



RAPID SCREENING FOR FENTANYL IN URINE USING THE ADVION expression^L CMS WITH THE TOUCH EXPRESS™ OPEN PORT SAMPLING INTERFACE (OPSI)

APPLICATION NOTE

Mass Spec: expression CMS
Sampling: Touch Express OPSI

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In this application note, a simple method for fentanyl screening in urine is introduced using the Advion Touch Express Open Port Sampling Interface (OPSI) coupled with the Advion expression Compact Mass Spectrometer (CMS).

INTRODUCTION

OPSI was developed by Gary Van Berkel and Vilmos Kertesz of Oak Ridge National Laboratory^[2,3,4]. Paired with the electrospray ionization (ESI) source of the Advion **expression** CMS, the Touch Express OPSI offers a fast assay benchtop solution in a small-footprint, easy-to-use system.

Fentanyl (1.0 mg/mL in methanol) and fentanyl-D₅ (100 µg/mL in methanol) were used as analytical standards. Samples for a calibration into fentanyl-free control urine with concentrations of 2.5, 5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL and fentanyl-D₅ at 100 ng/mL.

Samples were then prepared by liquid-liquid extraction (LLE). 1.5 mL of 1:1 ethyl acetate/hexane was added to 0.5 mL of urine sample and vortexed for 20 sec and left to stand for 2 min. Then 1 mL of supernatant was transferred to a clean 2 mL centrifuge vial and evaporated to dryness in a centrifuge vacuum evaporator. The dried extract was reconstituted with 250 µL methanol and then used for OPSI-CMS analysis.

METHOD

Solvent is delivered to the open port sampling area through the outer pair of concentric tubes at ~250 µL/min. The solvent forms a meniscus at the open port before being drawn down the inner path into the ion source of the mass spectrometer under the Venturi Effect of the nebulization gas in the ion source. The control and fentanyl-spiked urine samples are simply touched in sequence to the OPSI sampling port (Figure 1) and results are obtained in < 30 sec.

RESULTS

The mass spectra of fentanyl & fentanyl-D₅ (Figure 2) show protonated molecules were detected at m/z 337.2 for fentanyl and 342.2 for fentanyl-D₅. These ions are used in a Selected Ion Monitoring (SIM) method for the quantification of fentanyl in urine.

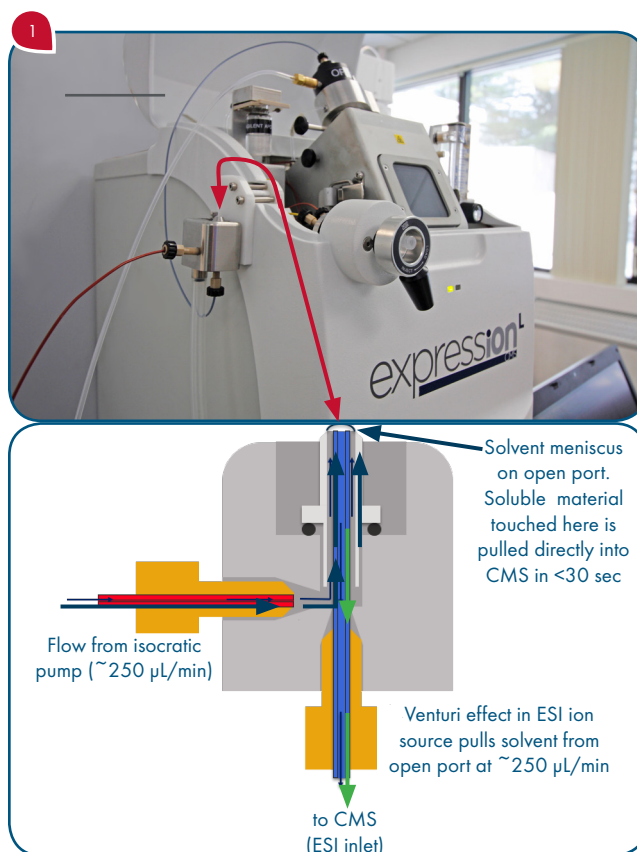


Figure 1: Experimental setup and schematic of the CMS and OPSI.

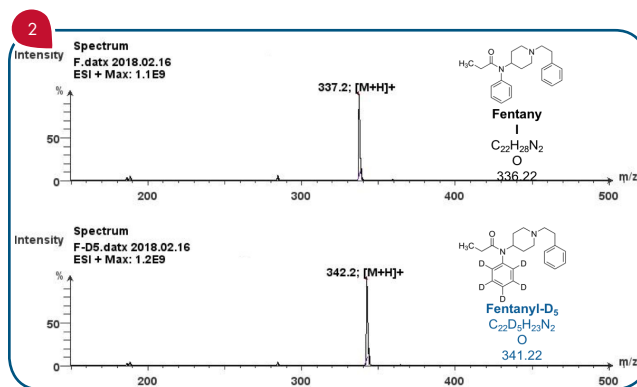


Figure 2: Mass spectra of Fentanyl & Fentanyl-D₅ in control urine.

