



A Combined Method for the Analysis of Barbiturates and 11-nor-9-carboxy Δ^9 THC in Urine by LC/MS/MS

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Introduction

Many laboratories are discovering the efficiency and ease of running larger advanced toxicology panels by liquid chromatography/tandem mass spectroscopy (LC/MS/MS) as opposed to the traditional screen then confirm model. Forgoing complicated sample preparation involving solid phase extraction (SPE) for a dilute and shoot methodology is another industry trend. The vast majority of the compounds in our 54-analyte advanced toxicology panel are run using positive mode; however, barbiturates and 11-nor-9-carboxy- Δ^9 -THC perform better in negative mode. With the rise in benzodiazepine use, abuse of barbiturates has declined, although not eliminated. Clinically, barbiturates are still commonly prescribed to treat seizure disorders and migraines and there is still a need to test for them. In addition, it is important to be able to test for both the synthetic version of cannabis that can be prescribed, and the natural version that in some jurisdictions can be legally prescribed, while remaining illicit in others. Traditionally, analyzing both barbiturates and 11-nor-9-carboxy- Δ^9 -THC would require separate sample preparation and two separate instrument runs. We developed an assay to combine five common barbiturates and a THC metabolite into one effective panel with minimal sample preparation.

Materials and Methods

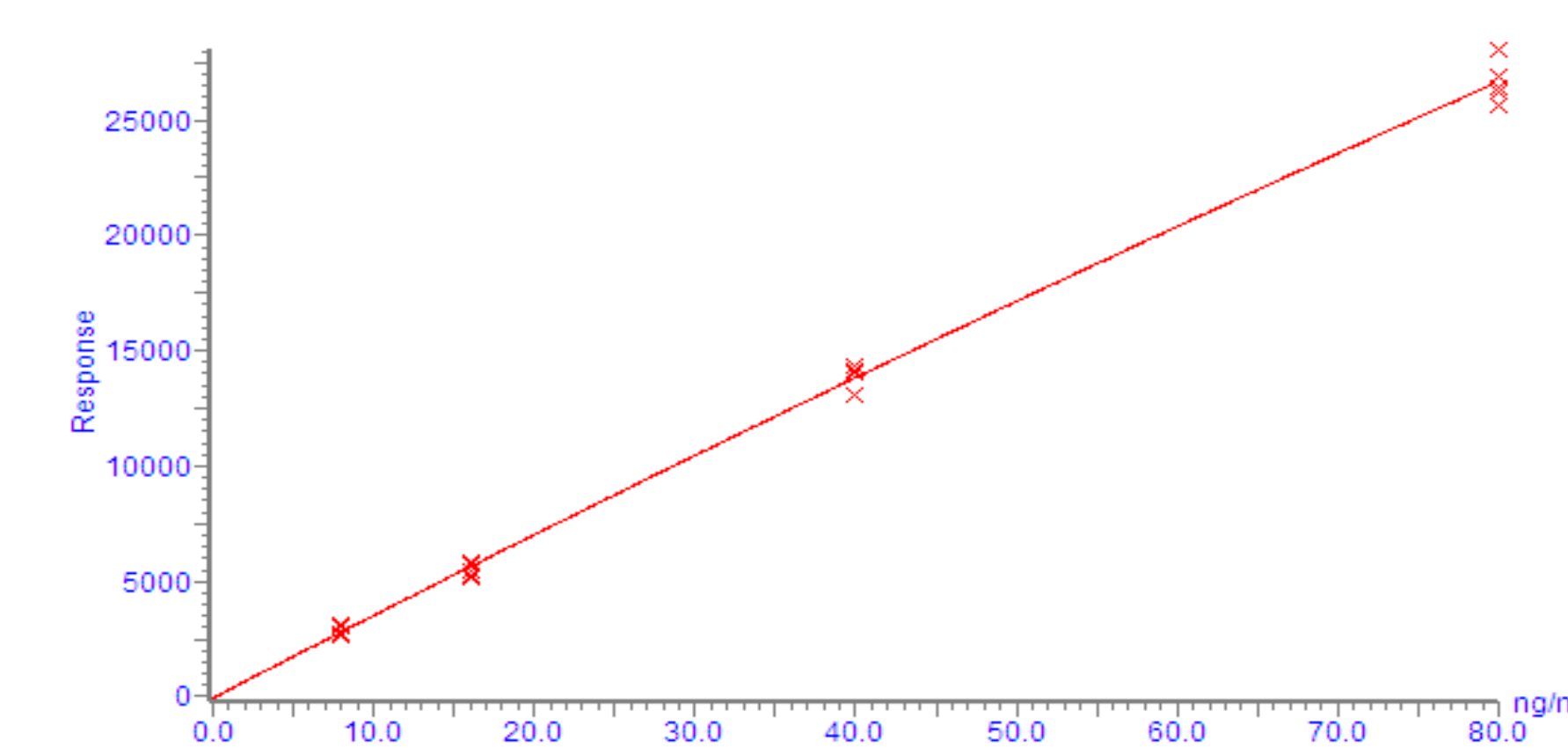
Patient urine samples arrived at our Troy, MI facility via second day air. An aliquot was then centrifuged at 220 x g for 5 minutes. Next, the urine was hydrolyzed with 2.5% β -Glucuronidase Type HP-2 enzyme from *Helix pomatia*. The samples were then diluted with a 50:50 water:methanol mixture spiked with the internal standards 11-nor-9-carboxy- Δ^9 -THC-d₉ and pentobarbital-d₅ from Cerilliant.

