

# High Throughput Nanospray Chip for Robust Molecular ID Using Direct Infusion

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## Overview

With the increasing need to detect sub-femtomole concentrations in complex samples, the use of nanospray mass spectrometry has become the technique of choice. What makes nanospray attractive is that it offers higher sensitivity over conventional electrospray (10x), solvent consumption is minimal, and it can tolerate samples in complex matrices, such as DMSO.

Typical nanospray applications use a nanospray emitter with a very small bore (<5µm). These emitters are prone to clogging due to their small diameter and the spray can be interrupted by air bubbles. As a result, care must be taken in removing any particulates from a sample. Also, it can be time consuming to determine the optimal voltage for the specific emitter and for solvent being used.

This work presents a system that overcomes many of the short comings of conventional nanospray sources by demonstrating the use of the AutoNanoDi system, which automatically perform nanospray of direct infusion with patented robust and anti-clogging nozzles.

## Chip Design and AutoNanoDi

All experiments were performed on the AutoNanoDi system (Phoenix S&T, PA) with the nanospray chip. The chip is designed for automated nanospray that allows up to 32 nozzles to be sprayed.



- Each chip consists of 16 nozzles, which can be stacked to make a 96 nozzles. In this experiment, two chips are stacked to make 32 nozzles.

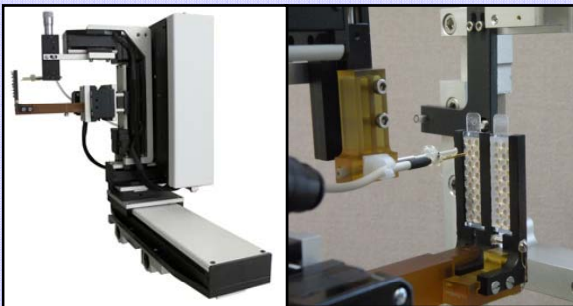
- Conical nozzle structures 0.5 mm to 3.5 mm in height

- 20µm orifice, 50µm o.d.

- Reservoirs can hold up to 15µL

- A silicone mat is placed on the back of the nozzles to hold the samples in the reservoirs and protects against cross contamination between reservoirs

## AutoNanoDi System



- The AutoNanoDi System performs direct infusion with nanospray nozzles automatically.
- The lower arm is the chip holder and can hold as many as two nanospray chips (32 nozzles total).
- The upper arm contains a high voltage probe, as well as a nitrogen air probe for sample pumping.

- Nanospray is emitted from the nozzles by pressing the spring-loaded high voltage probe against the gold plated back of the nozzle to apply the high voltage (max 4.8kV).

- Nitrogen air is allowed to freely flow on the back of the nozzle up to 3.5psi.

- AutoNanoDi system automatically moves in a user-specified order to each nozzle to initiate spray.

- A spray sensor is used to detect spray. If no spray is detected, the system goes through a series of automated responses to initiate the spray (e.g. suction of the sample to the nozzle orifice by vacuum and/or a high voltage spike)

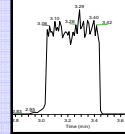
## Spray Control

- The current sensor is positioned near the inlet to detect the current of the spray. High and low preset current thresholds are used to determine the voltage required for the spray (going below the lower threshold initiates an increase in voltage and vice versa). Based on this feedback loop mechanism, the voltage is automatically adjusted to ensure a continuous spray.

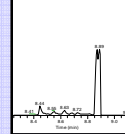
- Another way to control the spray is using an external trigger on the mass spectrometer. The voltage is automatically increased until a total ion count threshold has been achieved (i.e.  $1 \times 10^5$ ). This method is available on the Thermo MS.

- Using the trigger is more sensitive in that the spray is detected at a lower ion count, but dipping below that count, even briefly, initiates an increase in the voltage until the spray has increased to produce just enough ions to cross the lower threshold.

### Current sensor



### MS trigger



## Methods

- Nozzles on the chips were filled with 10µL of sample by puncturing the silicone mat on the back of the nozzles with a pipette tip.

- Prior to inserting the chips into the chip holder on the AutoNanoDi system, each nozzle was vacuumed with a small vacuum pump. This ensured that the sample was at the tip of the nozzle to produce a spray quickly.

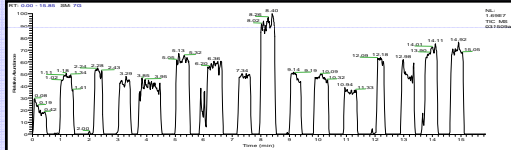
- A nanospray was produced from each nozzle by applying a high voltage between 2.8kV and 4.8kV and with an air pressure of 0.5psi.

- To ensure spray from each nozzle, a series of automated responses were incorporated into the system based on a feedback loop from either the current sensor or a mass spectrometer trigger.

