Fully automated forensic screening of dried bloodspots with MRM Spectrum Mode

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Overview

Fully automated forensic screening of dried bloodspots was achieved by coupling a DBS-MS 500 system (CAMAG, Switzerland) to an LCMS system (Shimadzu, Japan)

Introduction

An increasing amount of prescription and illicit drugs drive clinical laboratories to more cost effective and faster screening methods in workplace drug testing, roadside testing, rehabilitation programs or post-mortem investigations without compromising established false positive or negative detection rates. Therefore, the increasing sample amount with a large panel of analytes needs to be handled in shorter analysis time and with simple sample preparation steps. In this study we present a fully automated dried bloodspot (DBS) extraction procedure coupled to an LC-MS/MS-system for forensic and toxicology screening of drugs and their metabolites. We use a new approach, the MRM Spectrum Mode, by measuring at least 5 MRM-transitions of each compound to generate a high confidence for the identification of the results.

Figure 1   Instrumental setup: DBS-MS 500 (CAMAG, Switzerland),
Nexera X2 UHPLC (Shimadzu, Japan), LCMS-8060 (Shimadzu, Japan)

Methods

The DBS-MS 500 system (CAMAG, Switzerland) was attached as front end to a modular Nexera X2 UHPLC system from Shimadzu (Kyoto, Japan). Analysis was performed in positive multiple reaction monitoring (MRM) mode on ESI tandem mass spectrometry systems LCMS-8040 and LCMS-8060 (Shimadzu, Kyoto). Data interpretation was performed by LabSolutions Insight Screening software. Analytical standards were purchased from Lipomed (Switzerland). DBS cards (Ahlstrom TFN filter paper) were provided by CAMAG (Muttenz, Switzerland). Chromatography was performed on Shim-pack GIST (2.1 x50 mm, 2 µm C18) (GL Science, Japan) and Kinetex (2.1 x 100 mm, 2.6µm, XB-C18) (Phenomenex, USA) analytical columns using a gradient of 10 mmol/L ammonium formate + 0.1% formic acid in water and methanol as mobile phases A and B.
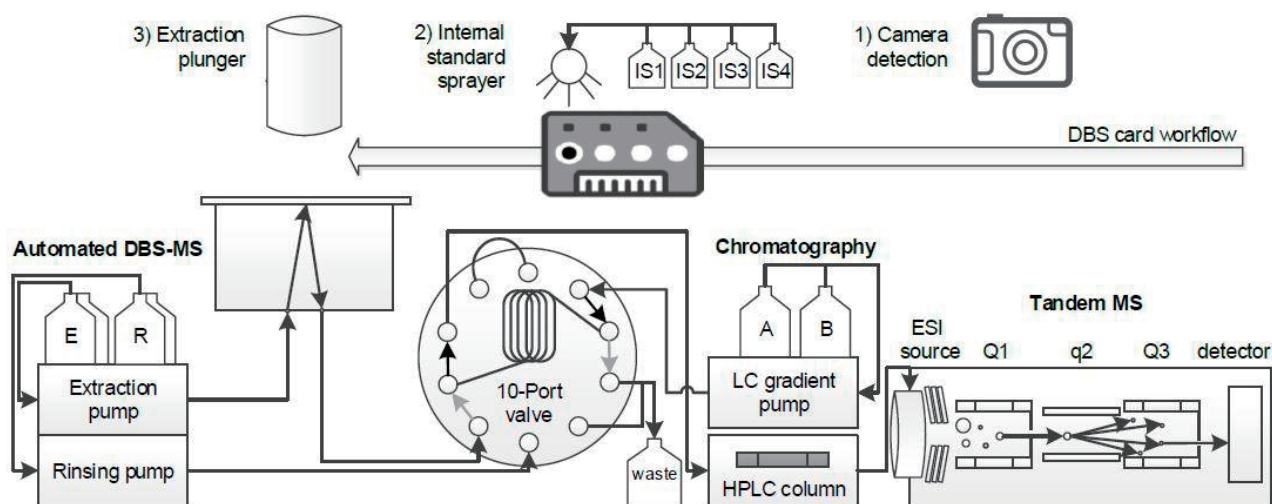

