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Effect of Accurate Mass MS Data Acquisition Rate on Data Quality in Metabolic Phenotyping Studies

Lisa Reid, Robert S. Plumb

Imperial College, Waters Corporation

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief evaluates the effect of mass spectrometric acquisition speed on LC-MS data quality in metabolic phenotyping studies of biological fluids.

Benefits

The derived data quality in metabolic profiling is significantly impacted by the MS data acquisition rate

Introduction

The MS data acquisition rate or scan time selected is usually determined by the design of the mass spectrometer and the MS resolution required for the analysis.

Fourier-transform mass spectrometers (FTMS) such as Fourier-transform ion cyclotron resonance mass spectrometry (FTICR) and Orbitraps have the potential to reach extremely high MS resolution, greater than 200,000 full width at half maximum (FWHM). In order to achieve such values, they require acquisition times in excess of 1 second, and therefore these high-resolution MS spectral results are most beneficial for structural elucidation experiments. Such slow data acquisition times can be acceptable when the liquid chromatography (LC) analysis time is greater than 45 minutes and when LC peak widths are greater than 10 seconds at the base. As the data acquisition time is reduced (faster acquisition) on a FTMS, there is commensurate reduction in MS resolution; this was demonstrated by Olah et al.[1] where the use of MS data acquisition rate of 100 milliseconds resulted in an effective mass resolution of 10,000 FWHM.

The MS data acquisition rate can have a significant effect on the quality of the data obtained in complex mixture analysis experiments such as metabonomics, metabolomics and metabolic phenotyping. Metabolic phenotyping studies require the analysis of biological samples from a large cohort ranging from several hundred to several thousand samples. Therefore in these studies, both short run times and high chromatographic resolution are required. When operated in gradient mode, modern sub-2-µm particle LC systems produce chromatographic peaks with widths of 1–3 seconds at the base.[2,3] It is therefore necessary to have a data acquisition rate that allows for accurate analyte quantification and identification when peaks are eluting rapidly and/or overlapping. For reliable analyte quantification, it is necessary to have 8–10 data-points across the chromatographic peak; therefore for a peak width of 1–3 seconds, a data acquisition rate of

50–100 milliseconds is required. Unlike FTMS instruments, Time-of-flight (Tof) mass spectrometers acquire MS data at virtually the same resolution independent of the data acquisition or scan rate.

Results and Discussion

The ability to derive meaningful biological results in metabolic phenotyping studies is highly dependent upon the capability of the analytical measurement apparatus to detect, identify and quantify the endogenous metabolites in biological samples. The analysis of large cohort studies by LC-MS increases this challenge as it requires the instrument to perform these tasks in a high throughput mode. To achieve this, the chromatography system needs to deliver a high resolution separation in a 10–15 minute time scale and the mass spectrometry platform needs to be able to acquire high resolution data with a fast acquisition rate.

UltraPerformance Liquid Chromatography (UPLC) proved to be the ideal chromatography platform for this task, delivering separations with peak capacities of 300–700 in just 10 minutes.[2,3] These high resolution separations generate chromatographic peaks with widths in the order of 1–3 seconds at the base. In order to accurately identify and quantify these peaks, it is necessary to operate the mass spectrometer with a data acquisition rate of 10–20 spectra per second, "scan" speeds of approximately 0.036–0.1 seconds.

The effect of data acquisition rate on the chromatographic resolution is demonstrated by the reversed phase LC-MS analysis of human urine. This analysis was performed by injecting 2 µL of diluted human urine onto a 2.1 x 150 mm Waters HSS T3 Column. The column was operated at 40 °C and compounds eluted with a 0–55% acidified aqueous-acidified acetonitrile gradient at 600 µL/min. The column effluent was monitored by positive ion ESI using a Xevo G2-XS QTof. The data displayed in Figure 1A and 1B represents the use of a data acquisition rate of 0.0036 seconds and 1.0 second respectively. A critical inspection of the data reveals that the use of the 1.0 second acquisition rate results in a reduction of observed chromatographic resolution with closely eluting peaks being merged and the fine detail of the separation lost. This effect is illustrated by the shaded region in the chromatogram between 6.00 and 7.25 minutes; it is clear that when using a 1.0 second data acquisition (1B) there are less discernable peaks in the chromatogram and those that are detected are broader. This observation is further illustrated by the data in Figure 2, which details the chromatographic resolution for four pairs of endogenous peaks. As can be seen from the data in Figure 2, there is a significant reduction in observed LC resolution as the data acquisition time increases beyond 0.05 seconds after which there is plateau followed by a further drop in observed resolution when the acquisition rate increases over 0.5 seconds.

