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Quick turnaround analysis of 8 drugs for driver under influence using LDTD-MS/MS system

Serge Auger, Jonathan Rochon, Jean Lacoursière and Pierre Picard
Phytronix Technologies, Québec, Canada
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Introduction

Each year, commonly abused drugs, such as Cannabinoids, Amphetamines, Cocaine, or Opioids become more easily available. As a consequence, there are an increasing number of individuals driving under the influence of these drugs. The recent judgment of the French Department of Justice specifies a cut-off (decision point) for the screening of 8 drugs in saliva (**Table 1**). A fast and effective method for sample extraction in saliva could provide a realistic and efficient approach for on-site drug screening using mobile laboratories.

A generic extraction method combined with LDTD®-MS/MS analysis was developed for fast turnaround screening of drugs in saliva. This new method could give police officers rapid and accurate answers in less than 10 minutes allowing on-site screening during a police roadblock. High Throughput capability of 400 samples per hour enable by LDTD-MS/MS run time of 9 seconds.

LDTD-MS/MS System

Figure 1 - LDTD-MS/MS system

Sample Preparation Method

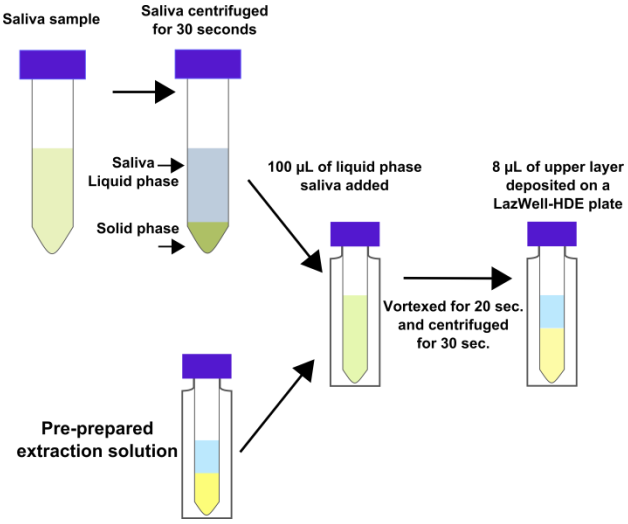

Figure 2 - Workflow from saliva collection to analysis

Saliva sample preparation:

- Approximately 1 mL of whole saliva was collected
- Centrifuge for 1 min. at 3000 rpm
- Spike at 50%, 100% and 150% of decision point concentration.

Pre-prepared extraction solution:

- In a 300 µL fused glass insert vial
- Add 100 µL of internal standard solution in acetonitrile.
- Add 75 µL of Extraction buffer

Saliva sample extraction:

- Add 50 µL of saliva
- Vortex 20 seconds
- Centrifuge 30 seconds at 5000 rpm
- Spot 8 µL of upper layer in a LazWell™ plate HDE
- Evaporate to dryness
- LDTD-MS/MS screening analysis after complete solvent evaporation

LDTD®-MS/MS Parameters

LDTD
Model: Phytronix, SH-960
Carrier gas: 3 L/min (air)
Laser pattern: 6 seconds ramp to 55% power.

MS/MS
Model: Shimadzu LCMS- 8060
Dwell Time: 3 msec
Pause Time: 3 msec
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method:

- Positive MRM transition

Table 1 - MRM transition

Drugs/Internal standard	Q1	Q3	CE	Cut-off (ng/mL)
Amphetamine	136.10	119.15	-15	50
Amphetamine-d5	141.10	124.10	-15	NA
Methamphetamine	150.15	119.15	-16	50
Methamphetamine-d9	159.15	125.20	-16	NA
MDMA	194.00	163.10	-14	50
MDMA-d5	199.00	165.10	-14	NA
Morphine	286.15	165.15	-40	10
Morphine-d3	289.18	165.15	-40	NA

Benzoylecgonine	290.15	168.15	-20	10
Benzoylecgonine-d8	298.26	171.19	-20	NA
Cocaine	304.15	182.15	-20	10
Cocaine-d3	307.15	185.19	-20	NA
THC	315.25	193.1	-25	15
THC-d3	318.25	196.14	-25	NA
6-AM	328.15	165.15	-36	10
6-AM-d6	334.25	165.15	-38	NA

Results and Discussion

LDTD®-MS/MS screening Method

Drug extract obtained were analyzed using a MRM method in positive mode. After a fast desorption, fortified and blank samples are evaluated using peak area ratio. All samples having a peak area ratio higher than the cut-off standard are classified as drug positive samples.

