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Application Note

# Analysis of Drugs in Blood for Toxicological Investigation of Drug-Impaired Driving in the U.S.A

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

*For forensic toxicology use only.*

## Abstract

This application brief details the sample preparation and UPLC-MS/MS methods applied to support the analysis of suspected drug-impaired driving samples to meet the U.S.A. Tier I recommendations.[1] Here we describe a single, robust sample preparation method which uses the Waters Ostro™ Pass-Through Sample Preparation Plate. This preparation method allows for all analytes detailed in the recommendations to be quantified, using one of two UPLC-MS/MS methods, at concentrations lower than the blood thresholds stated in the recommendations. The UPLC-MS/MS methods highlight how the chromatographic resolution of the ACQUITY™ UPLC I-Class coupled with the sensitivity of the Xevo™ TQ Absolute Mass Spectrometer provides a simple, robust platform for this analysis.

## Benefits

- Single sample preparation protocol using Ostro Pass-Through Sample Preparation Plate (p/n: 186005518 <

https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186005518-ostro-protein-precipitation--phospholipid-removal-plate-25-mg-1-.html> )

- Procedure based on just 100 µL of blood which is advantageous as the amount of specimen available can be limited
- Multiple drug classes separated on an Waters ACQUITY UPLC BEH™ $C_{18}$ Column (p/n: 186002352 < https://www.waters.com/nextgen/global/shop/columns/186002352-acquity-uplc-beh-c18-column-130a-17--m-21-mm-x-100-mm-1-pk.html> ) using an ACQUITY UPLC I-Class with Flow-Through-Needle (FTN)
- Excellent sensitivity of the Xevo TQ Absolute Mass Spectrometer allows for the analytes to be detected at concentrations relevant to the Tier 1 recommendations

## Introduction

Many illicit and prescribed medications have been reported to impair a driver's control of their vehicle and to increase the potential of road traffic accidents. In 2007, the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID) first published a list of drugs which were considered essential for the scope of testing in driving under the influence of drugs (DUID) investigations in the U.S.A.[2,3] These recommendations have been updated multiple times since, with the most recent being the 2021 update.[1] In the recommendations drugs of concern are divided into two groups, Tier I and Tier II. The Tier I group encompasses the most frequently encountered drugs in DUID investigations and are therefore deemed essential for inclusion in routine testing workflows, at or below the recommended blood threshold cutoffs (Table 1).[1]

| Cannabinoids | Blood threshold (ng/mL) |
|---|---|
| Δ9-THC 1 | 1 |
| Carboxy-THC | 5 |
| 11-hydroxy-THC | 1 |

| CNS stimulants | Blood threshold (ng/mL) |
|---|---|
| Methamphetamine | 20 |
| Amphetamine | 20 |
| MDMA | 20 |
| MDA | 20 |
| Cocaine | 10 |
| Benzoylecgonine | 50 |
| Cocaethylene | 10 |

| CNS depressants | Blood threshold (ng/mL) |
|---|---|
| Carisoprodol | 1000 |
| Meprobamate | 500 |
| Zolpidem | 10 |
| Alprazolam | 10 |
| Clonazepam | 10 |
| 7-Aminoclonazepam | 10 |
| Lorazepam | 10 |
| Diazepam | 10 |
| Nordiazepam | 10 |
| Oxazepam | 10 |
| Temazepam | 10 |

| Narcotic analgesics | Blood threshold (ng/mL) |
|---|---|
| Codeine | 10 |
| 6-Acetylmorphine | 5 |
| Buprenorphine | 0.5 |
| Norbuprenorphine | 1 |
| Fentanyl | 0.5 |
| Hydrocodone | 10 |
| Hydromorphone | 5 |
| Methadone | 20 |
| Morphine | 10 |
| Oxycodone | 10 |
| Oxymorphone | 5 |
| Tramadol | 50 |
| O-Desmethyltramadol | 50 |

*Table 1. Recommended compound groups together with the specified controlled drugs and individual thresholds for Tier 1 testing in blood.*

Analytical testing for these investigations requires quantitation for a range of drugs from differing drug classes which include a range of chemical properties. This can present analytical challenges for forensic toxicology laboratories to achieve a single, simple workflow that can detect all relevant compounds optimally and at the recommended blood concentrations.

