University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences

Development of a quantitative LC-MS/MS method with dried blood spot sampling for forensic toxicology

Stampfli Yvan

Master thesis, Master of Science in Life Sciences, Molecular Technologies

Principal: Prof. Dr. Schlotterbeck Götz, School of Life Sciences FHNW
Expert: Dr. Ehrat Markus, EK Biosciences
Supervisor: Assoc. Prof . Dr. Oberacher Herbert, Medical University of Innsbruck

INTRODUCTION

In forensic toxicology, a wide variety of substances, both legal and illegal, need to be identified and quantified every day. One type of compounds that is often encountered are recreational drugs such as Cocaine or Heroin as well as therapeutic substances such as Morphine. Since most of those substances are illegal or at least under strict control, an efficient, easy and reliable sampling and quantification method for abuse monitoring is required. Over the past few years, LC-MS analysis gained in significance for the quantitative analysis of blood samples. A common sampling procedure for forensic toxicology is venepuncture which is often done in a hospital. A more convenient alternative might be dried blood spot sampling which is often used in neonatal screening.[1][2]

Fig. 1: Example of dried blood spots (DBS)

Analytes of interest

The most commonly encountered drugs of abuse and their methabolites have been analysed. The following substances have been investigated:

Heroin, 6-Monoacetylmorphine (6-MAM), Morphine, Codeine, Dihydrocodeine, Buprenorphine, Norbuprenorphine, Methadone, (2Z)-2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), Cocaine, Benzoylecgonine, MDMA, MDA, Methamphetamine, Amphetamine

Methods

An LC-MS/MS method was developed using an Agilent 1100 LC system with a Phenomenex biphenyl column for separation. The MS was a QTRAP 4000 system by AB Sciex in MRM mode and with ESI in positive mode. For identification an quantification purpose, deuterated internal standards have been used.

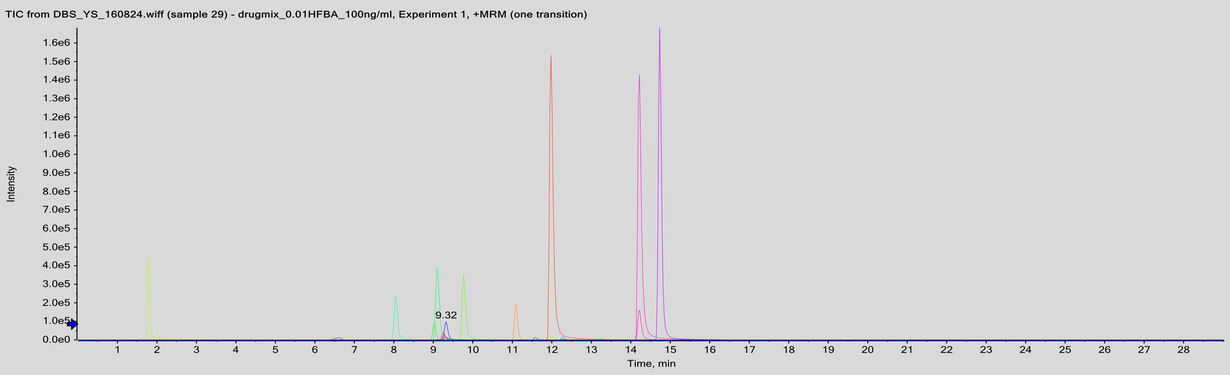

Fig. 2: Example Chromatogram of all analytes (c=50.0ng/mL)

DBS samples have been extracted using a phosphate buffer (1M, pH=6) and ultrasonic extraction followed by SPE using Strata-X Reversed phase cartridges from Phenomenex.

