Dried Blood Spots in Tips Targeted Drug Screening in 9 Seconds per Sample Using Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS)

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Introduction

- Dried blood spot technique is widely used in blood collection for clinical or medical analysis.
- High-throughput mass spectrometry is used to analyze a sample in seconds.
- Combination of Dried Blood Spot and High-throughput mass spectrometry could save time and lower the cost for targeted screening approach.

Dried Blood Spots

Advantages:

- Quick and simple collection of blood sample.
- Stability when the sample is dried.
- Low level training to collect sample.

Challenges:

- Whole blood analysis results on higher background.
- Efficiency of extraction.
- Low sensitivity due to small sample volume.
- Hematocrit induces variability in sample volume analyzed.

DBS in Tips

- Fiber material contained in a standard 200 µL tip.
- Drying and extraction is done directly in the tip. No need for further manipulation of the fiber.

Advantages:

- No Hematocrit effect since the whole blood volume is contained in the fiber.
- Requires only 2 µL of blood.

Objective

Objective: Evaluate the DBS in Tips technique with a wide selection of drug compounds (122) with a screening approach.

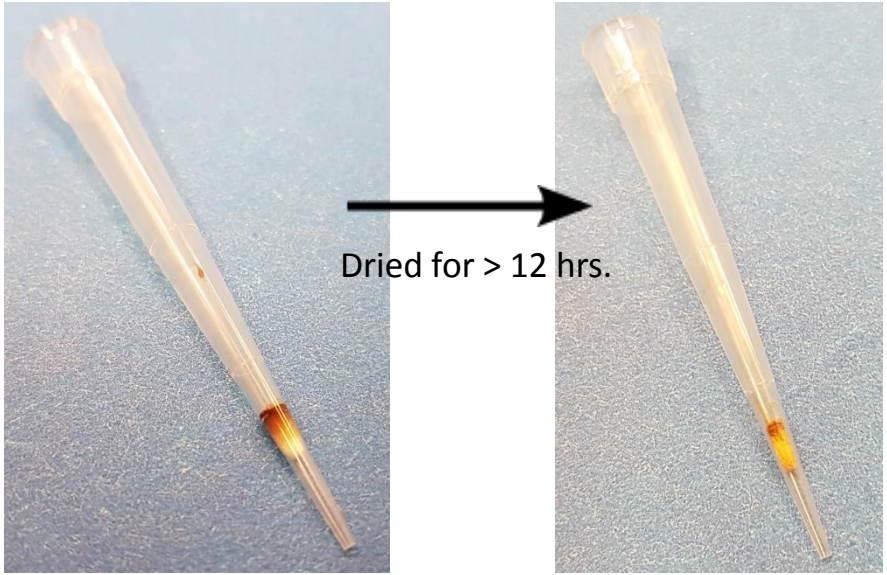
Drugs panel: Amphetamines, Barbiturates, Opioids, Benzodiazepines, Selective serotonin reuptake inhibitors (SSRI), Serotonin-norepinephrine reuptake inhibitors (SNRI), Tetracyclic antidepressants, Carbamates, Cannabinoids and others.

Experimental:

- Drug panel is separated in 7 groups and analyzed with 7 deuterated internal standards with a LDTD®- Sciex Q-Trap 5500® MS/MS system.
- The LDTD ion source is used to monitor all 122 transitions in 9 seconds.

Method - Blood Sampling

1) 2 µL of water mixed with the group of IS are deposited on the fiber to pre-wet it.
2) 2 µL of blank blood or of spiked blood at 25, 50 and 100 ng/mL are deposited on the fiber.
3) 8 replicates for each concentration, a double blank and a blank are prepared.
4) The blood is dried for at least 12 hrs.

