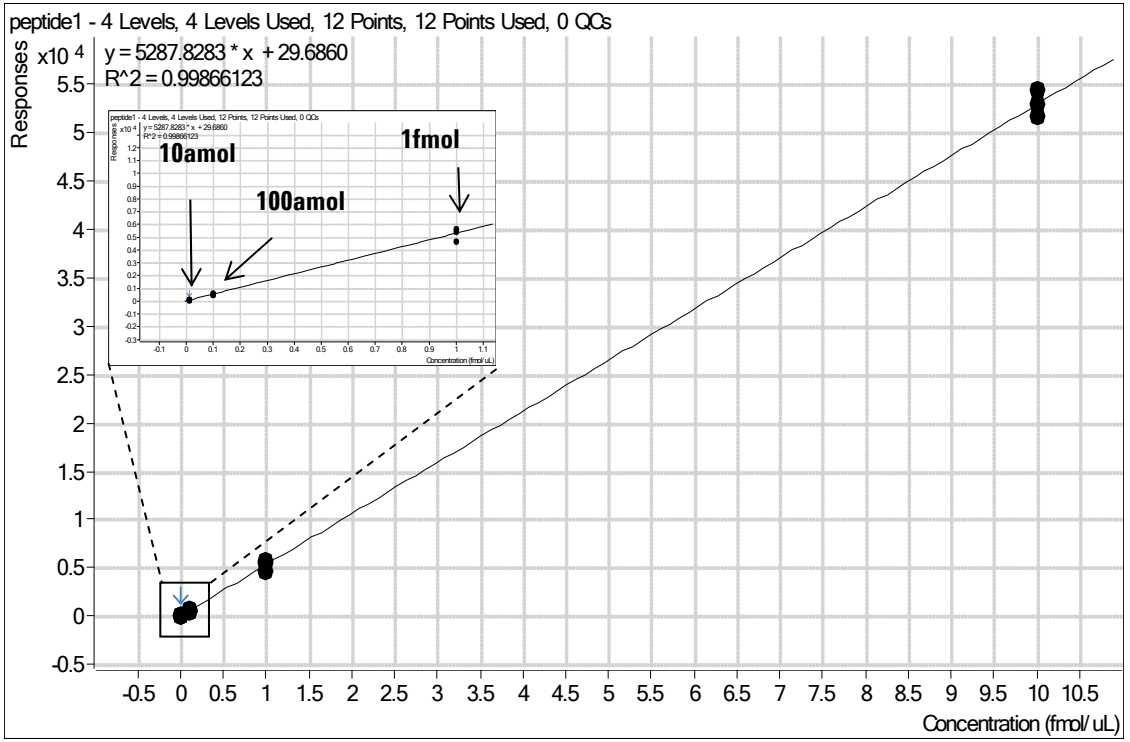




**Stable spray at 99.5 to 10% Aqueous**

# External quantitation curve of peroxidase peptide DTIVNELR from 10 amol to 10 fmol spiked in human serum

- External calibration
- Linear fit
- 3 order of magnitude
- $R^2 > 0.998$
- Lowest amount on column is 10 amol
- Peptides spiked into depleted human serum (1 ug on column)

**Peptide LOQ in serum matrix: 10 femotgram!**

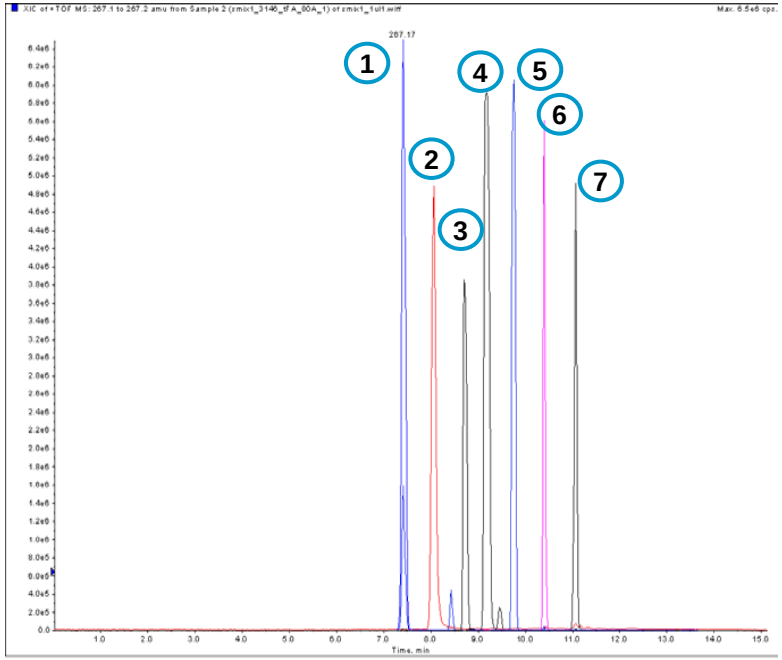


# Small molecule chip:

## Useful over a wide polarity range using Zorbax C-18 SB 80A

**amount injected: 100-400 pg**
**1 uL injection volume**

| Compound | logP | m/z (+1) |
|---|---|---|
| Attenolol | 0 | 267 |
| Caffeine | | 195 |
| Quinine | | 325 |
| Atropine | | 290 |
| Metoprolol | | 268 |
| Propranolol | | 260 |
| Imipramine | 4.2 | 281 |



