Generic SLE extraction method for ultra-fast drug screening in saliva using LDTD-MS/MS analysis.

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INTRODUCTION

Testing for drugs of abuse in oral fluids can strongly benefit the criminal justice field as a less invasive and cost-effective approach for drug detection when compared to blood or urine sampling. Oral fluid analysis has facilitated laboratory analysis for many drugs of abuse and is a constantly evolving analysis procedure which benefits from increasingly sensitive methods of detection. The Laser Diode Thermal Desorption (LDTD) source combined with Mass spectrometry is presented as a new screening tool for drug analysis in Oral Fluids. Analysis speed of LDTD provides accurate results in seconds in combination with exceptional specificity of MS instruments make a powerful platform for the screening of different drugs of abuse and new emerging drugs. Different extraction procedures are available; however those methods depend on specific drug conditions: basic or acid drugs / hydrophilic or hydrophobic. A new extraction approach, Supported Liquid Extraction (SLE+) is evaluated as generic extraction procedure.

LDTD Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support which is a 96 well plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in an APCI region. High efficiency protonation with strong resistance to ionic suppression characterize the ionization due to the absence of solvent and mobile phase. This allows very high throughput capabilities of 9 seconds sample-to-sample analysis time, without carry over.

METHOD

Sample preparation:

Supported Liquid Extraction (SLE):
ISOLUTE-SLE+ (400µL)
- 200 µL sample
- 10 µL of internal standard (IS)
- 200 µL Ammonium hydroxide (1% in water)

Load onto SLE cartridge. A pulse of vacuum or positive pressure is applied to initiate flow and allow the sample to adsorb for 5 minutes.
- Addition of 1 ml of ethyl acetate to elute. Wait 5 minutes.
- Addition of 1 ml of dichloromethane/IPA (95/5) and allow to flow under gravity.

6 µL of elution is directly deposited in the Lazwell plate.
Dry samples prior to analysis

Instruments setting:

Mass spectrometer AB Sciex 5500 QTrap operated in APCI positive mode.

MRM transitions used with DP=100, 3 µA discharge current and 5 msec dwell time

LDTD model S-960 operated with a gas flow rate of 3 L/min and a laser pattern ramp from 0 to 45% in 3 seconds, maintaining this power level for 2 seconds before dropping it back to 0.

Drug cut-off

Standard solutions are spiked in Orasure buffer at 0.5X, 1X and 2.5X the cut off level. The following cut-off levels are reported in **Table 1.**

Compound	Cut-Off
	(ng/ml)
Amphetamine	10
Butalbital	20
BZE	10
Cocaine	2
Cotinine	10
Fentanyl	10
MDA	10
MDEA	10
MDMA	10
Methamphetamine	10
Methadone	5
Phenobarbital	20
Secobarbital	20

Table 1 Screening cut-off

Figure 1: LDTD system on AB SCIEX 5500 Qtrap® Mass Spectrometer

Compound	Q1	Q3	CE
Amphetamine	136.0	119.0	10
Butalbital	225.0	98.0	30
BZE	290.0	168.0	20
Cocaine	304.0	182.0	20
Cotinine	177.0	80.0	30
Fentanyl	337.0	188.0	22
MDA	180.0	133.0	20
MDEA	208.0	163.0	12
MDMA	194.0	163.0	12
Methamphetamine	150.0	119.0	15
Methadone	310.0	265.0	20
Phenobarbital	233.0	162.0	15
Secobarbital	239.0	98.0	20
Amphetamine-d5	141.0	124.0	10
BZE-d8	298.0	171.0	20
Cocaine-d3	307.0	185.0	20
Cotinine-d3	180.0	80.0	30
Fentanyl-d5	342.0	188.0	22
MDMA-d5	199.0	165.0	12
Methamphetamine-d9	159.0	125.0	15
methadone-d9	319.0	268.0	20
PCP-d5	249.0	164.0	20

Table 2 MRM transitions

RESULTS

Intra-Run reproducibility and accuracy

Reproducibility and accuracy intra-run result at cut-off level are reported in **Table 3**.

