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Application Note

UPLC-oa-Tof MS with One-Minute Separation Times Applied to a Metabonomics 90-Day Tox Study

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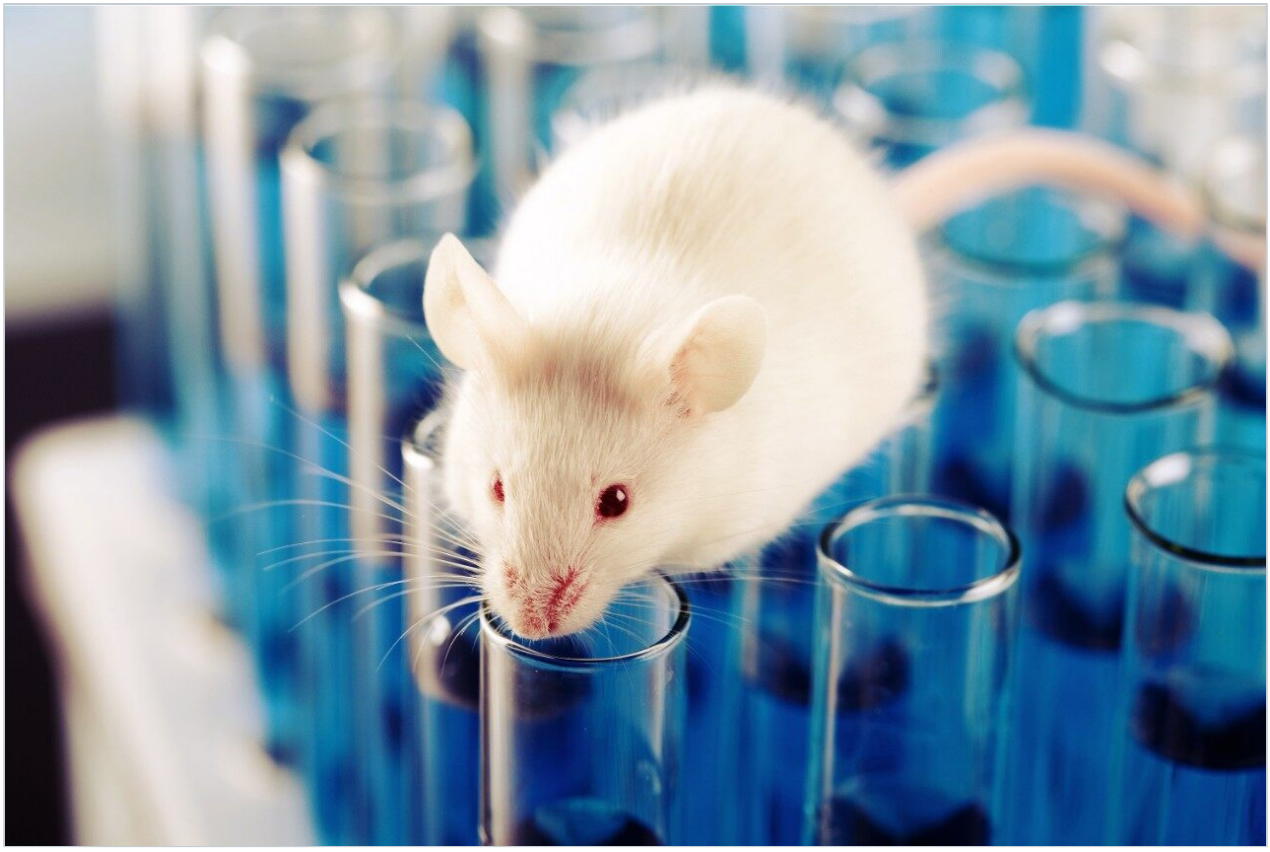

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Abstract

This application note employs UPLC coupled to oa-Tof MS with one-minute separation times for the analysis of rat urine following the oral administration of a candidate pharmaceutical over a 90-day study.

