

Introduction

The use of Novel Psychoactive Substances (NPS) is cause of health concern. Manufactured as synthetic alternatives to traditional drugs, NPS often exhibit similar effects but with heightened potency and legally evasive potential. The increasing number of these drugs represents a challenge in clinical test settings trying to maximise the detection of a large number of these compounds in a sample. Biochip array technology allows the multi-analytical screening of NPS and related analytes from a single sample. By employing simultaneous immunoassays, this technology increases the detection capacity, which is important when facing this opioid epidemic. Rapid development of such assays is also necessary to ensure relevance in a market which is constantly changing.

The objective of this study was to evaluate a biochip array, which enables the simultaneous detection of fentanyl and opioid novel psychoactive substances from a single urine sample.

Methodology

Competitive chemiluminescent immunoassays defining discrete test regions on a biochip and applicable to the Evidence series analysers, were employed.

Test Menu on the Biochip Array	
AH-7921	Mitragynine
Buprenorphine metabolite	MT-45
Designer Benzodiazepine Assays: Etizolam and clonazepam	Naloxone
Designer Fentanyl Assays: Furanylfentanyl, acetylfentanyl, carfentanil, sufentanil, ocfentanyl	U-47700
	W-19

Results

Results presented were obtained with the Evidence Investigator analyser.

Cut-offs and Limits of Detection (LOD) in urine

Cut-offs (ng/mL)	
Furanylfentanyl	1
Acetylfentanyl	1
Carfentanil	0.25
Sufentanil	1
Ocfentanyl	2
AH-7921	1
MT-45	2
U-47700	10
W-19	2
Etizolam	2
Clonazepam	2
Mitragynine	1
Naloxone	1
Buprenorphine Metabolite	0.5

LOD (ng/mL)		LOD (ng/mL)	
AH-7921	0.02	Sufentanil	0.04
Norbuprenorphine	0.03	Ocfentanyl	0.12
Clonazepam	0.01	Mitragynine	0.11
Etizolam	0.06	MT-45	0.17
Furanylfentanyl	0.06	Naloxone	0.06
Acetylfentanyl	0.02	U-47700	1.14
Carfentanil	0.03	W-19	0.17

Specificity (Cross-reactivity, CR ≥20%)

Designer Benzodiazepine Assays: Etizolam	Designer Benzodiazepine Assays: Clonazepam
Compound (CR%)	Compound (CR%)
Etizolam (100.0)	Clonazepam (100.0)
Brotizolam (69.6)	N-Desmethyflunitrazepam (128.2)
Alpha-OH Etizolam (34.0)	Delorazepam (41.0)
Estazolam (33.5)	7-Aminoclonazepam (40.6)
Deschloroetizolam (33.2)	Nitrazepam (38.9)
	Phenazepam (29.7)

Designer Fentanyl Assays: Furanylfentanyl	
Compound (CR%)	Compound (CR%)
Ocfentanyl (100.0)	Cyclopentylfentanyl (60.5)
Furanylfentanyl (105.5)	Para Fluoroisobutyrylfentanyl (FIBF) (57.1)
Thiofentanyl (250.4)	Benzylfentanyl (43.0)
Methoxyacetyl Fentanyl (222.5)	Norfentanyl (37.2)
Fentanyl (186.2)	Acrylfentanyl (36.3)
Butrylfentanyl (150.2)	Thienylfentanyl (36.1)
Alpha-Methylfentanyl (140.7)	Isobutyrylfentanyl (34.2)
Fluranylethylfentanyl (140.3)	Meta-Hydroxy-Acrylfentanyl (32.7)
Parafluorofentanyl (115.9)	Norfuranylfentanyl (22.4)
Tetrahydrofuran Fentanyl (112.2)	Valerylfentanyl (20.1)
Ortho-Fluorofentanyl (69.4)	4-Fluoro-isobutyrylfentanyl (20.0)