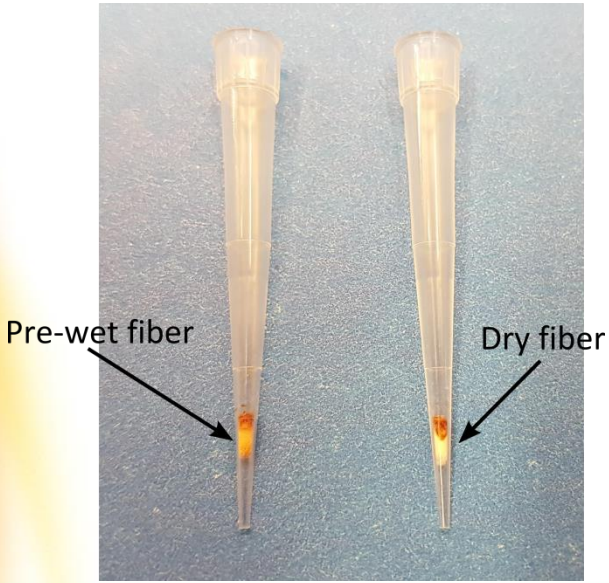


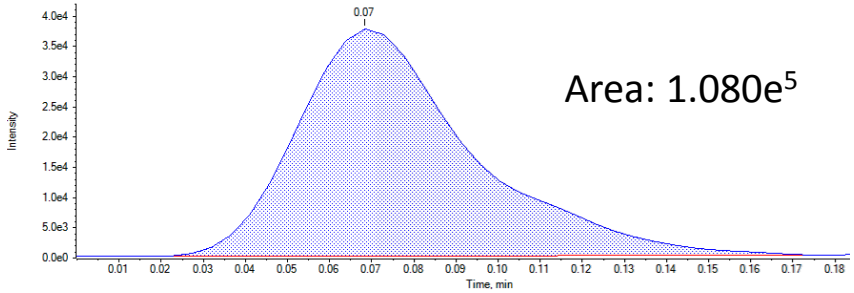
Wet Blood

Dry Blood

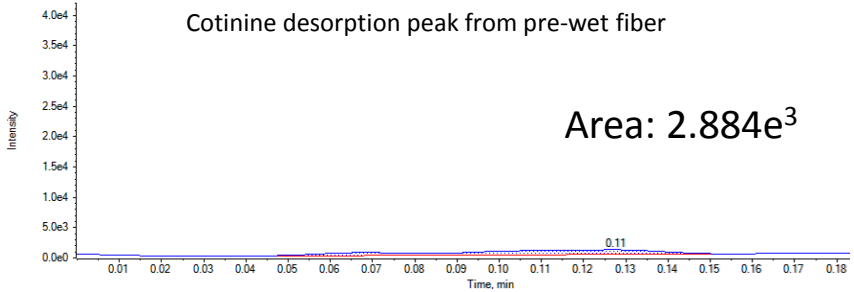
Method

- Pre-wetting the fiber ensures an efficient extraction.





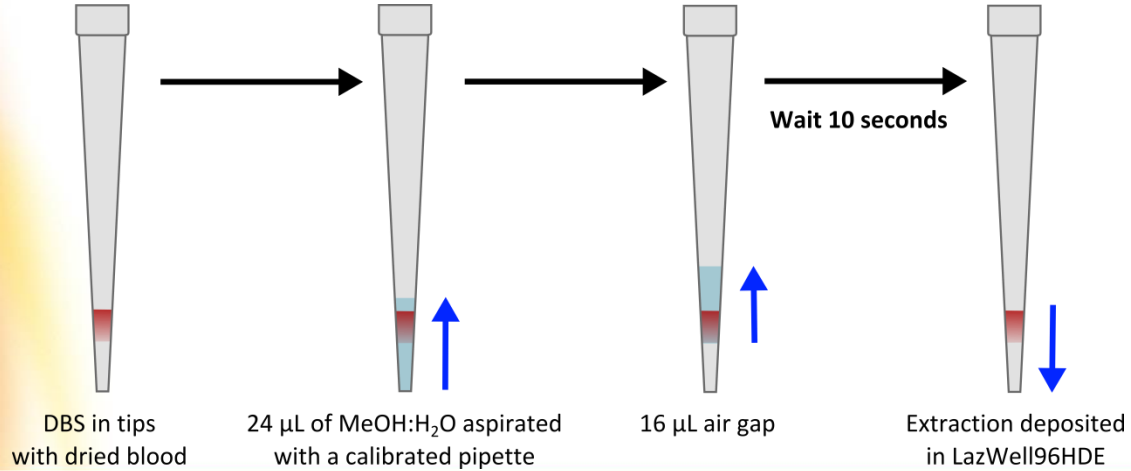
Cotinine desorption peak from pre-wet fiber