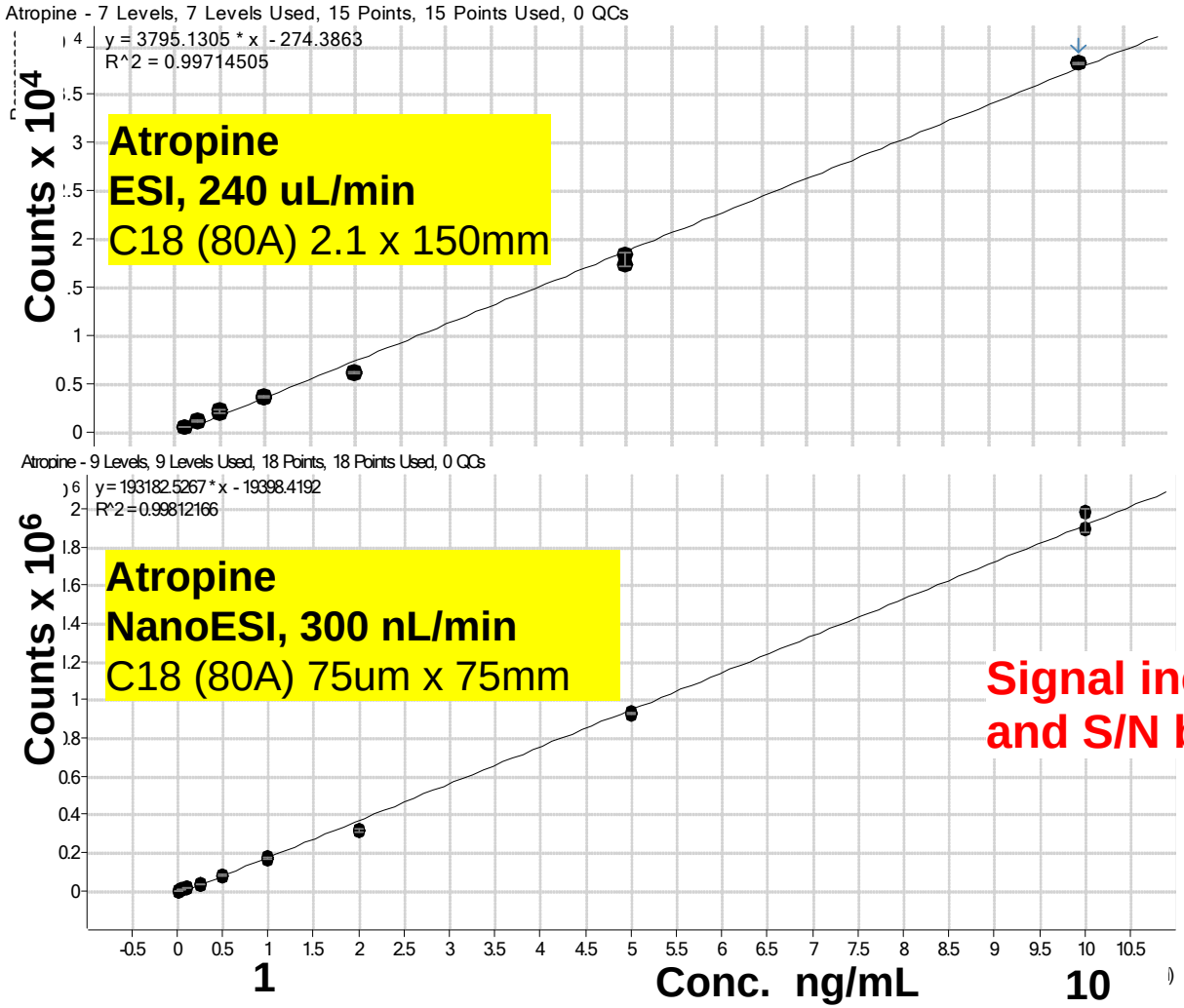

**Optimized XXL enrichment column**

# Nanoelectrospray retention time and area precision for HPLC-Chip Triple Quad LC/MS

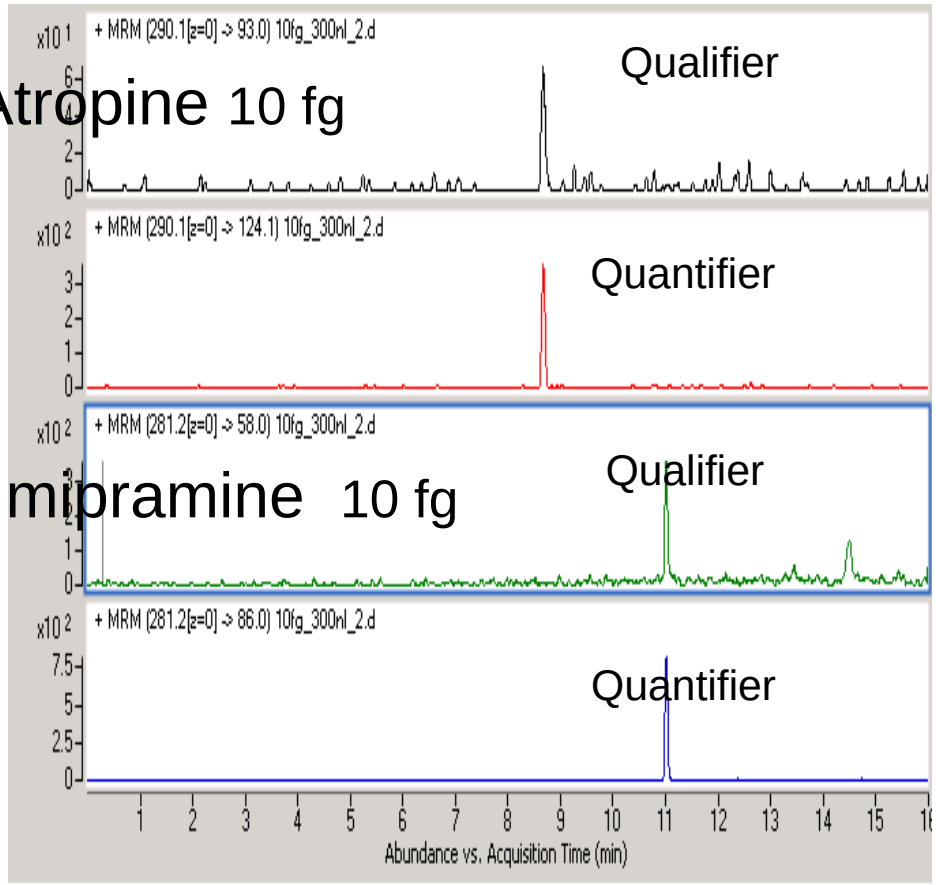
| | | Atenolol | | Atropine | | Metoprolol | | Imipramine | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. abs. | Acq. Date-Time | RT | Area | RT | Area | RT | Area | RT | Area |
| | | | | | | | | | |
| 1000fg | 2007-8-22 4:56 AM | 6.88 | 95529 | 8.52 | 443254 | 9.06 | 93983 | 10.82 | 273000 |
| 1000fg | 2007-8-22 5:17 AM | 6.86 | 102953 | 8.53 | 448162 | 9.06 | 83812 | 10.82 | 217364 |
| 1000fg | 2007-8-22 5:38 AM | 6.90 | 92080 | 8.50 | 470489 | 9.04 | 82884 | 10.80 | 223430 |
| 1000fg | 2007-8-22 5:59 AM | 6.89 | 96450 | 8.51 | 450958 | 9.04 | 83632 | 10.81 | 219541 |
| 1000fg | 2007-8-22 6:20 AM | 6.89 | 92895 | 8.51 | 438218 | 9.03 | 83738 | 10.83 | 231995 |
| 1000fg | 2007-8-22 6:41 AM | 6.90 | 88769 | 8.51 | 467724 | 9.04 | 82322 | 10.82 | 220997 |
| 1000fg | 2007-8-22 7:02 AM | 6.87 | 95403 | 8.51 | 447268 | 9.04 | 80319 | 10.81 | 257066 |
| 1000fg | 2007-8-22 7:23 AM | 6.91 | 90578 | 8.51 | 459767 | 9.04 | 80128 | 10.81 | 257715 |
| 1000fg | 2007-8-22 7:44 AM | 6.90 | 96525 | 8.51 | 465399 | 9.04 | 80480 | 10.82 | 248859 |
| 1000fg | 2007-8-22 8:05 AM | 6.91 | 95338 | 8.51 | 421649 | 9.05 | 79537 | 10.81 | 238361 |
| **Average** | | 6.89 | 94652 | 8.51 | 451289 | 9.04 | 83084 | 10.82 | 238833 |
| **Stdev** | | 0.01 | 3920.88 | 0.01 | 15091.37 | 0.01 | 4168.93 | 0.01 | 19414.74 |
| **RSD %** | | **0.21%** | **4.1%** | **0.08%** | **3.3%** | **0.10%** | **5.0%** | **0.07%** | **8.1%** |

