Fentanyl Analogs in Dried Plasma Spots; A Novel Technique

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ABSTRACT

Dried plasma sports (DPS) have potential to become suitable matrices for drug detection. These matrix spots offer many advantages over common biological matrices by reducing analyst infection rates (1), by consuming small sample volumes (2), and by minimizing time for sample preparation and cost of equipment. The Noviplex plasma preparation cards, shown in Figure 1, avoid the Hematocrit effect, commonly observed with the use of dried blood spots, by filtering the plasma from the red blood cells (3). Once dried, the blood is discarded, and drugs are extracted from the plasma disc. This research introduces a novel technique for extracting 13 fentanyl analogs from DPS using a simple extraction technique and robust instrumentation.

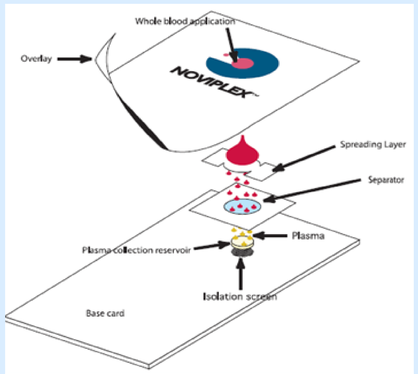

Figure 1: Diagram of a plasma preparation card for dried plasma spots (4)

MATERIALS AND METHODS

Sample preparation

- Whole bovine blood (250µL) was fortified with high quality control solution (10µL) containing 13 fentanyl analogs at a final concentration of 80 ng/mL in blood
- Extraction solvent; 1% formic acid in a 50:50 mixture of methanol and acetonitrile

Optimized Extraction

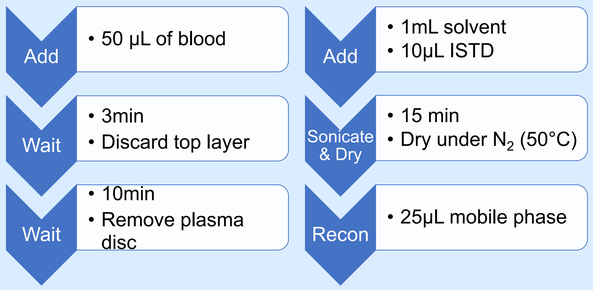

Figure 2: Flow chart of optimized solvent extraction for fentanyl analogs in dried plasma spots

RESULTS AND DISCUSSION

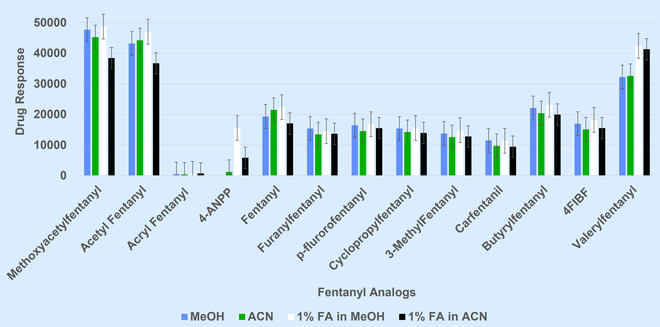

Figure 3: Average drug response of solvent comparison (methanol, acetonitrile, 1% formic acid in methanol, and 1% formic acid in acetonitrile) using 200µL extraction solvent

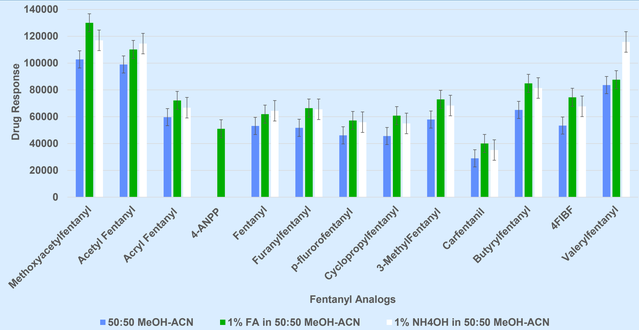

Figure 4: Average drug response of 1mL solvent comparison (50:50 MeOH:ACN, 1% formic acid in 50:50 MeOH:ACN, and 1% ammonium hydroxide in 50:50 MeOH:ACN)

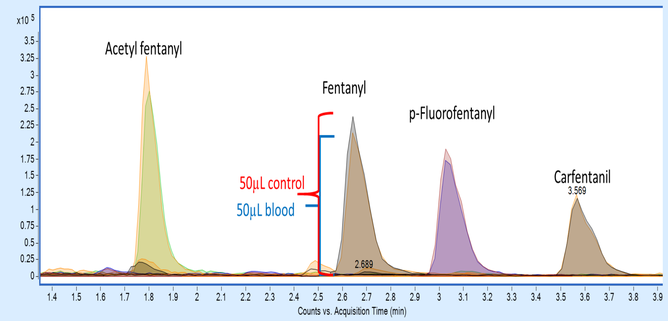

Figure 5: Extracted ion chromatogram of aqueous control vs blood sample extracted with 1% formic acid in 50:50 MeOH:ACN to determine if matrix hinders drug transfer through device

