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Evaluation of Immunoassays for the Determination of MDMA and Cannabinoids in Urine Samples

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ABSTRACT

Methylenedioxymethamphetamine (MDMA) is structurally related to methamphetamine (MA). There are many different commercially available immunoassay (IA) reagents for the initial screening of amphetamine and/or methamphetamine. These reagents may be employed to detect MDA/MDMA in urine samples. In order to select a suitable reagent for the initial screening of MDMA in urine samples, we evaluated 7 different amphetamine immunoassay reagents: Emit d.a.u. Monoclonal Amphetamine/Methamphetamine; Emit II Plus Monoclonal Amphetamine/Methamphetamine; Emit d.a.u. Amphetamine Class; DRI Amphetamine; AxSYM Amphetamine/Methamphetamine II; Abuscreen Online Amphetamine and Cedia Amphetamine/Ecstasy. We also determined the cross reactivity of these reagents with MDA, MDMA, MBDB, MDEA and other phenethylamines. These IA reagents were employed to screen a group of 146 urine samples collected from pub patrons. Results of the initial screening were compared with results obtained with gas chromatography/mass spectrometry (GC/MS). Five of the IA assays were acceptable for the initial screening of MDMA, except the Emit II Plus Monoclonal Amphetamine/Methamphetamine reagent and Emit d.a.u. Class Amphetamine reagent. Results obtained with Emit II reagent showed high false negatives, while results obtained with Emit d.a.u. Class reagent showed high false positives.

We evaluated 5 different IA for cannabinoids. Results of the initial screening of 74 urine samples collected from pub patrons were compared with results obtained by GC/MS. There are 12 confirmed positives with GC/MS. Results obtained with DRI reagent showed no false negatives, while results obtained with Emit, Abuscreen Online, AxSYM and Cedia reagents have 4, 2, 3 and 4 false negatives, respectively.

Key words: MDMA, cannabinoids, immunoassay, urine samples, pub

INTRODUCTION

Amphetamines are sympathomimetric drugs, which can be used to treat narcolepsy, attention deficit disorder and obesity[(1)]. Amphetamines are powerful central nervous system stimulants that lead to euphoria, and so are widely abused[(2)]. The most commonly available amphetamines are amphetamine (A) and methamphetamine (MA)[(1,2)]. There are many designer amphetamines, such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy methamphetamine (MDMA, Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butamine (MDB) and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)[(3,4)]. They are psychotropic drugs[(4-8)]. Among the amphetamine designer drugs, MDMA is the most popular. MDMA was widely abused in the U. S. A. college campuses in the 1980s[(9)]. Its use in the U. S. A. peaked in the middle of the 80s and declined in the early 1990s[(7,9)]. However, MDMA has regained its popularity in a different setting. In England, MDMA is commonly abused at "rave" parties where electronic music was mixed with video and laser shows. This counter culture with the combination of rave party and MDMA has been exported worldwide[(10)]. MDMA causes intensification of feeling, drop in defense mechanism, fear response and inhibition, increased esteem, desire to communication, empathy for others and euphoria[(4-8)]. MDMA has been used as an adjunct to psychotherapy[(5,8)]. Adverse effects of MDMA include tachycardia, ataxia, jaw clenching, nystagmus, anorexia, nausea and vomiting. In higher doses, MDMA can cause paranoid psychosis, panic attack and seizures[(11-16)]. Because of its mood and perception altering property, MDMA is classified as an entactogen that produces a pleasant state of introspection and reduces anxiety. Death due to MDMA abuse usually is associated with hyperthermia and dehydration[(7,17)].

Urine samples from persons suspected of abusing drugs are usually screened with an immunoassay (IA). The presumptive positives are then confirmed with gas chromatography/mass spectrometry (GC/MS)[(18-21)]. Most of the amphetamine immunoassay reagents available are directed toward the detection of amphetamine and/or methamphetamine. Although cross reactivities to structurally related metabolites can be found in the product

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inserts provided by the manufacturer, there are many minor metabolites that will also contribute to the final screening results. Whether the commercially available amphetamine reagents are suitable for the screening of MDA and/or MDMA need to be evaluated with urine samples from persons that abused MDMA.

