

Poster Reprint

**ASMS 2022**  
**Poster number ThP256**

# Impact of Quadrupole Mass Resolution on Analytical Performance of MS/MS Assays using a triple quadrupole LC/MS system

Peter Weidner<sup>a</sup>, Behrooz Zekavat<sup>a</sup>, Anabel Fandino<sup>a</sup>, Karen E. Yannell<sup>a</sup>, Linfeng Wu<sup>a</sup>, and Patrick Batoon<sup>a</sup>

<sup>a</sup>Agilent Technologies, Santa Clara, CA

## Introduction

One of the challenges of high throughput and routine targeted LC/TQ analyses of complex biological, environmental and food matrices is ensuring that interferences from matrix components will not have a deteriorating effect on analytical results. To achieve this, labor-extensive sample preparation and clean-up methods are generally applied. Recent advances in hardware and software have allowed operating LC/TQs in higher mass resolution with good sensitivity and precision. Performing sample analyses at higher quadrupole mass resolution can improve the assay performance while maintaining data quality and assay precision.

In this work, we present results from performance evaluation of an LC/TQ operated in two different mass resolution settings:

- 1) **Unit resolution** (MS1: Unit, 0.7 Da FWHM; MS2: Unit, 0.7 Da FWHM)
- 2) **Enhanced resolution** (MS1: Narrow, 0.4 Da FWHM; MS2: Unit, 0.7 Da FWHM)

## Experimental

### Sample Preparation

Two different matrices, reconstituted bovine plasma and organic black tea extract, were prepared by previously described methods using the Agilent Bravo Platform<sup>1</sup> and Agilent QuEChERS dispersive SPE<sup>2</sup> respectively.

The plasma matrix was spiked with Agilent LC/MS Forensic Toxicology Calibration Mixture (P/N 5190-0470), whereas the Agilent LC/MS Pesticide Comprehensive Mix (P/N 5190-0551) was added to the black tea extract. Sets of samples were prepared by serial dilution in the concentration range of 0.05 – 500 ng/mL.

Each sample was run in 10 replicates using both Unit and Enhanced mass resolution settings.

### Instrumentation

Data was collected using an Agilent 1290 Infinity LC system coupled to a 6475 triple quadrupole LC/MS (Figure 1) with MassHunter 12 acquisition software.

LC/TQ conditions and system parameters were based on previously published application notes,<sup>2,3</sup> as listed in Table 1 and Table 2.

## Experimental



Figure 1. Agilent 6475 triple quadrupole LC/MS

Table 1. LC conditions

Parameter	Value	
Matrix	Plasma	Black Tea
Column	Poroshell C18, 2.1x100 mm, 2.7 µm at 35 °C	Eclipse Plus C18, 3x100 mm, 1.8 µm at 40 °C
Injection Vol.	1 µL	1 µL
Mobile Phase	A: 5mM NH <sub>4</sub> -formate + 0.01% formic acid in H <sub>2</sub> O; B: 0.01% formic acid in ACN	A: 5mM NH <sub>4</sub> -formate + 0.1% formic acid in H <sub>2</sub> O; B: 5mM NH <sub>4</sub> -formate + 0.1% formic acid in MeOH
Flow Rate	0.5 mL/min	0.4 mL/min
Gradient	0.5 min 15%B 3.0 min 30%B 4.0 min 95%B 6.0 min 95%B	0.5 min 5%B 2.0 min 40%B 13.0 min 98%B 14.5 min 98%B 14.6 min 5%B

Table 2. TQ source parameters

Parameter	Value	
Matrix	Plasma	Black Tea
Ion Source	AJS	AJS
Polarity	Positive	Positive
Drying Gas Temp. & Flow	320 °C 8 L/min	225 °C 11 L/min
Sheath Gas Temp. & Flow	380 °C 12 L/min	350 °C 12 L/min