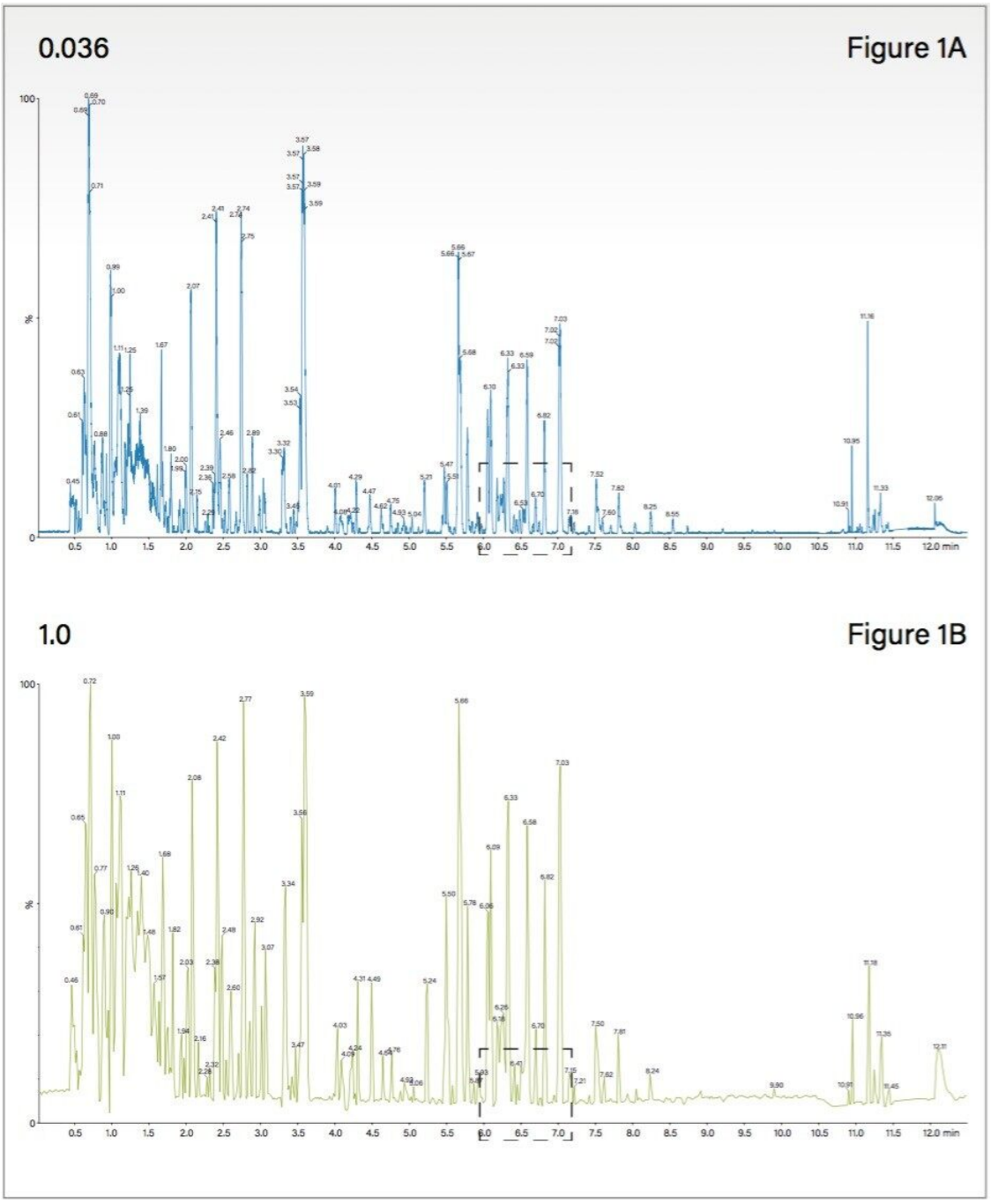

Figure 1. Effect of MS data acquisition rate on observed LC resolution.