The extracted ion currents (XIC) of protonated fentanyl and fentanyl-D₅ (Figure 3) show no interferences were detected at the SIM m/z values in the fentanyl-free control urine following LLE cleanup.

The signal-to-noise ratio (S/N) for 2.5 ng/mL fentanyl is ~ 135 , so the limit of quantitation (S/N = 10) is estimated to be lower than 1 ng/mL.

The urine sample spiked with 2.5 ng/mL of fentanyl was tested with OPSI-ESI-SIM analysis and the XIC shows a signal to noise ratio about 133.2 (Figure 4). The concentration with S/N at 10 can be the limit of quantitation, therefore the quantitation level for fentanyl can be estimated to be lower than 1 ng/mL.

From figure 5a, the analysis time for each sample can be finished within 30 seconds with the peak width about 12 sec. meanwhile, no carryover was detected with the present setup which is practically in favor of the high throughput environment for fentanyl screening.

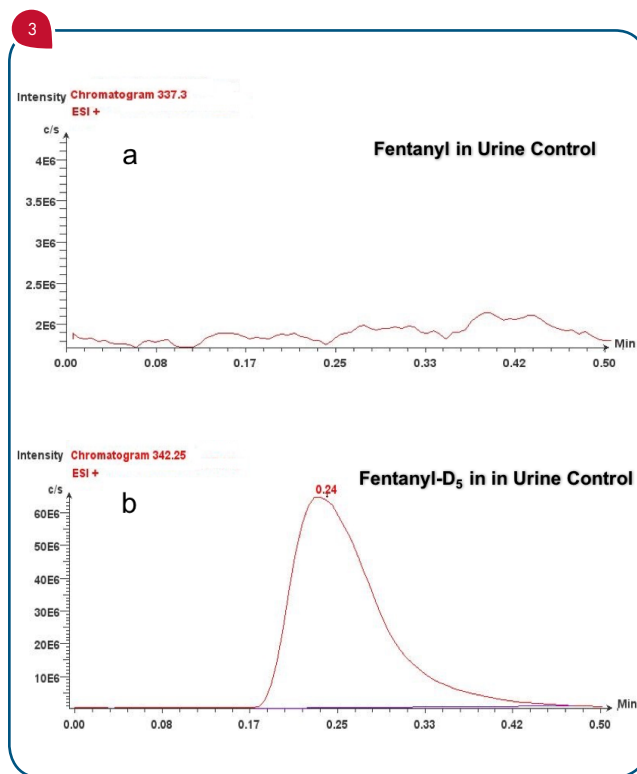


Figure 3: XIC of protonated fentanyl and fentanyl-D₅ in control urine.