Introduction

The two major reasons that candidate pharmaceuticals fall out of the drug development pipelines are toxicity and lack of efficacy. These typically occur late in the development process, when a significant amount of time, effort, and funding has already been invested in the compound. The ability to predict the potential for toxic effects earlier in the drug discovery timeline would allow development of an unpromising compound to be attenuated, allowing resources to be channeled into more potentially successful areas. Predicting the toxicity in mammalian systems relies on the detection and identification of the biomarkers related to these effects. The two “omics” sciences of proteomics and metabonomics are focused on detecting these biomarkers of disease, efficacy, and toxicity. In proteomics, we look for changes in the expression of proteins due to a disease state or toxic event, while metabonomics focuses on changes in the relative concentration levels of endogenous metabolites as a result of the same. These changes are typically measured using high-resolution mass spectrometric (LC-MS) or high-resolution spectroscopic (proton NMR) techniques combined with data reduction and chemometric analysis.

The use of liquid chromatography combined with oa-Tof mass spectrometry has become the mainstay of proteomics analysis and is evolving as an essential part of the science of metabonomics. The ability to detect important biomarkers relies upon the sensitivity and selectivity of this analytical technique. UltraPerformance LC (UPLC) uniquely employs sub-2 µm stationary phases and high backpressures. When operating with these sub-2 µm materials, two chromatographically advantageous effects are observed: i.) the efficiency of the LC process is improved, and ii.) the van Deemter plot takes on a very flat nature allowing fast analysis without compromising the efficiency of the separation process.

This increased chromatographic efficiency results in a reduction in peak widths and a proportionate increase in peak height. This not only has the effect of increasing sensitivity in the MS, but also reduces spectral overlap and ion suppression. To demonstrate, we have employed UPLC coupled to oa-Tof MS with one-minute separation times for the analysis of rat urine following the oral administration of a candidate

pharmaceutical over a 90-day study.

Experimental

UPLC Conditions

System:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column, 1.7 µm, 2.1 x 50 mm
Flow rate:	1 mL/min
Gradient:	0–95% B from 0–1.5 min, where A = 0.1% formic acid, B = acetonitrile, 0.1% formic acid
Injection volume:	2 µL
Sample temp.:	10 °C
Column temp.:	40 °C

MS Conditions

System:	Waters Micromass LCT Premier Mass Spectrometer
Ionization mode:	Positive ion ESI
Data range:	50–850 *m/z* with Dynamic Range Enhancement (DRE) and W optics resolution
Acquisition rate:	100 ms

Inter-scan delay:	100 ms
LockSpray:	Leucine enkephalin, 25 pmol/µL
Cone voltage:	60V
Capillary voltage:	3kV
Desolvation temp.:	250 °C
Source temp.:	120 °C

Animal Studies

The animals were divided into three groups containing 16 male and 16 female animals and housed in metabolism cages with access to food and water ad librium. They were orally dosed daily with either vehicle alone, or the candidate pharmaceuticals at 2 mg/kg or 18 mg/kg for a total of 90 days. Urine samples were collected twice daily (0–12 hr. and 12–24 hr.) on days 34 and 86 of dosing.

Data Processing

The UPLC-MS (Tof) data was processed by reduction and chemometric analysis using the Waters MarkerLynx Application Manager for MassLynx Software. The data was divided into gender, time and dose group sets for the purpose of analysis.

Results and Discussion

A total of 192 samples were analyzed over a period of five hours; a representative extracted ion chromatogram of control males from day 34, 0–8 hr. is given in Figure 1. We can see from this data that the sample is very complex. A 3-D plot (not shown) revealed that over 1000 peaks were detected in this 1-minute separation. The LC-MS data was divided into groups of sampling occasion, gender, or dosing group and then processed using MarkerLynx.

