ANALYSIS OF DRUGS IN BLOOD TO SUPPORT THE UK SECTION 5A DRIVING UNDER THE INFLUENCE OF DRUGS ACT

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INTRODUCTION

Many illicit and prescribed medications have been reported to impair a driver's control of their vehicle, and to increase the potential for road traffic accidents. In the UK, since March 2015, changes to legislation under the Section 5A of the Road Traffic Act were introduced, which make it an offence to have certain drugs, at blood concentrations above specified limits (Table 1).[1] The compounds fit broadly into two groups: one which contains prescribed drugs where the specified concentration in blood to be detected is relatively high, to ensure patients are not discouraged from taking their prescriptions whist driving. The other group of compounds are the illicit compounds - drugs of abuse where a zero-tolerance approach has been applied when setting the specified limits.

Analytical testing to support this legislation requires the quantitation of a panel of drugs from differing drug classes, involving a range of chemical properties. This can present some analytical challenges to achieve a simple workflow to detect all relevant molecules optimally and at their specified concentrations.

The aim of this study was to demonstrate the efficacy of a simplified sample preparation and UPLC-MS/MS methods[2] developed to meet the requirements of the UK Section 5A of the Road Traffic Act 1988.

SAMPLES

Whole blood samples spiked with the 17 drug substances listed in Section 5A of the Road Traffic Act (Table 1) were provided by Key Forensic Services (KFS). These consisted of 7 calibration levels, 3 quality control (QC) samples at low, medium and high concentrations. In addition, 3 external proficiency test (PT) samples at unknown concentrations were also provided; two were from LGC Forensic Blood Toxicology (QUARTZ) scheme and one from LGC Toxicology scheme. QC and PT samples were ran in duplicate.

SAMPLE PREPARATION

- One hundred microlitres of whole blood sample (calibrators, QCs and PTs) was added to 100µL 0.1M zinc sulphate/ammonium acetate solution in the wells of a Waters Ostro™ Pass-through Sample Preparation plate (Figure 1) and briefly vortex-mixed.
- Elution solvent (600µL of 0.5% formic acid in acetonitrile containing deuterated internal standards) was added to the samples and vortex -mixed for a further 3 minutes.
- The plate was placed onto a vacuum manifold and the elution solvent was drawn into a Waters 2mL square-well collection plate under full vacuum.
- Two separate aliquots (2 x 150µL) of the Ostro eluant were transferred to a Waters 1mL round-well collection plate and evaporated to dryness using an Ultravap Mistral® Evaporator.
- One dried aliquot, for the analysis of THC, was reconstituted in 50µL of 50% acetonitrile in 0.05% formic acid. The second dried aliquot, for the analysis of all other drugs, was reconstituted in 50µL of 10% acetonitrile in 0.05% formic acid. The plate was vortex-mixed for 3 minutes.

Figure 1. Ostro™ Pass-through Sample Preparation Plate (Waters)

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UPLC-MS/MS ANALYSIS

- Two different UPLC-MS/MS methods were applied in conjunction with a Waters Xevo™ TQ-S micro mass spectrometer which was operated in electrospray positive mode.
- Two MRM transitions were monitored for each of the 17 analytes and a single MRM transition for the deuterated internal standards.
- The UPLC conditions employed for both methods are displayed in Table 2, however each method employs a different chromatographic gradient.
- For the analysis for THC the initial starting condition was 50% mobile phase B, ramping to 90% over 4 minutes before re-equilibration.
- For the analysis of all other drugs the initial starting condition was 2% mobile phase B, ramping to 95% over 5 minutes before re-equilibration.

UPLC	ACQUITY™ UPLC™ I-Class PLUS
Mobile phase A	0.05% Formic acid in Water
Mobile Phase B	0.05% Formic acid in Acetonitrile
Column	ACQUITY™ BEH™ C_{18} (2.1x100mm, 1.7µm)
Column Temperature	30°C
Sample Temperature	10°C
Injection Volume	10µL
Run Time	7 minutes

Table 2. UPLC conditions used for both UPLC-MS/MS methods

Prescribed drugs	Blood Threshold (ng/mL)	Illicit drugs	Blood Threshold (ng/mL)
Clonazepam	50	LSD	1
Morphine	80	Delta-9-THC (THC)	2
Lorazepam	100	6-Monoacetylmorphine (6-MAM)	5
Oxazepam	300	Cocaine	10
Flunitrazepam	300	MDMA	10
Methadone	500	Methylamphetamine	10
Diazepam	550	Ketamine	20
Temazepam	1000	Benzoylecgonine	50
		Amphetamine	250