Previously we have described a simple clean-up protocol for whole blood based on Waters Ostro Pass-Through Sample Preparation Plate.[4] Ostro combines the removal of proteins and phospholipids and filtration in one device. The sample preparation method was applied in combination with UPLC-MS/MS methods for the analysis of a panel of 17 drugs to support the specific requirements of the Section 5A of the England and Wales Road Traffic Act 1988. In this study we applied this method to the Tier I panel of 35 drug substances to support DUID investigations in the U.S.A.

## Experimental

Control human whole blood (K2 EDTA, pooled) was obtained from Bio-IVT (Burgess Hill, West Sussex, UK).

Reference material for toxicologically relevant substances were obtained from Merck (Poole, Dorset, UK) or LGC

(Teddington, London, UK). These were supplied as individual 1 mg/mL solutions in either methanol or acetonitrile. The analytes were combined to prepare a mixed-drug spiking solution; subsequent dilutions were prepared using methanol. Stable-labelled internal standards for the analytes were also obtained from the same suppliers at a concentration of 0.1 mg/mL. These internal standards were combined to yield a mixed deuterated internal standard solution (ISTD) at a concentration of 1 ng/µL in methanol. All solutions were stored at -20 °C.

Whole blood was spiked with the mixed-drug solution to provide a series of concentrations, ranging from 0.25 to 1500 ng/mL. Aliquots of the blood was prepared as previously described.[4] In brief, an aliquot (100 µL) of control or spiked blood was added to 100 µL zinc sulphate/ammonium acetate solution in the well of an Ostro Sample Preparation 96-well Plate (p/n: 186005518 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186005518-ostro-protein-precipitation--phospholipid-removal-plate-25-mg-1-.html> ) and briefly mixed. Elution solvent (600 µL of 0.5% formic acid in acetonitrile plus 1 µL ISTD mixture) was added to the samples and the plate was further vortex-mixed for 3 minutes. The plate was placed onto a vacuum manifold and the elution solvent drawn into a Waters 2 mL Square-well Collection Plate (p/n: 186002482 < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186002482-96-well-sample-collection-plate-2-ml-square-well-50-pk.html> ) under full vacuum.

Two separate aliquots (150 µL) of the Ostro eluant were transferred to a 1 mL Round-Well Collection Plate (p/n: 186002481 <https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186002481-96-well-sample-collection-plate-800--l-round-well-50-pk.html> ) and taken to dryness using an Ultravap Mistral Evaporator (Porvair Sciences).

One dried aliquot, for analysis of THC and its metabolites, was reconstituted in 50 µL of 50% acetonitrile in 0.05% formic acid. The other dried aliquot, for the analysis of all other drugs, was reconstituted in 50 µL of 10% acetonitrile in 0.05% formic acid. The samples were quantified using one of the UPLC-MS/MS methods detailed below.

Both UPLC-MS/MS methods employ the same column and mobile phases ***i.e.,*** an ACQUITY BEH $C_{18}$ (2.1 x 100 mm, 1.7 µm) (p/n: 186002352 <https://www.waters.com/nextgen/global/shop/columns/186002352-acquity-uplc-beh-c18-column-130a-17--m-21-mm-x-100-mm-1-pk.html> ) and 0.05% formic acid in water (mobile phase A) and 0.05% formic acid in acetonitrile (mobile phase B). However, each method employed a different chromatographic gradient; for the analysis of THC and metabolites, the initial starting condition was 50% mobile phase B, while the initial starting conditions for the method to quantify all other drugs was 2% mobile phase B. The Xevo TQ-Absolute was operated in electrospray positive (ESI+) mode for both methods with two MRM transitions monitored for each analyte and a single MRM transition monitored for each internal standard.

## Results and Discussion

All analytes listed in the Tier I recommendations were investigated using the developed sample preparation procedure. This included various concentrations for each analyte which were used to prepare calibration curves; these ranged from two-fold lower than the recommended blood threshold concentration to five-fold higher than the recommended blood threshold concentration for most analytes.

All the Tier I analytes investigated were detected at two-fold lower than the recommended blood threshold concentration. Figure 1 shows the chromatograms for whole blood samples spiked at the blood threshold level with the compounds listed in the Tier 1 recommendations. There are three example chromatograms for each compound group *i.e.* Cannabinoids, CNS stimulants, CNS depressants and Narcotic analgesics.