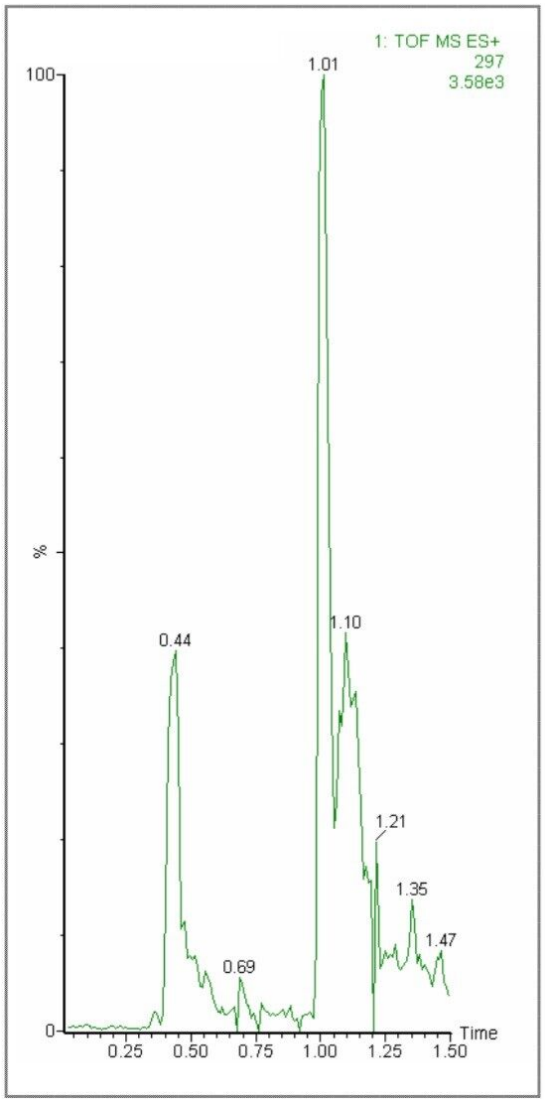


Figure 1.

Figure 2 shows the resulting PCA scores plot of the male samples on day 84, 0–8 hr. We can see that there is a clear differentiation between the control-, low-, and high-dose groups. The *m/z* and retention time pairs responsible for the group clustering are shown in the scores plot, Figure 3. The ion *m/z* = 455 at a retention time of 0.78 minutes is responsible for the low-dose group trend away from the control-dose group. Additionally, the ion *m/z* = 319 at 1.2 minutes contributes significantly to the high-dose group moving away from both the control-and low-dose group. The fact that the two different dose groups clearly move along different trajectories indicates that multiple biological responses are likely occurring in the animals. The low-dose animals could be experiencing a pharmaco dynamic effect from the administered compound, while the high-dose animals could be subjected to a toxic effect.

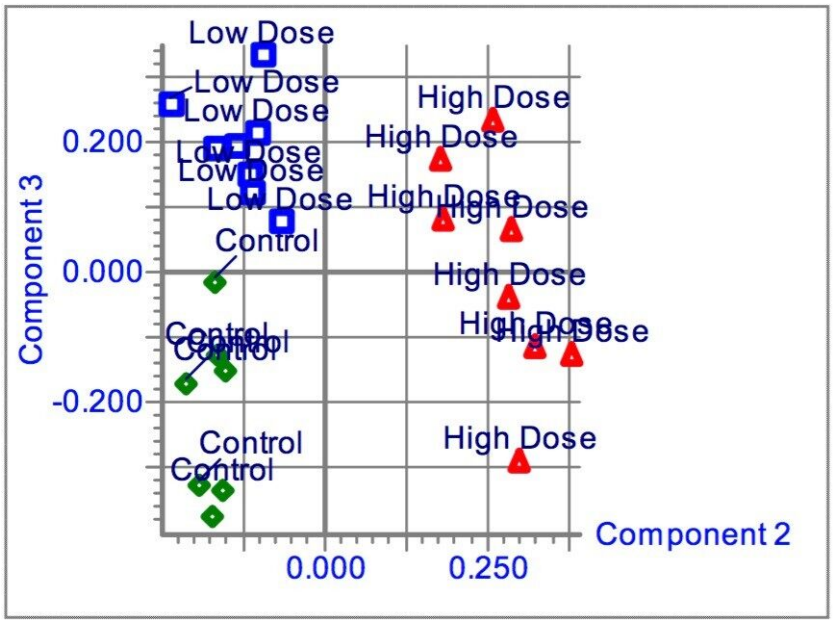


Figure 2.