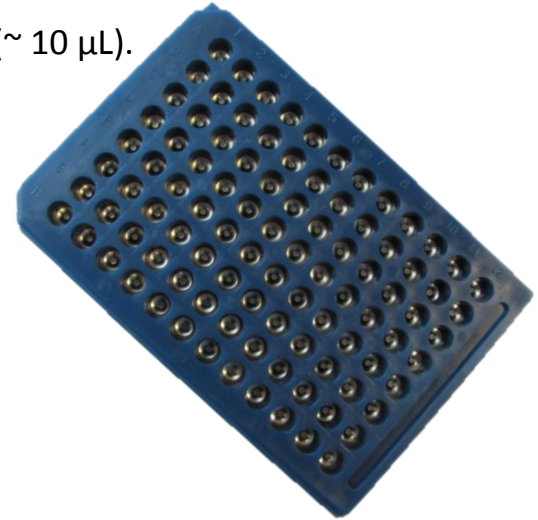


Cotinine desorption peak from dry fiber

Method - Extraction

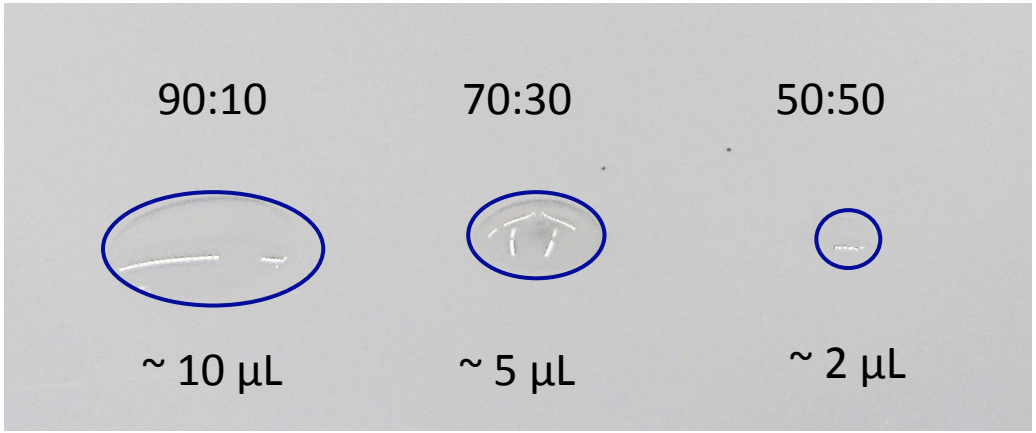
1) 24 µL of a solution of MeOH:H_2O (90/10, v/v) are aspirated in the tips with an automatic pipette.
2) 16 µL of air gap to allow all the MeOH:H_2O to penetrate the fiber.
3) Wait 10 seconds.
4) Deposit the whole volume on EDTA coated Lazwell™96HDE plate (~ 10 µL).





Extraction solution

The MeOH:H_2O ratio in the extraction solution is optimized to maximize the final volume extracted and lower the *S/N* ratio without extracting the red blood cells.



