Phytronix Breakaway Technologies

Sciex

bertin INSTRUMENTS MONITOR THE INVISIBLE

Toxicology

Application Note : 2214

Drug Panel Screening in Nails:

Drug Screening in Nails at 8 Seconds per Sample Using LDTD-MS/MS

Pierre Picard, Jean Lacoursière and Serge Auger
Phytronix Technologies, Québec, Canada
Keywords: High-Throughput, Nail, LDTD-MS/MS

Introduction

The analysis of nail clippings is a great alternative to hair analysis, in the case where hair is not available. They provide up to 6 months of drug usage history and are easily collected for testing.

Our goal for this application note is to develop a sample preparation method for a drug panel in nails using a single operation in LUXON-MS/MS and the Precellys 24 Touch (Bertin Technologies, Montigny-les-Bretonneux, France).

A proper sample preparation protocol is critical for MS-based analysis workflows. Indeed, the quality and reproducibility of the drug extraction can strongly influence MS results. This requires choosing an optimal protocol for the sample preparation step. The 3D-beating technology is considered the gold standard for sample pulverization. For this reason, Bertin Technologies has chosen 3-dimensional bead-beating technology to power the Precellys 24 Touch homogenizer (Bertin Technologies, Montigny-les-Bretonneux, France.

LUXON-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a quick and simple sample preparation. Fifteen drugs (different drug classes) are analyzed **simultaneously** with **quantitative** screening results obtained in less than 8 seconds per sample. Each drug has been screened based on the industry cut-offs required.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Sample Collection

Thirty milligrams of nail samples were transferred into the Precellys MK28R 2mL lysing kit (2 mL reinforced empty vials with 2.8 mm stainless steel beads from Bertin Technologies, Montigny-le-Bretonneux, France, p/n : P000917-LYSK0-A.0).

Decontamination and Pulverization

To remove undesired contaminants from the nail surface, 1 mL of dichloromethane (DCM) was added to the samples, and they were soaked for 1 minute at room temperature. The washing solution was then discarded. This washing step was repeated twice. Afterwards, samples were dried at 60°C / convection for 30 minutes. Next, the cleaned nails were pulverized into a fine powder (3 X 60 sec at 6500 rpm, 15 seconds pause time) using a Precellys 24 Touch system (**Figure 3**).

Figure 3 – Nail pulverization system (Precellys 24 Touch) from Bertin Technologies, Montigny-le-Bretonneux, France.

Sample Extraction

Samples were extracted as follows[1]:

- Add 1 mL of the internal standard solution (acetonitrile:water / 75:25) in a 2 mL vial.
- For the screening curve: 25 µL of the working solution of the standard were added.
  - Vials were capped, mixed and incubated at room temperature for 1.5 hours (horizontal mixing at 500 rpm).
- Add 0.5 mL of extraction buffer in extraction vial.
  - Vortex.
  - Centrifuge for 2 minutes at 5000 rpm.
- Mix 10 µL of desorption buffer with 90 µL of the upper layer phase.
- Spot 6 µL of the mixture onto a LazWell™96 plate
  - Dry 4 minutes at 40°C

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6 L/min (air)
Laser pattern:

- 6-second ramp to 65% power
- Hold 1 seconds at 65% power

MS/MS

MS model: Q-Trap System® 5500, Sciex
Scan Time: 5 msec
Total run time: 8 seconds per sample
Ionization: APCI
Analysis Method: MRM mode

Table 1 - Positive MRM transitions for LDTD-MS/MS

	Transition	CE
7-Amino Clonazepam	286.1 → 222.2	30
Alprazolam	309.1 → 274.1	35
Amphetamine	136.1 → 119.1	12
Benzoylecgonine	290.1 → 168.2	30
Clonazepam	316.0 → 214.0	50
Diazepam	285.1 → 154.1	45
Fentanyl	342.2 → 188.1	35
Codein / Hydrocodone	300.2 → 152.0	75
Morphine	286.1 → 152.0	75
Lorazepam	321.0 → 275.0	30
Methamphetamine	150.1 → 119.1	15
Nordiazepam	271.1 → 140.1	27
Oxazepam	287.1 → 104	40
Oxycodone	316.2 → 241.0	35
Temazepam	301.1 → 255.1	25
Oxazepam-d_5	292.1 → 109	40
Benzoylecgonine-d_3	293.1 → 171.1	30
Amphetamine-d_5	141.1 → 124.1	12
Methamphetamine-d_5	155.1 → 121.1	15
Morphine-d_6	292.1 → 152.0	75
Temazepam-d_5	306.1 → 260.1	25
Codeine-d_6	306.2 → 152.0	75
Oxycodone-d_6	322.2 → 247.0	35
Fentanyl-d_5	342.2 → 188.0	35
Alprazolam-d_5	314.1 → 286.1	35