In Taiwan, methamphetamine is still the most important drug of abuse, followed by the opiates[22,23]. There were many news reports of increasing abuse of MDMA in pubs. In this report, we collected urine samples from patrons of pubs in Taipei City. The samples were first screened with immunoassays for the presence of amphetamines, opiates, benzodiazepines, cannabinoids (THC) and cocaine metabolites. The presumptive positive samples with opiates, THC and cocaine metabolite were then confirmed with GC/MS. Every sample was also analyzed with GC/MS to determine the presence of A, MA, MDA and MDMA. The results of GC/MS and immunoassay were then used to calculate false positive and false negative rates of the various immunoassays.

MATERIALS AND METHODS

I. *Materials*

(I) *Samples*

Two groups of urine samples (146 and 74) were collected from participants of dancing pubs in Taipei, Taiwan, R. O. C. (referred to as Pub 1 and Pub 2, respectively). Samples were kept in the refrigerator at 4˚C until use.

(II) *Immunoassay reagents*

Immunoassay reagents for amphetamines [Syva Emit d.a.u. Amphetamine Class (Emit-P); Syva Emit d.a.u. Monoclonal Amphetamine/Methamphetamine (Emit-M); Syva Emit II Plus Monoclonal Amphetamine/ Methamphetamine (Emit II); DRI Amphetamine (DRI); Abbott AxSYM Amphetamine/Methamphetamine II (AxSYM); Roche Abuscreen Online Amphetamine (Online) and Microgenics Cedia Amphetamine/Ecstasy (Cedia)], and for marijuana metabolite (THC) (Emit d.a.u.; DRI; Abuscreen Online; AxSYM and Cedia) were all purchased through local distributors. Concentrations for the cutoff calibrator of the amphetamine reagents are as follows: Emit-P, d-A 300 ng/mL; Emit-M, d-MA 500 ng/mL; Emit II, d-MA 500 ng/mL; DRI, d-A 500 ng/mL; AxSYM, d-A 500 ng/mL; Online, d-A 500 ng/mL and Cedia, d-MA 500 ng/mL. Concentration of the cutoff calibrator for all the THC reagents is 11-nor-Δ9-THC-9-carboxylic acid at 50 ng/mL.

(III) *Calibrators*

Calibrators for the immunoassays were all purchased with the reagents. A, MA, MDA, MDMA, MBDB, MDEA and amphetamine-d_5 (A-d_5), methamphetamine-d_8 (MA-d_8) standards were obtained from Radian Corp. (Austin Texas, U. S. A.) Other phenethylamines were purchased from Sigma (St. Louis, U. S. A.)

(IV) *Chemicals*

Trichloroacetic anhydrides (Fluka, Switzerland), N,O-bis-(Trimethylsilyl) acetamide (BSA) (Pierce, U. S. A.) and other chemicals were purchased through local distributors.

II. *Methods*

(I) *Immunoassays*

All the IA reagents except AxSYM were adapted to a benchtop automated chemistry analyzer (Cobas Mira Plus, Roche Diagnostic Systems, Branchburg, NJ, U. S. A.) according to the manufacturer's recommendations. AxSYM reagent was analyzed with AxSYM analyzer according the manufacturer's recommended procedures. Urine samples were screened with 7 different IA for the presence of amphetamines and five different IA reagents for the presence of THC. Reaction rates of the unknowns were compared to the rate of cutoff calibrator. Samples with reaction rates greater than the cutoff calibrator were considered positives. Principle for Emit, DRI, AxSYM, Cedia and Online are explained in greater detail in the product inserts [24-28].