Table 1. Specified controlled drugs and specified limits for the purposes of Section 5A of the Road Traffic Act 1988.
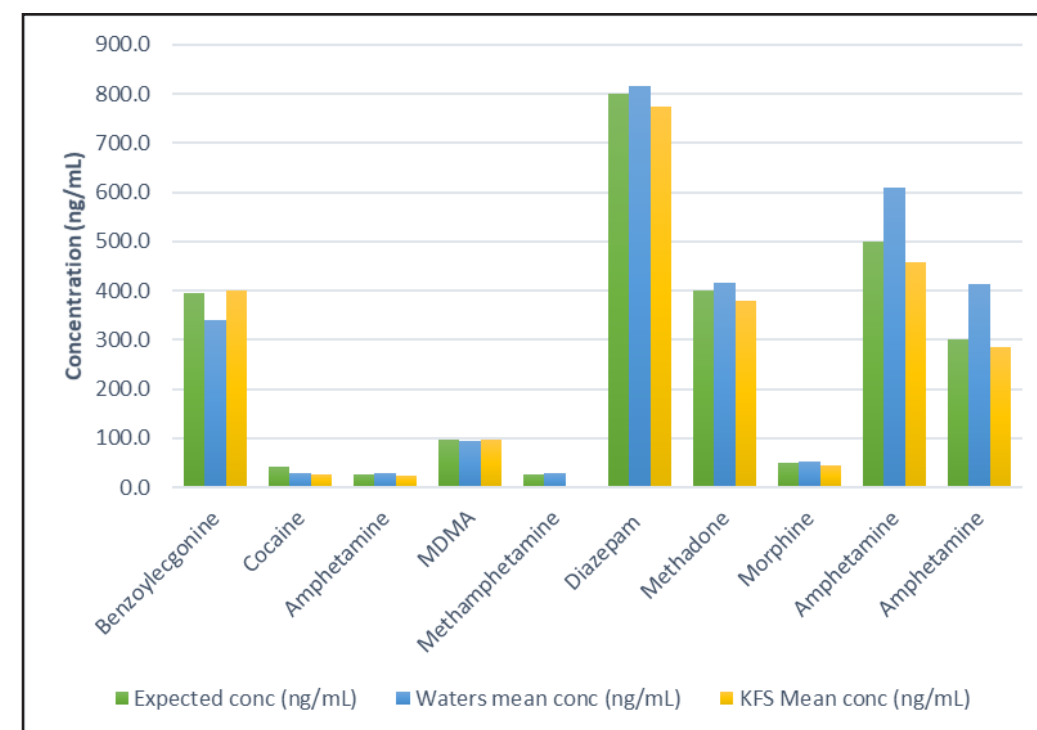

Figure 5. The concentration of the compounds detected in the PT samples from the developed method, compared with the concentrations obtained from the current method and the expected concentrations.

Sample	Low QC expected conc. (ng/mL)	Low QC % difference	Medium QC expected conc. (ng/mL)	Medium QC % difference	High QC expected conc. (ng/mL)	High QC % difference
6-MAM	-	-	5	0.1%	60	-1.1%
Amphetamine	40	20.2%	250	23.7%	1000	16.7%
Benzoylecgonine	40	0.3%	50	3.6%	800	1.6%
Clonazepam	40	-18.0%	50	-19.5%	320	-12.7%
Cocaine	20	-1.6%	10	6.6%	400	3.7%
Diazepam	200	-1.6%	550	-1.1%	1600	-7.9%
Lorazepam	40	-11.7%	100	5.4%	320	-11.2%
MDMA	20	-11.2%	10	-3.9%	800	-5.9%
Methadone	200	4.2%	500	7.6%	2000	8.2%
Methamphetamine	50	11.9%	10	2.4%	800	1.1%
Morphine	40	8.3%	80	7.2%	800	2.2%
Oxazepam	200	-12.9%	300	-12.0%	1600	-10.4%
Temazepam	200	2.3%	1000	4.3%	1600	-7.0%
Ketamine	15	4.2%	20	13.0%	800	9.7%
LSD	1.5	3.9%	1	-2.3%	8	26.2%
Flunitrazepam	200	-11.9%	300	-2.1%	1600	0.3%
THC	2	1.6%	-	-	22.5	3.4%

Table 3. Percentage difference in concentration between expected concentration and the calculated concentration using the developed method. Highlighted in green percentage difference within ±20%, highlighted in red percentage difference within ±30%

