

# High Throughput Mass Spectrometry with the Polypropylene Nanospray Plate

J. W. Ashmead, S. L. T. Staats, D. T. Chow, and S. Du, Phoenix S&T, Inc.

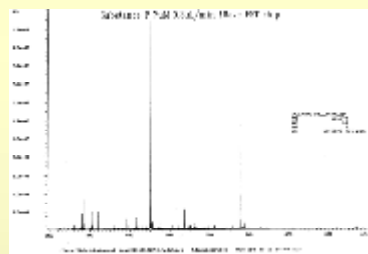
## Overview

The increasing size and complexity of compound libraries and the increasing number of targets due to the success of the Human Genome Project create an unprecedented urgency to increase the productivity of high throughput screening operations in the pharmaceutical industry. The key to boost productivity is to provide fast, efficient, non-radiometric assay systems that are miniaturized, accurate and have relatively fast assay development procedures. The automated polypropylene-based nanospray plate may potentially meet all the requirements for productivity enhancement as well as cost-effectiveness.

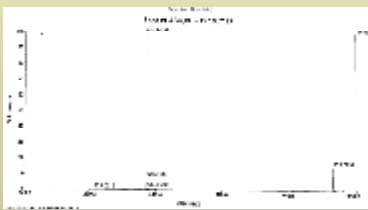
## Potential Applications in Automation

Numerous applications in drug discovery can benefit from automation, including ADMET assays, pharmacokinetic studies, combi-chem screening, proteomics, etc. Outside of pharmaceutical R&D, applications involving high throughput mass spectrometry include bioprocess monitoring, clinical diagnostics, environmental and petrochemical analysis, etc.

FT-MS may eventually be automated. The following mass spectrum was taken with the plastic nanospray chip in the manual mode of Substance P in a pure aqueous buffer with a Bruker Daltonic Apex FT-MS. The flow rate was 300 nL/min.



Compounds in pure DMSO from combinatorial chemistry library can be readily analyzed by nanospray chip-mass spectrometry without sample preparation. The following spectrum was taken from a 2  $\mu$ L drop of combi-chem compound in the A1 well of a plate and the sample was fed directly into the nanospray chip nozzle with a syringe pump without diluting or washing off the DMSO. Since the concentration was high (mg/mL), the nozzle was placed ~5 cm from the mass spectrometer inlet. Note that the peak height was still extremely high, > 7000 cps. The mass spectrometer used was a Mariner TOF.



## Nanospray nozzle and chip design



Nanospray chip: Nozzles side  
Nozzle spacing: 4.5 mm

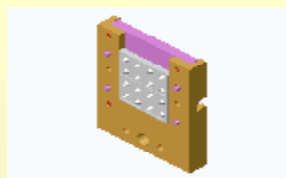


Nanospray chip:  
3D view showing  
nozzles and reservoirs



Nanospray chip: Reservoir side

- Conical nozzle structures 0.5 mm to 1.5 mm in height
- 20+/- 3  $\mu$ m i.d., 50  $\mu$ m o.d.
- A reservoir of a few microliters connects directly to each nozzle
- Four nozzles per chip
- Chips can be tiled to any configuration, up to a 384 microtiter-plate format



Four chips are tiled into a 16-nozzle configuration. The reservoirs in the back of the plate are sealed with a silicone sealing mat with slits that are reclosable.

## Sample Loading

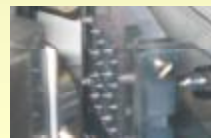


Samples are preloaded into the reservoirs with pipettes through the openings in the reclosable mat.

## Interface for chip automation



The chip and the pumping mechanism are mounted on a 3D positioner made of motorized stages with submicron accuracy.



Nozzle side



Sealed Reservoir side

The nozzle chip was placed at a 45° angle from the inlet of a mass spectrometer

## Sample Pumping



The preloaded sample is pumped out of the nozzle by a plunger pressing on the sealing mat acting as the diaphragm. During pumping, the high voltage contact pin transmits the high voltage to the sample through the gold coating on the wall of the reservoir. A con-jet spray resulted, as shown below.

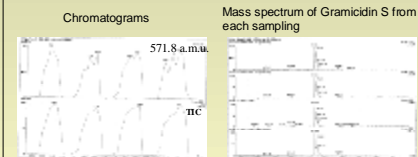
The flow rate can be regulated by changing the speed of the plunger. To calibrate the flow rates, a known amount of sample is loaded into the reservoir and the time it takes to empty the reservoir is measured for a particular speed of the plunger. The lowest flow rate we have achieved so far is 100 nL/min. Lower flow rates may be obtained since the speed of the plunger, which is controlled by a motorized stage, can be further reduced.



The cone-jet spray made by the automated plate

## Results

Gramicidin S, 2  $\mu$ g/mL in 50/50 MeOH/H<sub>2</sub>O  
2.7 KV, 400 nL/min, ~11s/sampling



Mass chromatogram of parahydroxycinnamic acid (m/z= 163) in 50/50 water/isopropanol  
Negative ion nanospray. Spray voltage: -1.8 KV. ~ 1minute/well, 400 nL/min. flow rate



For longer sprays, an initial burst of signal was observed. After the burst died down, a spray of even flow rate resulted.

## Summary

- A new plastic nanospray chip/plate with automated operation is presented
- By preloading the sample into the reservoir behind each nozzle, the dead time for switching the spray from nozzle to nozzle can be reduced to 1-2 seconds.
- Sample loading may be achieved through conventional microtiter plate robotics.
- The pumping mechanism is based on a plunger/diaphragm arrangement. Flow rates lower than 100 nL/min. will be achievable.
- Preliminary results indicated <10 s/sample will be possible with the automated nanospray plate.
- Numerous applications from drug discovery to clinical diagnostics may benefit from high throughput mass spectrometry using the automated nanospray plate.

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