



Introduction

Enzyme-linked immunosorbent assays (ELISAs), due to the ease of use, potential automation and application to different matrices, are useful analytical tools in forensic toxicology for the screening of drugs of abuse. Assay sensitivity is important to consider in the screening step, and assay accuracy and precision are analytical parameters that ensure reliability of the sample assessment.

This study presents the analytical evaluation of twelve Randox ELISA kits for the screening of drugs of abuse including analgesics, stimulants, hallucinogens, and tricyclic antidepressants in urine and blood. Assay sensitivity, accuracy and precision were evaluated to ensure optimal routine drugs of abuse screening results.

Methodology

Twelve Randox ELISA Kits were used for the routine drugs of abuse screening in urine and blood.

ELISAs and Cut -offs*						
Cut-off (urine) ng/mL Cut-off (blood) ng/mL						
Amphetamine	300/500	50				
Barbiturates	200	50				
Benzodiazepines	200	10				
Benzoylecgonine	300/150	50				
Cannabinoids	50	50				
Meprobamate	50	50				
Methadone	300	0				
Methamphetamine	300/500	50				
Phencyclidine	25	5				
Opiates	300	25				
Oxycodone	10	0				
Tricyclic antidepressants	100	25				

*For some assays in urine there are two possible cut-offs.

The limit of detection (LOD) was determined by the assessment of negative urine or blood samples (n=20), the mean concentration was calculated and 3 standard deviations added.

Recovery was determined by preparing spiked samples in negative matrix with the target material at three levels. The concentration of these samples was determined from the standard curve and expressed as percentage recovery of the expected concentration (n=12).

Intra-assay precision was determined by assessment of replicates (n=12) of different concentration levels for each target drug. Results were expressed as CV (%).

ANALYTICAL EVALUATION OF RANDOX ELISA KITS FOR A RELIABLE COMPREHENSIVE ROUTINE DRUGS OF ABUSE SCREENING IN URINE AND BLOOD

L. Keery, V. Anderson, L. Stewart, K. Buckley, S. Cardwell, D. Cameron, D. McKeown, A. Speers, J. Darragh, M.L. Rodríguez, R.I. McConnell, S.P. FitzGerald Randox Toxicology Ltd, Crumlin, Co Antrim, United Kingdom e-mail: scientific.publications@randox.com

Results

Sensitivity

The LODs (ng/mL) in urine (URN) and blood (B) were as follows:

Analyte	d-Amphetamine	Phenobarbital	Diazepam	Benzoylecgonine
LOD URN/B	108.2/11.7	42.7 /17.3	32.64 /6.62	I.I2 /2.96
Analyte	(-)-II-nor-9-carboxy-Δ9- THC	Meprobamate	Methadone	(+) Methamphetamine
LOD URN/B	I7.2 /23.9	4.78 /7.64	I.85 /0.18	37.7/0.0
Analyte	Phencyclidine	Morphine	Oxycodone	Nortriptyline
LOD URN/B	0.79 /0.69	5.53 /1.15	2.08 /3.85	33.6/10.9

Accuracy

The recovery (%) in urine (URN) and blood (B) was as follows:

500 /50	II0 /118	5000 /1000	I05 /I07	I00 /50	II2 /II5
I00 /I0	I06 /II3	2000 /400	91 /94	50 /25	I07 / 7
20 /2	94 /113	500 /100	94 /87	20 /10	II6 /108
Methadone (spiked sample URN/B) ng/mL	Recovery (%)	Methamphetamine (spiked sample URN/B) ng/mL	Recovery (%)	Phencyclidine (spiked sample URN/B) ng/mL	Recovery (%)
450 /450	II7 /121	75 /75	82 /70	75 /75	I 20 /158
I50 /150	93 /100	50 /50	88 /77	50 /50	I29 /171
45 /45	98 /116	25 /25	86 /81	25 /25	I26 /167
Benzoylecgonine (spiked sample URN/B) ng/mL	Recovery (%)	Cannabinoids (spiked sample URN/B) ng/mL	Recovery (%)	Meprobamate (spiked sample URN/B) ng/mL	Recovery* (%)
750 /75	92 /113	I000 /250	I07 /I03	500 /62.5	I 27 /96
500 /50	91 /130	500 /125	I08 /I08	300 /37.5	II6 /95
I00 /I0	95 /126	I00 /25	II5 /96	I00 /I2.5	II7 /109
Amphetamine (spiked sample URN/B) ng/mL	Recovery (%)	Barbiturates (spiked sample URN/B) ng/mL	Recovery (%)	Benzodiazepines (spiked sample URN/B) ng/mL	Recovery (%)