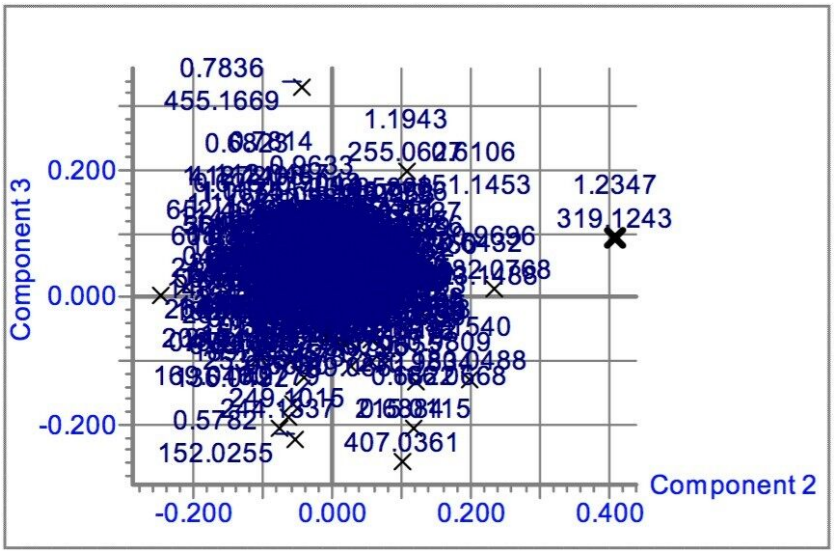


Figure 3.

The data generated by the 1.5-minute separation is similar to that generated by 10-minute separation in Figure 4, illustrating that the rapid separation generates the same high quality chemometric data.

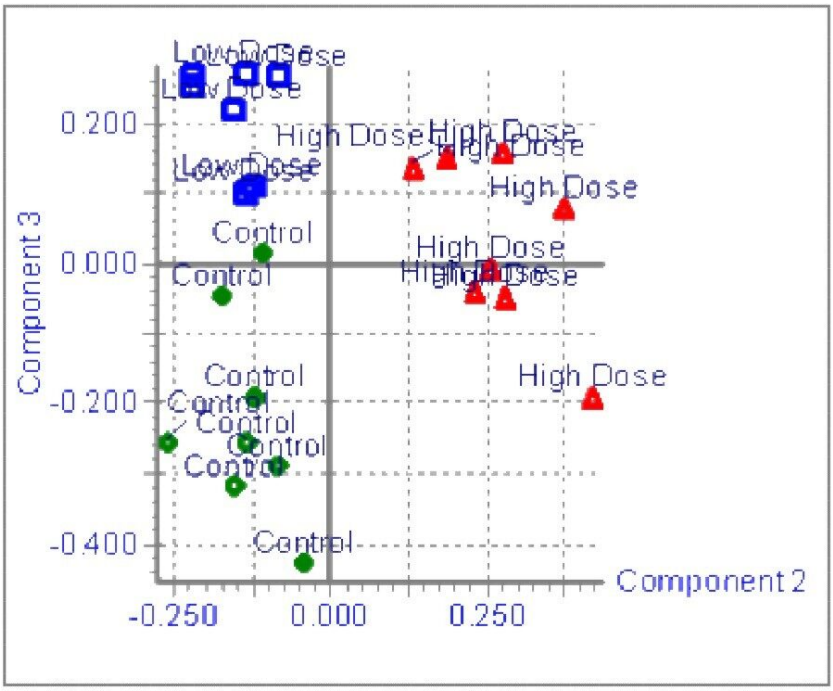


Figure 4.

By processing just the control animals on day 34, 0–8 hr., it is possible to differentiate the male and females by PCA analysis, Figure 5. While this may seem a trivial activity, it highlights the complex information-rich nature of the data and that care must be taken when interpreting, such that only true biochemical effects relating to the compounds under test are identified.

