MULTI-ANALYTICAL SCREENING OF FENTANYL, NEW OPIOID PSYCHOACTIVE SUBSTANCES, BUPRENORPHINE AND MITRAGYNINE IN URINE WITH A BIOCHIP ARRAY

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INTRODUCTION

Opioid use, abuse and dependence is a significant problem worldwide. Recently a new wave of illicit synthetic opioids including fentanyl derivatives has emerged. Fentanyl compounds have been observed in casework. Carfentanil is more potent than morphine and fentanyl. Buprenorphine, a semi-synthetic opioid, is used for the treatment of opiate dependence and mitragynine is an alkaloid present in kratom, responsible for the opioid effects. These compounds have abuse potential. The detection of these compounds in the screening step of drug testing is important.

Biochip array technology allows multi-drug screening from a single undivided sample. This study reports the applicability of a biochip array to the simultaneous detection of fentanyl, new opioid psychoactive substances, buprenorphine and mitragynine from a single urine sample.

METHODOLOGY

Simultaneous chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were applied to the analyser Evidence Investigator (EV4271, EV3602 Randox Toxicology, Crumlin, UK). This system incorporates the software to process, report and archive the data generated.

Test menu on the biochip array

- AH-7921
- Buprenorphine metabolite
- **Designer Benzodiazepine Assays:** Etizolam and clonazepam
- **Designer Fentanyl Assays:** Furanylfentanyl, acetylfentanyl, carfentanil, sufentanil, ocfentanyl
- Mitragynine
- MT-45
- Naloxone
- U-47700
- W-19

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

Biochip
(9mm x 9mm)

Biochip carrier
(3 x 3 biochips)

Semi-automated benchtop analyser Evidence Investigator

CONCLUSION

This biochip array is applicable to the simultaneous screening of new psychoactive substances, buprenorphine metabolite, mitragynine and naloxone in urine from a single urine sample. The result output is increased, which facilitates the drug testing process as the number of samples requiring confirmation is reduced.

RESULTS

AH-7921

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
AH-7921	0.02

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	0.3	0.8	1.2
CV (%)	5.3	6.4	4.5

Buprenorphine metabolite

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
Norbuprenorphine	0.03

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	0.4	1.0	1.6
CV (%)	6.9	11.5	7.1

Designer Benzodiazepine Assays: Etizolam and clonazepam

Specificity/Cross-reactivity (CR≥20%)

Etizolam Assay
Compound (CR%)
Etizolam (100.0)
Brotizolam (69.6)
Alpha-OH Etizolam (34.0)
Estazolam (33.5)
Deschloroetizolam (33.2)

Clonazepam Assay
Compound (CR%)
Clonazepam (100.0)
N-Desmethylflunitrazepam (128.2)
Delorazepam (41.0)
7-Aminoclonazepam (40.6)
Nitrazepam (38.9)
Phenazepam (29.7)

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
Etizolam	0.06
Clonazepam	0.01

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Etizolam Conc. (ng/mL)	0.6	1.7	2.5
CV (%)	7.4	5.8	5.9
Clonazepam Conc. (ng/mL)	1.1	2.2	4.0
CV (%)	11.9	9.9	6.6

Mitragynine

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
Mitragynine	0.11

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	0.6	1.6	2.4
CV (%)	5.0	6.0	5.8

MT-45

Specificity/Cross-reactivity (CR≥20%)

Compound	CR (%)
MT-45	100.0
3-Hydroxy MT-45	122.8

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
MT-45	0.17

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	0.7	1.3	1.9
CV (%)	4.9	6.4	5.7

Naloxone

Specificity/Cross-reactivity (CR≥20%)

Compound	CR (%)
Naloxone	100.0
Naloxone 3-Beta-D Glucuronide	70.6

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
Naloxone	0.06

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	1.2	2.9	4.6
CV (%)	4.6	10.7	8.9

Designer Fentanyl Assays: Furanylfentanyl (FNFNT), acetylfentanyl (ACFNT), carfentanil (CRFNT), sufentanil (SUFNT), ocfentanyl (OCFNT)

Specificity/Cross-reactivity (CR≥20%)

Furanylfentanyl Assay	
Compound (CR%)	
Ocfentanyl (100.0)	Cyclopentylfentanyl (60.5)
Furanylfentanyl (105.5)	Para Fluoroisobutrylfentanyl (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)
Furanylethylfentanyl (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)

Specificity/Cross-reactivity (CR≥20%)

Acetylfentanyl Assay	
Compound (CR%)	
Ocfentanyl (100.0)	Ortho-Fluorofentanyl (59.1)
Furanylfentanyl (65.4)	Cyclopentylfentanyl (129.2)
Acetylfentanyl (84.9)	Para Fluoroisobutrylfentanyl (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)	

Specificity/Cross-reactivity (CR≥20%)

Carfentanil Assay
Compound (CR%)
Carfentanil (100.0)
Remifentanil Acid (60.2)
Alfentanil (48.5)
Norcarfentanil (31.4)

Specificity/Cross-reactivity (CR≥20%)

Sufentanil Assay
Compound (CR%)
Sufentanil (100.0)
Alfentanil (92.2)
Norsufentanil (23.1)

Specificity/Cross-reactivity (CR≥20%)

Ocfentanyl Assay
Compound (CR%)
Ocfentanyl (100.0)
Methoxyacetyl Fentanyl (54.7)
Butrylfentanyl (20.2)

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
FNFNT	0.06
ACFNT	0.02
CRFNT	0.03
SUFNT	0.04
OCFNT	0.12

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
FNFNT Conc. (ng/mL)	1.2	2.9	4.7
CV (%)	5.8	7.5	4.8
ACFNT Conc. (ng/mL)	1.4	2.9	4.9
CV (%)	6.7	8.7	7.7
CRFNT Conc. (ng/mL)	0.2	0.5	0.9
CV (%)	6.2	6.7	5.0

	Intra-assay precision		
SUFNT Conc. (ng/mL)	0.3	0.8	1.3
CV (%)	7.8	5.8	5.2
OCFNT Conc. (ng/mL)	1.5	3.4	5.7
CV (%)	6.6	8.5	8.2

U-47700

Specificity/Cross-reactivity (CR≥20%)

Compound	CR (%)
U-47700	100
N-Desmethyl U-47700	24.3

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
U-47700	1.14

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	4.3	11.3	18.0
CV (%)	7.3	6.7	5.2

W-19

Specificity/Cross-reactivity (CR≥20%)

Compound	CR (%)
W-19	100
Deschloro W-19	661

Limit of Detection (human urine)

	Limit of Detection (ng/mL)
W-19	0.17

Intra-assay precision

	Intra-assay precision		
	Level 1	Level 2	Level 3
Concentration (ng/mL)	2.5	6.7	11.8
CV (%)	10.9	8.5	7.5

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