Amphetamine (spiked sample URN/B) ng/mL	Recovery (%)	Barbiturates (spiked sample URN/B) ng/mL	Recovery (%)	Benzodiazepines (spiked sample URN/B) ng/mL	Recovery (%)
I00 /I0	95 /126	I00 /25	II5 /96	I00 /12.5	II7 /109
500 /50	91 /130	500 /125	I08 /108	300 /37 . 5	II6 /95
750 /75	92 /113	I000 /250	I07 /I03	500 /62.5	I 27 /96
Benzoylecgonine (spiked sample URN/B) ng/mL	Recovery (%)	Cannabinoids (spiked sample URN/B) ng/mL	Recovery (%)	Meprobamate (spiked sample URN/B) ng/mL	Recovery* (%)
45 /45	98 /116	25 /25	86 /8	25 /25	I26 /167
I 50 /150	93 /100	50 /50	88 /77	50 /50	129 /171
450 /450	II7 /121	75 /75	82 /70	75 /75	I20 /158
Methadone (spiked sample URN/B) ng/mL	Recovery (%)	Methamphetamine (spiked sample URN/B) ng/mL	Recovery (%)	Phencyclidine (spiked sample URN/B) ng/mL	Recovery (%)
20 /2	94 /113	500 /100	94 /87	20 /10	II6 /108
I00 /I0	I06 / 3	2000 /400	9 1/94	50 /25	I07 / 7
500 /50	II0 / 8	5000 /1000	I05 /107	I00 /50	II2 /115
Opiates		Oxycodone		Tricyclic	

Opiates (spiked sample URN/B) ng/mL	Recovery (%)	Oxycodone (spiked sample URN/B) ng/mL	Recovery (%)	Tricyclic antidepressants (spiked sample URN/B) ng/mL	Recovery (%)
I0 /6	II5 /I25	5 /5	I00 / 3	56 /19	128 /122
50 /30	I05 /I23	IO /IO	I 20 /107	I67 /56	I09 /II8
250 /150	II6 /122	I5 /15	II3 /105	500 /167	I02 /I04

14.133,144,145,146,147,149,151,163,191,202,213.112RDFT, 15.029.125RDFT *In blood sample spiked 50% below cut-off read negative

Precision

Amphetamine	CV (%)	Barbiturates	CV (%)	Benzodiazepines	CV (%)
Level I	4.0	Level I	2.4	Level I	1.8
Level 2	5.0	Level 2	2.3	Level 2	2.0
Level 3	8.7	Level 3	2.3	Level 3	4.3
Level 4	4.6	Level 4	3.7	Level 4	5.3
Level 5	7.4	Level 5	3.8	Level 5	4.2
Level 6	8.5	Level 6	4.3	Level 6	5.5

Benzoylecgonine	CV (%)	Cannabinoids	CV (%)	Meprobamate	CV (%)
Level I	1.2	Level	7.6	Level I	5.5
Level 2	2.8	Level 2	3.5	Level 2	2.8
Level 3	3.1	Level 3	6.9	Level 3	4.5
Level 4	3.4	Level 4	7.9	Level 4	3.8
Level 5	4.1	Level 5	9.2	Level 5	6.1
Level 6	3.8	Level 6	10.0	Level 6	6.5

Methadone	CV (%)	Methamphetamine	CV (%)	Phencyclidine	CV (%)
Level I	4.6	Level I	5.6	Level I	3.6
Level 2	4.6	Level 2	7.0	Level 2	4.3
Level 3	5.9	Level 3	5.4	Level 3	3.4
Level 4	6.5	Level 4	10.0	Level 4	3.6
Level 5	9.0	Level 5	8.0	Level 5	4.2
Level 6	8.2	Level 6	8.8	Level 6	4.7

Opiates	CV (%)	Oxycodone	CV (%)	Tricyclic antidepressants	CV (%)
Level	3.1	Level	2.1	Level	4.0
Level 2	2.5	Level 2	2.9	Level 2	4.6
Level 3	3.6	Level 3	3.9	Level 3	6.4
Level 4	2.2	Level 4	5.0	Level 4	6.0
Level 5	3.7	Level 5	2.9	Level 5	5.3
Level 6	5.8	Level 6	4.5	Level 6	6.5

(AMP10002, BAR10004, BNZ10006, THC10008, MPB10020, MTD10012, PCP10018, OP110014, TCA10016)160516bi, BZG10010 170516ml, OXY10114 170516bi, MTH10000 190516ml, 15.018.125RDF

Conclusion

_ Data show optimal analytical performance of the twelve ELISA kits evaluated, which indicates their applicability _ to the reliable screening of a broad range of drugs of abuse including analgesics, stimulants, hallucinogens, and

The intra-assay precision (CV%) for different concentration levels of the target drug was as follows (n=12):