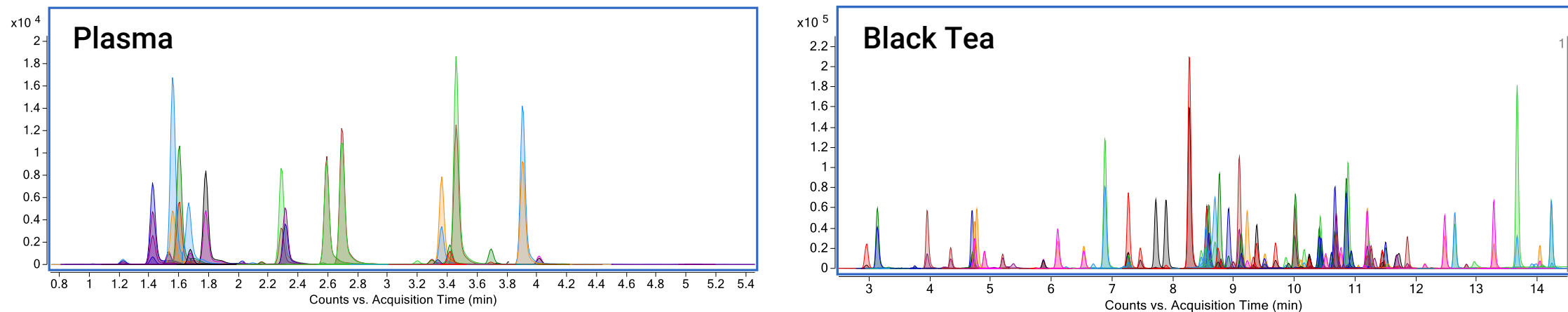


Figure 2. Extracted dMRM chromatograms of 24 compounds in plasma (left) and 80 analytes in black tea (right)

Operating the LC/TQ in enhanced resolution versus unit resolution mode leads to the following effects:

- 1) It can decrease background noise by filtering out isobaric matrix ions that otherwise would interfere with the analyte signal.
- 2) 2) By decreasing ion transmission through the quadrupole mass filter, it inevitably decreases the analyte signal as well. The overall effect depends on the balance between the reduction of noise and signal, which varies from analyte to analyte.

In this study, we observed approximately an average signal reduction of 30-40% in two different matrices over a total of 104 compounds (Figure 3).

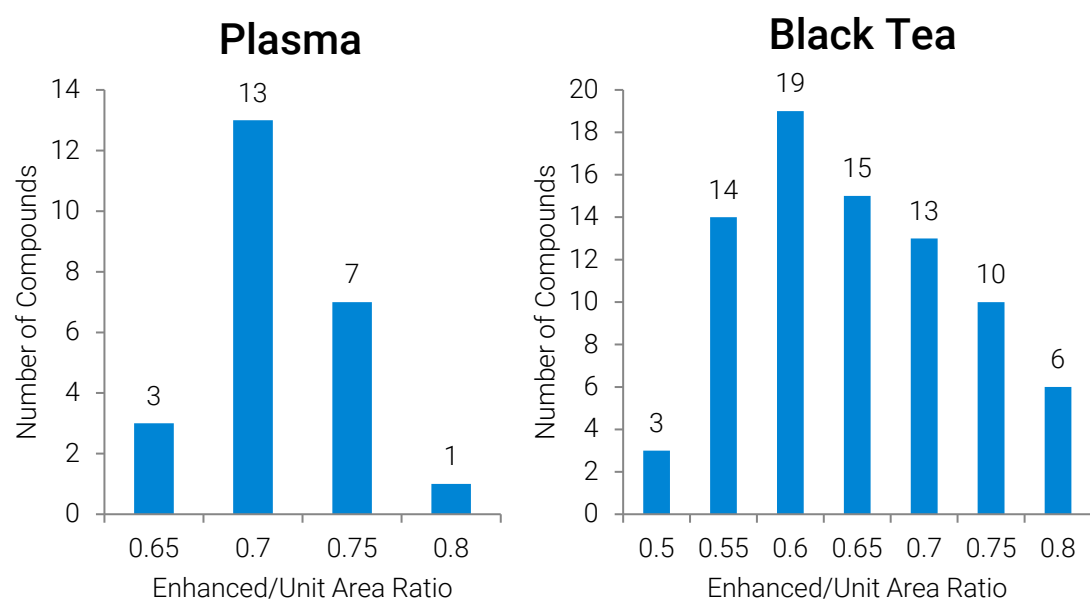


Figure 3. Distribution of Enhanced/Unit area ratios in plasma (left) and black tea matrix (right) at 5 ng/mL

The observed decrease in area response when operating the LC/TQ in enhanced resolution mode did not have a significant impact on performance or analytical results.

The majority of compounds maintained the criteria for quantitation (*i.e.*, both accuracy and precision) and calibration linearity (Figure 4).