Compound	Conc.	N	Mean	%RSD	%Nom
	(ng/ml)		(ng/ml)	(%)	(%)
Amphetamine	10	6	10,6	18,5	106,4
Butalbital	20	6	20,0	9,2	99,9
BZE	10	6	11,0	9,0	109,9
Cocaine	2	6	2,0	4,4	100,8
Cotinine	10	6	9,7	3,2	97,4
Fentanyl	10	6	10,2	4,1	102,1
MDA	10	6	10,4	9,9	103,9
MDEA	10	6	10,3	4,7	103,2
MDMA	10	6	10,3	6,5	102,6
Methamphetamine	10	6	10,0	5,9	100,5
Methadone	5	6	5,1	7,3	101,8
Phenobarbital	20	6	19,1	3,4	95,3
Secobarbital	20	6	20,5	6,2	102,3

Table 3 Intra-run reproducibility and accuracy.

Inter-Run reproducibility and accuracy

Reproducibility and accuracy inter-run result at cut-off level are reported in **Table 4**.

Compound	Conc.	N	Mean	%RSD	%Nom
	(ng/ml)		(ng/ml)	(%)	(%)
Amphetamine	10	18	10,6	13,8	105,5
Butalbital	20	18	21,0	12,5	105,2
BZE	10	18	10,6	8,9	105,7
Cocaine	2	18	2,0	5,1	100,3
Cotinine	10	18	10,0	5,0	100,2
Fentanyl	10	18	10,2	4,5	101,9
MDA	10	18	10,1	7,8	100,8
MDEA	10	18	10,2	4,0	101,5
MDMA	10	18	10,2	5,5	101,5
Methamphetamine	10	18	10,1	5,2	101,5
Methadone	5	18	5,0	5,6	99,8
Phenobarbital	20	18	20,0	9,8	99,8
Secobarbital	20	18	21,0	11,0	105,2

Table 4 Inter-run reproducibility and accuracy.

Recovery result

Recovery results are reported in **Figure 2**

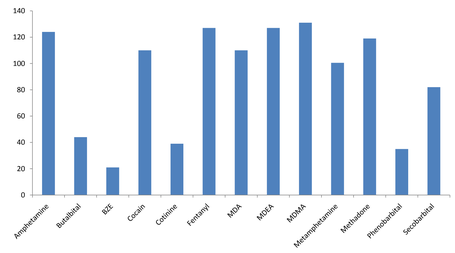

Figure 2 Extraction recovery result

Wet stability of extract sample

Following the extraction, sample are keep at 4°C. Samples were re-analyzed after 24 hours. Reproducibility and accuracy result are reported in **Table 5**.

Compound	Conc.	N	Mean	%RSD	%Nom
	(ng/ml)		(ng/ml)	(%)	(%)
Amphetamine	10	3	9,7	4,7	97,4
Butalbital	20	3	20,6	13,4	102,6
BZE	10	3	11,0	13,7	109,6
Cocaine	2	3	2,3	6,9	113,0
Cotinine	10	3	9,7	9,0	96,7
Fentanyl	10	3	11,4	1,9	114,4
MDA	10	3	10,7	7,4	107,2
MDEA	10	3	9,6	7,0	96,2
MDMA	10	3	10,0	11,2	100,1
Methamphetamine	10	3	9,6	9,5	96,3
Methadone	5	3	4,8	2,4	94,8
Phenobarbital	20	3	19,4	12,7	96,8
Secobarbital	20	3	19,6	8,6	98,5

Table 5 Wet stability result

Linearity

Inter-run linearity results are reported in **Table 8** and typical standard curve are presented in **Figure 3**.

Compound	Run 1	Run 2	Run 3
	r	r	r
Amphetamine	0,9966	0,9939	0,9950
Butalbital	0,9976	0,9923	0,9935
BZE	0,9959	0,9923	0,9944
Cocaine	0,9958	0,9969	0,9914
Cotinine	0,9975	0,9952	0,9962
Fentanyl	0,9956	0,9938	0,9970
MDA	0,9967	0,9973	0,9935
MDEA	0,9970	0,9983	0,9970
MDMA	0,9984	0,9957	0,9930
Methamphetamine	0,9985	0,9951	0,9923
Methadone	0,9977	0,9979	0,9934
Phenobarbital	0,9954	0,9908	0,9930
Secobarbital	0,9950	0,9926	0,9924

Table 8 Linearity result

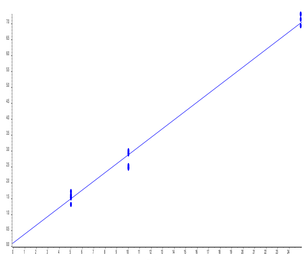

Figure 3 Typical standard curve

Dry sample stability in LazWell plate

Extracted samples are spotted in LazWell plate and kept 48 hours at room temperature before analysis. Reproducibility and accuracy result are reported in **Table 9**.

