Use of Automated Nanoelectrospray for Drug Metabolite Structure Elucidation

David J. Borts, Steven T. Cook, Gary D. Bowers, Michael J. O'Mara, Kate E. Quinn
GlaxoSmithKline, Development DMPK, Research Triangle Park, NC 27709

OVERVIEW

- LC/fractionation combined with automated nanoelectrospray has several advantages over the conventional LC/MS approach for drug metabolite structure elucidation
- These advantages include increased speed, improved data quality, and a reduction in analyte quantity required
- An automated nanoelectrospray source (Advion NanoMate 100) is used with Micromass Q-TOF 1, Finnigan LTQ, and Finnigan LTQ FT mass spectrometers in a variety of experiments aimed at elucidating the structures of drug metabolites
- Four examples are shown which illustrate the utility of this approach. These examples include: 1) signal summation with an extended infusion to generate higher quality spectra, 2) background subtraction using automated nanoelectrospray, 3) multiple fraction screening for components of interest, and 4) a rapid and sensitive product ion tree experiment

INTRODUCTION

Automated nanoelectrospray (nanoES) mass spectrometry offers several advantages over the conventional LC/MS approach for elucidating the structure of drug metabolites including increased speed, improved data quality, and a reduction in analyte quantity required.

In our experience, we are able to acquire mass spectrometry data necessary for drug metabolite structure elucidation approximately 4-5 times faster with automated nanoES compared to LC/MS. LC/MS analysis usually requires multiple injections of a sample to acquire the necessary data, while automated nanoES typically requires only a single infusion of a fraction of interest. It is also much easier to switch between MS instruments and projects with automated nanoES. With LC/MS it may take several hours to switch from one method to another.

Much less analyte is required with nanoES, compared to LC/MS, to generate equivalent quality spectra. LC/MS typically consumes 10s to 100s of nanograms of drug metabolite in acquiring spectra of sufficient quality to determine a metabolite structure. NanoES typically consumes picogram quantities. This corresponds to an average decrease in required analyte quantity of at least a factor of 100.

Finally, higher quality data can be acquired with the nanoES approach compared to LC/MS with less overall effort. To increase data quality in LC/MS, often a more concentrated sample must be injected. This usually requires labor-intensive sample preparation procedures. With nanoES, infusion/MS data can simply be acquired and signal averaged for a longer period of time until the data quality is fit for purpose.

METHODS

LC fractionation

Samples:	Biological fluid extracts containing radiolabeled or 'cold' drug and metabolites
HPLC systems:	Agilent 1100
96-well plate fraction collectors:	Gilson FC204
Automated pipetting:	Tecan Genesis RMP 150
96-well radio detector:	Wallac 1450 MicroBeta

Mass spectrometry

Spectrometer:	Micromass Q-TOF 1
Spectrometer:	Finnigan LTQ
Spectrometer:	Finnigan LTQ FT

Automated nanoelectrospray

Instrument:	Advion NanoMate 100 (Figure 1)

Figure 1. **Advion NanoMate 100 automated nanoelectrospray system installed on a Micromass Q-TOF 1 mass spectrometer**

RESULTS

Example 1. Signal summation to generate higher quality spectra

Figure 2(A) shows an LC/MS extracted ion chromatogram from a hepatocyte incubation of a drug. This data was acquired on a Micromass Q-TOF 1 mass spectrometer. The extracted ion is m/z M+16 of the drug and corresponds to mono-oxygenated metabolites. The component of interest was the metabolite peak at 17.5 minutes. Since the signal level was very low for this peak (≈ 25 counts above background), no attempt was made to perform an LC/MS/MS experiment to generate a structurally informative product ion spectrum. Instead, the peak at 17.5 minutes was fraction collected and infused for MS/MS analysis using the Advion NanoMate and the Q-TOF 1. Figure 2(B) shows a series of product ion spectra from this infusion. Signal was summed for 1 second, 1 minute, 5 minutes, and 40 minutes in these spectra. At 40 minutes, sufficient product ion signal had been accumulated to allow accurate mass measurement and to confirm that m/z 128 was the key ion required to assign the location of oxidation. Without nanoelectrospray and signal summation, additional labor-intensive and time-consuming sample preparation would have been required to generate a useful sample for LC/MS/MS analysis.

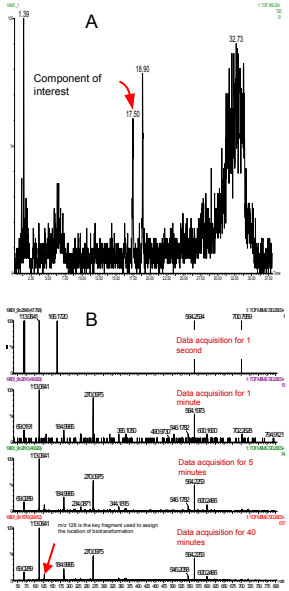

Figure 2. **LC/MS extracted ion chromatogram (A) and signal-summed product ion mass spectra from nanoES infusion of the component of interest (B)**

Example 2. Background subtraction with automated nanoelectrospray

Figure 3(A) shows a reconstructed LC/radio chromatogram from analysis of a plasma sample extract from a monkey dosed with radiolabeled drug. A background fraction and an analyte fraction were selected from this run. These fractions were infused by automated nanoelectrospray and data from both infusions were acquired to the same data file (Figure 3(B)). Figure 3(C) shows a summed spectrum from the analyte fraction infusion. No drug-related material (indicated by a characteristic ^{12}C:^{14}C ratio) is observed in this spectrum. The spectrum in Figure 3(D) is a result of subtracting a summed background spectrum from the summed analyte spectrum. Drug-related ions are now clearly visible.