(II) *Cross reactivity of MDA, MDMA, MBDB, MDEA and other phenethylamines*

MDA, MDMA, MBDB, MDEA calibrators and other phenethylamines were diluted with blank urine (normal urine that has been analyzed with GC/MS to be negative for the amphetamines) to 1 and 10 μg/mL and analyzed with respective immunoassay reagents. The cross reactivity is calculated as the percent ratio of the observed concentration to the expected concentration.

(III) *Determination of amphetamines and opiates with GC/MS*

All the samples were analyzed for the presence of A, MA, MDA and MDMA. Samples were extracted with organic solvent and followed by chemical derivatization as described[23]. Briefly, urine samples were adjusted to pH 9.5 with 1.5 M bicarbonate buffer and extracted with ethylacetate (EA). The organic layer was evaporated to dryness under a stream of nitrogen gas. The analytes were derivatized with trichloroacetic anhydride and analyzed with an Agilent GC (6890) coupled with MSD (5973), (Agilent, Palo Alto, CA, U. S. A.) equipped with a capillary column HP-5MS (5% Phenyl 95% dimethylpolysiloxane, 12 m x 0.2 mm i.d., 0.25 μm film thickness). The opiates positive samples were confirmed with GC/MS as described[23].

A-d_5 and MA-d_8 were employed as the internal

standards for the quantification of A /MDA and MA/MDMA, respectively. Mass data were collected in the full scan mode with scan range from *m/z* 45 to *m/z* 350. The retention time and quantitation ions are summarized in Table 1. The limit of detection (LOD) is 75 ng/mL for the amphetamines.

(IV) *Determination of THC with GC/MS*

The presumptive positive samples were extracted with Hexane: EA (9:1) and derivatized with N,O-bis-(Trimethylsilyl) acetamide (BSA), analyzed with GC/MS. Selected Ion Monitoring (SIM) mode was used to analyze samples. Quantitation ion for THC and the internal standard (THC-d_9) are *m/z* 371 and 380, respectively. The limit of detection is 3 ng/mL.

RESULTS AND DISCUSSION

I. *Comparison of Different Amphetamine Immunoassays*

A group of 146 pub samples (Pub 1) were screened with 7 different immunoassay reagents. Results are shown in Table 2. With results obtained by GC/MS as references, the number of true positive, false positive and false negative samples for Emit-P are 38, 15 and 0; for Emit-M are 38, 10 and 0; for Emit II are 31, 0 and 7; for DRI and AxSYM are 38, 0 and 0, for Online are 36, 0 and 2; for Cedia are 38, 2 and 0. The concordance between IA and GC/MS are as follows: Emit-P 89.7%, Emit-M 93.2%, Emit II 95.2%, DRI 100%, Online 98.6%, AxSYM100% and Cedia 98.6%. Concordance is calculated as the ratio of the number of true positive plus true negative samples divided by the total number of samples.

II. *Amphetamine Discordant Results by IA and GC/MS*

The discordant results between IA and GC/MS are presented in Table 3. GC/MS positive samples are defined as follows: A, MDA, MDMA ≧ 500 ng/mL; MA ≧ 500 ng/mL and A ≧ 200 ng/mL. There are many false positives when analyzed with Emit-P reagent, due to the low cutoff concentration (300 ng/mL) and the broad specificity of the antibody employed in the assay[25]. When

Table 1. Retention time and ions monitored for the amphetamines tested by GC/MS

Compound	Retention time (min)	Ions monitored (*m/z*) (quantitation ion underlined)
Amphetamine	4.64	<u>118</u> 188 190
Amphetamine-d_5	4.63	<u>123</u> 194 196
Methamphetamine	5.11	<u>202</u> 204 118
Methamphetamine-d_8	5.10	<u>209</u> 211 213
MDA	5.85	<u>162</u> 135 188
MDMA	6.31	<u>162</u> 135 202

Table 2. Comparison of amphetamine results between GC/MS and various immunoassays