Materials and Methods, cont.

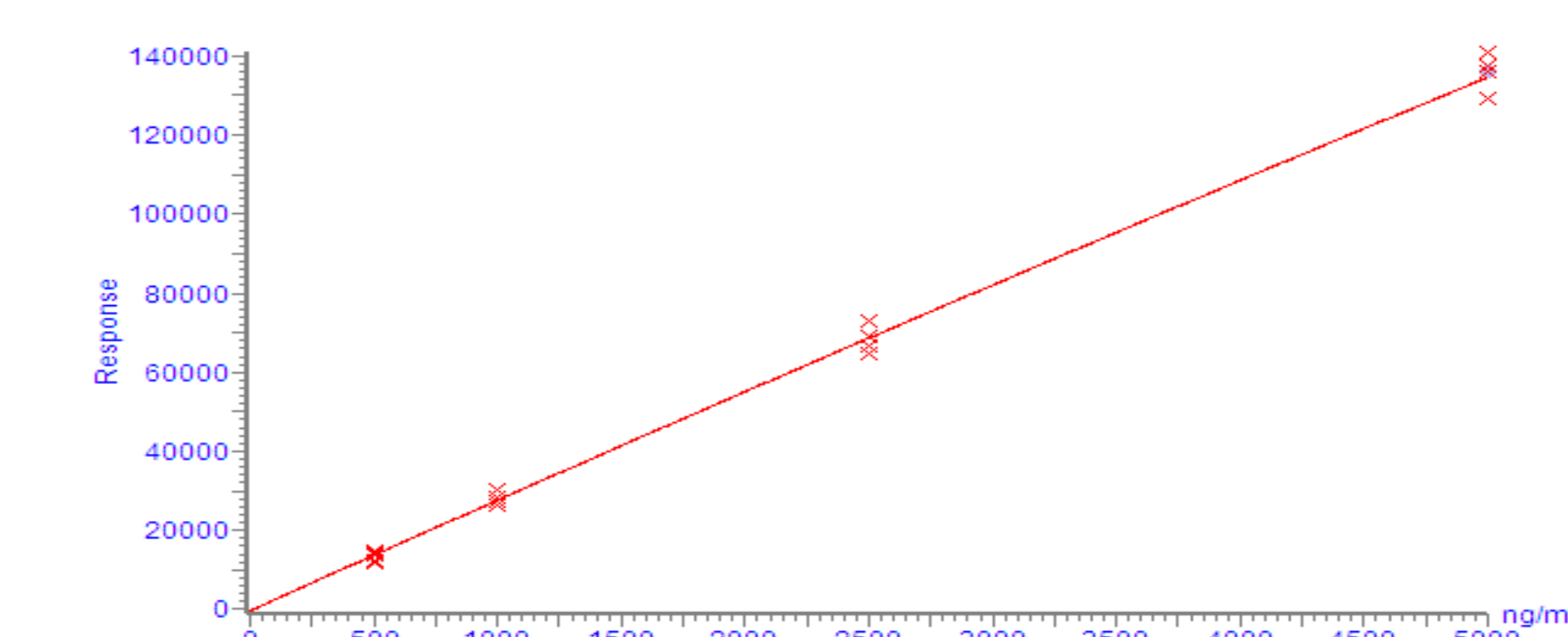
Barbiturates tested were: butabarbital, butalbital, pentobarbital, phenobarbital, and secobarbital. The THC metabolite was 11-nor-9-carboxy- Δ^9 -THC. Samples were run utilizing electrospray ionization and negative multiple reaction monitoring (MRM) mode on a MicroMass Ultima coupled to an Alliance 2795 HPLC Autosampler. Separations were performed using a Pinnacle® DB C18 column 5 μ m 150mm x 2.1mm and carried out at 45 C. The mobile phases consisted of water and acetonitrile with an ammonium hydroxide modifier. The run time was 10 minutes.