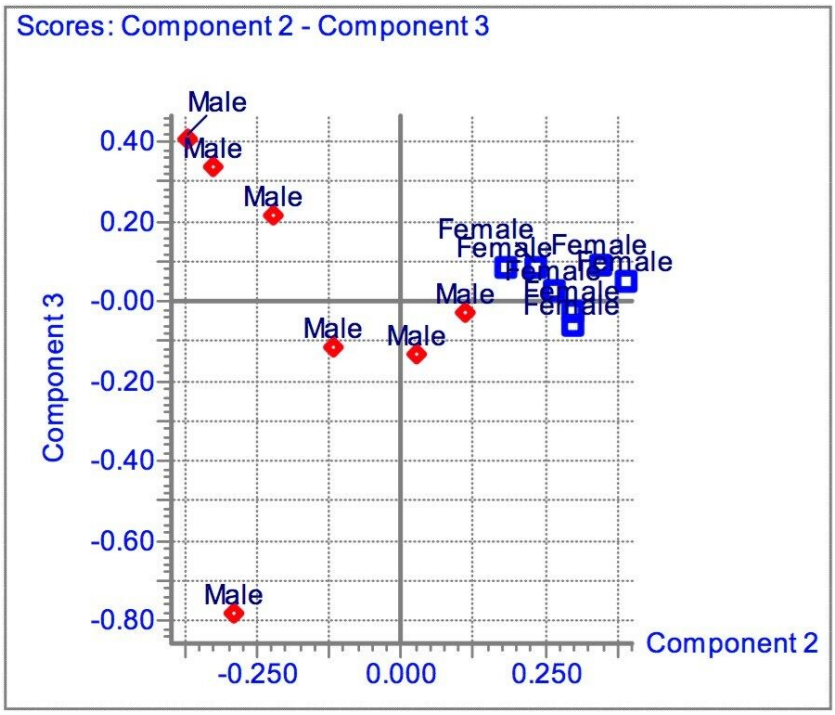
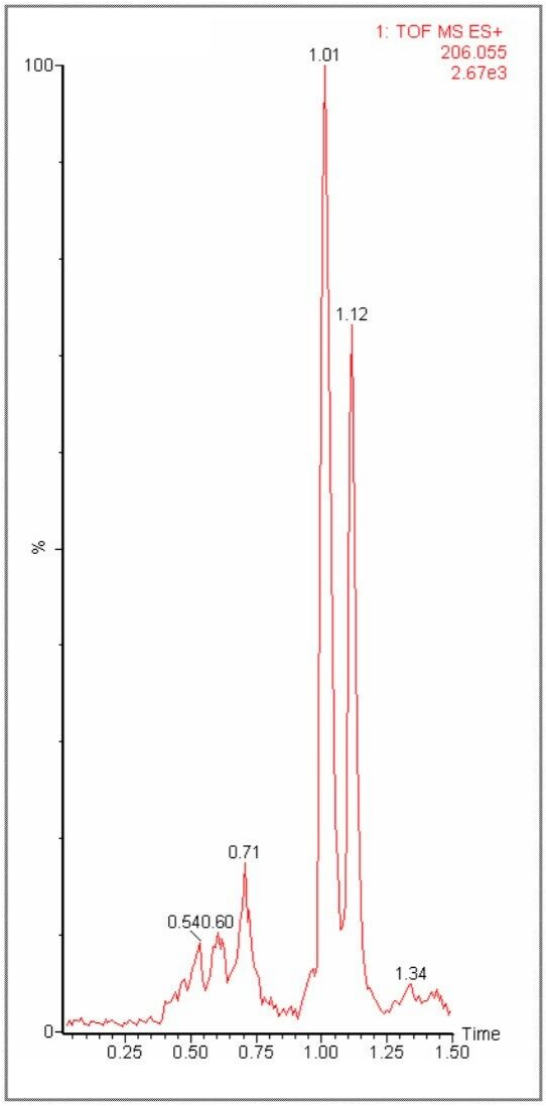


Figure 5.

The peak widths generated by the 1.5-minute separation are approximately 3 seconds at the base, yielding a total peak capacity of 25 for the separation. This is highlighted by the extracted ion chromatogram of xantheric acid (m/z = 206), Figure 6. Despite the very short analysis time, the MS spectra was of sufficient quality to detect several ions of interest, Figure 7a. Although these analytes are not completely resolved, there is adequate resolution to allow for the detection of kyneuric acid, xanthurenic acid, and panothenic acid. The intensities of these ions are significantly greater than that generated by infusion nanosprayMS, Figure 7b. The statistical data generated by the 1.5 minute separations show a similar trend in ion intensities as that generated by a 5 or 10 minute separation. Figure 8 again shows that although the 1.5-minute separation is less information-rich than a 10 minute separation, it still contains the necessary biomarker information to allow for quality statistical data generation.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Time

Figure 6.