RESULTS AND DISCUSSION

- To solve the challenge of acceptable peak shape for polar analytes while maintaining efficient reconstitution for THC, the single pass-through preparation procedure resulted in two aliquots of the Ostro eluant which were dried. One was reconstituted in a solvent suitable for the analysis of THC and the other reconstituted in a solvent suitable for the analysis of all of the other drugs. To ensure high throughput, two UPLC-MS/MS methods were developed which used the same column and mobile phases but different chromatographic gradients (Table 2). The results from the developed method were compared with established, accredited protocols, currently used by KFS, based on two different preparation methods (protein precipitation for basic drugs; liquid/liquid extraction for cannabinoids) and two separate LC-MS/MS methods.
- Figure 2 shows the chromatograms for the quantifier ions from a whole blood sample spiked at the lowest calibration level for all illicit drugs and prescribed medicines apart from THC. Figure 3 shows a chromatogram for a whole blood sample spiked at the lowest calibration level for THC. The THC protocol detailed is also suitable for the analysis of other cannabinoids i.e, hydroxy-THC, carboxy-THC, cannabinol and cannabidiol.
- Calibrators (six/seven levels per analyte) typically ranging from 10x below, to 2.5x above, the specified thresholds (Table 1), were analysed together with QC and PT samples. Responses were linear (1/x weighting applied) with R^2 values ranging from 0.98 to 0.999. Figure 4 shows the calibration curve (6 points) for 6-MAM.
- QCs met the applied ±20% from expected concentrations for all analytes, except Amphetamine and LSD (as highlighted in red in Table 3) which were still within 30%. The PT samples used in this study were from previous schemes, therefore it is possible that some analyte degradation has occurred. However, good agreement was achieved between the results obtained from the developed method, KFS current methods and expected concentrations provided by the test provider (Figure 5).
- The method detailed here is a single sample preparation for all required analytes, this could therefore save laboratories analysis time and enables a faster sample throughput when compared to the established methods.
- Further time can be saved due to the detailed method having a shorter run time (~15 minutes combined) than both established methods (~30 minutes combined) and also using the same UPLC conditions for both UPLC-MS/MS methods. This enables instruments within the laboratory to be more efficiently utilised.
- The streamlined method uses only 100µL of sample, compared to a ~1.5mL requirement currently for the established sample preparation methods. This is beneficial as sample volume received by laboratories can be limited, especially if repeat analysis or further tests are required.
- To further investigate the potential applicability of this method for the wider testing of UK drivers and for the testing of drivers in other geographies and other associated legislation, the developed method has also been applied to 155 analytes in whole blood.[3] These include analytes that are covered in the UK Section 4[1] and US Tier 1[4] recommendations. Figure 6 shows the chromatograms of the analytes included in the US Tier 1 recommendations at 5ng/mL.
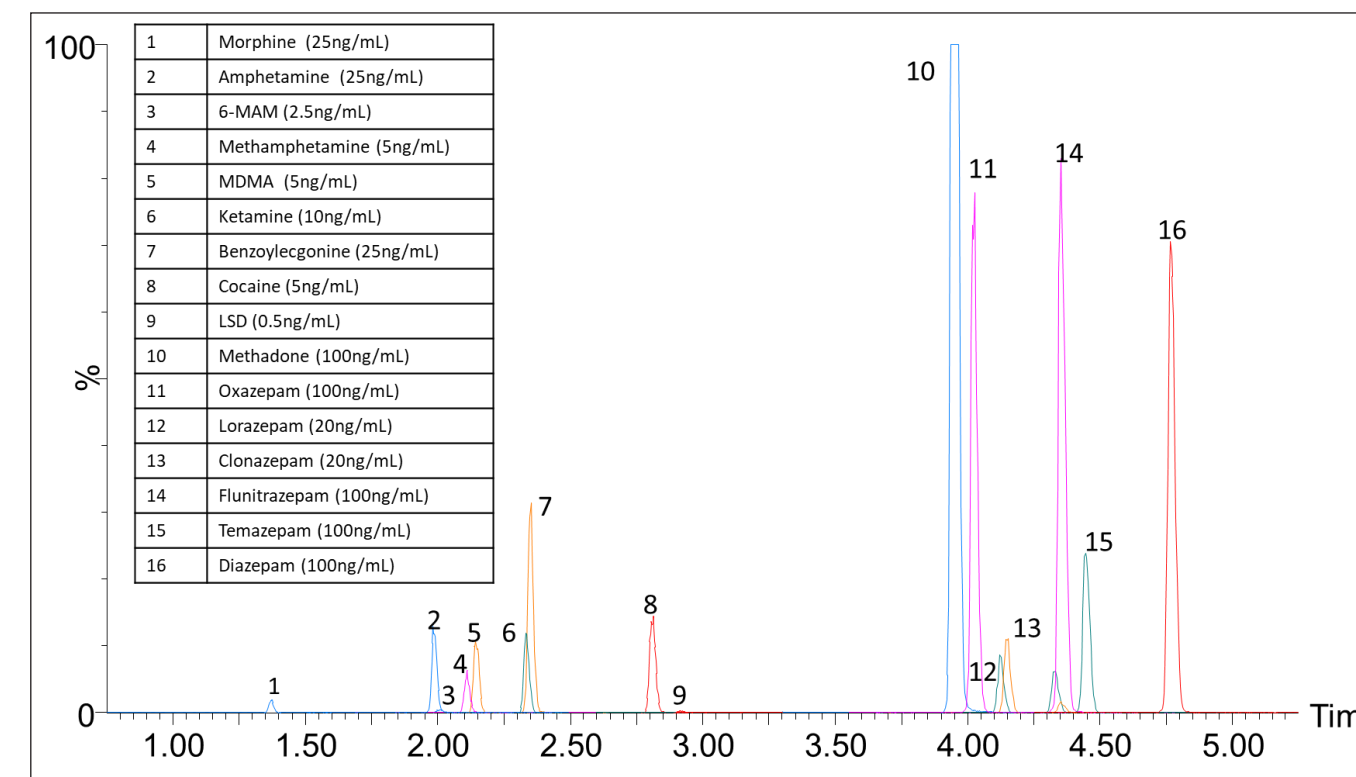