Results

Compound name: THC
Coefficient of Determination: R² = 0.997394
Calibration curve: -0.332127 * x² + 350.58 * x + -53.2912
Response type: External Std. Area
Curve type: 2nd Order, Origin: Include, Weighting: Null, Axis trans: None



Compound name: PHENOBARBITAL
Coefficient of Determination: R² = 0.995553
Calibration curve: -0.000238768 * x² + 28.1715 * x + -290.913
Response type: External Std. Area
Curve type: 2nd Order, Origin: Include, Weighting: Null, Axis trans: None

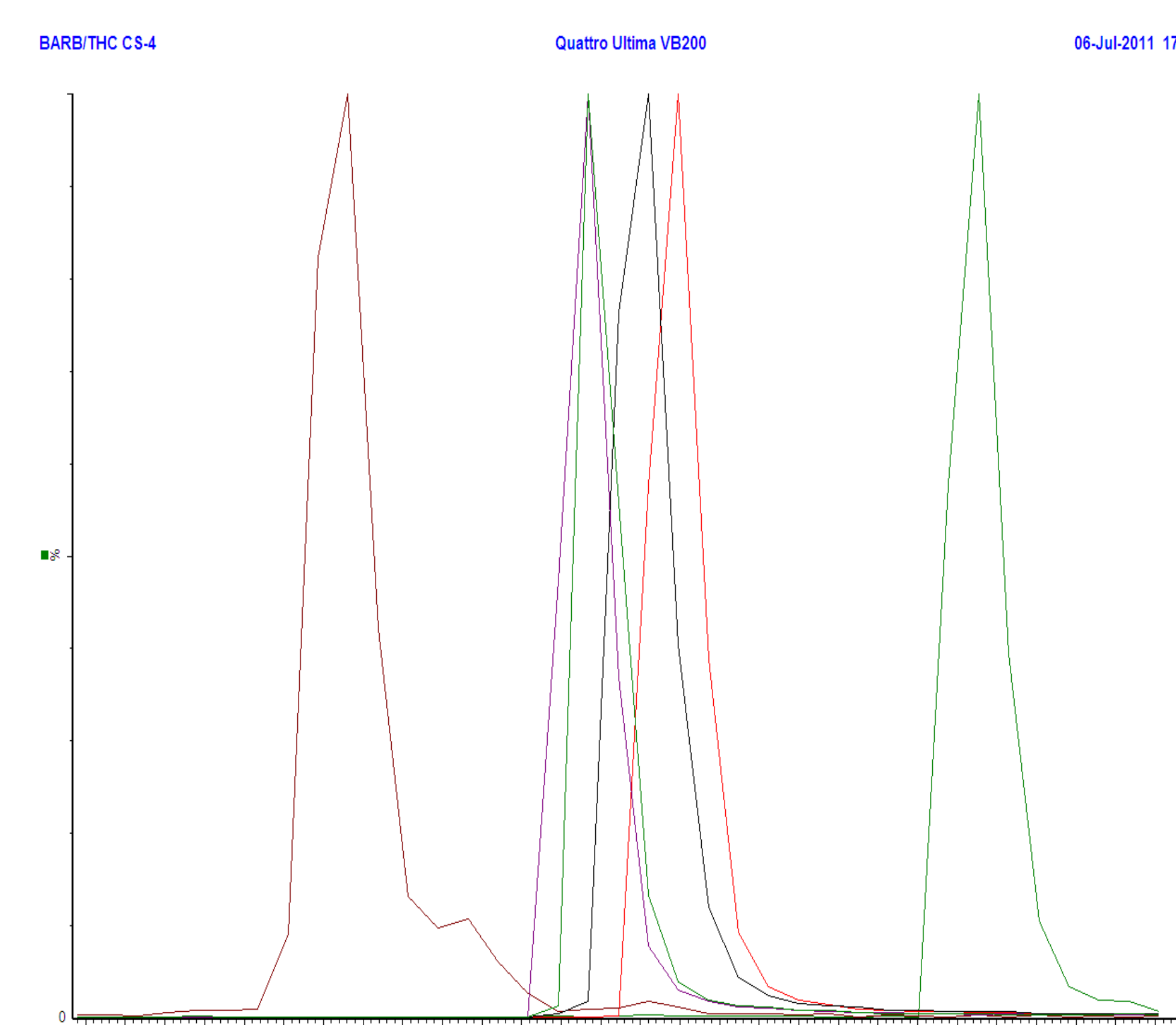


Graph 1: calibration curves for 11-nor-9-carboxy- Δ^9 -THC and phenobarbital, respectively.

Sample preparation was simple and efficient. Separation of the analytes was adequate (Graph 2). Butabarbital and butalbital did not separate chromatographically, but can easily be separated by their different mass-to-charge ratios and subsequent loss.

Results, cont.