# Enhanced sensitivity: NanoElectroSpray Ionization

## 30 fold increase in sensitivity with 75 u columns



# 6410 with HPLC Chip Cube
## breaking the Femtogram Barrier




Nanoelectrospray,
300 nL/min
1 uL injection

- LODs in solvent **< 1 fg**

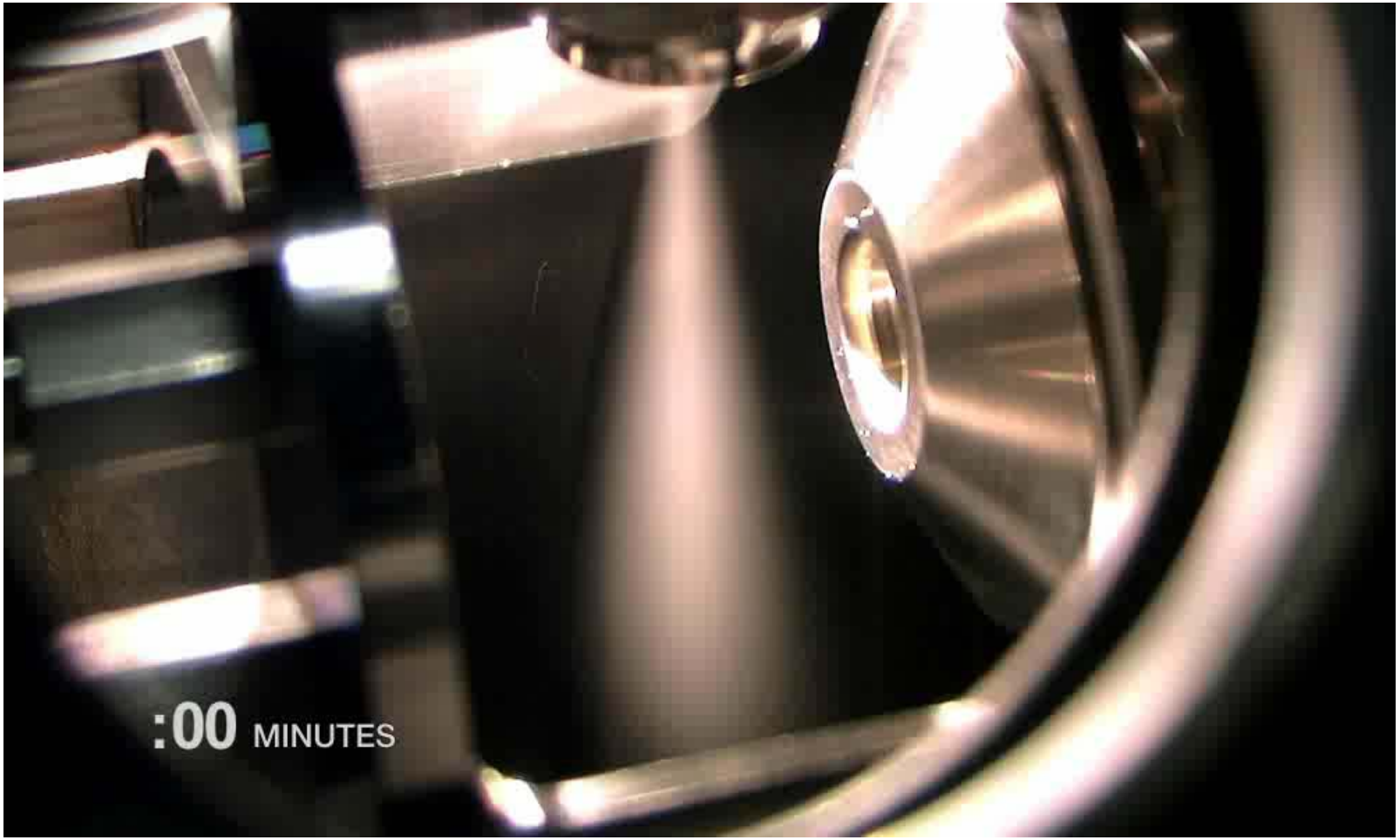
# 6400 Agilent Triple Quad LC/MS Family

**New 6460 Triple Quad LC/MS**
**> 5 x higher sensitivity with**
**Agilent Jet Stream Technology**
**100 millisec pos/neg switching**
**mass range to 3,000 m/z**

**Agilent Jet Stream Technology**

**6410B Triple Quad LC/MS**
**500 msec polarity switching**
**mass range to 2,000 m/z**

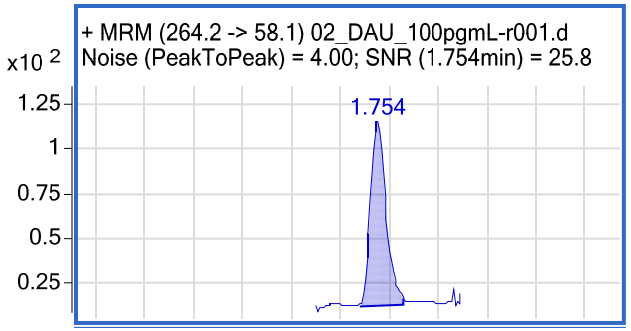
# Agilent Jet Stream Technology



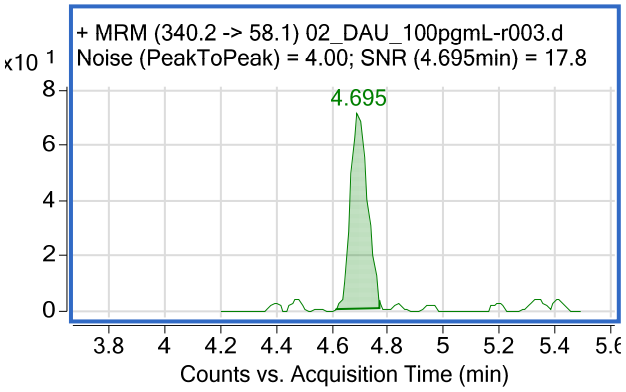
# 6460 Triple Quad compared to 6410 Triple Quad