Optimization Results:

- Solvent type: 1% formic acid in 50:50 MeOH:ACN
- Solvent volume: no statistically significant difference
- Internal standard: should be added to plasma disc or extraction tube for best response & reproducibility
- Preparation: no statistically significant difference
- Matrix type: matrix effects present with smaller sample volume; matrix does not hinder drug movement through device
- Blood volume: greater volume produces greater drug response

Analyte	Calibration Range (ng/mL)	R^2 (Range, n=6)	Bias (%, n=12)		Maximum With-In Run Precision (%CV, n=3)		Between-Run Precision (%CV, n=12)		Matrix Effects (%, n=3)		Recovery (%, n=3)	
			MQC	HQC	MQC	HQC	MQC	HQC	MQC	HQC	MQC	HQC
Methoxyacetyl fentanyl	5-100	0.926 - 0.998	0.5	7.5	25.7	17.7	20.9	19.7	104.8	41.2	7	6
Acetyl fentanyl	5-100	0.976 - 0.999	-2.3	6.5	24.5	17.8	17.9	20.5	184.1	24.4	7	7
Acryl fentanyl	5-100	0.967 - 0.997	5.3	14.2	20.3	18.0	17.3	21.6	91.7	29.7	7	6
4-ANPP	5-100	0.978 - 0.993	7.0	18.6	21.1	20.5	21.8	28.0	191.7	78.6	7	7
Fentanyl	5-100	0.954 - 0.996	2.5	8.4	23.9	18.9	17.4	21.8	97.2	32.9	7	6
Furanyl fentanyl	5-100	0.979 - 0.997	-1.9	10.1	22.2	25.9	20.4	24.8	98.5	31.1	6	6
p-Fluorofentanyl	5-100	0.964 - 0.998	0.5	11.4	18.1	11.0	19.4	23.0	91.9	28.0	7	6
Cyclopropyl fentanyl	5-100	0.974 - 0.999	-1.1	8.9	29.7	18.3	19.6	21.5	103.6	35.5	6	6
3-Methylfentanyl	5-100	0.980 - 0.998	14.2	28.2	22.6	15.7	18.1	15.9	107.0	40.1	6	6
Carfentanil	5-100	0.984 - 0.999	4.5	16.5	28.2	21.5	19.0	23.6	90.9	26.1	5	5
Butyryl fentanyl	5-100	0.980 - 0.999	0.2	12.8	20.8	19.3	16.9	21.3	110.2	37.8	6	6
4-FIBF	5-100	0.970 - 0.999	0.5	11.4	21.9	21.4	18.8	20.4	111.2	37.0	6	5
Valeryl fentanyl	5-100	0.987 - 0.999	-2.1	21.8	22.4	24.1	38.0	26.5	91.1	26.6	6	6

Table 2: Calibration information, bias, precision, matrix effects, and extraction recovery results for fentanyl analogs at medium (40 ng/mL) and high (80 ng/mL) quality control levels

CONCLUSION

- Fentanyl analogs can be extracted from DPS via solvent extraction (1% formic acid in 50:50 MeOH:ACN)
- The method is semi-quantitative and offers limited sensitivity (limits of detection 1-5 ng/mL)
- Potential to become a screening method
- Additional method optimization may mitigate matrix effects and recovery issues

MATERIALS AND METHODS

Optimization Type	Parameters Evaluated
Extraction solvent type	MeOH, ACN, (acidified versions of both) 50:50 MeOH:ACN (acidified and alkalized)
Solvent volume	200µL vs 1mL
Internal Standard Placement	Top layer of collection device vs plasma disc vs tube
Extraction Preparation	Incubation vs sonication
Matrix type	Blood vs plasma
Blood sample volume	30µL vs 50µL

Table 1: Assessed parameters of solvent extraction

Instrumentation

Analysis was performed using an Agilent 1290 Infinity liquid chromatograph coupled to an Agilent Technologies 6530 Accurate Mass Time-of-Flight mass spectrometer (LC/Q-TOF) according to Palmquist et. al. (5), with minor modifications:

Liquid Chromatography

- Injection volume: 5 µL
- Column: Poroshell 120 EC-C18 (2.1x100mm, 2.7 µm)
- Flow rate: 0.4 mL/min (gradient elution)
- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in acetonitrile

Quadrupole/Time-of-Flight

- Ionization type: ESI positive mode
- Acquisition mode: Targeted
- Collision energies: 5-30 eV
- Mass range: 100-1000 *m/z*

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ACKNOWLEDGMENTS

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