

Drug Metabolism Studies Utilizing A Nanosplitting Device with On-line Mass Spectrometric and Radiomatic Detection

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Previously in our laboratory, we designed a nanosplitting device that increases the sensitivity of liquid chromatography – mass spectrometry (LC-MS) analyses. This device incorporates a concentric split as part of the electrospray interface, which directs much of the sample to another detector or to collection. In drug metabolism, multiple detection methods are desirable to provide confirmation that peaks of interest are indeed metabolites of the parent drug. Preclinical animal dosing with a ¹⁴C drug candidate allows for selective radiomatic detection of metabolic conversions of the parent compound, while mass spectrometry provides sensitive detection and an additional separation method, since the masses of suspected metabolites can be separated into multiple chromatographic traces.

Rat models were dosed with a ¹⁴C-enriched version of Compound A. Urine was collected at subsequent time intervals and injected into the LC-MS-Radiometry system without further clean up. The LC-MS-Radiometry system consisted of a CTC PAL Autosampler (LEAP Technologies, Carrboro, NC), an Agilent Technologies 1100 LC Pump (Wilmington, DE), a ThermoFinnigan TSQ 7000 triple quadrupole mass spectrometer (San Jose, CA), and a Packard Instruments Radiomatic Flow-One Radiochromatography Detector (Downers Grove, IL). The flow rate through the LC column was 1.0 mL/min, which was split once post-column, providing 0.9 mL/min to the radiometric detector, and 0.1 mL/min to the mass spectrometer. Subsequently, when the nanosplitter was used, the MS flow was split again and only 200 nL/min entered the mass spectrometer. When the standard MS interface was used, the entire 0.1 mL/min flow was introduced to the MS.

Several metabolic pathways were investigated to determine the utility of the nanosplitter in drug metabolism. A rat urine sample collected 4 hours after dosing with a ¹⁴C-enriched compound was injected into the standard LC-MS-Radiometry system. This sample was then re-analyzed with the nanosplitter in place instead of the conventional ThermoFinnigan electrospray interface. All LC and MS scan parameters were kept the same regardless of the interface used, and only parameters specific to the interface (i.e. spray voltage, capillary temperature, sheath gas flow) were changed between the two analyses. A blank (time point zero) was analyzed in the same fashion, and those results were compared to the 4 hour time point to eliminate endogenous peaks that were not produced by metabolism of the parent compound. Additional verification that peaks of interest were produced by metabolism was provided by the specific radiomatic detection of ¹⁴C-enriched analytes. The radiomatic traces of each set-up (standard MS interface versus nanosplitter interface) were compared to ensure that changing the MS interface did not affect this method of detection. The peak shape and signal intensity of the

radiochromatographic detection achieved when the conventional MS interface was utilized were maintained in the nanosplitter system, as shown in Figure 1.

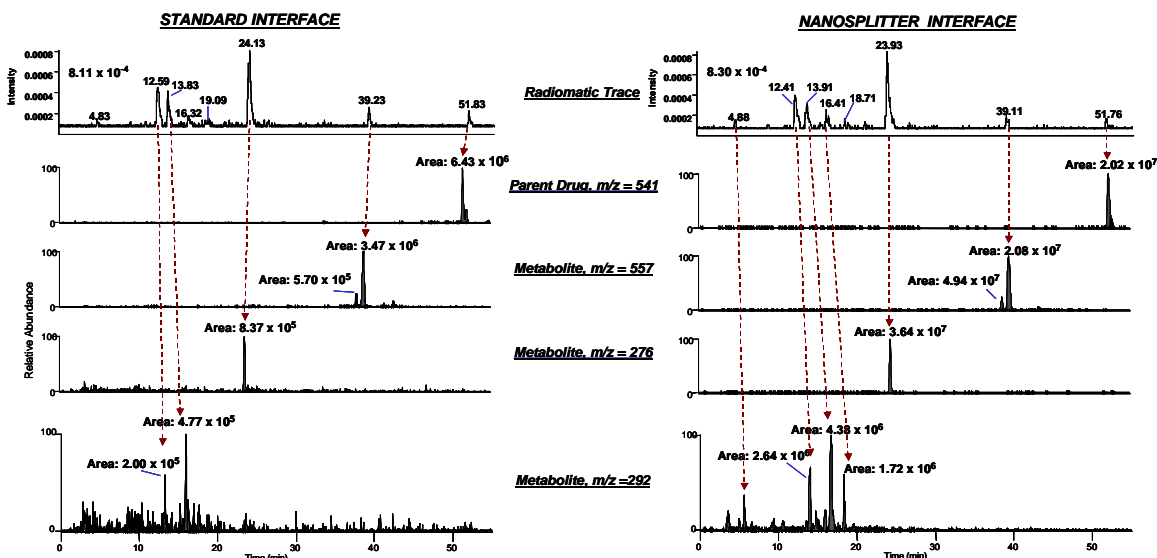


Figure 1: Comparison between the two analyses with the standard interface and the nanosplitter. The top trace is the radiomatic detection. Each MS trace is an extracted ion chromatogram for the parent drug or a specific metabolic pathway. In the m/z 292 trace, 2 additional peaks are fished out of the noise, and are matched to signals in the radiomatic trace.

By using the nanosplitter, signal increases were observed ranging from 200 to 4000% (2x to 400x the peak area) of the signal generated using conventional electrospray ionization, depending on the structure of the metabolite and its retention time. Additional metabolites not identified using conventional electrospray were detected in the m/z 292 trace using the nanosplitter interface. When using the standard interface, the additional metabolites generated radiomatic peaks, but the corresponding MS peaks were buried in the noise. The detection of these peaks when using the nanosplitter demonstrates the power of this interface for early drug metabolism studies.

It is normally difficult to couple radiomatic detection to a LC-MS analysis because a large portion of the LC eluent must be directed toward the radiomatic detector, thus decreasing the sensitivity of the MS detection. By utilizing the nanosplitting MS interface, it was possible to direct 90% of the LC column eluent to the radiomatic detector and still obtain a substantial increase in the signal intensity of each of the metabolites. Additionally, the radiomatic detector was coupled through a micro-tee before the nanosplitter. In this configuration, it would still be possible to recover almost 10% of the sample for further analysis. This set-up was chosen due to pressure fluctuations generated by the radiomatic cocktail pump necessary for ^{14}C detection.

This work demonstrates the successful utility of a nanosplitter LC-MS interface with multiple detection methods, including radiometry, in drug metabolism experiments. The radiomatic detection allows for specific detection of radiolabeled compounds, thus providing initial confirmation that peaks detected by the mass spectrometer are indeed metabolites. Samples recovered can then be re-analyzed, and peaks of interest can be further investigated using MS/MS.