Urine and serum Ultra-Rapid Analysis of Flakka (alpha-PVP) in 9 Seconds per Sample by Laser Diode Thermal Desorption Mass Spectrometry

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OVERVIEW

Purpose
- Ultra-Fast analysis of synthetic cathinone (α-pyrrolidinopentiophenone) in urine and serum samples using LDTD®-MS/MS

Method
- Standards, QC and samples using 2 different matrices
- Liquid-Liquid extraction
- Deposit of a small volume of the organic phase in LazWell™ plate
- Fast Analysis using LDTD®-MS/MS system

Results
- Excellent linearity over the calibration range (R^2 > 0.9936 for a total of 6 different calibration curves in each matrix)
- Good sample stability (Wet and Dry in LazWell™)
- **All samples are analyzed with a run time of 9 seconds using LDTD®-MS/MS system.**

INTRODUCTION

In April 2015, NIH published that the emerging drug alpha-pyrrolidinovalerophenone (alpha-PVP), also known as ''Flakka'', is surging in Florida and in other parts of the US. It is chemically similar to other synthetic cathinone drugs popularly called ''bath salts'', and takes the form of a white or pink, foul smelling crystal that can be vaporized, which sends the drug very quickly into the bloodstream. It is structurally related to pyrovalerone, a psychoactive drug that was used to treat chronic lethargy fatigue, which has amphetamine like effects. Alpha-PVP (**Figure 1**) is widely sold on the internet as a ''research chemical'', a ''legal high'' or as ''bath salts'', probably with the intention of using it as a recreational drug or as legal substitutes for illicit compounds such as MDPV.

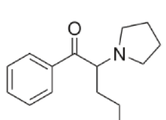

Figure 1 α-PVP

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.

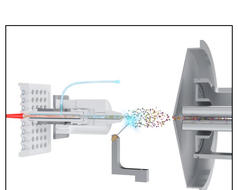

Figure 2 Schematic of the LDTD® ionization source.

METHOD

Liquid-liquid extraction

- 200 µL calibrator, QC or patient specimen
- 20 µL Internal standard (α-PVP-D8, 200 ng/mL in MeOH)
- 200 µL Borate buffer (68 mM pH 10)
- 400 µL hexane/ethyl acetate : 75/25 v/v
- Vortex and centrifuge at 5000 rpm for 5 minutes
- 100 µL of organic upper layer
- 10 µL HCl 0.01 N in MeOH
- Transfer 5 µL onto a HDE LazWell™ plate
- Analyze after complete solvent evaporation

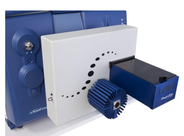

Figure 3 LDTD® model S-960 coupled with Sciex QTRAP® 5500

Instrumentation
- LDTD® model S-960, Phytronix Technologies
- QTRAP® 5500 Systems, Sciex

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 65 % in 6.0 s
  - ➢ Hold for 2 seconds
  - ➢ Decrease laser power to 0 %
- Gas flow: 3 L/min.

MS Parameters
- APCI (+) positive
- Scan time: 20 msec
- DP: 100
- Gas Flow: 3L/min
- MRM:

		CE
➢α-PVP (Quant):	232.1 → 126.0	35
➢α-PVP (Conf):	232.1 → 91.0	35
➢α-PVP-D8 (Quant):	240.1 → 134.0	35

RESULTS:

Linearity

As shown in **Table 1,** all calibration curves have an excellent linearity (R^2 > 0.9936) in both matrices with no signs of carry over effect within the quantification range **(2 to 200 ng/mL)**.

Table 1 Correlation for α-PVP in both matrices

Matrix	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Urine	0.99814	0.99498	0.99897	0.99873	0.99970	0.99362
Serum	0.99780	0.99911	0.99896	0.99590	0.99539	0.99827

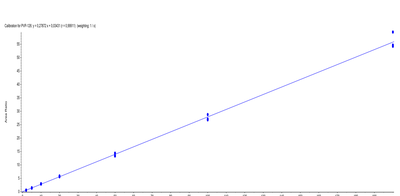

Figure 4 Typical Standard curve for α-PVP in urine

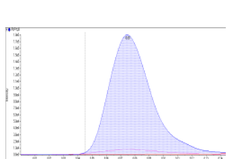

Figure 5 Typical α-PVP desorption peak in urine matrix

Intra-run Precision and Accuracy

Reproducibility and accuracy are calculated in both matrices. All results, from both matrices, have a precision between 0.9 and 9.9% and an accuracy between 90.7 and 109.5%. **Table 2** shows the QCs results in urine matrix. Similar results are obtained in serum matrix.