## 6410 Triple Quad: 200 femtograms of each compound injected on-column

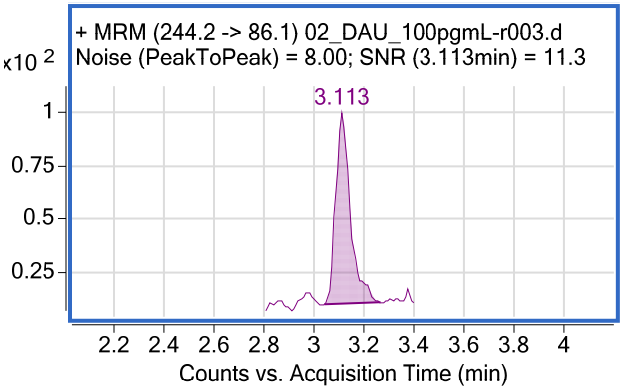
Tramadol **(LOD 23 fg)**




Propoxyphene **(LOD 33 fg)**

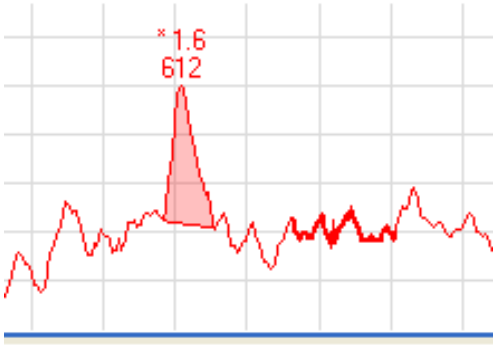



Phencyclidine **(LOD 54 fg)**



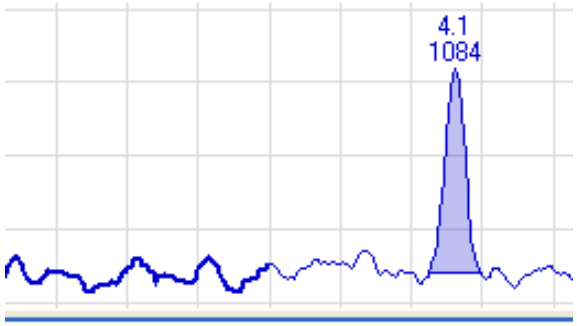

## 6460 Triple Quad: 5 femtograms of each compound injected on-column

Tramadol **(LOD 3 fg)**




**6460 8 x more S/N**

Propoxyphene **(LOD 2 fg)**