Results and Discussion

Initial Cut-off Test (pg/mg nail)

A screening cut-off of 20 pg/mg nail is reached for fentanyl. For all the other drugs, a screening cut-off of 40 pg/mg nail is obtained[2].

Precision / Accuracy

Three-point screening curves and two QCs (QC-0.5X and QC-2X) were prepared in negative nail powder and used to validate the method. The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell™ plate and dried before analysis.

The following acceptance criteria were used:

- Each standard concentration must not exceed 20% CV
- Each standard concentration must be ±20% of the nominal value (%Biais).
- QC-0.5X cut-off must be detected as negative.
- QC-2X cut-off must be detected as positive.

For the inter-run precision/accuracy experiment, each fortified sample set is analyzed in triplicate on five different days. **Table 2** shows the inter-run precision and accuracy results. For temazepam, the %CV and %Biais was below 20%. All QC-0.5X were detected as negative and QC-2X detected as positive. Similar results are obtained for the other drugs in the panel.

Table 2 - Inter-Run Precision / Accuracy

Temazepam	S1	S2	S3
Conc (pg/mg)	40	80	200
N	15	15	15
Mean (pg/mg)	39.9	80.4	199.8
%CV	4.9	5.8	3.3
%Biais	0.4	0.5	0.1

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of standards are reported in **Table 2**. All the results are within the acceptable criteria range for 1 day at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 60 minutes before analysis. The precision and accuracy results of standard samples are reported **Table 3**. All the results are within the acceptable criteria range for 60 minutes at room temperature.

Table 3 - Wet and Dry Stability of Temazepam

Parameters	Dry stability (60 min /RT)			Wet stability (1 day / 4°C)		
Conc.(pg/mg)	40	80	200	40	80	200
N	3	3	3	3	3	3
Mean (pg/mg)	39.3	81.7	198.9	37.6	86.3	196.1
%CV	4.9	8.4	5.1	5.0	3.4	10.4
%Biais	1.7	2.2	0.5	5.9	7.8	2.0

Multi-matrix validation

Negative nail clippings were collected from 6 volunteers. Samples were analyzed without spiking and spiked at QC-0.5x and QC-2X levels and screened using the LDTD-MS/MS method. Results were compared to LCMSMS. The method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy are verified as follows:

		LC-MS/MS	
		Yes	No
LDTD-MS/MS	Yes	TP (True positive)	FP (False positive)
	No	FN (False negative)	TN (True negative)

Where:
Sensitivity: (TP / (TP + FN))
Specificity: (TN / (TN + FP))
PPV: (TP / (TP + FP))
NPV: (TN / (TN + FN))
Accuracy: ((TP+TN) / (TP + FN+TN+FP))

Table 4 shows the analysis results of 18 spiked samples for temazepam.

Table 4 – Temazepam results

		LC-MS/MS	
		Yes	No
LDTD-MS/MS	Yes	TP=6	FP=0
	No	FN=0	TN=12

Validation results are reported in **Table 5** for temazepam. Similar results are obtained for the other drugs.

Table 5 – Validation results for Temazepam

Parameters	Temazepam
Sensitivity	1
Specificity	1
PPV	1
NPV	1
Accuracy	1

Conclusion

The Precellys®24 Touch can be successfully used to pulverize nail samples for mass spectrometry drug analysis. The Precellys 24 Touch combined with the Luxon Ion Source® and Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of different drug classes in nail clippings using a simple sample preparation method.

References

Cobo-Golpe et Al. (2020). J. of Pharmaceutical and Biomedical Analysis; 189:113443
Shu et Al. (2015). J of Analytical Toxicology; 39: 624–628