Analysis

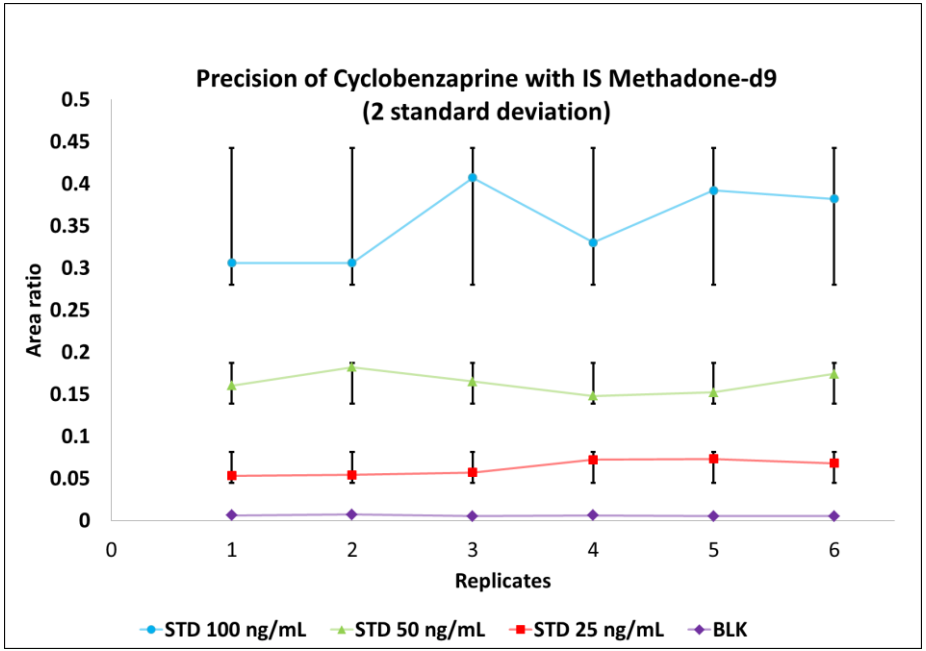
- Generic extraction with only 7 IS.

Internal Standard compensation:

- Difference in volume extract
- Variation due to ion suppression
- Difference in extraction efficiency
- Difference in desorption efficiency.

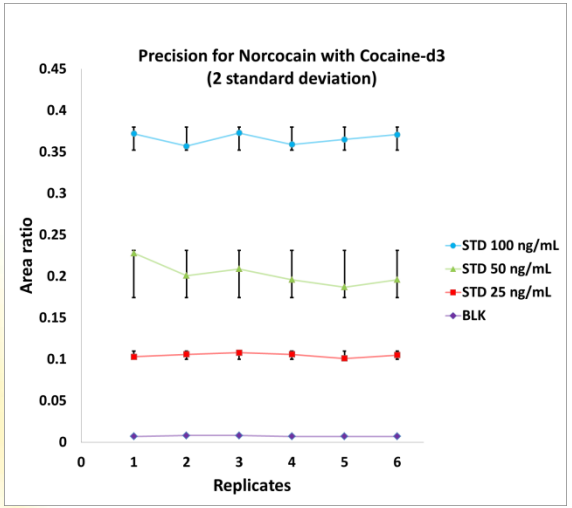
Selectivity criteria for decision point :

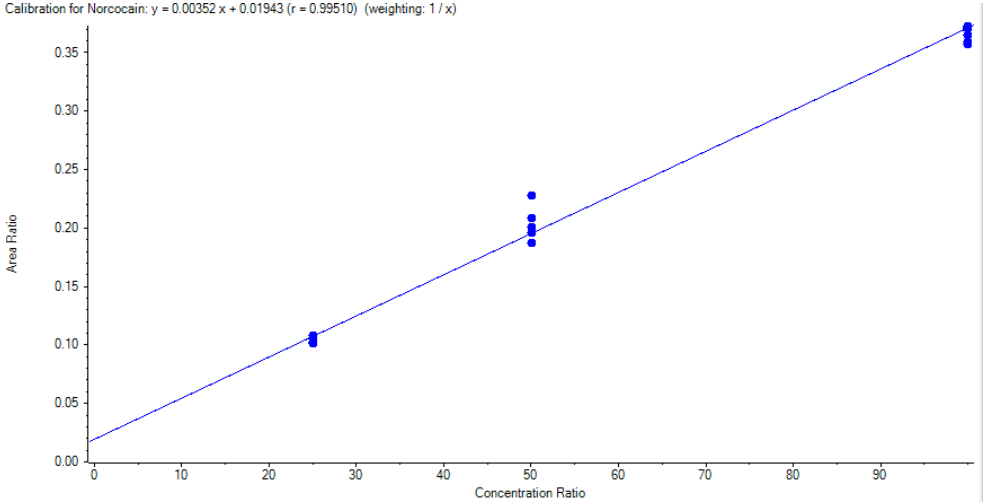
- Compounds must differentiate from blank by 2 standard deviations
- Curve should have a R > 0.90.