Table 2 Intra-run precision and accuracy results for α-PVP in urine matrix

Sample	N	Conc. (ng/mL)	Mean (ng/mL)	%RSD	%NOM
LLOQ	3	2	2.1	6.9	104.1
QCL	3	10	10.7	8.0	106.8
QCM	3	20	21.7	4.0	108.3
QCH	3	50	51.5	0.9	102.9
ULOQ	3	200	195.0	6.9	97.5

Inter-run Precision and Accuracy

Reproducibility and accuracy are calculated in both matrices. All results, from both matrices, have a precision between 6.9 and 14.7% and an accuracy between 94.2 and 105.8%. **Table 3** shows the QCs results in urine matrix. Similar results are obtained in serum matrix.

Table 3 Inter-run precision and accuracy results for α-PVP in urine matrix

Sample	N	Conc. (ng/mL)	Mean (ng/mL)	%RSD	%NOM
QCL	28	10	10.6	6.9	105.8
QCM	28	20	19.8	9.3	99.2
QCH	28	50	49.8	10.0	99.6

Wet stability

After the liquid-liquid extraction, samples are kept at 4°C. After 24 hours, sample extracts were spotted on HDE LazWell™ plate and analysed. Reproducibility and accuracy in urine are reported in **Table 4** for QCL sample. Wet stability was also done in serum matrix and all results are within the acceptable range (criteria %RSD ≤ 15% and %NOM 100 ± 15%.)

Dry stability

Extracted samples are spotted onto a HDE LazWell™ plate and kept 24 hours at room temperature before analysis. Reproducibility and accuracy in urine are reported in **Table 4** for QCL sample. Dry stability was also done in serum matrix and all results are within the acceptable range.

Table 4 Wet and dry stability results in urine matrix for QCL sample

Parameters	Dry stability	Wet stability
Time (h)	24	24
Temp. (°C)	22	4
Conc. (ng/mL)	10	10
N	3	3
Mean (ng/mL)	10.6	10.4
%RSD	9.0	5.8
%NOM	105.8	103.6

Drug interference

To verify the potential interference of other drugs of abuse on the quantification of α-PVP, five different drug pools (group 1 to 5) are tested (benzodiazepines, opiates, barbiturates, cannabinoids and amphetamines) for a total of 41 drugs that could interfere. Drugs are spiked in α-PVP QCM samples for a final interference test concentration of 1 µg/mL for each drug. Reproducibility and accuracy of drug interference spiked in QCM are evaluated. All tested pools have α-PVP quantification precision ranging from 1.2 to 6.5% and accuracy ranging from 105.2 to 115.5%. **Table 5** shows the results in urine matrix. Similar results are obtained in serum matrix.

Table 5 Precision and accuracy results for drug interference in urine matrix

Matrix	N	Conc. (ng/mL)	Mean (ng/mL)	%RSD	%NOM
Group 1	3	20	21.0	6.5	105.2
Group 2	3	20	21.6	3.3	107.9
Group 3	3	20	22.8	3.0	113.8
Group 4	3	20	22.0	1.3	110.2
Group 5	3	20	21.1	1.2	105.3

Matrix effect

α-PVP is spiked in 6 different matrices for a final concentration of 20 ng/mL. Samples are then analyzed and the precision/accuracy parameters are evaluated. All tested samples have α-PVP quantification precision ranging from 0.1 to 3.9% and accuracy raging from 96.7 to 117.5%. **Table 6** shows the results in urine matrix. Similar results are obtained in serum matrix.

Table 6 Precision and accuracy results for matrix effect in urine matrix

Sample	N	Conc. (ng/mL)	Mean (ng/mL)	%RSD	%NOM
M1	3	20	19.3	0.1	96.7
M2	3	20	22.7	3.5	113.5
M3	3	20	21.5	3.9	107.3
M4	3	20	21.8	2.7	108.8
M5	3	20	23.5	0.5	117.5
M6	3	20	22.8	1.6	113.8

CONCLUSIONS

- **Fast Extraction of α-PVP in Serum and Urine Matrices for concentration ranging from 2 to 200 ng/mL**
- **High Selectivity, Sensitivity and Accuracy Using Tandem Mass Spectrometry**
- **Versatility of LDTD® Technology proven with human samples**
- **LDTD® provides ultra-fast High-Throughput analysis of sample extract in 9 seconds sample-to-sample without any carry over**