

High Throughput Mass Spectrometry with the Polypropylene Nanospray Plate

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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.

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

The polypropylene nanospray plate is made of subunits of nanospray chips. The spacing of the nozzles is that of the 384-microtiter format. Each nozzle has a 50-micron o.d. and a 20- micron i.d.. A reservoir for storing 1 to 20 microliters of sample is connected to each nozzle through a microfluidic channel. A conducting pad of gold made by sputter-coating extends from the reservoir surface into the microfluidic channel for charging the sample for nano-electrospray. The reservoirs are sealed with a sealing mat to prevent sample evaporation. The plate used for the experiments has 16 nozzle-reservoir units, and can be expandable to 384. The automation robotics is built of fast translational stages with sub-micron resolution. Figure 1 is a schematic drawing of the 16 nozzle-reservoir plate in its holder.

Samples are preloaded into each reservoir by piercing the reclosable silicone sealing mat with a pipette tip. The seal prevents the sample from leaking out of the nozzle opening during sample loading, and also prevents sample evaporation before the MS analysis. The sample in the reservoir is pumped out of the nozzle by a plunger that pushes on the sealing mat acting as the diaphragm. The plunger mechanism provides a well-controlled pumping method from 100 nL/min. to several microliters/min. The nozzle-reservoir combination minimizes the cycle time of spraying from one nozzle to the next to about two seconds. Below are some of the preliminary results obtained with this automated plate. The mass spectrometer used was a Micromass Ultima Quattro triple quadrupole

Figure 2 shows the mass chromatogram result of four samplings of ~ 11 seconds each of gramicidin S (2 µg/mL in 50/50 Methanol/water). The cycle time from sampling to sampling was about 2 seconds, and the total mass spectrometer sampling time was about 8 seconds. The top panel of the $m/z = 571.8$ trace and the bottom panel of the total ion current trace indicate no cross-talk between samplings and good signal to noise ratio even for such a short scan.

Figure 3 shows the mass chromatogram of three samplings of parahydroxycinnamic acid ($m/z= 163$) in 50/50 10 mM aqueous ammonium acetate/isopropanol of slightly longer duration, about 1 minute each. The cycling time between nozzles was about 2 seconds. In the longer sprays, an initial burst of signal in the spray was observed. After the initial burst, the chromatographic trace indicated that the flow rate was even, estimated to be ~ 400 nL/min. It is possible that the almost "bell-shape" peaks in Figure 2 were due to the initial burst in the spray. Since the sampling time was short, only 8 seconds, the spray did not have time to reach the flat part of the equilibrium flow rate of 400 nL/min.

The results in this proceeding show that very high throughput mass spectrometry, <10 second/sample, may be feasible with the polypropylene-based nanospray plate. The high speed is enabled by the nozzle-reservoir combination that reduces sample cycling time to 2 seconds per sample or less. Future work will include further lowering the flow rate to take advantage of the ability of the plastic nozzle to spray complex matrices such as culture broth and serum without clogging. Automating MS analyses of processes involving these matrices will potentially not only improve the efficiency of the MS

measurements, but also simplify the sample preparation steps upstream from the final MS measurement step.

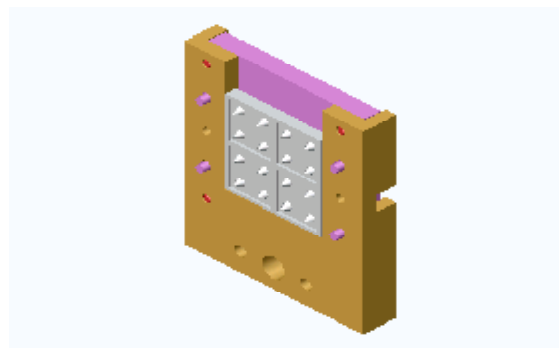


Figure 1: a 16-nozzle-reservoir unit for automated high throughput mass spectrometry. The unit is shown in its mounting frame for deployment in the motorized interface.

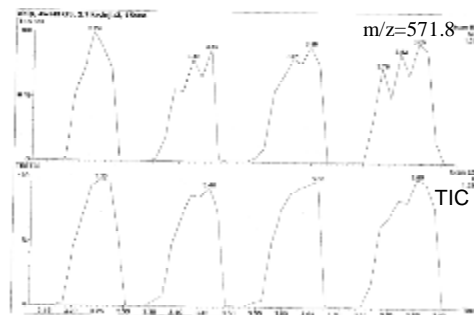


Figure 2: mass chromatogram of gramicidin S in 50/50 methanol/water. The spray voltage was 2.7 KV. The time intervals between peaks was ~11 S, two seconds of which was time needed to position the next nozzle and reset the pumping mechanism for a

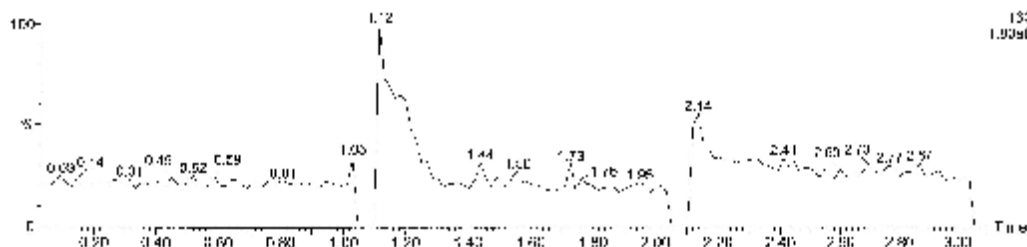


Figure 3: Mass chromatogram of three samplings of parahydroxycinnamic acid ($m/z=163$) in 50/50 water/isopropanol. Negative ion nanospray was carried out with a spray voltage of -1.8 KV. The duration per sampling was ~1 minute/spray, and the flow rate was 400 nL/min. The initial burst of signal in the first spray was not recorded.

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