Results

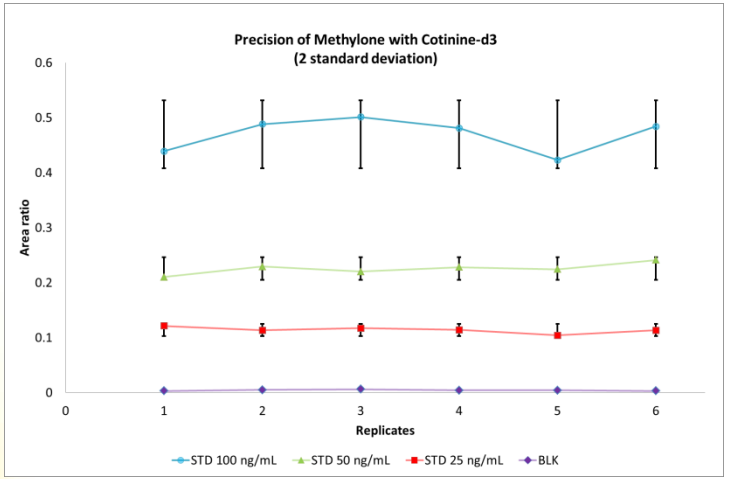
For compounds with their deuterated IS or from the same family (ex : Norcocaine – Cocaine-d_3), the decision point was established at 25 ng/mL with a R > 0.99.

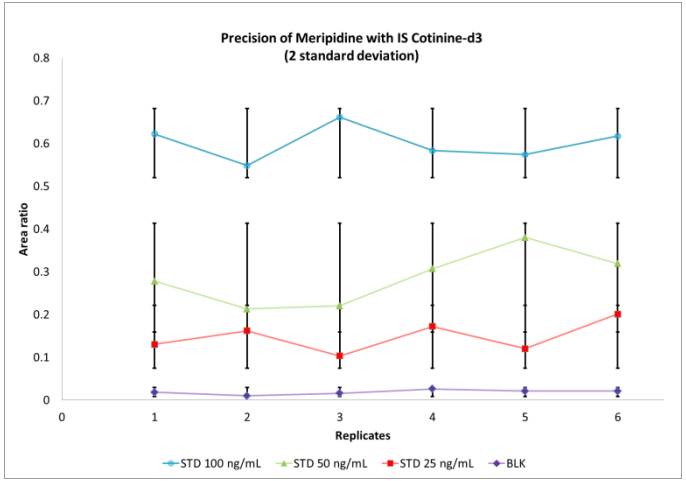




Results

For the other compounds, IS were associated with each compound in order to optimize the detection signal.