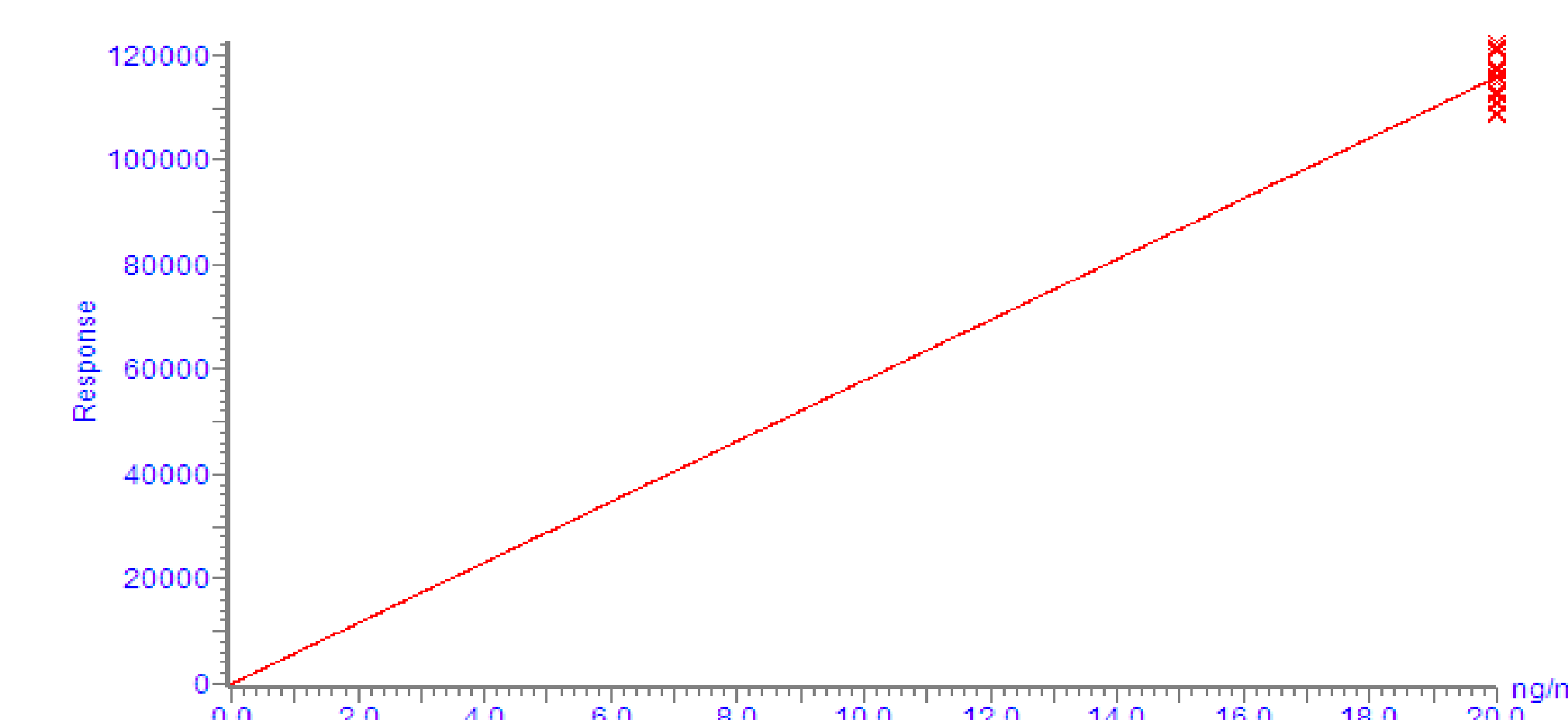
The linearity for all compounds was at least 0.995 (R²) for five repeat injections of the standards, with a calibration range for the barbiturates of 0-5000 ng/mL, and 0-80 ng/mL for 11-nor-9-carboxy- Δ^9 -THC (Graph 1). A series of 30 injections gave a %RSD of under 16% for each compound. Graph 3 shows the repeatability for 11-nor-9-carboxy- Δ^9 -THC which is 3.58% RSD. The lower limits of quantitation (LLOQ) varies for the barbiturates between 10-100 ng/mL, while the LLOQ for 11-nor-9-carboxy- Δ^9 -THC was 3 ng/mL. The single to noise ratio for the barbiturates and 11-nor-9-carboxy- Δ^9 -THC were both over 15 at the lower end of the calibration curve (Graph 4). No ion suppression studies were performed at this time. The results were comparable to our previous methods that involved running each compound class independently.



