A Comparative Study of ESI, Nano ESI, and HPLC-Chip/MS Ion Sources for Optimum Sensitivity and Sample Throughput

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Introduction

Nanoflow techniques generally offer excellent chromatographic resolution with high sensitivity, but problems with clogging have limited their use for high throughput analysis. With the introduction of microfluidic LC chips, many of the robustness issues with conventional nanoelectrospray (nanoES) sources have been overcome. In this study we compare the ionization efficiencies of an HPLC-Chip/MS interface vs. conventional nanoES and pneumatically assisted electrospray (ES) sources using a variety of samples.

Experimental

ES-TOF Tuning Mix (Agilent part# G1969-85000) was infused to produce singly charged ions at 118, 622, 922 and 1222 amu. The sample was delivered via a syringe pump at flow rates of 50-800 nL/min for the nanoES and 30-1500 nL/min HPLC-Chip sources and 100 µl/min for the ES source.

LC/MS System: A model 6140 quadrupole LC-MS system (Agilent Technologies) was configured with ES, NanoES and HPLC-Chip/MS interfaces (Figure 1).

Figure 1: Agilent 6140 LC-MS System

Experimental

ES Source: The Agilent G1948A electrospray ion source uses a grounded, orthogonal pneumatic nebulizer with a 0.12 mm (120 µM) I.D. needle. Variable nebulizer pressure from 0 to 60 psi allows effective nebulization at LC flow rates from 0.1 to 1.0 mL/min (Figure 2).

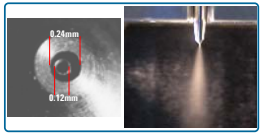


Figure 2: ES Pneumatic Nebulizer

NanoES Source: The Agilent G1982A nanospray ion source uses a grounded, orthogonal emitter which can be fitted with PicoTips (New Objective, Inc) with I.D.s ranging from 5 to 30 µM. For this investigation, a distal coated PicoTip with an I.D. of 15 µM was used (Figure 3).

Figure 3: NanoES Emitter Assembly

HPLC-Chip/MS: The Agilent HPLC-Chip/MS interface uses a polyimide microfluidic chip with an integral 15 µM I.D. emitter. The HPLC-Chip includes a metal cartridge that simplifies handling includes an RF tag for identification (Figure 4). For this investigation, a G4240-61002 infusion chip was used.

Experimental

Figure 4: HPLC-Chip Assembly

MS Conditions:

In order to compare the responses of each ion source, the following MS conditions were used for all experiments: MS Scan Range: 100 to 1350. MS Scan Speed: 2.912 sec/cycle, 433 u/sec.

Experiment 1: Pneumatically Assisted ESI Source

ESI-TOF Tuning Mix was introduced directly into the pneumatic nebulizer of the ES source at 100 µL/min flow rates using a 2.5 ml GasTight syringe (Hamilton, Inc).; **ES Source Conditions:** Nebulizer pressure: 15 psi; Drying gas: 7 L/min, 300 °C; Vcap: 4000 V; Fragmentor: 100 V.

Experiment 2: NanoES Source

ESI-TOF Tuning Mix was introduced directly into the nanoES emitter assembly at 300, 150, 100, 80, 60, 40, and 30 nL/min flow rates using a 100 µL GasTight syringe. The nanoES source was fitted with a 15 µM ID distal coated PicoTip emitter (New Objective, Inc); **NanoES Source Conditions:** Drying gas: 3 L/min, 250 °C; Vcap: 1900 V; Fragmentor: 100 V.

Experiment 3: HPLC-Chip/MS Interface

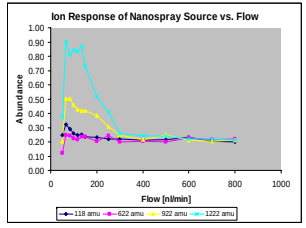
ESI-TOF Tuning Mix was introduced to the HPLC-Chip/MS interface at 1500, 1250, 1200, 750, 600, 500, 400, 300, 250, 200, 133, 117, 100, 83, 67, 50, and 30 nL/min using a 100 µL GasTight syringe. The HPLC-Chip/MS interface was fitted with an infusion chip (Agilent part # G4240-61002) which has a 15 µM ID tip.; **HPLC-Chip/MS Conditions:** Drying gas 3 L/min, 250 °C; Vcap 1700 V; Fragmentor: 100 V

Results and Discussion

The performance of the ES source was used as the reference for the nanoES source and HPLC-Chip/MS interface. In order to compare the results of this study graphically, the extracted ion response of the nanoES source and the HPLC-Chip/MS interface were both normalized to the ES source response at 100 µL/min.

NanoES Source:

The nanoES source showed significantly lower response vs. the ES source for all masses at flow rates above 200 nL/min. Below 200 nL/min. the response for higher molecular weight ions increased dramatically, reaching 90 % of the normalized API-ES source response at 100 µL/min. while the response of the lower molecular weight ions remained relatively constant throughout the investigated flow range.



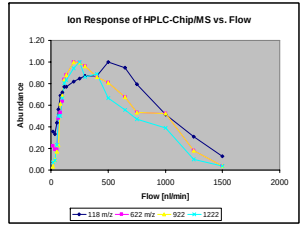
It has been observed by Mann et al. (Int. J. Mass Spectrom. Ion Processes **136** (1994), pp. 167–180) that ions with the highest surface concentration in the electrospray droplets are favored. The surface concentration is a function of the ion solubility and charge density of the analyte.

Results and Discussion

At the lower flow rates used in our experiments, the droplet size was decreasing while the charge concentration was increasing for all ions. This lessens the competition for ion formation, thus, the previously suppressed ions show an increase in response. In our experiments, this transition occurs below 200 nL/min of flow.

HPLC-Chip/MS Interface:

In contrast with the nanoES source, the response of the HPLC-Chip/MS did not show significant ion selectivity throughout the investigated flow range. The relative ion responses track quite closely with the reference ES source for singly charged ions. The overall ion response was also better than the nanoES source and was comparable to the reference ES source from 117 to 500 nL/min.



These results are consistent with previously reported observations that this type of microfluidic chip is less susceptible to Taylor cone variations and has a greater dynamic range that conventional nanoES sources.

Conclusions

- The HPLC-Chip/MS interface provided good sensitivity for small molecule ions without the ion suppression effects of conventional nanoES.
- The HPLC-Chip/MS interface exhibited superior response across a much wider flow range that conventional nanoES. With chromatographic applications this allows shorter run times and greater sample throughput.
- The HPLC-Chip/MS interface also provided greater ease-of use, yielding consistent response without the extensive optimization required for nanoES.