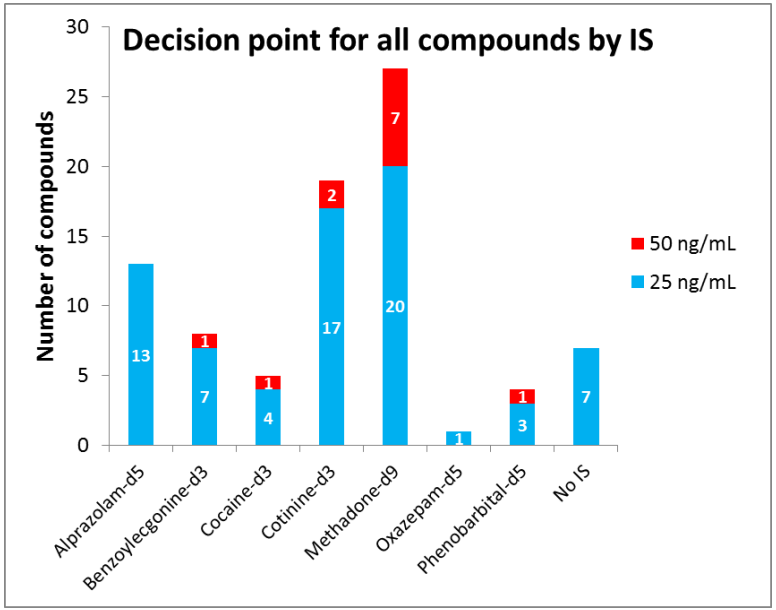




2 examples of compounds that have a decision point at 25 ng/mL

Results

- 122 compounds were analyzed
- 84 compounds complied with our screening criteria
  - 12 compounds with a decision point of 50 ng/mL
  - 72 compounds with a decision point of 25 ng/mL
  - 8/84 overlap in concentration but differentiate from the blank



Data acquired with a LDTD ion source and a Sciex Q-Trap 5500 MS

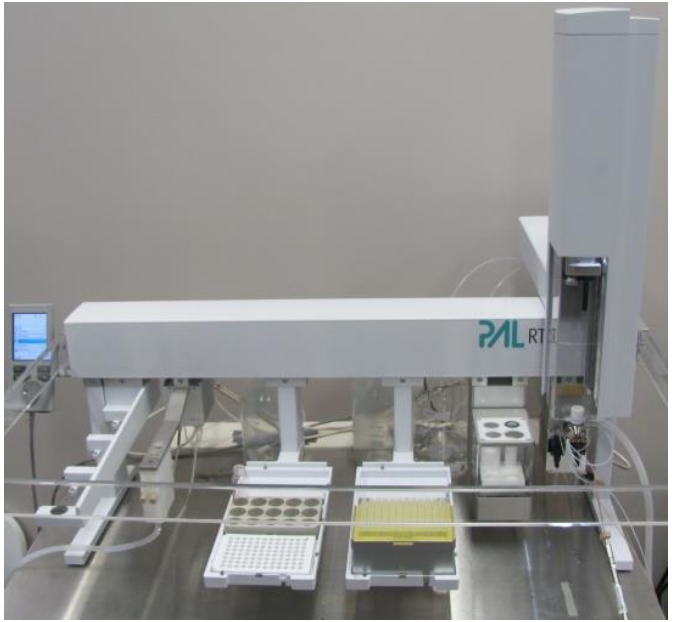
Observation

Challenges:

- Reproducibility issue, because the pre-wetting (IS), the blood spotting, the extraction and the deposition on a LazWell are all done manually and introduce a precision problem.
- The fiber can move during manual aspiration of extraction solution.
- Deposition on the LazWell plate is challenging because of the air gap.

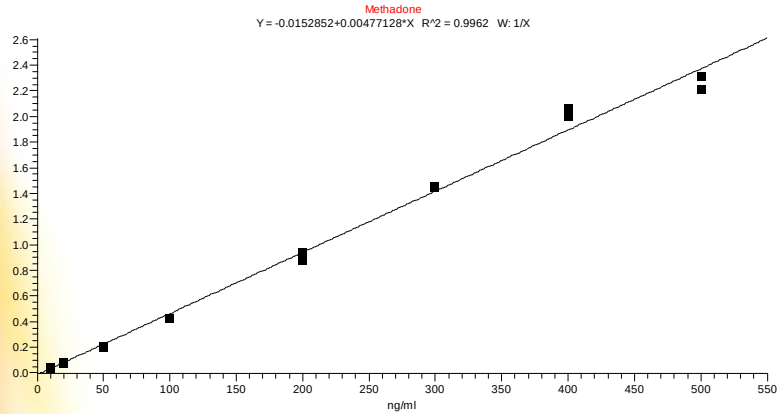
Automation

To reduce variability, the extraction process and the deposition/injection can be completely automated.