Figure 2   Fully automated workflow for the DBS-MS 500

Results

Comparison of DDA and MRM spectrum mode

The classic approach for identification in routine drug screening workflows is based on data dependant acquisition (DDA). After a certain threshold of a given MRM transition is exceeded the MS acquires automatically three product ion scans (PIS) at different CE levels. These spectra are merged and compared to a library. The novel approach is the acquisition of at least five MRM transitions per compound, which are also merged to one spectrum (MRM spectrum) and compared to the corresponding library entries. All MRM transitions are optimized with the automatic method optimization tool implemented in the LabSolutions Software (Shimadzu, Kyoto). Even at low concentrations high correspondence between experimental and reference spectra are achieved with the MRM spectrum mode, resulting in a better similarity index (SI). Also the duty cycle time was reduced with this approach.
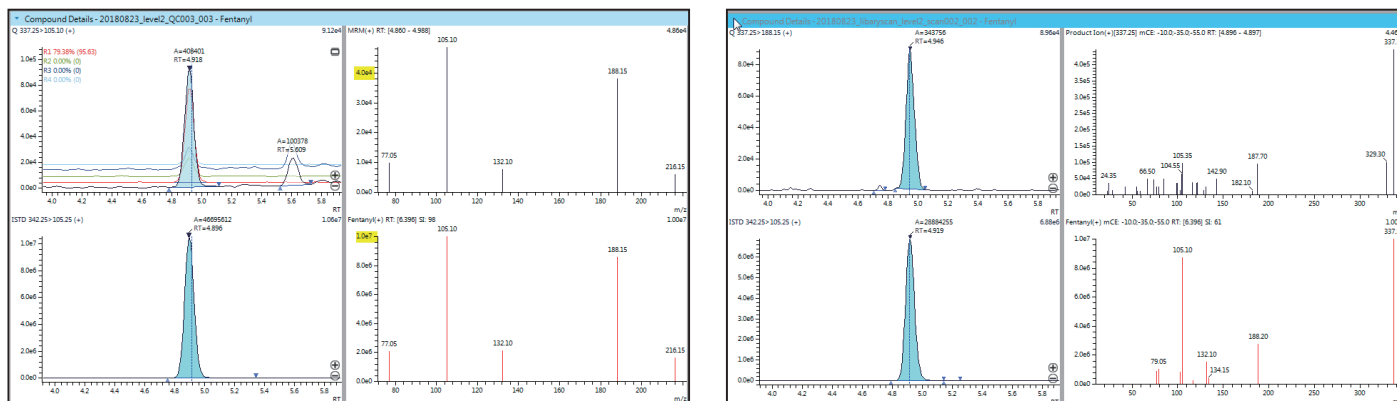
Figure 3   Comparison of MRM spectrum mode and product ion scan (DDA)

MRM spectrum (black) of 5 ng/ml Fentanyl in whole blood compared to its reference spectrum (red)
SI = 98; cycle time = 30 ms

Product ion spectrum (black) of 5 ng/ml Fentanyl in whole blood compared to its reference spectrum (red)
SI = 61; cycle time = 95 ms

Correlation and precision

The calibration levels were measured 6-fold on two different days to determine the method robustness and validity. The relative standard deviation of the internal standard was below 15 % for all target compounds by comparing the data through all levels (5-1000 ng/mL). The correlation and inter-day variations of all target compounds are listed in Table 1. The target to internal standard ratio was used to compare the results and to develop the calibration line. Excellent correlation was obtained for all target compounds. All points of the calibration functions were sufficiently precise with relative standard deviations below 15 % and 20 % at LOQ, respectively. THCA-A, 6-MAM and Diazepam constitute an exception, which has to be further investigated and validated. Table 1 also includes the cut-off and toxicological levels from literature for blood samples. The goal of the analysis method was to detect the cut-off concentration with confidence.

