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Note d'application

Analysis of Drugs in Blood to Support the UK Section 5 Driving Under the Influence of Drugs Act

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For forensic toxicology use only.

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

Abstract

This note details the sample preparation and UPLC-MS/MS methods developed to meet the requirements of the UK Section 5A of the Road Traffic Act 1988.[1]

A single, robust sample preparation method using the Waters Ostro Pass-Through Sample Preparation Plate was developed. This preparation method allows for all analytes detailed in the legislation to be quantified, using one of two UPLC-MS/MS methods, at concentrations lower than those stated in the act. The UPLC-MS/MS methods highlight how the chromatographic resolution of the ACQUITY UPLC I-Class coupled with the sensitivity of the Xevo TQ-S micro 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 is based on just 100 µL of blood which is advantageous as the amount of specimen available can be limited
- Multiple drug classes separated on a 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-S micro Mass Spectrometer allows for the analytes to be detected at concentrations relevant to the legislation

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.[2,3] Since March 2015, changes to the existing legislation were introduced which made it an offence for a vehicle driver to have certain drugs, including legal medication, at blood concentrations above specified limits (Table 1).[1] The compounds fit broadly into two groups: the first group contains prescribed drugs (medicines) where the concentration in blood (threshold) to be detected is relatively high so as not to discourage patients from taking their prescriptions whilst still driving. The second group of compounds are the illicit compounds; drugs of abuse where a zero-tolerance approach has been applied to setting their thresholds.

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.

Analytical testing to support this legislation requires the quantitation of a panel of drugs from differing drug classes, and as such, involves a range of chemical properties, from polar substances such as morphine, to the non-polar THC. This diversity in chemical properties can present some analytical challenges if one is to achieve a simple workflow to detect all relevant molecules optimally, and at their specified concentrations.

Experimental

Control human whole blood was supplied by Bio-IVT (Burgess Hill, West Sussex, UK).

Reference material for the 17 analytes of interest 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; further 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). All standard solutions were stored at -20 °C.

Whole blood was spiked with the mixed-drug solution to provide a range of concentrations.

Control or spiked blood (100 µL) was added to 100 µL of a 0.1 M zinc sulphate/ammonium acetate solution in the wells of the Ostro Sample Preparation Plate. Internal standards were added and the samples briefly vortex-mixed. Elution solvent (600 µL of 0.5% formic acid in acetonitrile) was added to the samples and the plate further vortex-mixed for 3 minutes. The plate was placed onto a vacuum manifold and the elution solvent was 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 (2 x 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, 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 employed the same column ***i.e.,*** an ACQUITY BEH C_{18} (2.1 x 100 mm, 1.7 µm) and

the same mobile phases, 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 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-S micro was operated in electrospray positive (ESI+) mode for both methods with 2 MRM transitions monitored for each analyte and a single MRM transition monitored for the internal standards.

Results and Discussion

To solve the challenges of efficient reconstitution for THC and acceptable chromatography for morphine and the other early eluting basic compounds, two aliquots of the Ostro Eluant were dried. The first aliquot was reconstituted in a solvent suitable for the analysis of THC and the other aliquot was reconstituted in a solvent suitable for all the other drugs. To ensure high throughput, two UPLC-MS/MS methods were developed using the same column and mobile phases. The first, to quantify THC and the other, for the quantitation of all the other analytes.

Figure 1 shows a chromatogram for whole blood spiked with THC at 1 ng/mL. Note the described THC protocol is also suitable for the analysis of several other cannabinoids *i.e.,* hydroxy-THC, carboxy-THC, cannabinol and cannabidiol. Figure 2 shows the data for a whole blood sample spiked with all remaining illicit drugs and prescribed medications at a concentration of 10 ng/mL.