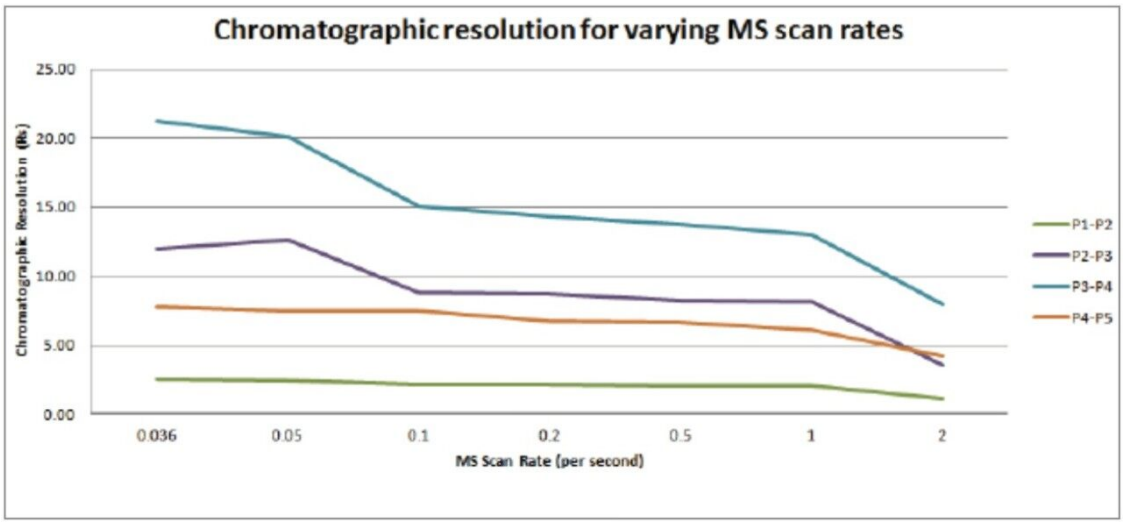

Figure 2. Effect of data acquisition rate on chromatographic resolution.

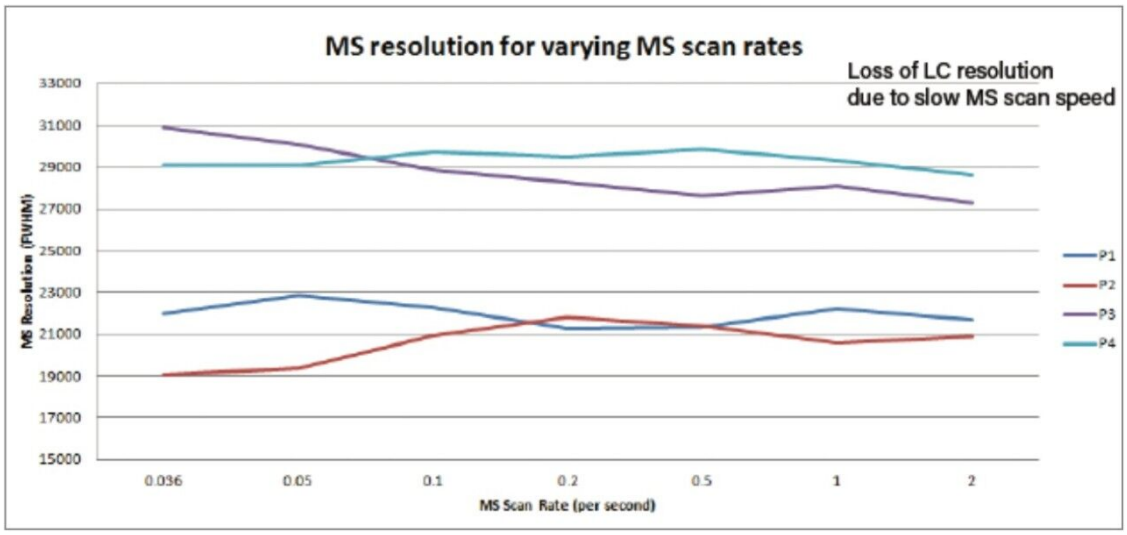

Figure 3. Effect of data acquisition rate on MS resolution.

Conclusion

Metabolic phenotyping of clinical or animal studies for biomedical research relies on the generation of high quality, information rich data from which meaningful biological data can be derived. UPLC-Accurate Mass

MS has become a foundation technology for such studies, due to its sensitivity, analysis speed and ability to identify potential biomarkers from the MS spectral information. However, the ability to perform the analysis of large cohort studies requires an analytical platform that can operate in a high-throughput environment without losing spectral quality or analyte specificity. The Waters Xevo G2 XS Mass Spectrometer is ideally suited to this task. The time-of-flight design of the instrument and high speed QuanTof Detector allows for very fast data acquisition rates to be employed without losing mass resolution and thus allowing for the retention of the fine chromatographic detail. This prevents spectral overlap, and reduces the potential for missing closely eluting isobaric analytes.

References

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