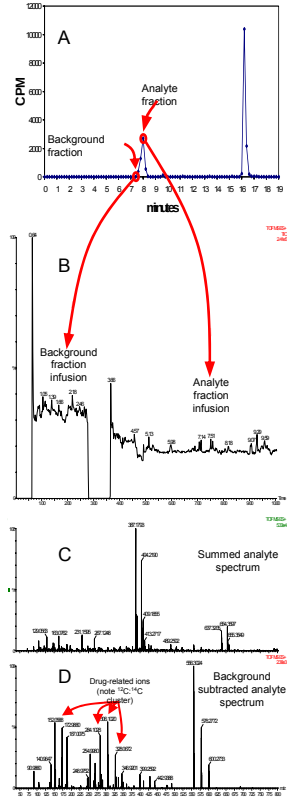

Figure 3. **Reconstructed LC/radio chromatogram (A), total ion chromatogram for background and analyte infusions (B), and summed and background subtracted analyte mass spectra (C) and (D)**

Example 3. Multiple well screening for components of interest

In this example, an LC run with a non-radiolabeled sample was fractionated into a 96-well plate. Each fraction was infused without further manipulation using the Advion NanoMate automated nanoelectrospray source and a Finnigan LTQ FT mass spectrometer. Full scan high resolution data were acquired from 75 fractions in just over an hour to a single data file. Figure 4(A) shows the total ion 'infusagram' for this experiment. Figure 4(B) shows an accurate mass extracted ion 'infusagram' from this same experiment. An ion of interest at m/z 595 was extracted the same way as with conventional LC/MS data. Figure 4(B) clearly shows that a fraction at about 29 minutes contains a component that gives an ion at m/z 595. Figure 4(C) shows the full scan mass spectrum from the 29 minute fraction. The inset shows the isotope cluster for m/z 595 and the high mass resolution obtained. The fraction could easily be re-visited using the NanoMate and reinfused for further, more detailed, MS analyses.

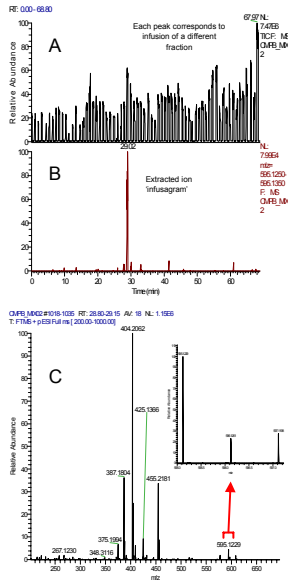

Figure 4. **Total ion 'infusagram' (A), extracted ion 'infusagram' (B), and mass spectrum of fraction of interest (C)**

Example 4. Rapid product ion tree experiment

Figure 5 shows the full scan MS and MS^2 through MS^6 product ion spectra for a drug metabolite analyzed in an automated product ion tree experiment. These data were acquired using an Advion NanoMate and a Finnigan LTQ mass spectrometer. These data were acquired very rapidly (under 4 seconds) and with high sensitivity (approximately 17 pg of drug metabolite was consumed to generate these spectra).

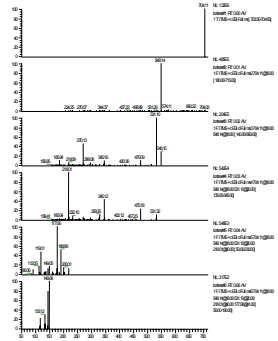

Figure 5. **Full MS and MS^2 through MS^6 product ion spectra from an automated product ion tree experiment**

CONCLUSIONS

Automated nanoES mass spectrometry offers several advantages over the conventional LC/MS approach for elucidating the structures of drug metabolites. These advantages include:

- A factor of 4-5 increase in experimental work throughput
- Ability to signal average to generate higher quality data
- A reduction in the quantity of analyte required, at least 100 times less material required to generate equivalent quality data
- Rapid switching between projects on a mass spectrometer (no LC system changeover is required)
- Rapid switching between ionization polarities
- Rapid switching between MS platforms with the same sample fraction
- The ability to modify the analyte molecule and/or spray solvent
- Default matching of metabolite structures and spectra to retention time (no issues with matching between different LC systems)
- Capability to make use of very low intensity product ions for structural assignment

Future work plans include integrating automated nanoelectrospray with automated data dependent data acquisition strategies.