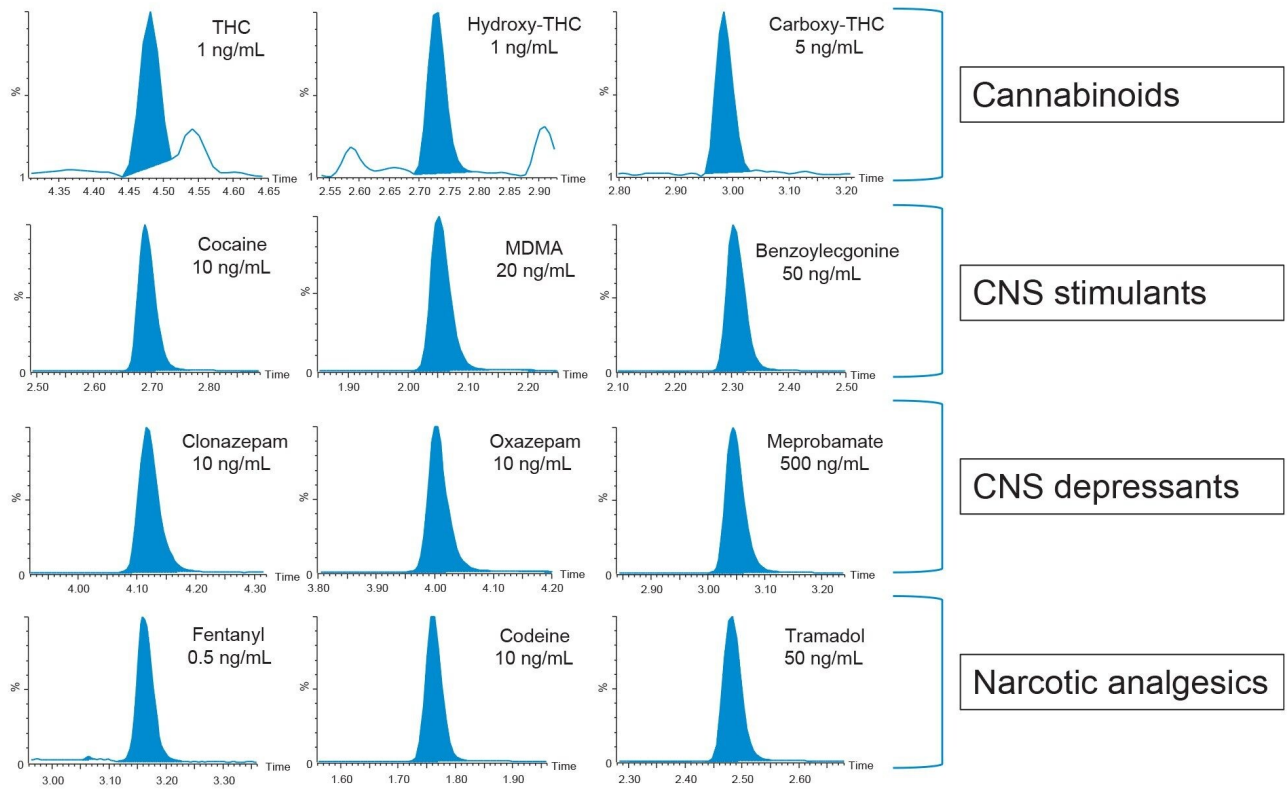


*Figure 1. Three MRM representative chromatograms, for the quantifier trace, for a whole blood sample spiked at the blood threshold level, from each compound group as specified in the Tier I recommendations.*

The calibration curves for the analytes investigated showed good linearity, all with $R^2$ >0.98. Figure 2 shows an example calibration curve from each compound group.