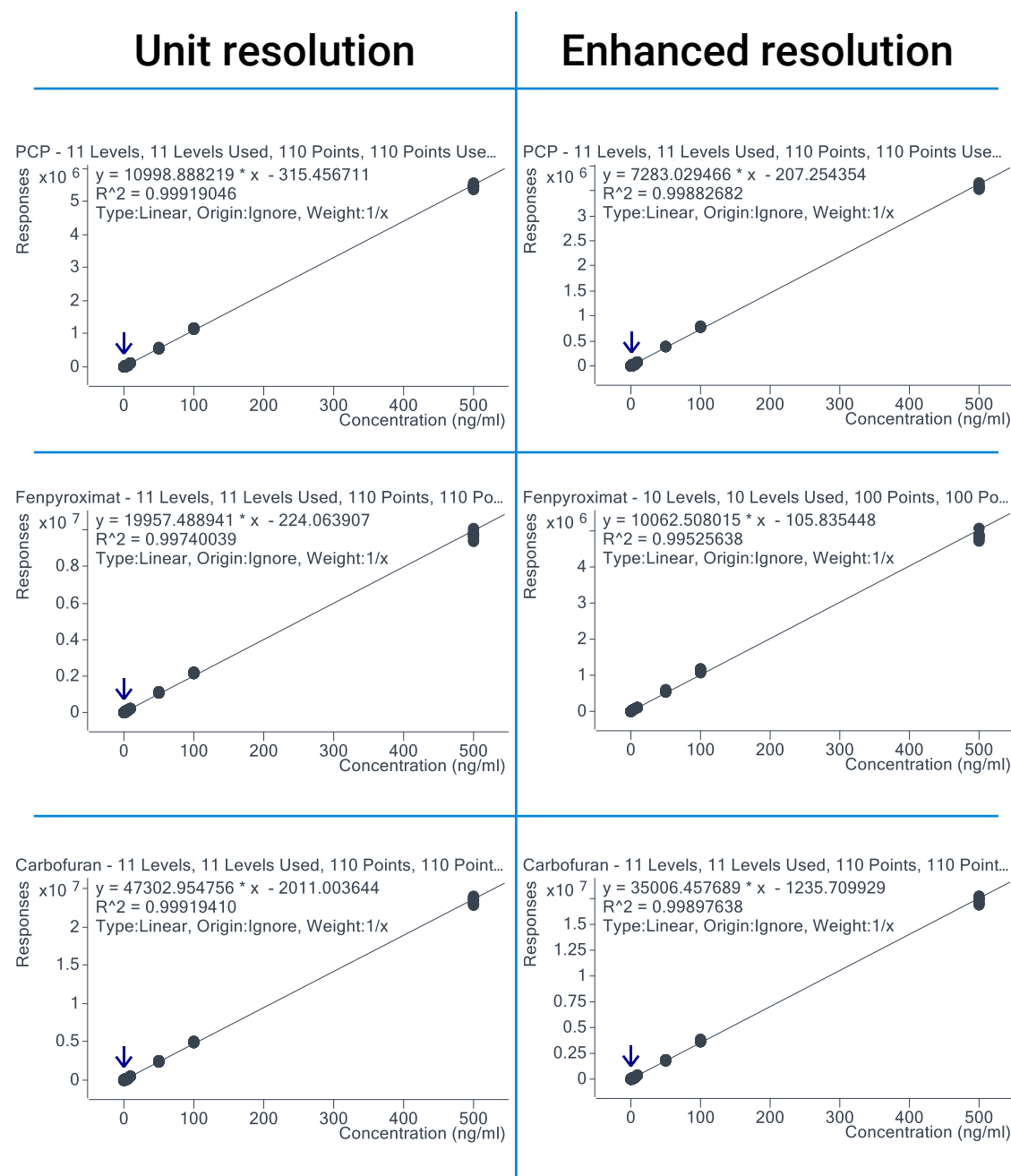


Figure 4. Calibration curves obtained in Unit (left) and Enhanced resolution (right) for three compounds: PCP in plasma (top), Fenpyroximat and Carbofuran in black tea (middle and bottom)

Unit and Enhanced resolution experiments resulted in similar area RSD% distributions (Figure 5), further supporting that operating the 6475 triple quadrupole LC/MS in Enhanced resolution provides reliable and high-quality data similar to Unit resolution.