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Table 1 Relative inter-day standard deviation for 27 target compounds at different concentration levels;
correlation coefficients (R²) of the linear fit; cut-off and toxicological levels

|	Relative inter-day standard deviation				Correlation R²			
	5 ng/ml [%]	25 ng/ml [%]	250 ng/ml [%]	500 ng/ml [%]	5-500 [ng/ml]	25-1000 [ng/ml]	cut-off [ng/ml]	tox. Level [ng/ml]
Codeine	10.4	10.5	9.7	11.6	0.992	0.997	50	1100
6-MAM	22.3	9	13.4	15.6	0.992	n/a	10	n/a
MDMA	14.3	8.5	6.8	6.3	0.995	0.999	100	1000
Amphetamine	12.6	9.6	4.9	7.1	0.996	0.999	100	1000
Methamphetamine	13.2	11.3	8	9	0.995	0.998	100	n/a
MDEA	11.8	8.7	6.9	6.6	0.994	0.997	100	n/a
MDA	9.4	5.1	5.3	5.8	0.995	0.998	100	n/a
Heroin	4.8	7.4	4.6	5.6	0.995	0.997	50	200
Cocaine	14.8	13.3	14.7	7.7	0.999	n/a	20	1000
Morphine	18.6	9	8.2	8.6	0.993	0.998	50	200
7-Aminoclonazepam	12.6	7.7	7.4	6.6	0.995	n/a	25	n/a
Chlorphenamine	11.6	3.4	1.9	7.1	0.999	n/a	25	n/a
Clozapine	11.2	5.1	6.5	3.9	0.994	n/a	25	n/a
Bromazepam	15.2	7.6	6.5	5.7	0.998	n/a	25	n/a
Midazolam	10	4.5	3.1	4	0.995	n/a	25	n/a
Clonazepam	14.7	6.9	5.7	8.2	0.995	n/a	25	80
Clobazam	13.3	6.6	5.8	8.1	0.997	n/a	25	n/a
Oxazepam	13.8	13.3	11.1	12.6	0.999	n/a	25	1400
Alprazolam	11.9	3.9	4.5	7.1	0.991	n/a	25	350
Chlorpromazine	16.6	13.9	13.1	5.6	0.999	n/a	25	n/a
Nordiazepam	13	11.1	6.9	7	0.999	n/a	25	n/a
Diazepam	19.4	29.9	24.8	31.8	0.995	n/a	25	3000
11-nor-Δ9-THC-COOH	15.4	11.8	11.9	7.4	0.998	n/a	10	n/a
MAM-2201	16.8	11.1	10	5.6	0.996	n/a	10	n/a
THCA-A	13.6	11	15.4	8.7	0.998	n/a	10	200
Δ9-THC	13.2	12.3	6.8	8.7	0.995	n/a	10	n/a
Cannabidiol	10.5	8.4	12.1	9	0.996	n/a	10	n/a
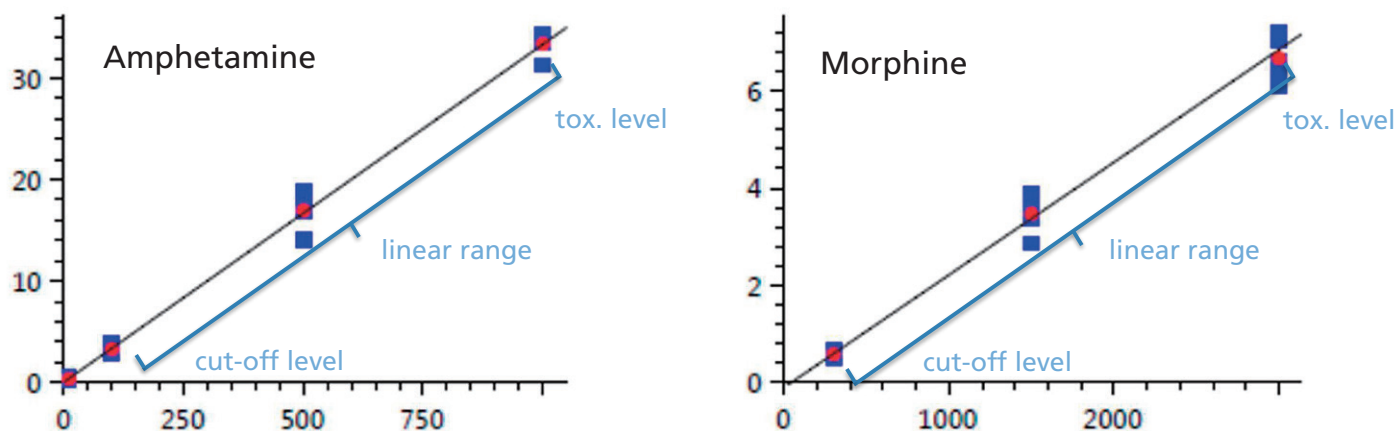
Figure 4   Representative calibration curves (Amphetamine and Morphine; curve type: linear; zero: not forced; weighting: $1/C^2$; quant. method: internal standard)