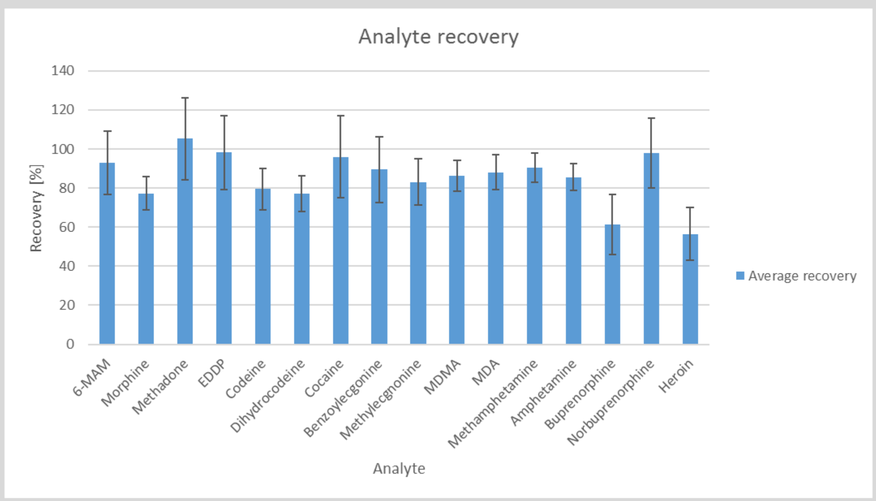

Fig. 3: Recovery values for DBS extraction

RESULTS

The extraction procedure developed in this method allows extraction with recovery values of >50% for all analytes of interest and adequate addition of internal standards. The LC-MS/MS method allowed to quantify all analytes, mostly with Limits of quantification (LOQ) below 10ng/mL except for Buprenorphine and Heroin as shown in Table 1. Accuracy (Bias <±15%) as well as interday and intraday precision (RSD <15%) were sufficient and stability of the DBS samples was proven for up to 72 hours at room temperature and up to 21 days at -18°C. By application to casework samples it was shown that the new method showed similar results as the validated GC-MS methods of the casework unit at the Institute for Legal Medicine of the Medical University of Innsbruck. Occurring differences between the two methods can be explained by varying experimental factors such as different extraction methods and derivatisation as well as long storage periods for the DBS samples. Only in case of Methylecgonine, problems with the used standard were encountered which lead to the conclusion that all validation and casework results for Methylecgonine needed to be declared invalid.

Substance	LOD [ng/mL]	LLOQ [ng/mL]	ULOQ [ng/mL]
Heroin	5.0	10.0	500.0
6-MAM	0.5	2.0	500.0
Morphine	1.0	2.0	500.0
Methadone	0.5	5.0	500.0
EDDP	0.5	1.0	500.0
Codeine	1.0	2.0	500.0
Dihydrocodeine	0.5	2.0	500.0
Cocaine	0.5	0.5	500.0
Benzoylecgonine	0.5	1.0	500.0
MDMA	0.5	1.0	500.0
MDA	1.0	2.0	500.0
Methamphetamine	0.5	1.0	500.0
Amphetamine	0.5	1.0	500.0
Buprenorphine	50.0	50.0	500.0
Norbuprenorphine	5.0	5.0	500.0

Table 1: Analytical limits for all Analytes

CONCLUSION

A novel LC-MS/MS method was developed, allowing simultaneous quantification of 15 drugs of abuse in dried blood spot samples down to less than 10ng/mL. The developed extraction workflow allows simultaneous extraction of all analytes with sufficient recovery values of more than 50%. The Method has been validated an was successfully applied to casework samples. The developed method has the potential to simplify sampling and analysis in forensic toxicology as it performs better than existing methods in terms of analytical working range as well as sampling simplicity.

REFERENCES

[1] Forensic Toxicology Council, 2015, What is Forensic toxicology?, viewed January 18. 2017, http://www.swgtox.org/documents/WHAT_IS_FORENSIC_TOXICOLOGY.pdf

[2] Deglon, J., Thomas, A., Magnin, P., Staub, C., 2012, ‘Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications’ *Analytical and Bioanalytical Chemistry,* Vol. 402, 2485-2498

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