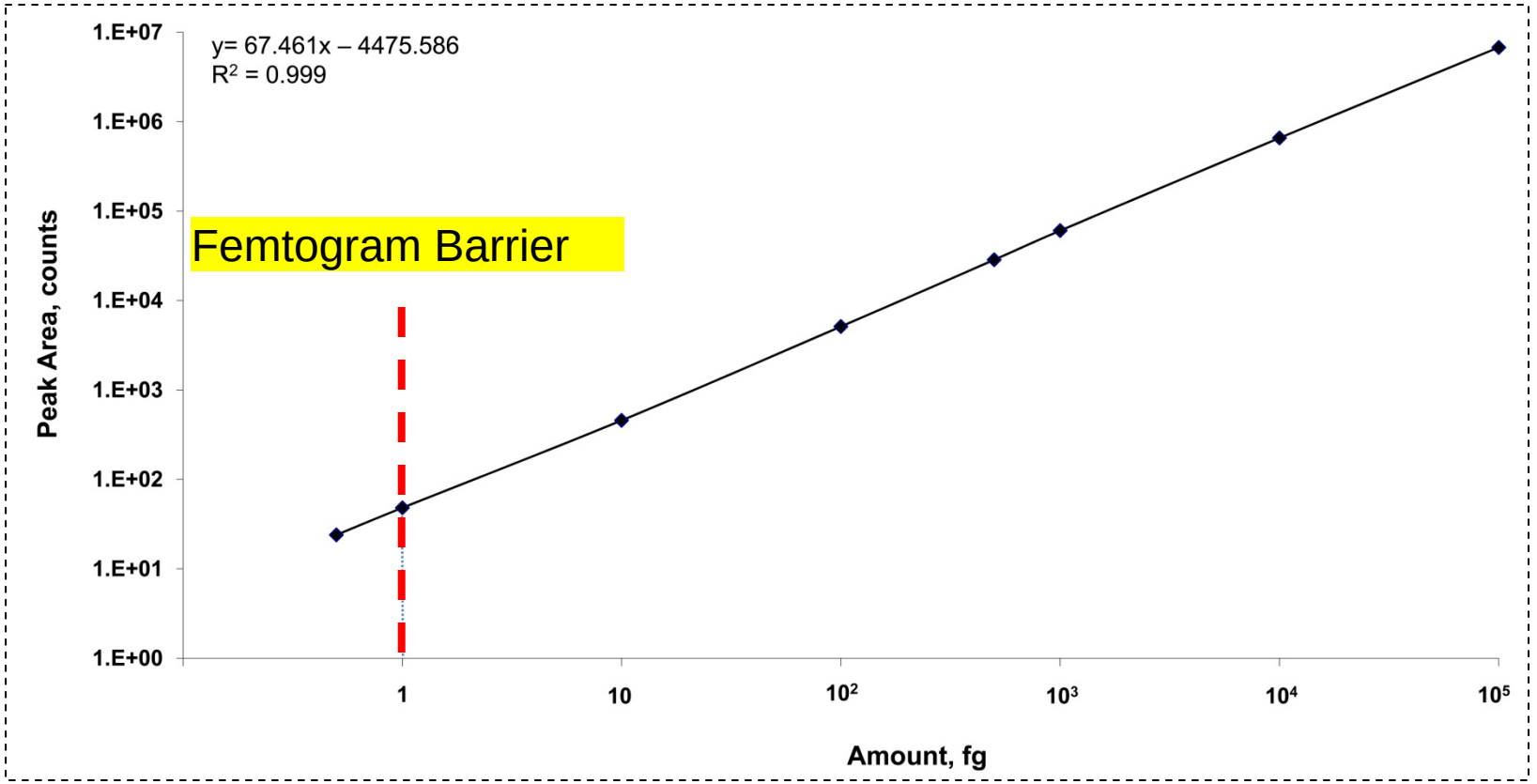

**6460 16 x more S/N**

Phencyclidine **(LOD 3.5 fg)**

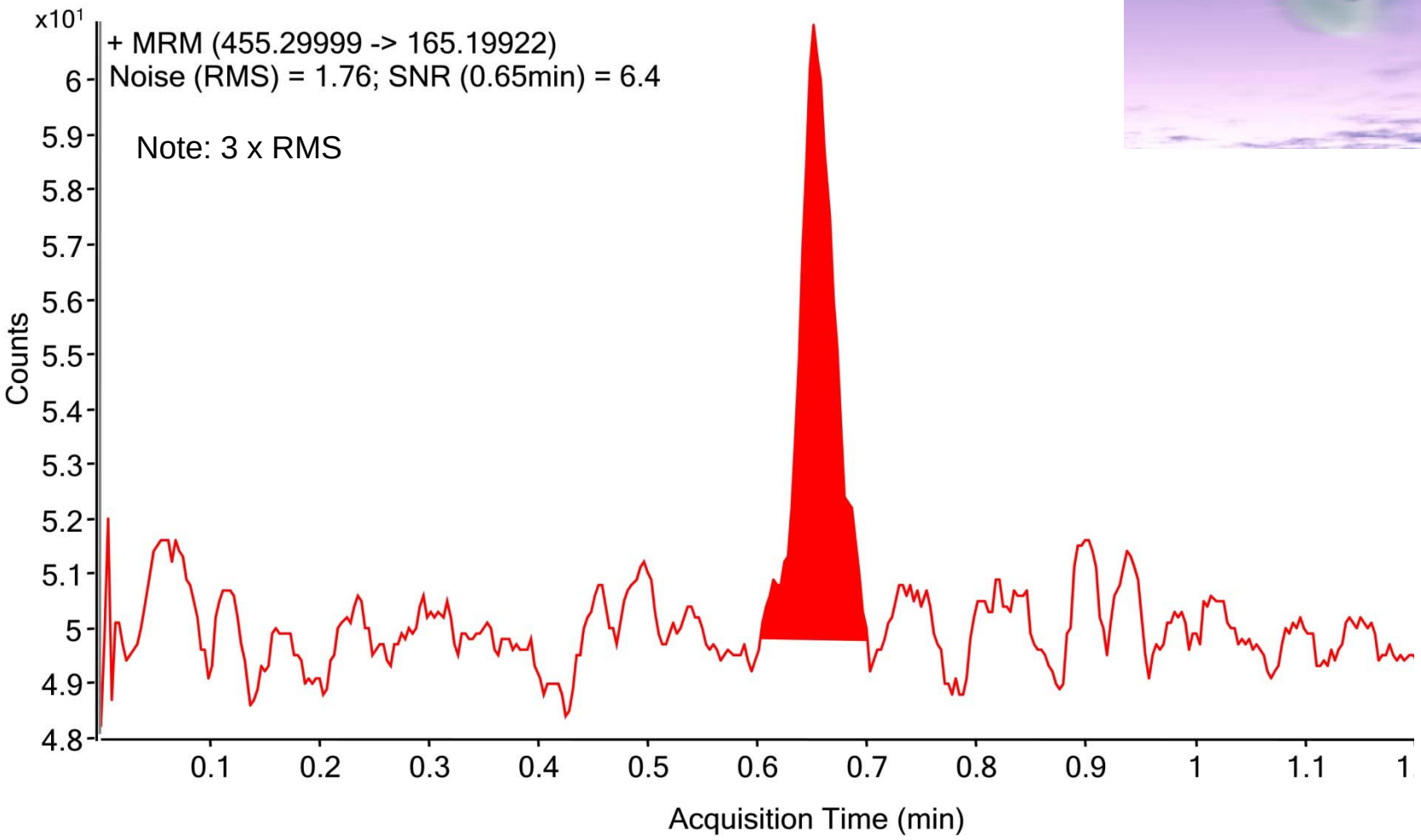

**6460 15 x more S/N**

# Five Orders of linearity with Agilent's 6460 Triple Quad with Agilent Jet Stream technology

**Outstanding linearity and sensitivity from sub-femtogram to 100 pg verapamil**



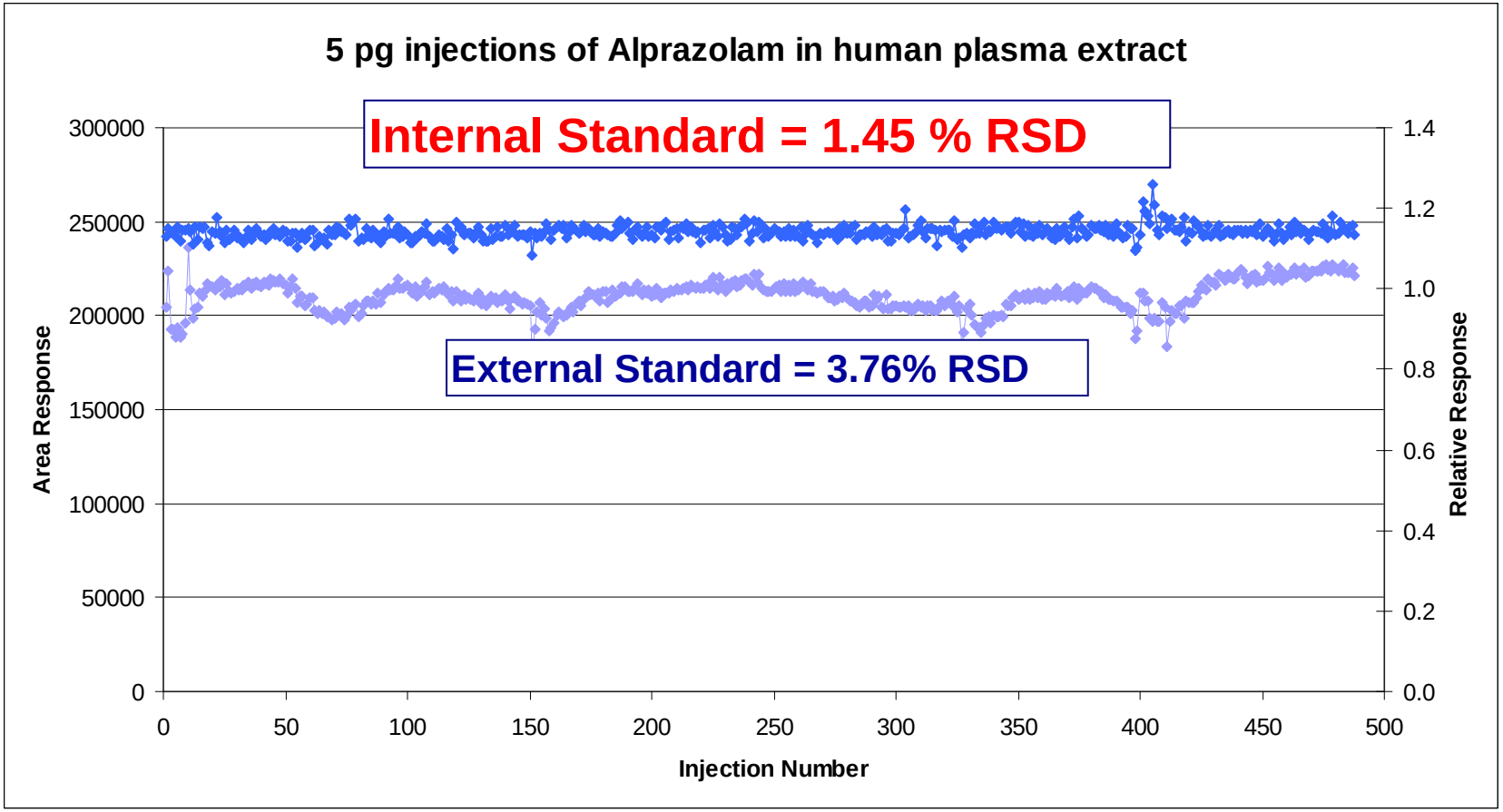
# Breaking the fg sensitivity barrier
## 500 Attograms verapamil on-column



