Screening of amphetamines in hair using a homogeneous immunoassay procedure

IMMUNALYSIS

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G. Wang, M. Vincent, A. Agrawal, C. Castro, C. R. Henry, C. Moore Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767

Introduction

Hair is a useful specimen for the detection of long-term drug use. In general, drug concentrations in hair are lower than those identified in other matrices such as urine and blood. Further, specimen volume may be limited, so sensitive screening methods are necessary. Following METH use, METH itself is generally found in hair, but AMP may be present as a metabolite, or as an independent drug.

Methods

- Hair from METH users (n = 20; 10mg); Drug free hair (n = 20; 10 mg)
- Cut into small pieces; 0.1 M phosphate buffer added (pH 2.7; 0.5 mL)
- Incubated (3 hrs/75°C).
- Supernatant was analyzed using two ELISA and two homogeneous immunoassays (EIA) on an Olympus 400 platform
- One for METH; One for AMP
- For ELISA, the supernatant was diluted 1:5 with PBS before plating
- For EIA, 10μL of supernatant was used, making the process compatible with most commercial chemistry analyzers

Cross-reactivity

AMP kit	XR (%)		
S(+)-AMP	100		
S(+)-METH	2		
R(-)-METH	<1		
(+/-)-MDMA	1.5		
R(-)-AMP	2.6		
(+/-)-MDA	40		
(+/-)-MDEA	N.D.		
Fenfluramine	7.5		
1S, 2R(+)-Ephedrine	<0.1		
1R, 2S(-)-Ephedrine HCI	N.D.		
(+/-)-PPA	<0.1		
Phentermine	2.4		

METH kit	XR (%)		
S(+)-METH	100		
R(-)-METH	4		
(+/-)-MDMA	80		
S(+)-AMP	<1		
(+/-)-MDA	<1		
(+/-)-MDEA	52		
PMA HCI	2		
Fenfluramine	15		
1S, 2R(+)-Ephedrine	N.D.		
1R, 2S(-)-Ephedrine HCI	4		
(+/-)-PPA	N.D.		
Phentermine	<0.1		

Results

A screening cutoff of 500 pg/mg was used, (recommendation from the Proposed Federal Guidelines 2004) Precision at 250, 500, 1000, 2500 pg/mg

AMP EIA Intra-assay: 9.9%, 8.2%, 6.6%, 3.7%; METH EIA Intra-assay: 11.6%, 8.3%, 7.1%, 2.7%

All the negative specimens screened negatively using both ELISA and EIA; positive specimens are shown below

Sample	ELISA	EIA			GC/MS (pg/mg)	
	AMP	METH	AMP	METH	AMP	METH
1	Р	Р	Р	Р	930	>10,000
2	Р	Р	Р	Р	103	6121
3	Р	Р	Р	P	283	2360
4	Р	Р	N	P	257	8671
5	N	Р	N	P	233	9273
6	N	Р	N	P	105	2046
7	Р	Р	N	P	2883	>10,000
8	Р	Р	Р	P	311	7953
9	Р	Р	N	Р	219	2707
10	Р	Р	Р	P	108	6665
11	Р	Р	Р	P	2390	>10,000
12	Р	Р	Р	P	5944	>10,000
13	Р	Р	Р	P	1089	9850
14	Р	Р	Р	P	1825	>10,000
15	Р	Р	Р	P	3689	>10,000
16	Р	Р	Р	P	1130	>10,000
17	Р	Р	Р	P	1135	>10,000
18	Р	Р	Р	P	1115	9512
19	Р	Р	Р	P	775	7088
20	Р	Р	Р	P	645	5560

Summary: The assay is precise, sensitive and conducive to rapid hair screening using commercial chemistry analyzers