Figure 2. Chromatographic separation of a whole blood sample spiked at the lowest calibration level, with a range of illicit drugs and prescribed medications. The data is scaled to the most intense peak in the chromatogram.
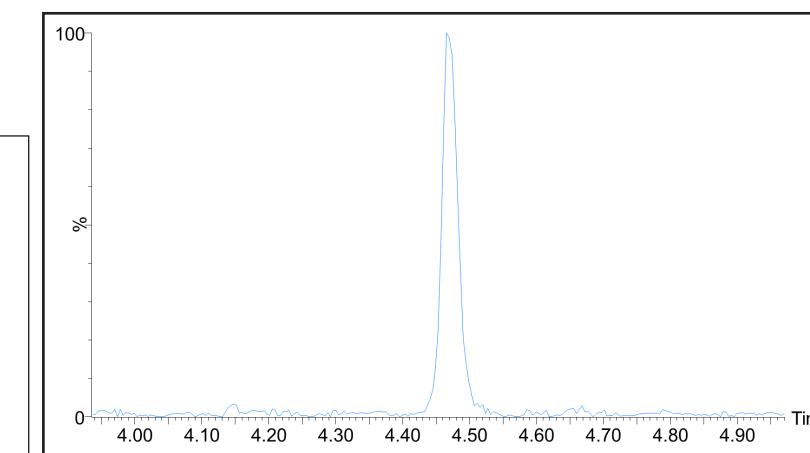

Figure 3. Chromatogram for the quantifier trace for THC in a whole blood sample spiked at the lowest calibration level, 0.5ng/mL.
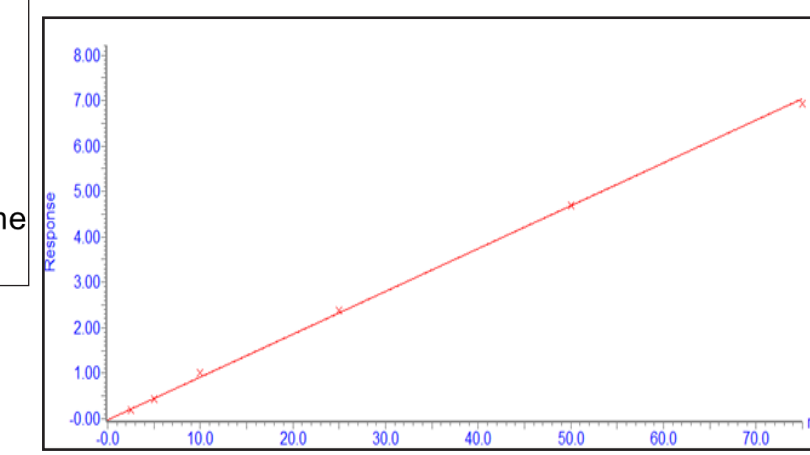

Figure 4. Calibration curve for 6-MAM. Response is linear (1/x weighting applied), $R^2 = 0.999$