# Agilent Jet Stream Performance

## *Ruggedness & Reproducibility – 6460 QQQ*

500 Injections of Alprazolam in Spiked Human Plasma Extract, ~ 10hrs.



# Agilent Jet Stream Fundamentals

Alex Mordehai,

R&D manager

LC/MS Division

Agilent Technologies

Santa Clara, CA

# Our Agenda…

- Product specifications.
- Learn about Agilent Jet Stream Technology

# Agenda…

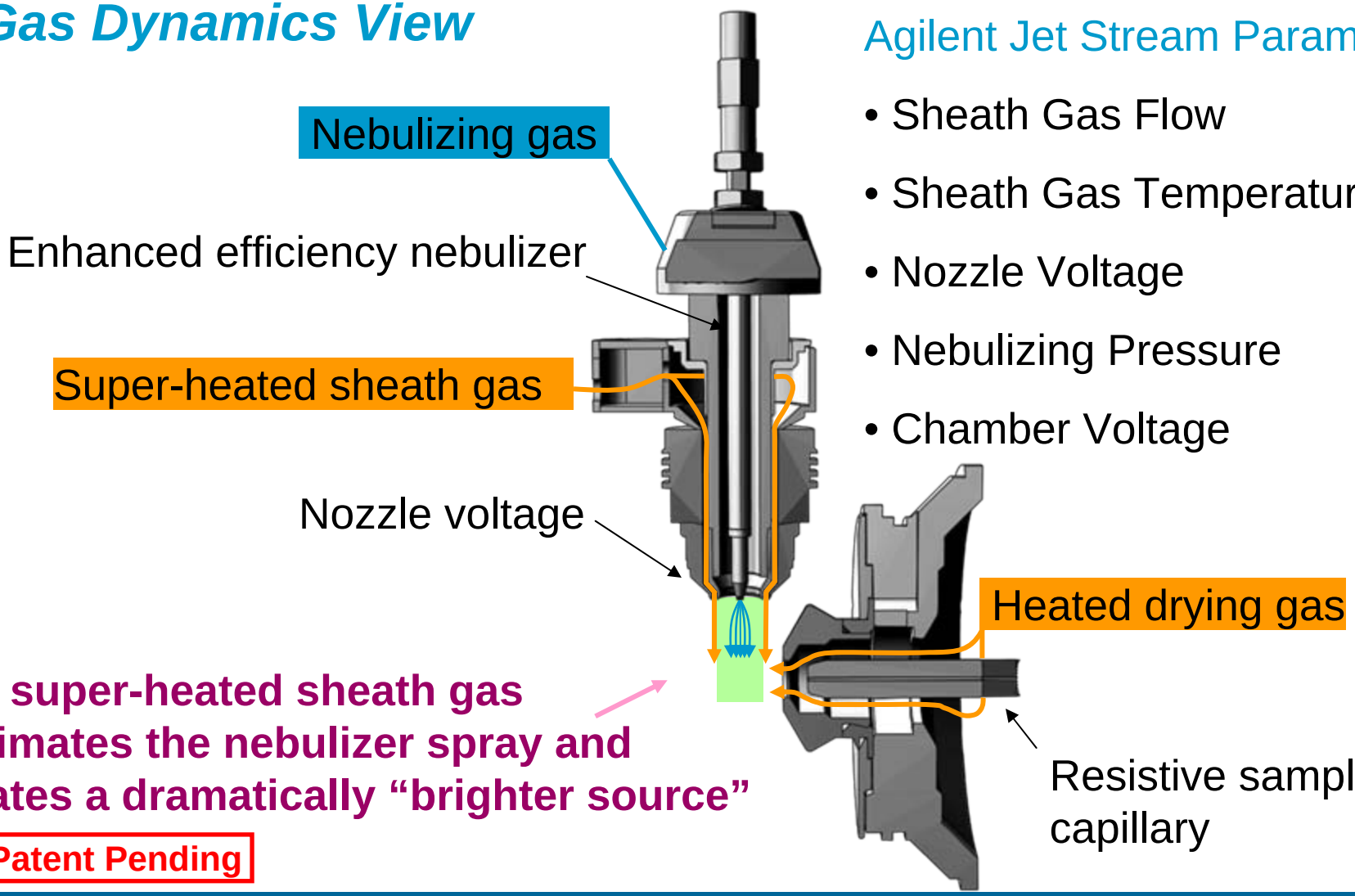
- Learn about Agilent Jet Stream Technology
- Project goals
- Ease of use/ new parameters
- Physics and fundamentals of the technology

# Project Goals

- 5x Performance Improvement
- 100ul/min – 2.5ml/min Flow Rates
- Position/flow Independent
- Easy to tune – smooth function of the parameters
- Easy to use – same orthogonal ESI fill
- Previous Methods Compatibility
- Excellent chromatographic performance – no peak tailing
- Low RSDs – below 10% at the check out
- Rugged and Reliable

# Agilent Jet Stream Ion Generation

## *Gas Dynamics View*




Agilent Jet Stream Parameters

- Sheath Gas Flow
- Sheath Gas Temperature
- Nozzle Voltage
- Nebulizing Pressure
- Chamber Voltage

**The super-heated sheath gas collimates the nebulizer spray and creates a dramatically "brighter source"**

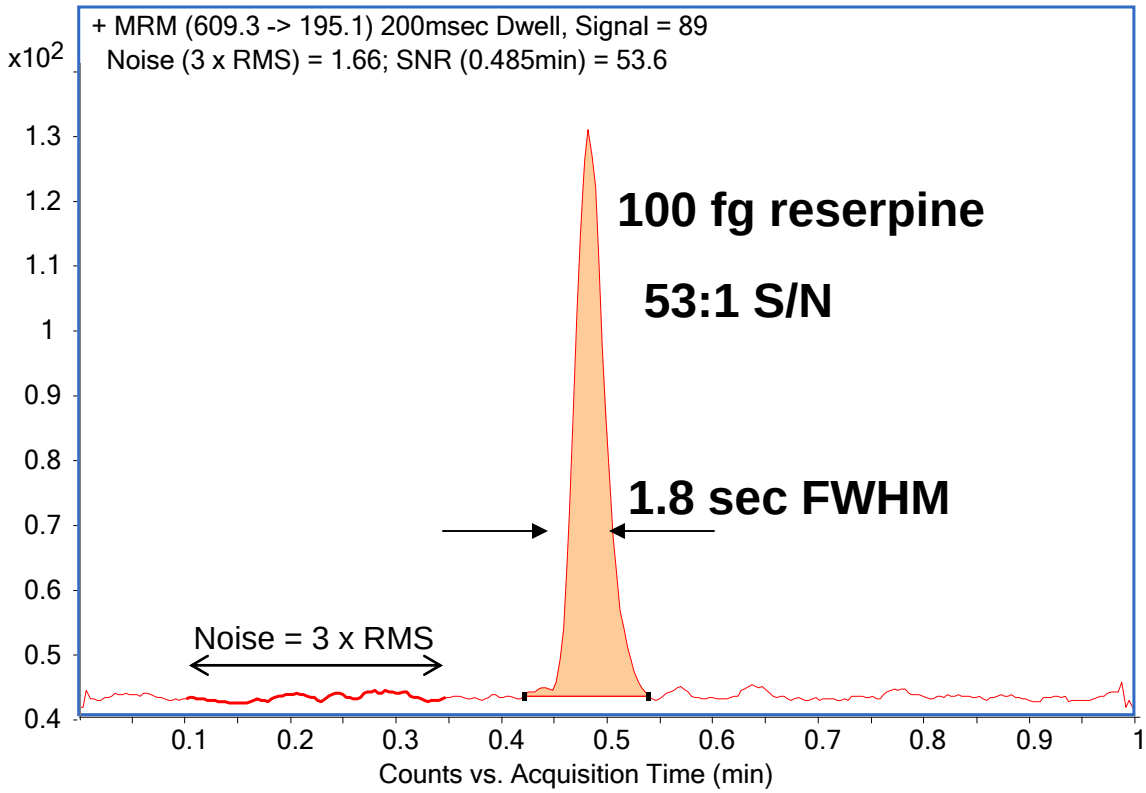
**Patent Pending**

# Default Agilent Jet Stream Parameters vs. Flow Rate

| Flow Rate, mL/min | Position | Sheath Gas Flow | Sheath Gas Temp | Nozzle Voltage | Nebulizing Pressure | Chamber Voltage |
|---|---|---|---|---|---|---|
| 0.1 | fixed (n/a) | 11 L/min | 300C | 600V | 30psi | 4kV |
| 0.5 | fixed (n/a) | 11 L/min | 300C | 600V | 30psi | 4kV |
| 2.0 | fixed (n/a) | 11 L/min | 300C | 600V | 30psi | 4kV |

- Sensitivity is proportional to the Sheath Gas Temperature
  cold – ESI equivalent, 400C – Agilent Jet Stream (5x – 10x)