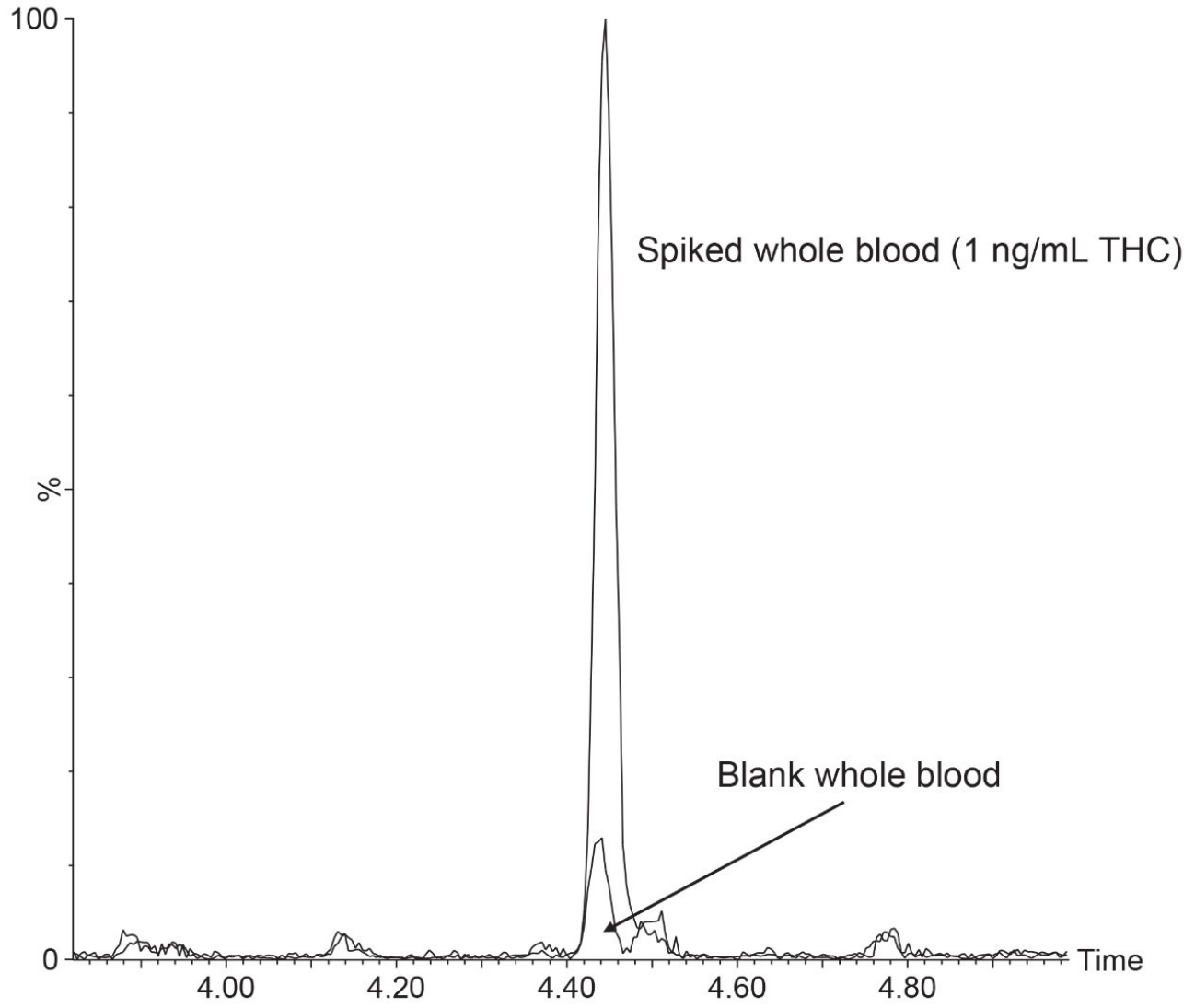

Figure 1. Chromatogram for the quantifier trace for THC in spiked whole blood. The response for the quantifier trace for the blank whole blood sample is also displayed for comparative purposes.

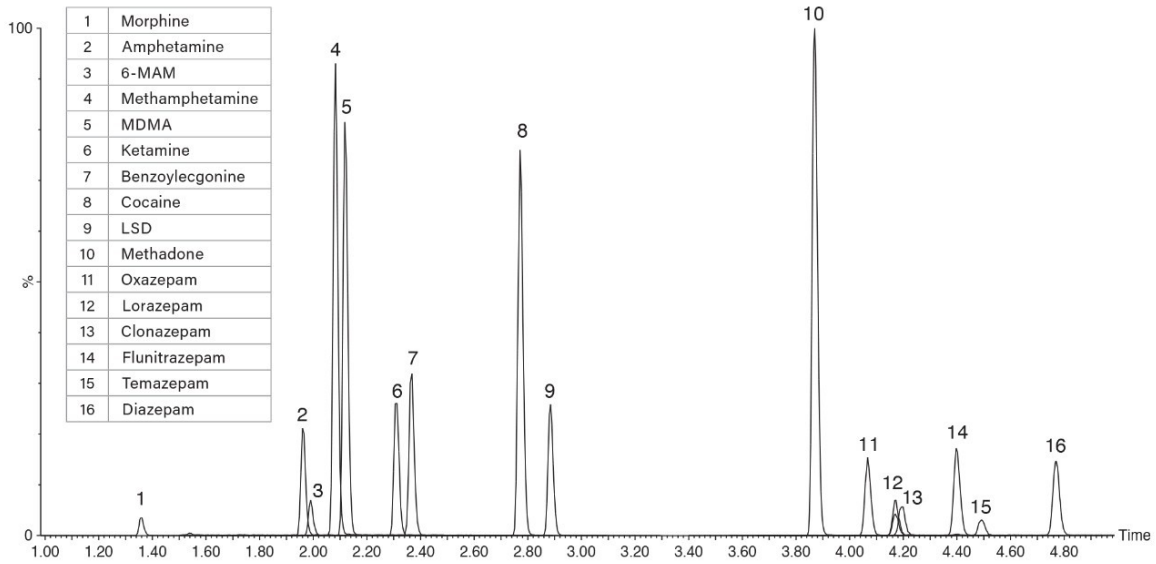

Figure 2. Chromatographic separation of a whole blood sample spiked with a range of illicit drugs and prescribed medications at a concentration of 10 ng/mL. The data is scaled to the most intense peak in the chromatogram.

Conclusion

The increased testing for drugs in drivers has highlighted the need for quick, accurate, reliable, and robust methods to quantify these compounds. This note 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. The described procedure utilizes just 100 µL of blood which is beneficial as the amount of available sample can be limited, particularly if the sample requires multiple analyses.

The excellent sensitivity of the Xevo TQ-S micro Mass Spectrometer allows for the analytes to be detected at concentrations relevant to the legislation, while the use of the Ostro Plate allows the sample preparation protocol to be automated for any laboratories requiring higher sample throughput.

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. Honkanen, R *et al*. Role of Drugs in Traffic Accidents. *British Medical Journal*, 281, 1309–1312 (1980).

3. Drummer, OH *et al*. The Involvement of Drugs in Drivers of Motor Vehicles Killed in Australian Road Traffic Crashes. *Accident; Analysis and Prevention*, 36, 239–248 (2004).

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