Compound	Conc.	N	Mean	%RSD	%Nom
	(ng/ml)		(ng/ml)	(%)	(%)
Amphetamine	10	3	10.1	6.7	100.9
Butalbital	20	3	19.2	2.3	96.3
BZE	10	3	10.1	8.7	101.1
Cocaine	2	3	2.0	6.3	99.5
Cotinine	10	3	9.9	5.0	99.0
Fentanyl	10	3	10.2	3.7	101.8
MDA	10	3	9.7	4.8	96.8
MDEA	10	3	10.0	3.8	100.3
MDMA	10	3	10.1	4.9	100.6
Methamphetamine	10	3	10.1	4.6	99.9
Methadone	5	3	4.9	5.0	98.1
Phenobarbital	20	3	20.4	8.3	101.5
Secobarbital	20	3	18.6	11.6	93.4

Table 9 Dry stability result

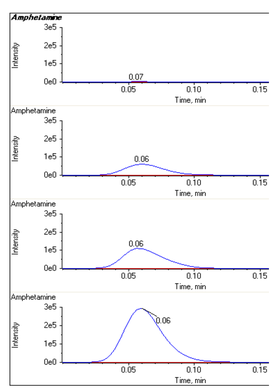

Figure 4 Typical desorption peak

Matrix selectivity and Matrix effect evaluation

The % of interferences at lower standard for six different matrices was evaluated as measurement of selectivity.

A interference less than 20% are obtained for each matrix. Similarly, matrix effects were evaluated by spiking QC in six different negative urines. Results are reported in **Table 6**.

Compound		M1	M2	M3	M4	M5	M6
Amphetamine	Mean	11,80	11,20	9,20	9,10	8,80	8,50
	%RSD	12,5	3,3	7,4	15,2	14,0	7,8
	%Nom	118,0	112,0	92,0	91,0	88,0	85,0
Butalbital	Mean	18,90	20,70	21,30	22,05	20,10	24,90
	%RSD	9,9	14,2	10,6	14,9	3,6	5,7
	%Nom	94,5	103,5	106,5	110,3	100,5	124,5
BZE	Mean	9,00	9,20	9,60	8,80	9,90	10,80
	%RSD	10,4	14,0	4,5	12,6	15,8	10,0
	%Nom	90,0	92,0	96,0	88,0	99,0	108,0
Cocaine	Mean	2,12	2,18	2,08	2,04	2,14	2,32
	%RSD	8,4	1,8	1,4	4,2	2,4	3,4
	%Nom	106,0	109,0	104,0	102,0	107,0	116,0
Cotinine	Mean	10,10	10,30	9,60	10,20	10,60	10,70
	%RSD	3,8	4,6	0,5	6,2	4,9	3,7
	%Nom	101,0	103,0	96,0	102,0	106,0	107,0
Fentanyl	Mean	9,80	9,70	9,50	9,60	9,80	10,20
	%RSD	10,0	3,4	3,3	1,8	3,7	3,5
	%Nom	98,0	97,0	95,0	96,0	98,0	102,0
MDA	Mean	10,10	11,20	10,90	10,70	10,90	11,00
	%RSD	10,6	5,8	0,7	0,9	3,9	6,0
	%Nom	101,0	112,0	109,0	107,0	109,0	110,0
MDEA	Mean	11,10	11,80	10,70	11,40	10,90	11,90
	%RSD	2,5	2,4	1,8	6,1	3,7	4,4
	%Nom	111,0	118,0	107,0	114,0	109,0	119,0
MDMA	Mean	11,00	11,50	11,10	10,90	10,90	11,00
	%RSD	8,8	1,6	3,6	2,7	1,4	2,3
	%Nom	110,0	115,0	111,0	109,0	109,0	110,0
Methamphe.	Mean	10,70	10,90	10,00	10,00	9,30	9,90
	%RSD	4,1	13,0	6,7	5,7	9,1	12,4
	%Nom	107,0	109,0	100,0	100,0	93,0	99,0
Methadone	Mean	5,05	5,30	5,15	5,20	5,15	5,35
	%RSD	2,8	5,1	1,9	6,1	1,6	1,6
	%Nom	101,0	106,0	103,0	104,0	103,0	107,0
Phenobarbital	Mean	20,93	19,05	19,65	20,10	16,80	20,48
	%RSD	6,6	8,5	12,9	12,7	10,1	3,6
	%Nom	104,6	95,3	98,3	100,5	84,0	102,4
Secobarbital	Mean	20,40	20,60	20,60	18,60	18,20	21,00
	%RSD	9,9	14,2	10,6	14,9	3,6	5,7
	%Nom	102,0	103,0	103,0	93,0	91,0	105,0