Precision of Screening Method

Spiked samples at 50%, 100% and 150% of the decision point and blank solutions (one with IS solution and one without) are used to validate the precision of the screening method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against IS ratio was used to normalize the signal. Triplicate extractions, analyzed twice, are deposited on a LazWell96HDE plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15%. Results using the ± 2 STD overlay are plotted. **Figure 3** shows the results for the all the drugs of abuse tested.

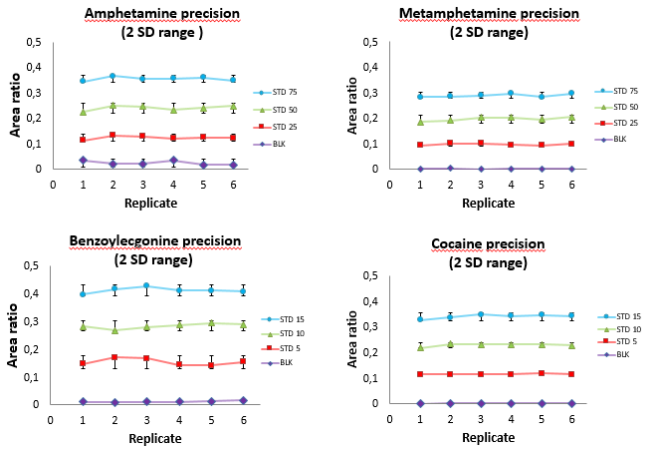

Figure 3 - Precision Screening curve

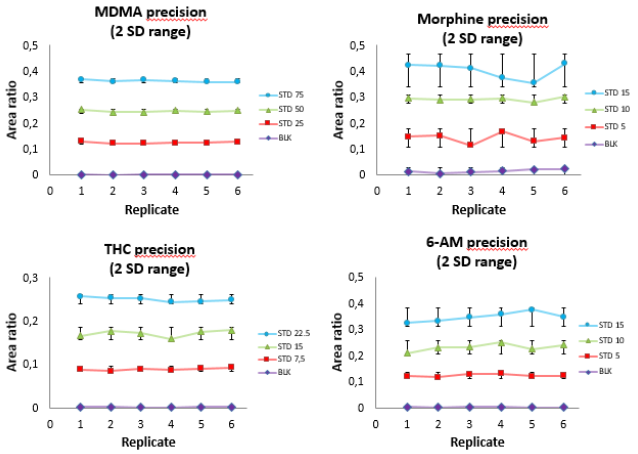

Figure 4 - Precision Screening curve

Matrix effect evaluation

6 different matrices were collected. Each sample was divided in two parts. One part was used as a blank sample to validate **Negative** samples and the other part was spiked at 150% of decision point to validate **Positive** results for the 8 drugs of abuse. Example for the Benzoylecgonine is shown in Table 2 where the ratio of 150% spiked sample (15 ng/mL) clearly discriminates from the value of the cut-off ratio (0.28 at 10 ng/mL). No false negative or false positive results were observed.

Table 2 - Benzoylecgonine area ratio for blank and spiked samples in different matrix

Matrix samples	Area ratio for Blank samples	Area ratio for Spiked samples
M1	0.0074	0.3774
M2	0.0045	0.3636
M3	0.0039	0.3850
M4	0.0040	0.4103
M5	0.0049	0.3868
M6	0.0087	0.4054

Conclusion

LDTD technology combined with the Shimadzu LCMS-8060 mass spectrometer system allows ultra-fast (**9 seconds per sample**) and specific drug screening in saliva samples with single and generic sample preparation.