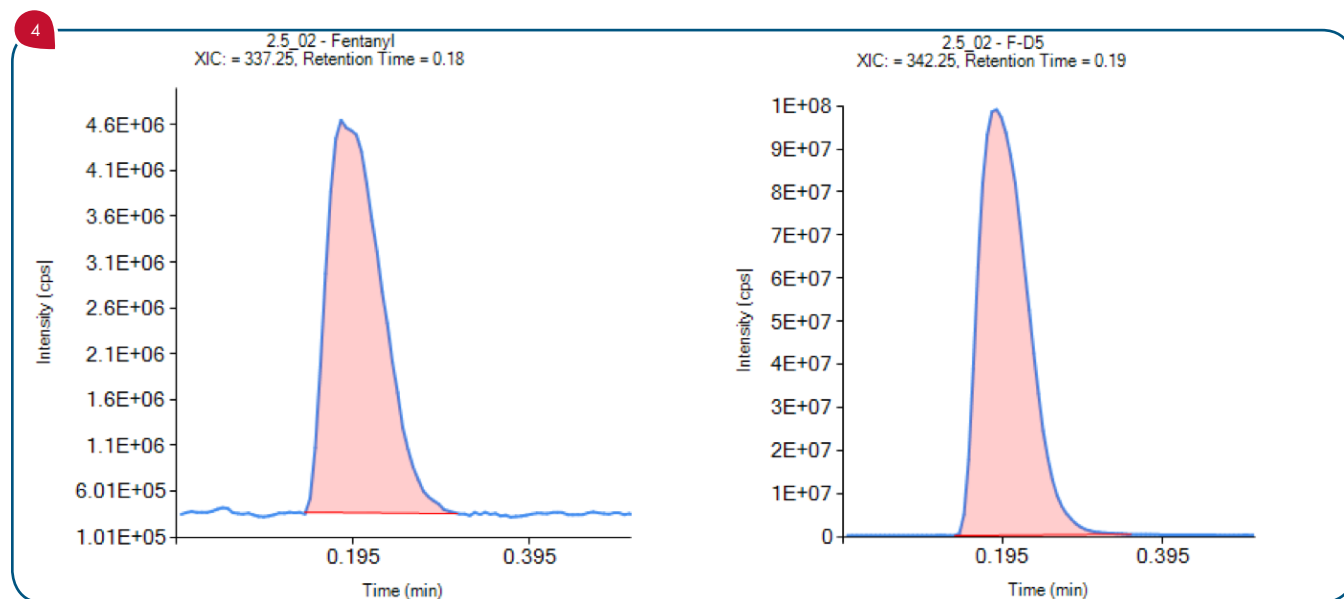


Figure 4: Touch Express SIM analysis of fentanyl, 2.5 ng/mL (left) and fentanyl-D₅, 100 ng/mL in control of urine (right).

The calibration curve shows a linear regression R^2 value of 0.997 over a linear dynamic range of 2.5 to 500 ng/mL.

The statistical summary (Table 1) shows the average accuracy and precision (CV%) over concentrations from 2.5 to 500 ng/mL are measured at 87.5 $\pm$ 0.17% to 106.5 $\pm$ 3.36%, respectively; within the acceptable range of 85–115% for accuracy and 0–15% for precision.

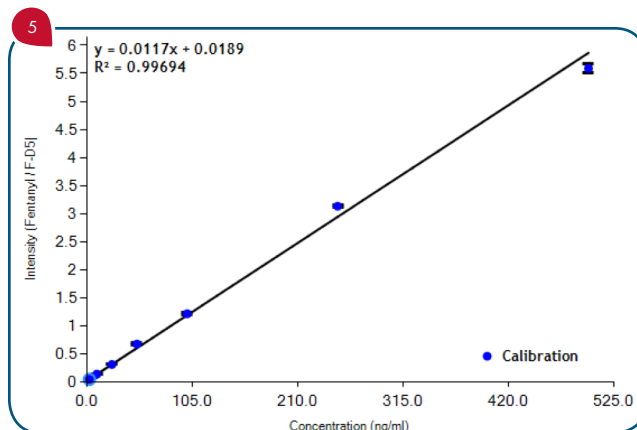


Table 1: Statistical Summary of Fentanyl in Urine Analysis

Samples TypeT	Samples Included	Concentration (ng/mL)	Measured Concentration (ng/mL)	Averaged Accuracy (%)	Analyte/ISTD (Fentanyl/F-D ₅)	Calculated Analyte/ISTD (Fentanyl/F-D ₅)	CV (%)
Cal 1	3/6	2.5	2.2	87.5	0.045	0.048	0.17
Cal 2	3/3	5	3.8	96.7	0.076	0.077	0.40
Cal 3	3/3	10	10.1	100.7	0.137	0.136	2.21
Cal 4	3/3	25	24.8	99.1	0.309	0.311	0.78
Cal 5	3/3	50	56.0	112.0	0.674	0.604	3.36
Cal 6	3/3	100	102.2	102.2	1.214	1.188	2.02
Cal 7	3/3	250	266.1	106.5	3.131	2.943	0.55
Cal 8	3/3	500	476.3	95.3	5.589	5.866	1.41

Figure 5: Calibration curve of fentanyl in urine.

CONCLUSION

The Touch Express OPSI provides:

- Simple, robust operation – no mechanical parts
- Fast analysis – results in <30 sec
- Direct samples – just touch and assay
- No carryover or contamination - the port is continuously self cleaning
- A cost-effective, high throughput screening technique

REFERENCES

- [1] "Fentanyl And Analogues". LiverTox. 14 December 2017.
- [2] Van Berkel G., Vilmos K.; An open port sampling interface for liquid introduction atmospheric pressure ionization mass spectrometry; Rapid Comm. Mass Spec., 2015 29(19) 1749-1756
- [3] Gary J V. B., Vilmos K.; Rapid sample classification using an open port sampling interface coupled with liquid introduction atmospheric pressure ionization mass spectrometry, Rapid Comm. Mass Spec. 2017 31(3) 281–291
- [4] Gary J V. B., Vilmos K., Harry B.; Immediate drop on demand technology (I-DOT) coupled with mass spectrometry via an open port sampling interface; Bioanalysis, 2017, 9(21),1667-1679