Table 6 Matrix effect evaluation result

Commun drug interferences

Common drug interferences are evaluated by adding a cocktail of known drugs to the QC .Results are reported in **Table 7**.

Compound		Pool 1	Pool 2	Pool 3
Amphetamine	Mean	9,53	9,65	10,02
	%RSD	14,2	7,8	6,7
	%Nom	95,3	96,5	100,2
Butalbital	Mean	22,76	21,81	23,42
	%RSD	8,6	8,8	14,8
	%Nom	113,8	109,1	117,1
BZE	Mean	10,26	9,72	10,40
	%RSD	15,0	9,0	4,2
	%Nom	102,6	97,2	104,0
Cocaine	Mean	2,06	2,02	2,27
	%RSD	7,3	8,5	5,3
	%Nom	102,8	101,2	113,4
Cotinine	Mean	10,61	9,94	11,05
	%RSD	8,6	5,9	3,4
	%Nom	106,1	99,4	110,5
Fentanyl	Mean	10,43	9,99	10,97
	%RSD	3,1	5,4	7,4
	%Nom	104,3	99,9	109,7
MDA	Mean	10,57	9,29	10,82
	%RSD	10,0	9,1	4,6
	%Nom	105,7	92,9	108,2
MDEA	Mean	11,04	10,48	11,43
	%RSD	4,8	4,9	6,1
	%Nom	110,4	104,8	114,3
MDMA	Mean	11,15	10,36	11,31
	%RSD	5,5	5,2	5,0
	%Nom	111,5	103,6	113,1
Methamphe.	Mean	10,10	10,68	11,30
	%RSD	7,2	9,7	4,3
	%Nom	101,0	106,8	113,0
Methadone	Mean	5,38	5,05	5,38
	%RSD	5,3	3,6	5,1
	%Nom	107,6	101,1	107,6
Phenobarbital	Mean	21,28	21,83	21,33
	%RSD	8,4	12,3	13,5
	%Nom	106,4	109,1	106,7
Secobarbital	Mean	21,22	20,76	23,01
	%RSD	1,3	13,3	13,7
	%Nom	106,1	103,8	115,1

Table 7 Potentially drug interference evaluation result

Pooled interference drugs are:

Pool 1: phentermine; THC; 11-nor-9-carboxy-THC; piroxicam; norpropoxyphene; norfentanyl oxalate; eslicarbazepin; EDDP
Pool 2: codeine; hydrocodone; hydromorphone; morphine; norcodeine; oxycodone; oxymorphone; norhydrocodone
Pool 3: nordiazepam; diazepam; oxazepam; estazolam; temazepam; alprazolam; lorazepam; triazolam

CONCLUSIONS

- **A generic extraction method of different drug class was achieved using SLE 96- well array and was rapidly analyzed by LDTD-MS/MS in under 9 seconds per sample.**
- **Good signal to noise (S/N) was achieved for the compounds at the required cut-off levels.**
- **Intra and inter days run reproducibility and accuracy are within requirement.**
- **Stability, matrix selectivity, common drug interference and matrix effect fulfil validation requirement**
- **The evaluation of this new workflow to provide a fast and generic screening method for analysis of drugs in oral fluid provided excellent quantitation data at ultra-high-throughput speed and was a success.**