Automation

Calibration curve for Methadone with IS Methadone-d_9.



	LLOQ	QC-Low	QC-Med	QC-High	ULQC
Nominal conc. (ng/mL)	10	30	150	350	500
N	4	4	4	4	4
Mean (ng/mL)	10.9	29.6	158.0	351.9	477.7
RSD (%)	3.4	3.1	4.1	11.6	2.6
%Nom. conc.	109.4	98.7	105.3	100.5	95.5

Data acquired with a HPLC Shimadzu *Nexera X2*™ and a Thermo *TSQ Vantage*™ MS

Discussion

- 70% of the compounds were differentiated from the blank with a generic extraction method.
- Keeping the same extraction method, some compounds could have a lower decision point.
- Lowering the decision point of the other compounds would require to:
  - use the deuterated IS to enhanced the precision,
  - use an optimized laser pattern to increase the desorption rate,
  - use a specific extraction solution to enhance extraction efficiency.

Conclusion

- Multiple compounds analyzed with DBS in Tips.
- Rapid extraction in only 10 seconds.
- Low volume of blood required (2 µL).
- Applications in other fields such as pharmacokinetics:
  - Do multiple replicates with the same animal;
  - Use the same animal multiple times;
  - Lower cost.

Acknowledgment

The team

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Serge Auger

Pr. Réal Paquin

Pr. Jacques Corbeil

Pierre Picard

Funding resources

CRSNG-FQRNT

Mitacs

Special thanks for support

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Questions

Annexe

Table of all compounds that were extracted

Name	IS	Cut-off (ng/mL)	R	MS Mode
2-OH-Ethylflurazepam	Alprazolam-d5	25	0.989	Positif
4-Methylephedrine	Cotinine-d3	25	0.948	Positif
5-Fluoro-ABD	BZE-d3	25	0.992	Positif
7-Aminoclonazepam	Alprazolam-d5	25	0.992	Positif
7-Aminoflunitrazepam	Alprazolam-d5	25	0.975	Positif
Alpha-OH-Alprazalom	Alprazolam-d5	25	0.976	Positif
Alpha-OH-Midazolam	BZE-d3	25	0.984	Positif
Alpha-Triazolam	Alprazolam-d5	25	0.989	Positif
Alprazalom	Alprazolam-d5	25	0.988	Positif
Amitriptyline	Methadone-d9	50	0.978	Positif
Amobarbital	Phenobarbital-d5	50	0.938	Négatif
Benzoylecgonine	BZE-d3	25	0.995	Positif
Butabarbital	No IS	25	0.901	Négatif
Butalbital	Phenobarbital-d5	25	0.982	Négatif
Butylone	Cotinine-d3	25	0.991	Positif
Carisoprodol	No IS	25	0.989	Positif
Chlorodiazepoxide	Alprazolam-d5	25	0.992	Positif
Citalopram	Methadone-d9	25	0.991	Positif
Clomipramine	Methadone-d9	25	0.994	Positif
Clonazepam	Oxazepam-d5	25	0.983	Positif
Clozapine	Methadone-d9	25	0.986	Positif