## Results and Discussion

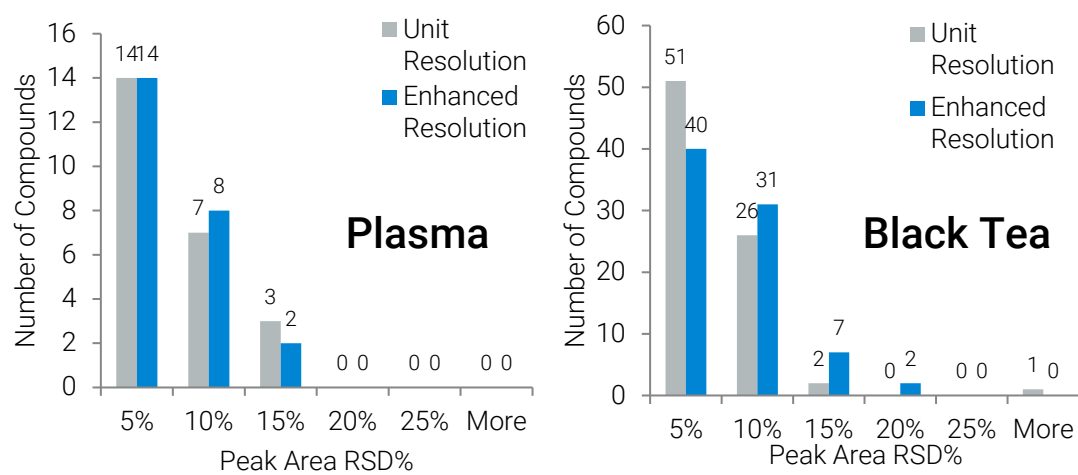


Figure 5. Area RSD% distributions in plasma (left) and black tea (right) at 5 ng/mL concentration level

Similar to Unit resolution, LLOQ values for all compounds remained at or below 2.5 ng/mL in the plasma matrix, and below the maximum residue limit (MRL) for pesticides (i.e., 10 ng/mL) in the black tea matrix, when the LC/TQ was operated in Enhanced resolution. Significant deterioration of LLOQs was not observed. Furthermore, 13% of all analytes in plasma and 10% in black tea achieved lower LLOQ levels in Enhanced mass resolution setting. (LLOQ defined as:  $RSD\% < 20\%$  and at least 7 out of 10 replicates have accuracy of 80%-120%). LLOQ distributions in the two different matrices are depicted on Figure 6.

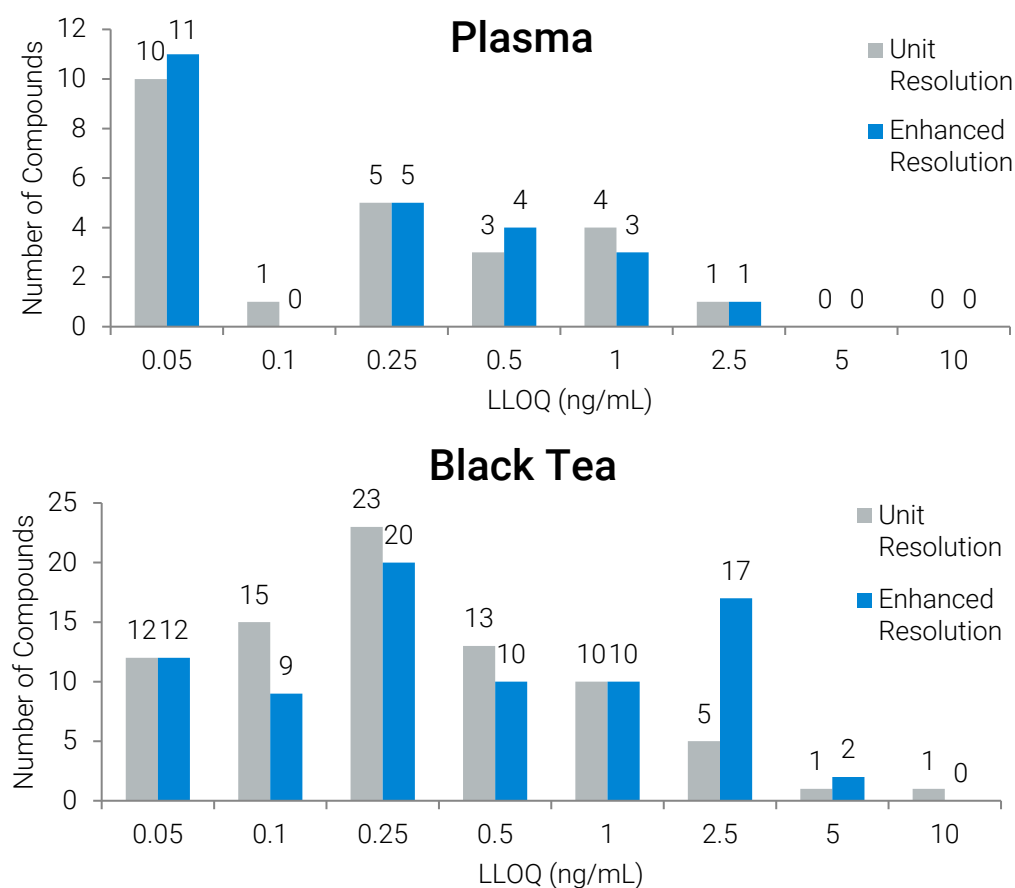
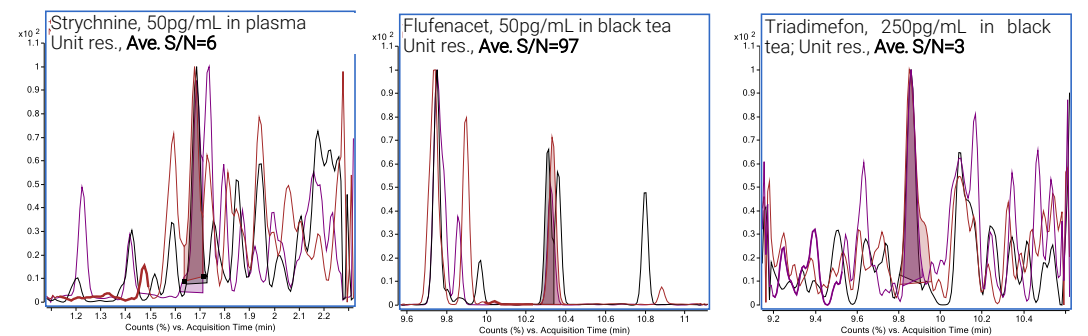