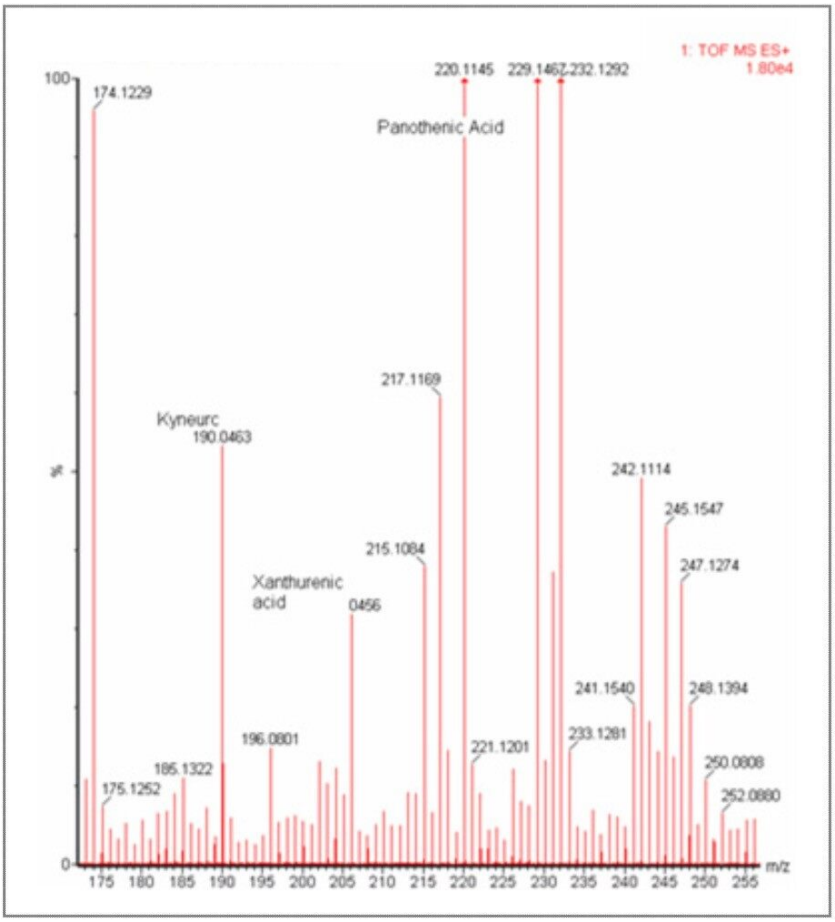

Figure 7a.

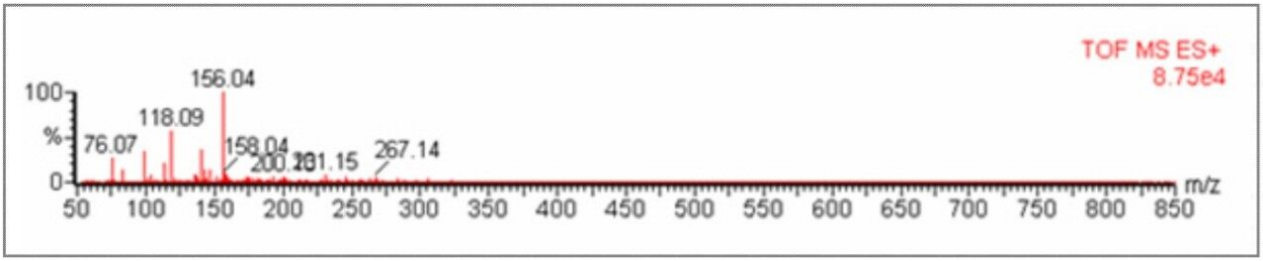

Figure 7b.

