Generic Extraction Method for screening of 8 drugs of abuse in Saliva with a quick turnaround analysis by LDTD-MS/MS

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OVERVIEW

Purpose
- Quick analysis for 8 drugs of abuse in saliva for on-site drug screening using LDTD-MS/MS.

Method
- Liquid-Liquid extraction of saliva sample
- LazWell plate spotting. evaporated to dryness and LDTD-MS/MS analysis

Validation
- Precision at the decision point.

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® Ionization Source:

The LDTD® uses a Laser Diode to produce and control heat on the sample support **(Figure 2)** which is a 96-wells plate**.** The energy is then transferred through the sample holder. The sample gets dried and vaporized prior being carried by a gas in a corona discharge region. This type of ionization is characterized by a strong resistance to ionic suppression because of the absence of solvent. LDTD® ionization reduces sample-to-sample analysis time to 9 seconds and allows high throughput capabilities without carry over.

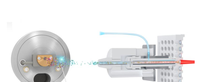

Figure 1 Schematic of the LDTD® Ion Source

Figure 2 LDTD® Ion Source on a Shimdazu LCMS-8060

METHOD

Saliva sample preparation:
- 2 mL of Human saliva
- Saliva centrifuged for 1 min at 3000 RPM
- Saliva was spiked at 50%. 100% and 150% of the decision point concentration

Pre-prepared extraction solution:
- In a 300 µL fused insert vial
- 100 µL of Internal Standards (**Table 1**) in Acetonitrile at 4 times the decision point concentration
- 75 µL of Extraction Buffer

Saliva sample extraction:
- 50 µL of saliva sample added to the extraction solution
- Vortexed for 20 seconds
- Centrifuged for 30 seconds at 5000 RPM
- 8 µL of upper layer were deposited on LazWell96HDE plates and evaporated to dryness (3-5 min).

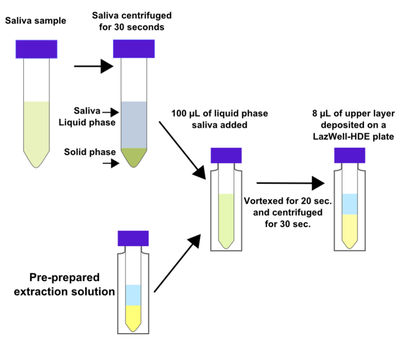

Figure 3 Workflow from saliva collection to analysis

INSTRUMENTAL CONDITIONS

Instrumentation
- LDTD model: SH-960
- MS: Shimadzu LCMS-8060

LDTD Parameters
- Laser power pattern :
  - ➢Increase laser power to 55 % in 6 sec
  - ➢Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

MS Parameters
- APCI (+)
- Dwell: 10 msec
- MRM mode

Table 1 Cut-off concentrations and MRM transitions for drugs of abuse and IS

Drugs	Cut-off (ng/mL)	Q1	Q3	CE (V)
Amphetamine	50	136.10	119.15	-15
Amphetamine-d_5	-	141.10	124.10	-15
Methamphetamine	50	150.15	119.15	-16
Methamphetamine-d_9	-	159.15	125.20	-16
MDMA	50	194.00	163.10	-14
MDMA-d_5	-	199.00	165.10	-14
Morphine	10	286.15	165.15	-40
Morphine-d_3	-	289.18	165.15	-40
Benzoylecgonine	10	290.15	168.15	-20
Benzoylecgonine-d_8	-	298.26	171.19	-20
Cocaine	10	304.15	182.15	-20
Cocaine-d_3	-	307.15	185.19	-20
THC	15	315.25	193.1	-25
THC-d_3	-	318.25	196.14	-25
6-AM	10	328.15	165.15	-36
6-AM-d_6	-	334.25	165.15	-38

RESULT

Validation parameters to be assessed

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 with 2 replicates are deposited on a LazWell96HDE plate and analyzed. No overlapping at the decision point is observed for all curves and the CV% were below 15%. Results using the ± 2 STD overlay are plotted. **Figure 3** shows the results for the all the drugs of abuse tested.

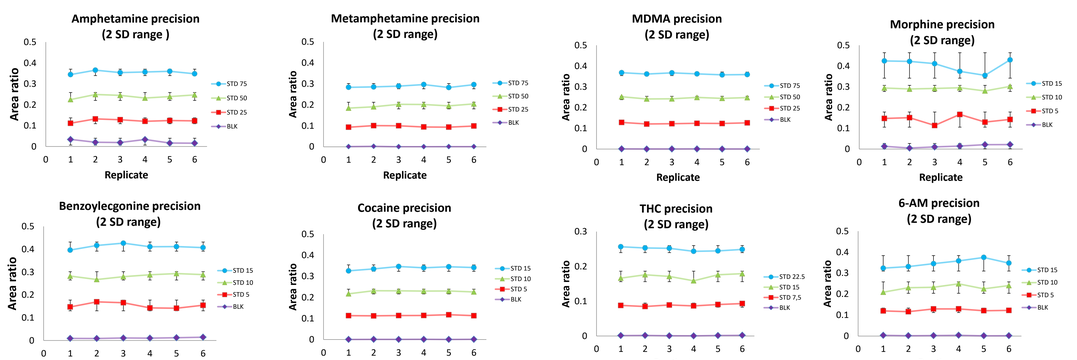

Figure 3 Accuracy and precision curves for all drugs tested.

Comparison of different matrices

6 different matrices are collected. Each sample is divided in two parts. One part is 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 (results for the Benzoylecgonine in **Table 2**). No false negative or false positive results are observed.

Table 2 Benzoylecgonine area ratio for blank and spiked samples in different matrices.

Matrix samples	Area ratio for Blank samples	Area ratio for Spiked samples
M1	0.0074	0.3773
M2	0.0045	0.3636
M3	0.0039	0.3849
M4	0.0040	0.4103
M5	0.0049	0.3868
M6	0.0087	0.4054

CONCLUSION

- **Validation parameters showed no overlapping at the decision point (cut-off) for all the drugs tested**
- **No false negative or false positive are observed**
- **Complete screening workflow for 100 samples in less than 15 minutes for drugs of abuse in saliva**
- **High-Throughput capability with LDTD-MS/MS analysis of 9 seconds sample to sample**
- **Realistic approach for on-site drug screening using mobile laboratories**