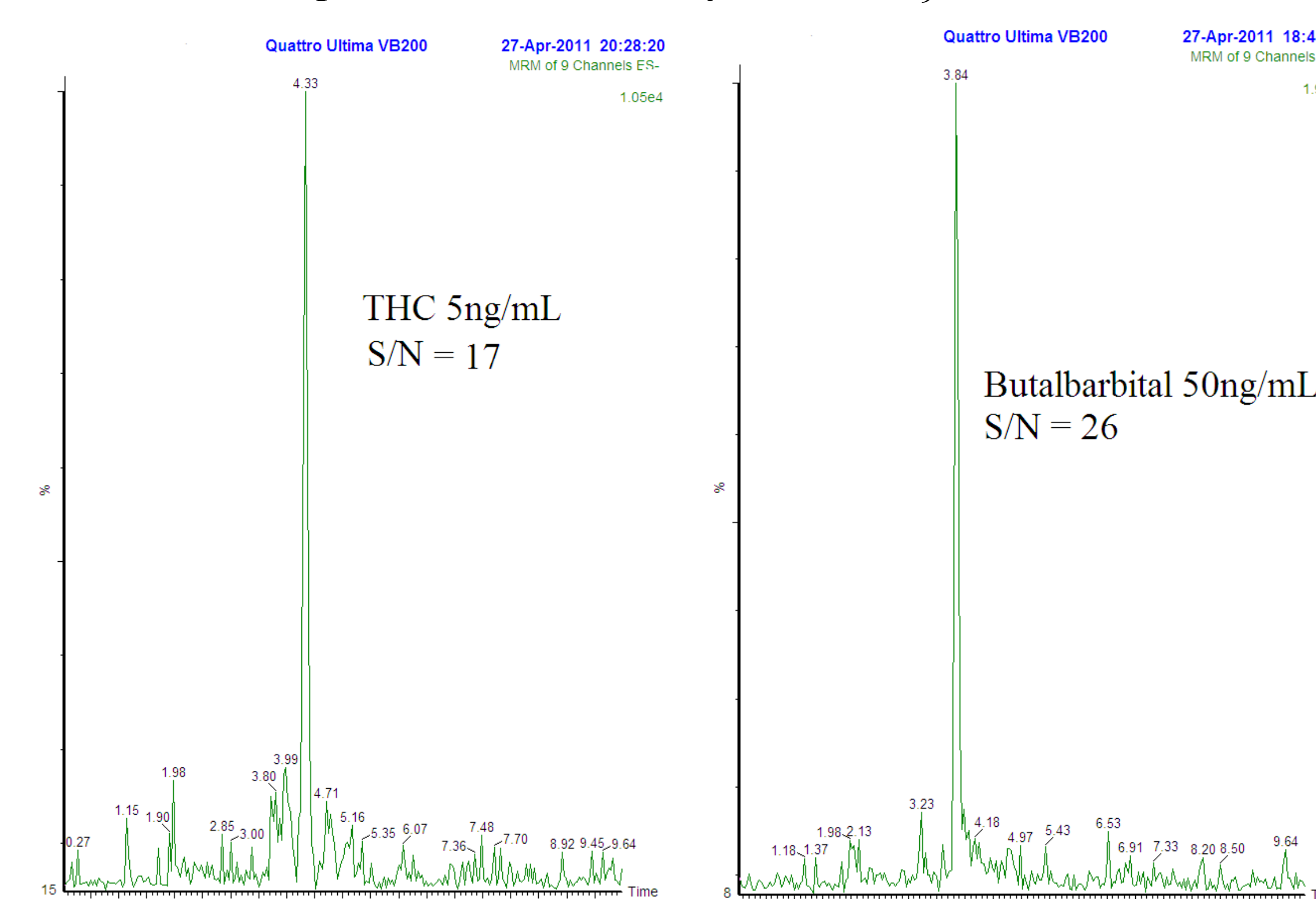
Graph 2: XIC trace, L to R, phenobarbital, butalbital, butabarbital, pentobarbital, secobarbital, and 11-nor-9-carboxy- Δ^9 -THC

Results, cont.

Compound name: THC-D9
Response Factor: 5789.42
R²: 0.997413, % Relative SD: 3.58262
Response type: External Std. Area
Curve type: RF



Graph 3: 11-nor-9-carboxy- Δ^9 -THC-d₉, %RSD



Graph 4: Signal to noise ratio for THC and Butalbital, respectively

Summary and Conclusion

In conclusion, we were able to successfully combine two commonly run negative mode assays into one efficient panel with minimal sample preparation, and low limits of quantitation.

Acknowledgements

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