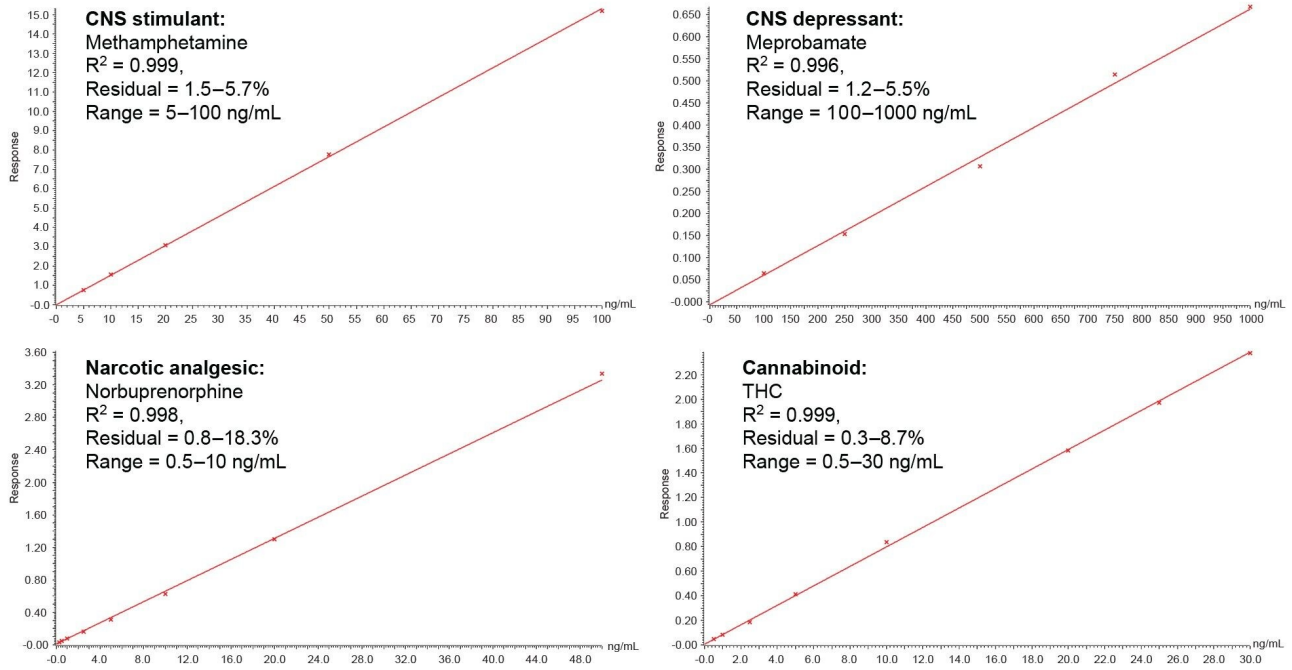


*Figure 2. An example calibration curve for an analyte in each compound group included in the Tier I recommendations. Calibration curves are linear with a weighting of 1/x.*

## Conclusion

The increased testing for drugs in drivers has highlighted the need for quick, accurate, reliable, and robust methods to quantify these compounds. This proof of principle method details a complete workflow that can be used to determine a large panel of drugs from whole blood using the Ostro Pass-Through Sample Preparation Plate. This procedure combined with the excellent sensitivity of the Xevo TQ-Absolute Mass Spectrometer allows for the 35 listed analytes detailed in the U.S.A. Tier I recommendations to be detected at relevant concentrations, especially for analytes with particularly low thresholds e.g. buprenorphine. In addition, the use of the Ostro Plate allows for the sample preparation protocol to be automated for any laboratories requiring higher sample

throughput.

## References


1. A.L D'Orazio ***et al.*** Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2021 Update. *Journal of Analytical Toxicology* 2021, 45(6), 529–536.
2. L.J. Farrell, S. Kerrigan, B.K. Logan. Recommendations for Toxicological Investigation of Drug Impaired Driving. *Journal of Forensic Sciences* 2007, 52, 1214–1218.
3. National Safety Council–Alcohol, Drugs and Impairment Division https://www.nsc.org/workplace/get-involved/divisions/alcohol-drugs-impairment-division <https://www.nsc.org/workplace/get-involved/divisions/alcohol-drugs-impairment-division> (accessed 12 May 2023).
4. M. Wood and R. Lee. Analysis of Drugs in Blood to Support the Section 5A Driving Under the Influence of Drugs Act. Waters Application Note, 720007451, 2021.


## Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ Absolute Triple Quadrupole Mass Spectrometry </nextgen/hr/en/products/mass-spectrometry-systems/xevo-tq-absolute.html>

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

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