Designer Fentanyl Assays: Acetylfentanyl	
Compound (CR%)	Compound (CR%)
Ocfentanyl (100.0)	Ortho-Fluorofentanyl (59.1)
Furanylfentanyl (65.4)	Cyclopentylfentanyl (129.2)
Acetylfentanyl (84.9)	Para Fluoroisobutyrylfentanyl (FIBF) (132.3)
Thiofentanyl (69.0)	Acrylfentanyl (89.8)
Methoxyacetyl Fentanyl (163.9)	Isobutyrylfentanyl (83.5)
Fentanyl (99.3)	Valerylfentanyl (191.1)
Butrylfentanyl (124.8)	(±)-cis-3-methylfentanyl (27.7)
Alpha-Methylfentanyl (31.4)	Cis-Mefentanyl (23.7)
Furanylethylfentanyl (23.6)	Ω-Hydroxyfentanyl (84.7)
Parafluorofentanyl (118.2)	(±)-trans-3-methylfentanyl (32.8)
Tetrahydrofuran Fentanyl (221.8)	Para methoxy-Butyryl fentanyl (HCl) (116.9)
	4-Fluoro-isobutyrylfentanyl (80.4)

Designer Fentanyl Assays: Carfentanil	Designer Fentanyl Assays: Sufentanil
Compound (CR%)	Compound (CR%)
Carfentanil (100.0)	Sufentanil (100.0)
Remifentanil Acid (60.2)	Alfentanil (92.2)
Alfentanil (48.5)	Norsufentanil (23.1)
Norcarfentanil (31.4)	

Designer Fentanyl Assays: Ocfentanyl
Compound (CR%)
Ocfentanyl (100.0)
Methoxyacetyl Fentanyl (54.7)
Butrylfentanyl (20.2)

Inter-assay precision and recovery

AH-7921	Below cut-off	Cut-off	Above cut-off
Recovery (%)	119	110	114
CV(%)	8.1	5.3	8.0

Buprenorphine metabolite	Below cut-off	Cut-off	Above cut-off
Recovery (%)	106	84	80
CV(%)	7.5	5.4	7.9

Designer Benzodiazepine Assays	Below cut-off	Cut-off	Above cut-off
Etizolam Recovery (%)	101	101	98
CV(%)	5.9	11.0	12.6
Clonazepam Recovery (%)	80	82	80
CV (%)	10.7	9.3	13.7

Designer Fentanyl Assays	Below cut-off	Cut-off	Above cut-off
Furanylfentanyl Recovery (%)	118	111	105
CV (%)	8.5	5.9	8.0
Acetylfentanyl Recovery (%)	119	117	111
CV (%)	11.9	6.4	10.3
Sufentanil Recovery (%)	108	111	122
CV (%)	7.2	5.7	12.4
Carfentanil Recovery (%)	98	103	110
CV (%)	10.9	5.9	6.7
Ocfentanyl Recovery (%)	122	107	113
CV (%)	12.0	9.8	12.1

Mitragynine	Below cut-off	Cut-off	Above cut-off
Recovery (%)	138	133	124
CV(%)	6.3	6.8	6.6

MT-45	Below cut-off	Cut-off	Above cut-off
Recovery (%)	127	112	104
CV (%)	5.5	6.3	9.9

Naloxone	Below cut-off	Cut-off	Above cut-off
Recovery (%)	99	88	82
CV (%)	11.6	9.4	14.1

U-47700	Below cut-off	Cut-off	Above cut-off
Recovery (%)	119	110	105
CV (%)	10.9	7.1	8.5

W-19	Below cut-off	Cut-off	Above cut-off
Recovery (%)	116	95	87
CV (%)	12.8	12.7	15.0

Conclusion

This biochip array, by simultaneously detecting multiple NPS and related analytes from a single urine sample, is relevant for the current NPS market, doubling as both a screening method and indication of treatment. It is an anticipated answer for many laboratories facing the crisis of unknown drug combinations and concentration.