# A Comparison of Nominal Mass and High-Resolution Technologies for the Generation of Simultaneous Quantitative and Qualitative Metabolism Data

Angus Nedderman, Mark Savage, Michelle Gleave and Julian Haynes

**Unilabs York Bioanalytical Solutions, Metabolism and Discovery Services, Discovery Park, Ramsgate Road, Sandwich, Kent, CT13 9ND, UK**

## INTRODUCTION

In recent years, the revolution in automated bioanalytical approaches has enabled the routine generation of rapid ADME data, providing key information on the metabolism of novel compounds. Amongst the most recent initiatives within the DMPK arena is a drive towards the simultaneous generation of quantitative and qualitative metabolism data, known as 'qual/quan' approaches.[1,2] This work describes an investigation of the benefits and limitations of different mass spectrometric platforms and chromatographic approaches to the generation of qual/quan metabolism data.

## EXPERIMENTAL

Verapamil, terfenadine and haloperidol were incubated (1µM) in human liver microsomes and aliquots of the incubates were precipitated with acetonitrile at various time points. The supernatant was evaporated to dryness and the samples were reconstituted in 25% acetonitrile prior to MS analysis.

Quantitative clearance data were obtained on API5000, LTQ Orbitrap and QToF instruments using MRM or HRMS approaches. Qualitative information was obtained via predicted MRM transitions (API5000) or by acquiring full scan HRMS data ± data-dependent $MS^2$ (varying the MS resolution/IT fill time) or MSE acquisition.

## RESULTS

### *Quantitative analysis of model compounds*

LC-MS analysis on both triple quadrupole and high-resolution instruments yielded very similar intrinsic clearance data for all the model compounds (Figure 1; Table 1).

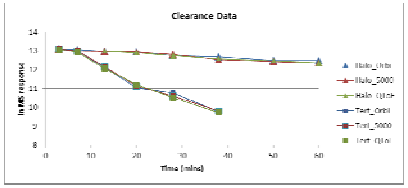


*Figure 1: Illustration of the correlation in Clint data generated on TQ and HRMS instruments*

| Compound | Half-life (mins) | | |
|---|---|---|---|
| | API5000 | Orbitrap | Q-ToF |
| Verapamil | 8.4 | 8.2 | 8.5 |
| Terfenadine | 6.2 | 5.7 | 6.4 |
| Haloperidol | 51.0 | 49.1 | 48.5 |

*Table 1: comparison of metabolic half-life data for the model compounds on the 3 MS instruments*

Using MS resolution settings of 12000 and 7500 on the Q-ToF and Orbitrap respectively allowed sufficient scan speeds to generate robust quantitative data generation with relatively short LC run-times (~2mins).

### *Qualitative analysis of model compounds*

Regardless of the MS platform used for the analysis, typical LC-MS/MS methodologies used for clearance screens resulted in significantly compromised chromatographic resolution, as evidenced for verapamil, where demethylated metabolites could not be fully resolved without extending the run-time to approx 6 minutes (Figure 2).

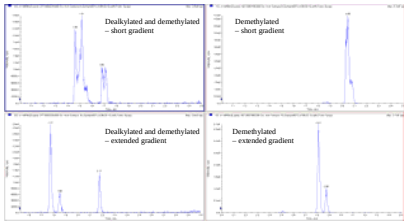


*Figure 2: LC resolution of verapamil metabolites*

Using the extended gradient, adequate qualitative data could be generated using all MS platforms. With the API5000, predicted MRM transitions followed by data-dependent MS/MS data generation yielded useful data for many key metabolites. However, major components involving unusual pathways were missed, such as the dehydrated/aromatised metabolite of haloperidol (Figure 3).

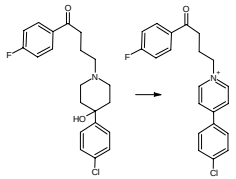

*Figure 3: Dehydration and aromatisation of haloperidol*

Qualitative analysis on the LTQ Orbitrap via extraction of accurate mass chromatograms resulted in detection of all major metabolites (see for example Figure 4) with the resolution setting used for quantitation (7500).

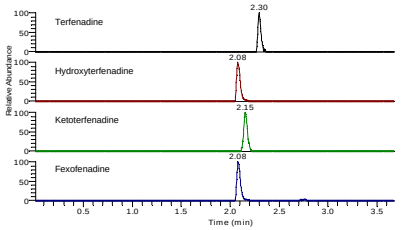


*Figure 4: Example XICs for terfenadine derived from HRMS*

In addition, simultaneous product ion spectra could be acquired on the LTQ Orbitrap using data-dependent scanning, enabling partial structural elucidation of metabolites (see for example Figure 5).

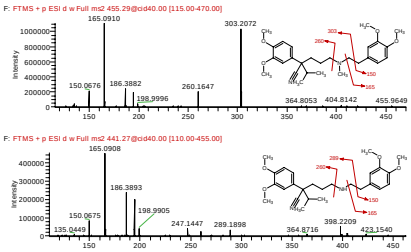


*Figure 5: MS/MS data for verapamil & N-desmethyl verapamil*

Qualitative analysis on the Q-ToF instrument was achieved via extraction of accurate mass chromatograms and simultaneous generation of MSE (pseudo-precursor) data (Figure 6), followed by additional MSMS analysis.

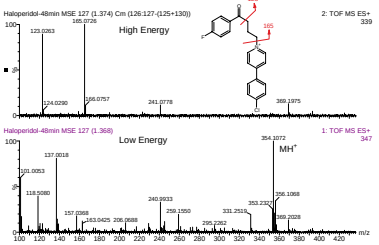


*Figure 6: MSE data for the dehydrated and aromatised metabolite of haloperidol*

## DISCUSSION

This analysis shows that comparable metabolic clearance data can be obtained using both MRM and HRMS approaches. As expected, LLOQs were higher using HRMS, whilst suitable MS resolution values could be chosen to allow relatively short run-times. All instruments were able to provide additional qualitative information, although high-throughput conditions significantly compromised chromatographic resolution. Qualitative data on the API5000 relies on metabolite prediction, which may require expert guidance or may miss key metabolites, whereas full scan HRMS data can be interrogated to detect metabolites and information on their structure can be obtained from data-dependent MS/MS data acquisition or by MSE scanning.

## CONCLUSIONS

This work demonstrates the feasibility of using qual/quan approaches to provide simultaneous information on the rate and routes of metabolic clearance. Three key discussion points arise from the investigation:

- Qual/quan analysis inevitably involves a compromise between speed and the thoroughness of the qualitative data
- Biotransformation and bioanalytical specialists need to collaborate to ensure optimal study design and data output
- A compromise study design, involving high-throughput intrinsic clearance data generation with selected samples taken for more detailed qualitative investigation might represent the most appropriate balance between speed and value in early discovery

### References

1. Hopfgartner G, Tonoli D, Varesio E. *Anal. Bioanal. Chem.* 402, 2587-2596 (2012).
2. Korfmacher W. *Bioanalysis* 3, 1169-1171 (2011).

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