Quality control cards and internal standard

Prior the analysis, high and low control samples were measured. The low control reflects a concentration close to the cut-off of the THC derivates and the high control gives a value significantly higher to allow controlling of the analysis performance. The control cards were prepared by the laboratory using the DBS-MS 500 spraying module. 10 µl of the deuterated alprazolam or cocaine standard is sprayed as 0.1 µg/mL solution in methanol directly on the DBS prior extraction. The internal standard solution can be used for up to two week if stored in the fridge overnight. Alprazolam-D5 was used during method development phase and cocaine-d3 for the method implementation due to standard availability.
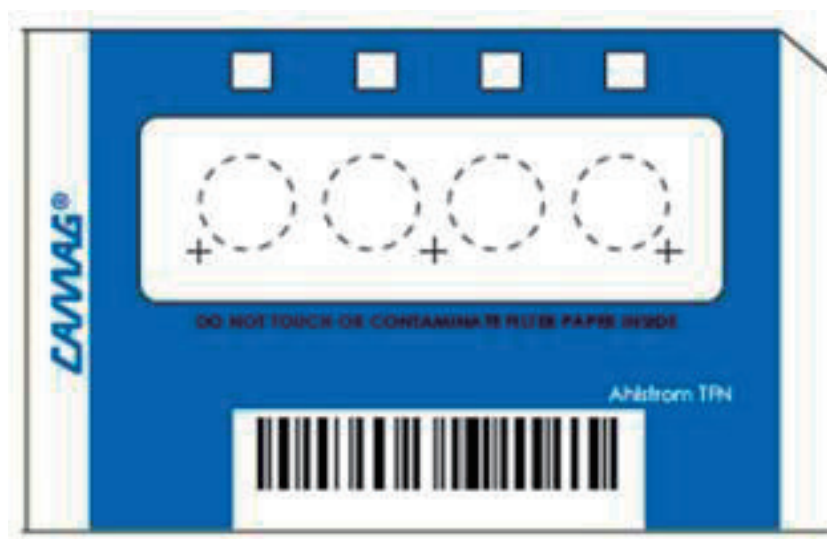
Figure 5   DBS card (CAMAG, Switzerland)

Method transfer to routine laboratory

The method described was developed in a research laboratory using a Shimadzu LCMS-8060 system and subsequently transferred to a routine laboratory equipped with an LCMS-8040. After transferring the method and adjusting the MRM voltages of the target compounds by the LabSolutions optimization tool, a five point calibration each with three repetitions was measured in the range of 10 to 500 ng/mL target drug in blood, since this was the region of interest for the routine laboratory. Pre-sprayed quality control samples were measured before and after the calibration samples according to the described workflow. Excellent correlation >0.995 and standard deviation below 15 % was obtained for all compounds except for the THC derivates, where the correlation was above 0.985 and standard deviation below 25 %. The sensitivity of the THC derivates is at its limit on the 8040 LCMS system, however, when the cut-off is put at 25 ng/mL rather than at 10 ng/mL the result can be detected reliably.

Conclusions

- A fast (5 min per sample) screening method of dried bloodspots was developed for 28 high abundant illicit drugs
- The method was extended to 20 min, enabling the usage of a Forensic Toxicology Database to screen over 1200 drugs
- MRM spectrum mode provides more confidence in results and reduces false positives and negatives due to better spectral quality, no need of an intensity threshold faster cycle times
- The drug screening method was successfully transferred to a routine laboratory and shows good correlation and precision

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