Name	IS	Cut-off (ng/mL)	R	MS Mode
Cocaethylene	Cocaine-d3	25	0.996	Positif
Cocaine	Cocaine-d3	25	0.994	Positif
Codeine	Methadone-d9	25	0.971	Positif
Cotinine	Cotinine-d3	25	0.993	Positif
Cyclobenzaprine	Methadone-d9	25	0.995	Positif
Desipramine	Cotinine-d3	50	0.931	Positif
Dextromethorphan	No IS	25	0.986	Positif
Dextrorphan	No IS	25	0.982	Positif
Diazepam	BZE-d3	25	0.987	Positif
Doxepin	Methadone-d9	50	0.971	Positif
Duloxetine	Methadone-d9	25	0.984	Positif
EDDP	Cotinine-d3	25	0.987	Positif
Fentanyl	Methadone-d9	25	0.994	Positif
Flunitrazepam	Methadone-d9	25	0.990	Positif
Fluoxetine	Cotinine-d3	50	0.983	Positif
Flurazepam	Methadone-d9	25	0.986	Positif
Haloperidol	Methadone-d9	50	0.951	Positif
Hydroxybuprion	Cotinine-d3	25	0.986	Positif
Imipramine	Methadone-d9	25	0.980	Positif
JHW-018-OH	Alprazolam-d5	25	0.986	Positif
JHW-073-OH	Alprazolam-d5	25	0.994	Positif

Annexe

Table of all compounds that were extracted

Name	IS	Cut-off (ng/mL)	R	MS Mode
Ketamine	Cotinine-d3	25	0.925	Positif
Levitracetam	Cotinine-d3	25	0.994	Positif
Lorazepam	BZE-d3	25	0.953	Positif
MDA	Cotinine-d3	25	0.976	Positif
MDEA	Cotinine-d3	25	0.993	Positif
MDMA	Cotinine-d3	25	0.995	Positif
MDPV	Methadone-d9	50	0.957	Positif
Meprobamate	Cocaine-d3	25	0.976	Positif
Merperidine	Cotinine-d3	25	0.962	Positif
Meta-chlorophenylpiperazine	Cotinine-d3	25	0.993	Positif
Methadone	Methadone-d9	25	0.996	Positif
Methylone	Cotinine-d3	25	0.997	Positif
Methylphenidate	Cotinine-d3	25	0.986	Positif
Midazolam	Alprazolam-d5	25	0.986	Positif
Mirtazapine	Cotinine-d3	25	0.974	Positif
Naphyrone	Methadone-d9	25	0.989	Positif
Naxolone	Methadone-d9	50	0.970	Positif
N-desmethyl citalopram	Methadone-d9	25	0.978	Positif
N-desmethyl mirtazapine	Cotinine-d3	25	0.960	Positif
N-desmethyl-clozapine	Methadone-d9	50	0.989	Positif
Norcocain	Cocaine-d3	25	0.996	Positif

Name	IS	Cut-off (ng/mL)	R	MS Mode
Nordiazepam	BZE-d3	50	0.970	Positif
Norfentanyl	Methadone-d9	25	0.994	Positif
Norhydrocodone	BZE-d3	25	0.989	Positif
Norperidine	Methadone-d9	25	0.991	Positif
Norquetiapine	No IS	25	0.979	Positif
Nortriptyline	Methadone-d9	25	0.985	Positif
O-desmethyltramadol	Methadone-d9	25	0.990	Positif
O-desmethylvenlafaxine	Cocaine-d3	50	0.975	Positif
Oxymorphone	Methadone-d9	25	0.949	Positif
PB-22	Alprazolam-d5	25	0.983	Positif
Phenobarbital	Phenobarbital-d5	25	0.958	Négatif
Propoxyphene	No IS	25	0.984	Positif
Quetiapin	Methadone-d9	25	0.984	Positif
Secobarbital	Phenobarbital-d5	25	0.993	Négatif
Termazepam	BZE-d3	25	0.987	Positif
Topiramate	Methadone-d9	50	0.953	Positif
Tradamol	Cotinine-d3	25	0.993	Positif
Venlafaxine	No IS	25	0.978	Positif
XLR-11 4-Hydroxypentyl	Alprazolam-d5	25	0.988	Positif
Zaleplon	Alprazolam-d5	25	0.992	Positif
Zolpiem	Methadone-d9	25	0.960	Positif