DRUG SCREENING IN HAIR AT 8 SECONDS PER SAMPLE USING THE PRECELLYS 24 TOUCH AND LUXON-MS/MS

BENZODIAZEPINE DRUG PANEL SCREENING IN HAIR

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CONTEXT

During the last few decades, research work has been done to investigate the different drugs of abuse in different types of biological samples (urine, saliva, hair, etc). One of the advantages of hair sample is the noninvasive collection.

Our goal for this application note is to develop a **sample preparation method for a drug panel in hair** 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. Twenty drugs 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

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SAMPLE PREPARATION METHOD

Sample Collection

Ten milligram hair samples (approximately 1 cm length) 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, ref : P000917-LYSK0-A.0)

Decontamination and Pulverization

To remove undesired contaminants from the hair surface, 1 mL of methanol was added to the samples, and it was soaked for 5 minutes at room temperature. The washing solution was discarded then samples were dried at 60°C / convection for 30 minutes. Samples were pulverized into a fine powder (3X 60sec at 6500 rpm, 15 seconds pause time) using **Precellys 24 Touch system** (Figure 3).

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

Sample Extraction

Samples were extracted as follows:

- Add 1 mL of the internal standard solution in a 2 mL vial.
- For the screening curve: 10 µL of working solution of the standard were added.
- Vials were capped, mixed and incubated at 60°C for 1h45 followed by 15 minutes in the sonication bath.
- Centrifuge for 5 minutes.
- Mix 6.5 µL of desorption buffer with 20 µL of upper layer phase
- Spot 6 µL of the mixture onto a LazWell™96 plate
- Dry 6 minutes at 40°C

	Transition	CE
Meprobamate	219.1 → 158.1	10
Carisoprodol-d_7	268.2 → 183.2	10
Nordiazepam	271.1 → 140.1	27
7-Aminoflunitrazepam	284.0 → 226.0	50
Diazepam	285.1 → 154.1	32
7-Amino Clonazepam	286.1 → 222.2	30
Oxazepam	287.1 → 241.1	30
7-Amino Clonazepam-d_4	290.1 → 226.0	30
7-Amino Flunitrazepam-d_7	291.0 → 230.0	50
Oxazepam-d_5	292.1 → 246.1	30
Temazepam	301.1 → 255.1	25
Temazepam-d_5	306.1 → 260.1	25
Zaleplon	306.1 → 264.1	30
Zolpidem	308.1 → 236.1	35
Alprazolam	309.1 → 274.1	35
Alprazolam-d_5	314.1 → 286.1	35
Clonazepam	316.0 → 214.0	50
Lorazepam	321.0 → 275.0	30
(Alpha) Hydroxyalprazolam	325.1 → 205.1	54
Midazolam	326.1 → 291.1	35
Clozapine	327.1 → 270.1	30
2-Hydroxyethylflurazepam	333.1 → 194.1	30
Alpha-OH-Midazolam	342.2 → 203.1	35
Etizolam	343.1 → 314.0	25
(Alpha) Hydroxytriazolam	359.0 → 331.0	36
Flurazepam	388.1 → 315.0	27

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

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6 L/min (air)
Laser pattern:
6-second ramp to 55% power
Hold 2 seconds at 55% 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

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RESULTS AND DISCUSSION

Initial Cut-off Test (pg/mg hair)

A screening cut-off of 500 pg/mg hair is reached for **7-aminoflunitrazepam**, **7-aminoclonazepam** and **nordiazepam**. For all other drugs, a screening cut-off of 250 pg/mg hair is obtained.

Precision

Spiked samples around the decision point (50% cut-off: QC-L, cut-off: CO and 200% cut-off: QC-H) and blank solutions are used to validate the precision of the method. The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited onto a **LazWell™** plate and dried before analysis.

The following acceptance criteria were used:
- Each concentration must not exceed 20% CV
- The mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the cut-off.

For the inter-run precision experiment, each fortified sample set is analyzed in triplicate on five different days. **Table 2** shows the inter-run precision results. No overlapping at the cut-off is observed for 2-Hydroxyethylflurazepam and the %CV was below 20%. Similar results are obtained for the other drugs in the panel.

2-Hydroxyethylflurazepam	QC-L	CO	QC-H
Conc (pg/mg hair)	125	250	500
N	15	15	15
Mean (pg/mg hair)	123.7	246.2	505.6
SD	17.5	15.2	21.3
%CV	14.1	6.2	4.2
Mean – 2SD (pg/mg hair)	88.8	215.7	463.1
Mean + 2SD (pg/mg hair)	158.7	276.7	548.2

Table 2 – Inter-Run Precision

For the intra-run precision experiment, each fortified sample is extracted and analyzed in 8 replicates. Area ratio results are plotted using the ± 2 STD error bars. **Figure 4** shows the intra-run results for **2-Hydroxyethylflurazepam**. No overlapping is observed for each concentration and the %CV was below 20%. Similar results are obtained for the other drugs in the panel.

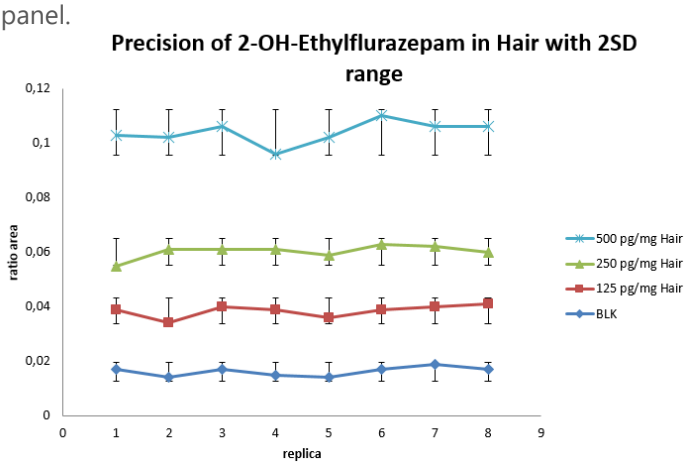

Figure 4 – Intra-Run Precision Curves for 2-Hydroxyethylflurazepam

Multi-matrix validation

Hair samples are collected from ten different volunteers. Samples are screened to verify the presence of each analyte (all samples were negative). Drugs are spiked 200% cut-off (QC-H) and screened as unknown to verify the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

		Spiked sample	
		Yes	No
Luxon-MSMS	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))

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Table 3 shows the analysis result of non-spiked/spiked samples for 2-Hydroxyethylflurazepam.

2-Hydroxyethylflurazepam		Spiked sample	
		Yes	No
Luxon-MSMS	Yes	TP = 10	FP = 0
	No	FN = 0	TN = 10

Table 3 – 2-Hydroxyethylflurazepam results

Validation results are reported in **Table 4** for 2-Hydroxyethylflurazepam. Similar results are obtained for the other drugs.

Parameters	2-Hydroxyethylflurazepam
Sensitivity (%)	100
Specificity (%)	100
PPV (%)	100
NPV (%)	100
Accuracy (%)	100

Table 4 – Validation results for 2-Hydroxyethylflurazepam

CONCLUSION

The Precellys 24 Touch can be successfully used to pulverize hair 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 Benzodiazepine drug panel in hair using a simple sample preparation method.

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