

Luxon Ion Source Fentanyl Analogs Screening:

Fentanyl Analogs in Urine at 8 Seconds per Sample Using LLE

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Introduction

Many countries worldwide are currently experiencing a public health crisis due to the abuse of illicitly manufactured fentanyl (IMF) and its analogues. Unfortunately, IMF and its analogues are not always part of routine toxicology testing. Thus, there is an urgent need for developing sensitive screening tools for urine samples.

Our goal for this application note is to use a Liquid-Liquid Extraction sample preparation method for the screening of all fentanyl analogues in a single operation in LDTD-MS/MS.

LDTD-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 preparation method. Fourteen (14) illicitly manufactured fentanyl (IMF) are analyzed simultaneously with quantitative screening results obtained in less than 9 seconds per sample. Specific cut-off values were attained for each individual drug.

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 unmatched thermal uniformity giving 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

- Add 50 µL urine sample
- Add 5 µL internal standard (Fentanyl-d₅ and Norfentanyl-d₅, 100 ng/mL and 300 ng/mL in MeOH:Water/1:1).
 - Vortex
- Add 50 µL NaOH (0.1N)
- Add 200 µL MTBE
 - Vortex
- Spot 5 µL upper-layer phase on a LazWell™96 plate
 - Dry 1 minute at room temperature
- LDTD-MS/MS analysis

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix

Carrier gas: 3 L/min (air)

Laser pattern:

- 3-second ramp to 65% power
- Hold 2 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: Positive MRM mode

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

	LDTD	CE
Acetyl Norfentanyl	219.1 → 84.1	15
Norfentanyl	233.2 → 84.2	25
Norfentanyl-d ₅	238.2 → 84.0	25
Butyryl norfentanyl	247.2 → 84.2	25
cis-3-Methyl norfentanyl	247.2 → 98.2	25
4-ANPP	281.2 → 188.1	15
Norcarfentanyl	291.2 → 113.2	25
Acetyl fentanyl	323.2 → 188.1	30
U-47700	329.2 → 204.0	35
Acryl fentanyl	335.2 → 188.1	30
Fentanyl	337.2 → 188.1	25
Fentanyl-D ₅	342.2 → 188.1	25
Butyryl fentanyl	351.2 → 188.1	32
cis-3-Methyl fentanyl	351.2 → 202.2	32
Furanyl fentanyl	375.2 → 188.2	30
Carfentanyl	395.2 → 246.0	22

Results and Discussion

Precision

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. 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. No overlapping at the decision point is observed for all curves and the CV% was below 15% for intra-run experiments. Results using the ± 2 STD overlay are plotted in **Figure 3**. The results of intra-run test for Fentanyl allow a cut-off value of 1 ng/mL in urine. All cut-offs of different drugs are determined this way.

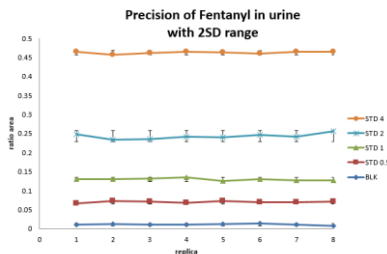


Figure 3 – Intra-Run Precision Curves for Fentanyl

For the inter-run precision experiment, each fortified sample sets are analyzed in triplicate on five different days. **Table 2** shows the inter-run precision results.