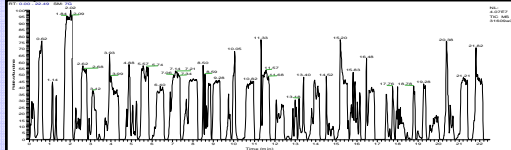
- All results were collected with an Thermo-Finnigan LCQ Advantage Mass Spectrometer

## Results

a) 16 nozzle spray of 1µg/mL of erythromycin in 50/50 v/v acetonitrile/water using an analysis time of 20s for each nozzle



b) 32 nozzle spray of 1µg/mL of erythromycin in 50/50 v/v acetonitrile/water using an analysis time of 20s for each nozzle



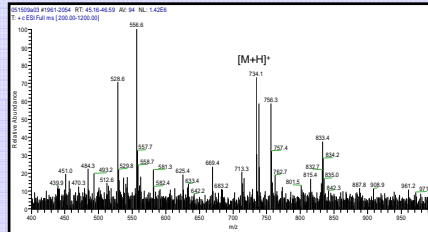
## Spray with DMSO

Erythromycin was analyzed using pure dimethyl sulfoxide (DMSO) as the solvent. DMSO is typically for drug synthesis applications and it is not typically used in conventional electrospray due to its high boiling point (189°C). Using nanospray, the drug molecules can potentially be analyzed directly from synthesis.

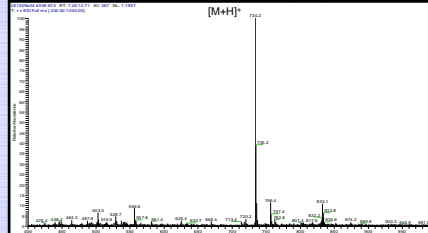
The nanospray observed from the nozzle while analyzing 6µg/mL of erythromycin in DMSO at 4.7kV.



a) 1µg/mL of erythromycin in DMSO



b) 6µg/mL of erythromycin in DMSO

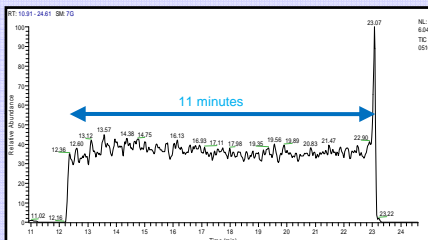


- The s/n ratio observed for the 1µg/mL of erythromycin in DMSO was 15, where as the s/n ratio for 6µg/mL of erythromycin in DMSO was 203.

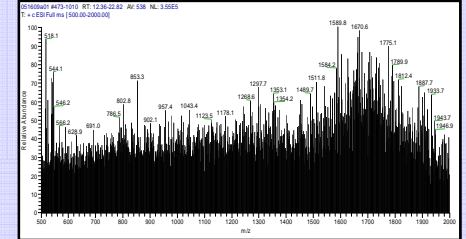
## Unfiltered Fetal Bovine Serum

Fetal bovine serum, used for growth media for cells, was centrifuged for 10min and then diluted 200x with 50/50 v/v methanol/water.

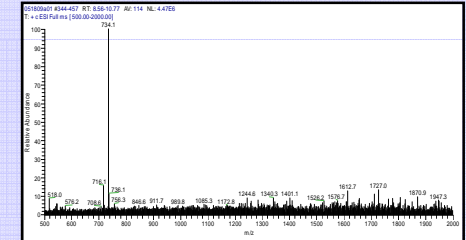
Total ion chromatogram of the spray of the 200x diluted fetal bovine serum indicating a flow rate of 900nL/min.



## Spectrum of centrifuged, unfiltered, 200x diluted fetal bovine serum



## Mass spectrum of 100ng/mL erythromycin spiked in the 200x diluted fetal bovine serum



## Summary and Discussions

This work has shown the benefits of using the AutoNanoDi system for a variety of applications including drug discovery and the potential for use in biomarker research. The benefits that were found within this work include:

- Spray control can be done with either the current sensor or a mass spectrometer trigger. Both offer automated responses from the AutoNanoDi system, such as voltage spikes, vacuuming, and air pressure increases. This makes initiating the spray, as well as finding an optimal voltage easier and less time consuming.

- Automated samples can be acquired from 32 nozzles currently. By stacking the nozzles, there is potential to go as high as 96 or even 384 nozzles. Analysis can be done within seconds of achieving a spray from the nozzle.

- The sensitivity and the robustness of the nozzles have been shown with the data collected with the fetal bovine serum. The spiked fetal bovine serum shows that a molecule of interest can be detected in a complex matrix using the nanospray nozzles.

- The nozzles can be used to directly analyze products with a mass spectrometer directly from a synthesis, using DMSO as the solvent. However, it appears to inhibit ion formation, and more concentrated solutions can be utilized during analysis.

These nozzles, in combination with the AutoNanoDi system provides a robust, time saving, sensitive technique that may be beneficial to many research applications.

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