Acknowledgement to Key Forensic Services for their kind donation of samples and data from established methods.
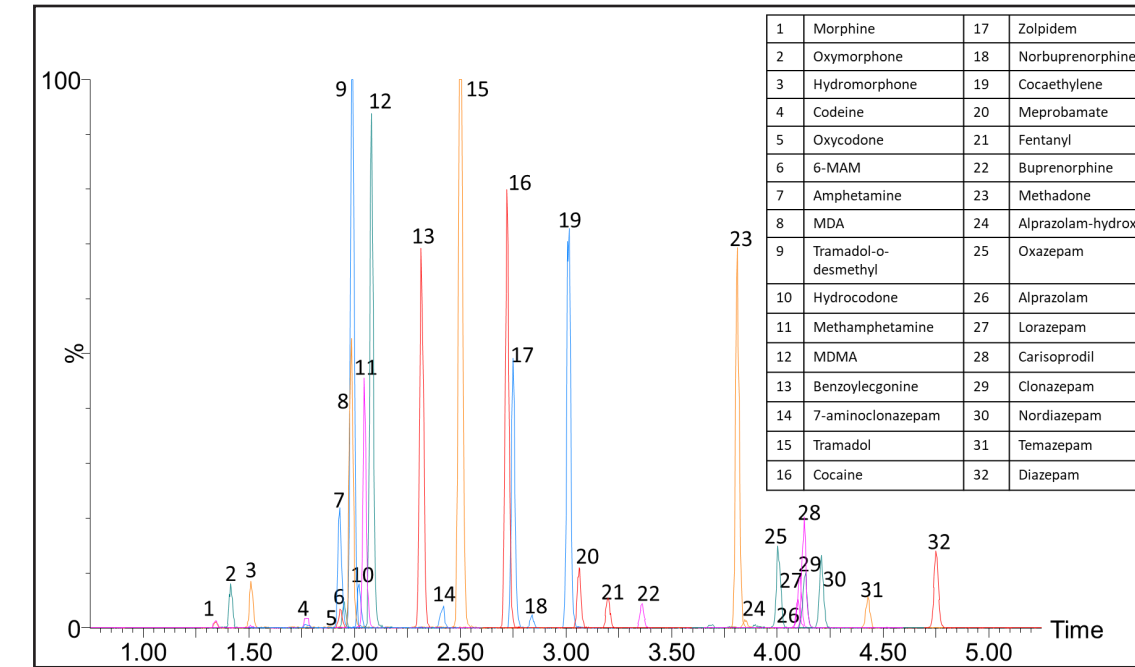

Figure 6. Overlaid chromatograms of quantifier ions of Tier 1 recommended analytes spiked at 5ng/mL

FUTURE WORK

- The detailed method was developed using a plate format with a view to automating. Forensic toxicology laboratories receive large volumes of drug driving samples, thus automation could be of great benefit.
- The presented work focuses on addressing Section 5A requirements, however a wider range of analytes are also of interest for testing of drivers—both in the UK and worldwide. Thus future testing will evaluate the potential of the protocol to meet the legislative thresholds for the UK Section 4 analytes and the Tier 1 recommended panel which is relevant to the US. These recommendations include more analytes, some at much lower concentrations.

CONCLUSIONS

- As there has been an increase in testing for drugs in drivers, the need for quick, reliable and robust methods to quantify the specified analytes has become apparent.
- The developed sample preparation method has been applied in combination with UPLC-MS/MS for analytes that are covered in Section 5A of the UK Road Traffic Act 1988, and satisfies the legislative requirements.
- Comparison with established sample preparation and LC-MS/MS methods shows good agreement. The new method has increased analytical sensitivity, thus requiring a lower volume of sample, which is beneficial if further testing is required.
- The procedure also offers a more streamlined workflow and has been found to have potential for replacing two separate methods with one simplified procedure with a significantly reduced run time.
- Preliminary work has been performed to evaluate the detailed protocol for use with Section 4 (UK) and Tier 1 (US), and this will be further assessed with future work which will be performed to assess the ability to meet the legislative thresholds.

References
1. The Drug Driving (Specified Limits) (England and Wales) Regulations 2014 (as amended, Road Traffic Act, England and Wales, 2015 no.2015 (2014)
2. M. Wood and R. Lee; Analysis of Drugs in Blood to Support the UK Section 5 Driving Under the Influence of Drugs Act. Waters Application Note Library Number 720007451
3. M. Wood and E. Lee; Determination of Drugs of Abuse in Whole Blood Using the Ostro™ Pass-through Sample Preparation Plate. Waters Application Note Library Number 720007699
4. B.K. Logan et al. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 Update. Journal of Analytical Toxicology 2021: 45: 529-536