Table 2 - Inter-Run Precision

	Grand mean (ng/mL)	Grand mean – 2SD	Grand mean + 2SD		Grand mean (ng/mL)	Grand mean – 2SD	Grand mean + 2SD
4-ANPP (ng/mL)				Cis-3-methyl fentanyl (ng/mL)			
1	1.027	0.902	1.153	0.25	0.226	0.216	0.236
2	2.106	1.929	2.284	0.5	0.515	0.502	0.527
4	3.973	3.590	4.355	1	1.046	0.996	1.097
Acetyl fentanyl (ng/mL)				Cis-3-Methyl norfentanyl (ng/mL)			
0.25	0.239	0.222	0.256	0.5	0.501	0.460	0.542
0.5	0.508	0.473	0.542	1	1.036	0.956	1.117
1	1.021	0.964	1.079	2	1.979	1.818	2.139
Acetyl Norfentanyl (ng/mL)				Fentanyl (ng/mL)			
2.5	2.463	2.013	2.914	0.5	0.477	0.421	0.532
5	5.192	4.695	5.689	1	1.010	0.931	1.090
10	9.991	9.391	10.591	2	2.048	1.940	2.156
Acryl fentanyl (ng/mL)				Furanyl fentanyl (ng/mL)			
0.25	0.237	0.220	0.253	0.25	0.243	0.220	0.266
0.5	0.506	0.475	0.536	0.5	0.497	0.464	0.531
1	1.031	0.971	1.091	1	1.016	0.943	1.089
Butyryl fentanyl (ng/mL)				Norcarfentanyl (ng/mL)			
0.25	0.234	0.226	0.241	1	0.993	0.872	1.115
0.5	0.508	0.488	0.528	2	2.046	1.855	2.237
1	1.031	0.991	1.071	4	4.052	3.621	4.484
Butyryl norfentanyl (ng/mL)				Norfentanyl (ng/mL)			
2.5	2.419	1.811	3.027	0.5	0.481	0.412	0.551
5	5.081	4.693	5.470	1	1.033	0.912	1.154
10	10.080	9.549	10.611	2	2.048	1.866	2.231
Carfentanyl (ng/mL)				U-47700 (ng/mL)			
0.25	0.256	0.230	0.281	2.5	2.436	2.168	2.704
0.5	0.500	0.452	0.547	5	5.079	4.664	5.494
1	0.996	0.935	1.057	10	10.158	9.120	11.197

Linearity

Blank urine sample is spiked to get different concentration to generate a calibration curve. **Table 3** shows the inter-day correlation coefficients for all drugs. Values greater than 0.99 are obtained for all drugs. **Figure 4** shows typical calibration curve results for Fentanyl.

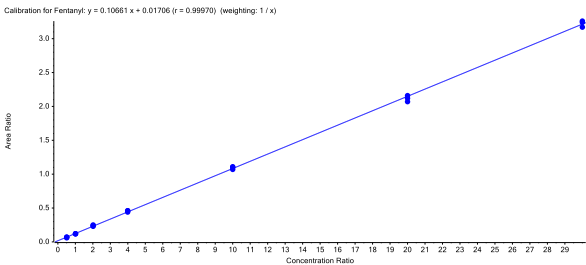


Figure 4 - Standard Curve for Fentanyl

Table 3 – Inter-day calibration curve correlation coefficient

	Curve 1	Curve 2	Curve 3	Curve 4	Curve 5
4-ANPP	0.99914	0.99919	0.99942	0.99905	0.99663
Acetyl fentanyl	0.99966	0.99977	0.99944	0.99986	0.99980
Acetyl Norfentanyl	0.99902	0.99990	0.99961	0.99912	0.99925
Acryl fentanyl	0.99972	0.99972	0.99988	0.99983	0.99973
Butyryl fentanyl	0.99907	0.99902	0.99929	0.99930	0.99916
Butyryl norfentanyl	0.99958	0.99941	0.99940	0.99914	0.99948
Carfentanyl	0.99938	0.99962	0.99951	0.99957	0.99973
cis-3-Methyl fentanyl	0.99859	0.99885	0.99988	0.99923	0.99763
cis-3-Methyl norfentanyl	0.99931	0.99888	0.99945	0.99939	0.99898
Fentanyl	0.99977	0.99970	0.99970	0.99980	0.99973
Furanyl fentanyl	0.99976	0.99957	0.99925	0.99939	0.99958
Norcarfentanyl	0.99822	0.99894	0.99855	0.99875	0.99748
Norfentanyl	0.99748	0.99901	0.99946	0.99946	0.99853
U-47700	0.99895	0.99965	0.99962	0.99962	0.99759

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 4 days, sample extracts were spotted on a LazWell™ plate and analyzed. Precision at 50% cut-off standard is reported in **Table 4** for Fentanyl. All the results are within the acceptable range (criteria %CV $\leq 20\%$) for 4 days at 4°C. Similar results are obtained for the other drugs.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate and kept at room temperature before analysis. Precision at 50% cut-off standard is reported in **Table 4** for Fentanyl. All the results are within the acceptable range (criteria %CV $\leq 20\%$) for 2 hours at room temperature. Similar results are obtained for the other drugs.

Table 4 - Wet and Dry Stability Fentanyl

Parameters	Dry stability	Wet stability
Time	2 hours	4 days
Temp. (°C)	22	4°C
Conc. (ng/mL)	0.5	0.5
N	3	3
Mean (ng/mL)	0.473	0.489
%CV	4.8	7.1

Conclusion

Luxon Ion Source® combined to Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of illicitly manufactured fentanyl (IMF) and its analogue drugs in urine sample using a simple generic sample preparation method.