# 6460 QQQ Sensitivity with Agilent Jet Stream




**10 injections**

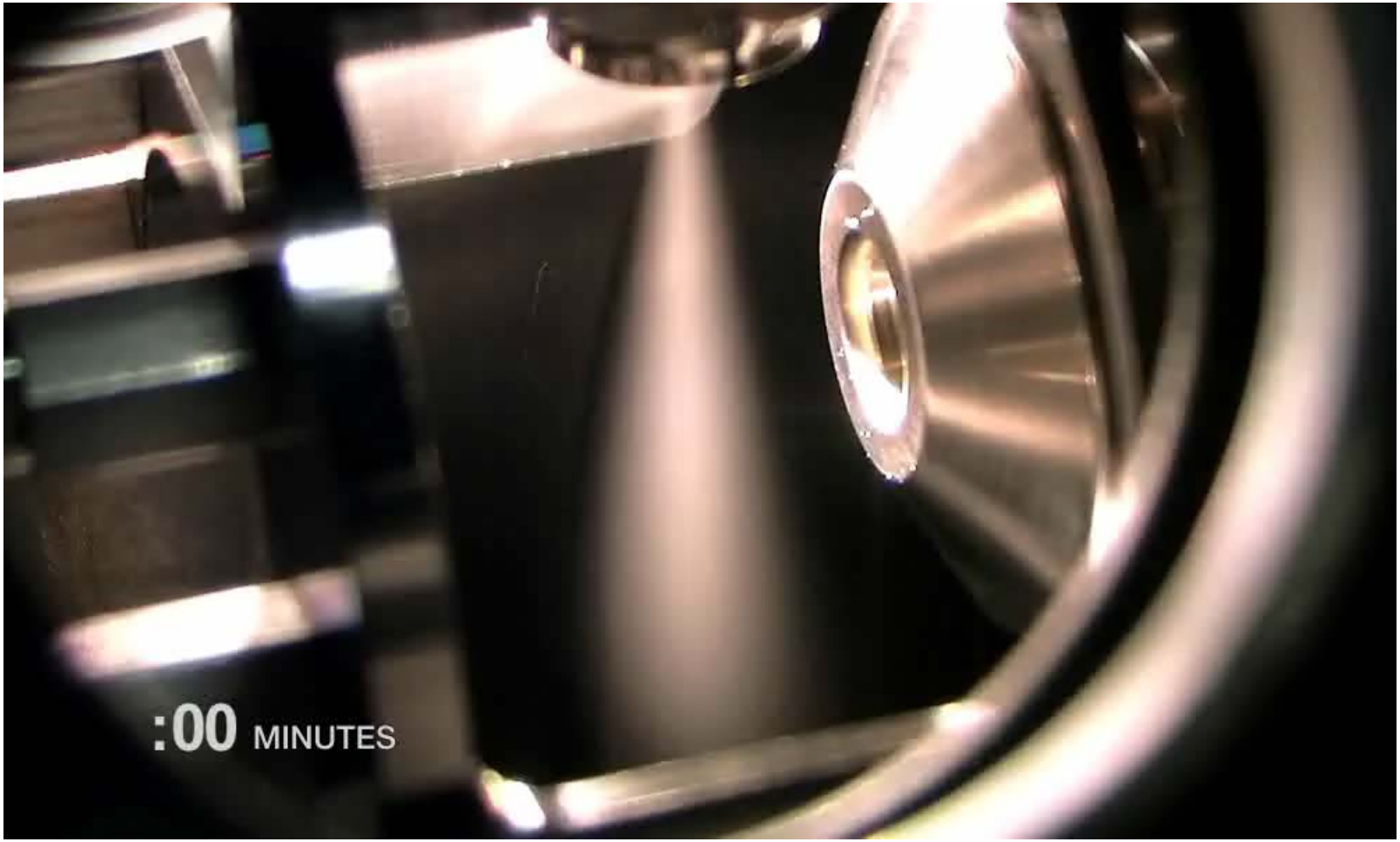
**S/N = 69 ± 15 (obs.)**

**RSD = 4 % (obs.)**

| | |
|---|---|
| ***Column*** | **1.8 µm , 2.1 x 50mm, ZORBAX SB-C18** |
| ***Flow rate*** | **0.4 mL/min** |
| ***Mobile phase*** | **70% acetonitrile / water, 0.1% formic acid** |

# Agilent Jet Stream In Action

## *Observing Thermal Gradient Focusing*




Start temperature = 25 ºC

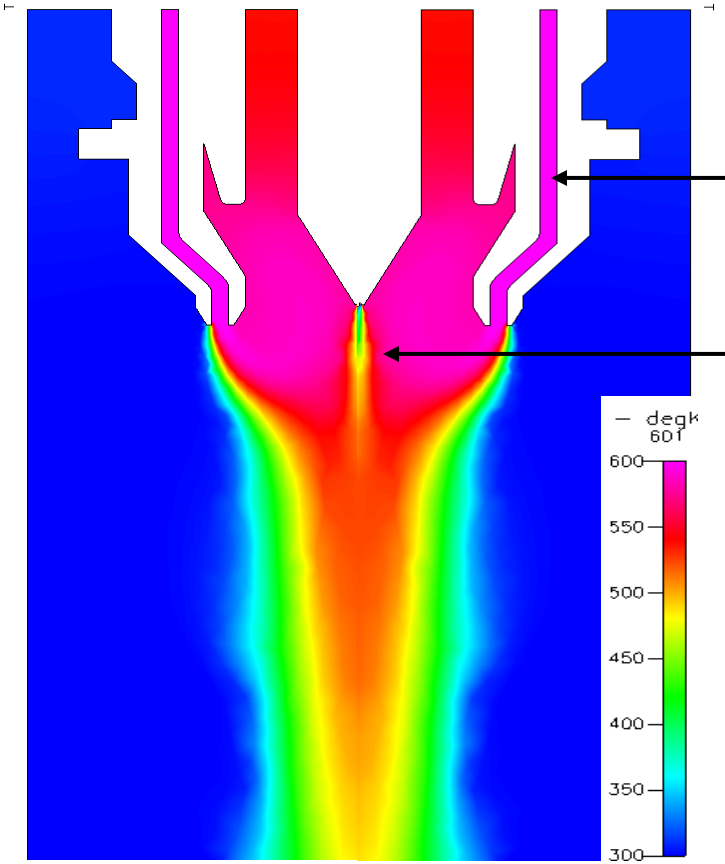
Stop temperature = 400 ºC

# Agilent Jet Stream Ion Generation

## *Thermal Dynamics View*

This plot is a simulation showing the **thermal profile** of the Agilent Jet Stream Nebulizer