Figure 6. LLOQ distributions of 24 compounds in plasma (top) and 80 analytes in black tea (bottom)

Additionally, Enhanced mass resolution measurements can provide improved signal-to-noise ratio (S/N) in cases where isobaric background interference is reduced to a higher extent than signal from the analyte (Figure 7).

### Unit Resolution



### Enhanced Resolution

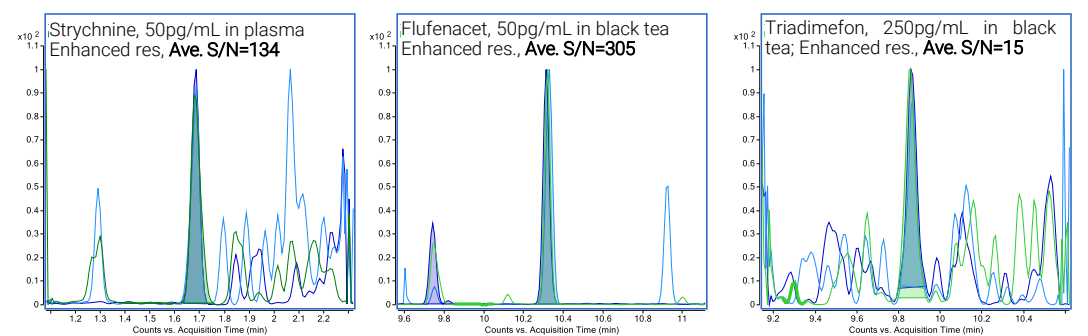


Figure 7. Extracted MRM chromatograms (n=3) for selected analytes in plasma (left) and black tea matrix (middle and right) using Unit (top) and Enhanced resolution (bottom)

## Conclusions

The comparison of two mass resolution settings (Unit and Enhanced resolution) was studied in two different matrices using a total of 104 analytes.

Data suggest that the peak area responses were reduced when using Enhanced mass resolution as compared to Unit resolution; however, operating the 6475 triple quadrupole LC/MS in Enhanced mass resolution mode can provide MRM chromatographic data with less interference and increased S/N, while maintaining data quality for quantitation.

## References

- <sup>1</sup>Agilent Application Note 5994-2156EN
- <sup>2</sup>Agilent Application Note 5994-1973EN
- <sup>3</sup>Agilent Application Note 5990-4254EN

<https://explore.agilent.com/asms>

This information is subject to change without notice.

DE49169329

© Agilent Technologies, Inc. 2022  
Published in USA, May 20, 2022