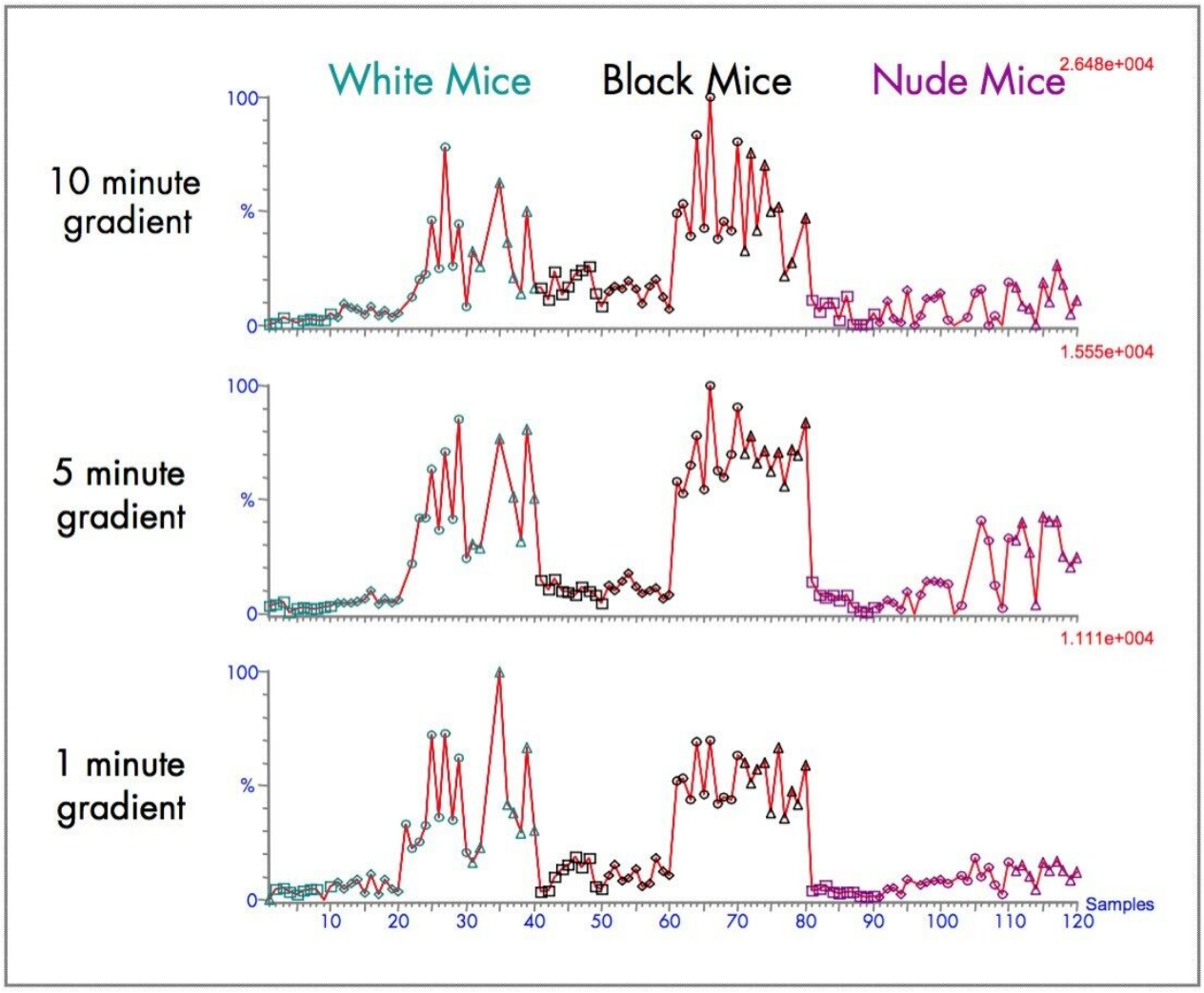

Figure 8.

Conclusion

The flat nature of the van Deemter plot with sub-2 µm particles combined with the ability of the ACQUITY UPLC System to operate at pressures up to 15,000 psi allows ultra-fast separations with good chromatographic resolution to be produced. The low system dead volume allows MS-compatible 2.1 mm internal diameter columns to be employed without the need for post-column splitting. These two features allow the rapid analysis of metabonomics samples in under 1.5 minutes by LC-MS. The data generated yields comparable statistical results to that achieved with a longer 10-minute separation. Thus, this 1.5-minute

analysis using ACQUITY UPLC System coupled to fast-scanning oa-Tof MS is the ideal screening tool for high throughput metabonomics.

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