Thermal energy is focused to the nebulizer spray



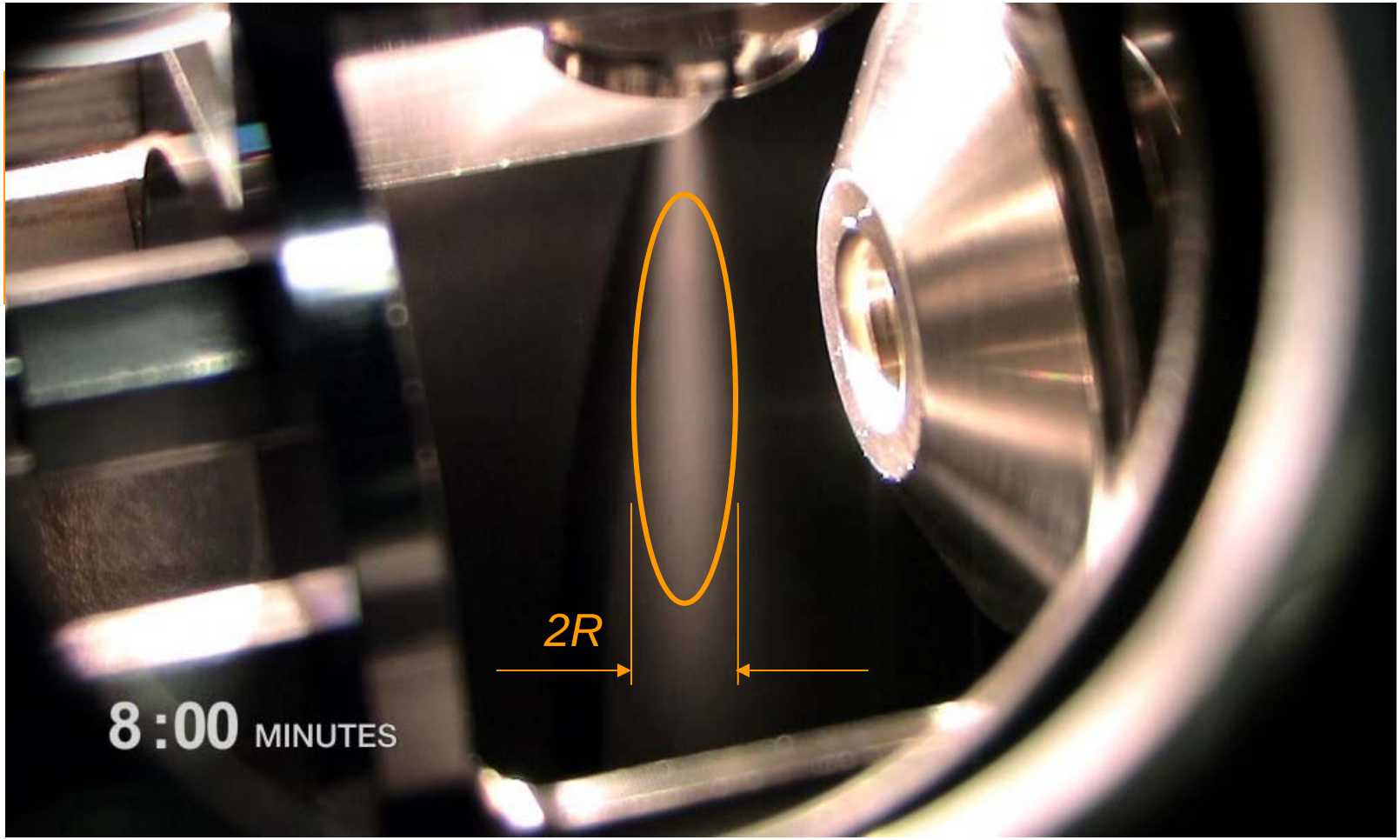

Super-heated $N_2$ sheath gas

Nebulizer $N_2$ gas (near sonic velocity)

**Patent Pending**

# Agilent Jet Stream In Action

## *Observing Thermal Gradient Focusing*




Start temperature = 25 ºC

Stop temperature = 400 ºC

# Collimation of the Spray with Thermal Gradient Focusing

$$Q \sim \Delta T S$$

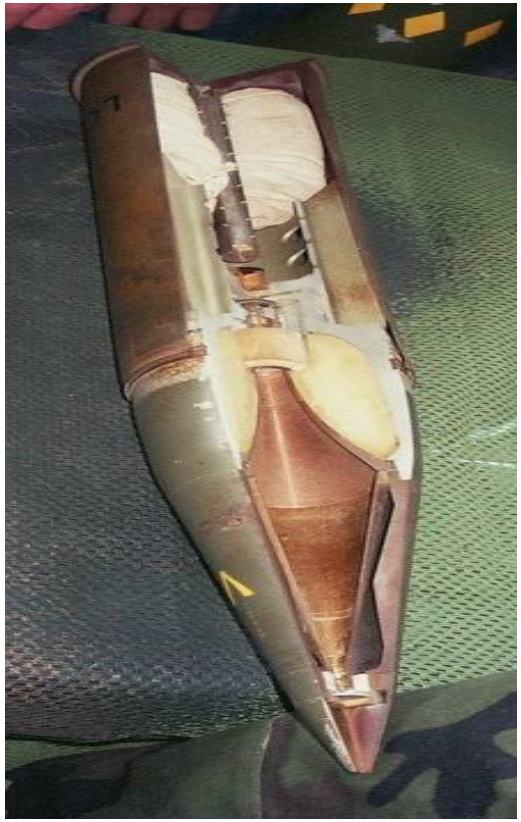
$$S \sim R^{\alpha}$$

where $\alpha=1$ for cylindrical geometry and $\alpha=2$ for elliptical geometry

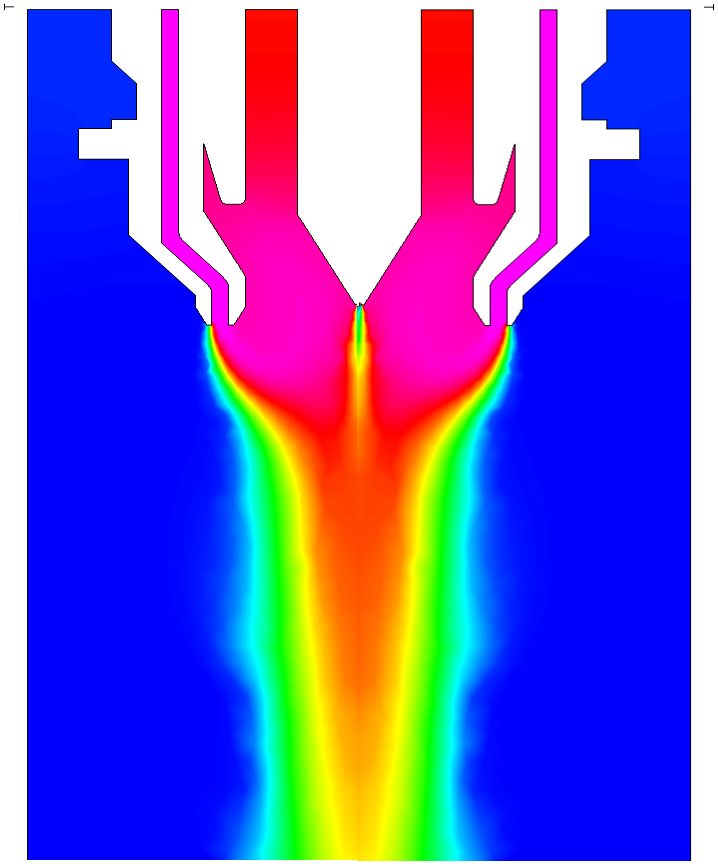
$R \sim 1/\Delta T$ (cylindrical)  $R \sim 1/\sqrt{\Delta T}$ (elliptical)

# Military vs. Civilian

Shape Charge



Agilent Jet Stream



# Other Improvements

## Resistive Sampling Capillary

- The resistive inner bore surface is highly inert
- Virtually instantaneous field equilibration within capillary bore during polarity switching
- Highly reproducible and stable ion transmission due inertness and lack of surface charge build up along capillary bore

## Increased Stage 2 Conductance

- Modified pump out within stage 2 allows more pumping speed behind the skimmer into the first octopole vacuum region
- Improved ion transmission

## Improved High Efficiency Nebulizer

# Jet Stream Goals - Summary

- 5 - 10x Performance Improvement for the ESI
- 100ul/min – 2.5ml/min Flow Rates
- Position/flow Independent
- Easy to tune – smooth function of the parameters
- Easy to use – same orthogonal ESI fill
- Previous Methods Compatibility
- Excellent chromatographic performance – no peak tailing
- Low RSDs – below 10% at the check out
- Rugged and Reliable

# Acknowledgments

## Agilent R&D Team