IA Test*	IA Status	GC/MS Status		Concordance**
		+	–	
1	+	38	15	89.7%
	–	0	93	
2	+	38	10	93.2%
	–	0	98	
3	+	31	0	95.2%
	–	7	108	
4	+	38	0	100%
	–	0	108	
5	+	36	0	98.6%
	–	2	108	
6	+	38	0	100%
	–	0	108	
7	+	38	2	98.6%
	–	0	106	

*: Number represents different reagents:
1. Emit d.a.u. Amphetamine Class Assay (Emit-P)
2. Emit d.a.u. Monoclonal Amphetamine/Methamphetamine Assay (Emit-M)
3. Emit Ⅱ Plus Monoclonal Amphetamine/Methamphetamine Assay (Emit II)
4. DRI (Synchron System) Amphetamines Enzyme Immunoassay (DRI)
5. Roche Abuscreen Online Amphetamine (Online)
6. Abbott AxSYM Amphetamine/Methamphetamine II (AxSYM)
7. Microgenics Cedia Amphetamine Assay (Cedia)

**Concordance = (True positive + True Negative) / All Samples

Table 3. Amphetamines discordant samples tested by GC/MS and IA

GC/MS	GC/MS (ng/mL)				IA Status				
Status	A	MA	MDA	MDMA	1*	2*	3*	5*	7*
–	0	0	0	0	+	–	–	–	–
+	0	0	392	2152	+	+	–	+	+
+	535	0	0	0	+	+	–	–	+
–	0	0	0	0	+	–	–	–	–
+	0	0	214	2552	+	+	–	+	+
–	0	0	0	0	+	–	–	–	–
–	0	0	0	0	+	+	–	–	–
–	0	0	0	0	+	+	–	–	–
–	0	0	0	0	+	+	–	–	+
–	0	0	0	0	+	+	–	–	+
–	0	0	0	0	+	+	–	–	–
–	0	0	0	0	–	+	–	–	–
+	0	0	0	3950	+	+	–	+	+
–	0	0	0	0	+	+	–	–	–
+	0	0	0	5920	+	+	–	+	+
–	0	0	0	0	–	+	–	–	–
–	0	0	0	0	+	+	–	–	–
–	0	0	0	0	+	+	–	–	–
+	0	0	82	2610	+	+	–	+	+
–	0	0	0	0	+	–	–	–	–
+	0	0	0	1356	+	+	–	–	+
–	0	0	0	0	+	–	–	–	–
–	0	0	0	0	+	–	–	–	–
–	0	0	0	0	+	–	–	–	–

*: Number represents different reagents as in Table 2. There is no discordant sample between GC/MS and DRI or AxSYM immunoassay reagents.

Emit II is employed, there are many false negatives samples. Emit II is not acceptable as screening reagent for samples containing MDMA.

III. *Positive Rates of Amphetamine, Methamphetamine, MDA and MDMA in Urine Samples*

Every Pub 1 sample was analyzed with GC/MS. The positive rates for A, MA, MDA and MDMA are presented in Table 4. There are 27% of the samples positive for one of the amphetamines, the majority (76%) of the amphetamines positive samples are positive for MDA and/or MDMA. Only 24% of the amphetamines positive samples are positive for amphetamine and/or methamphetamine alone. The high positive rate of MDMA is in contrast to that found in the urine samples from drugs abusers in the general public[22,23]. In the general public, MA is the most prevalent drugs of abuse in Taiwan.

IV. *Comparison of Different THC Immunoassays*

A group of 74 Pub 2 samples were screened with 5 different THC immunoassay reagents. Results are presented in Table 5. With results obtained by GC/MS as references, the number of true positive, false positive and false negative samples for DRI are 12, 2 and 0; for Emit are 8, 0 and 4; for Online are 10, 1 and 2; for AxSYM are 9, 1 and 3; for Cedia are 8, 0 and 4. The concordance between IA and GC/MS are as follows: DRI 97.3%, Emit 94.6%, Online 95.9%, AxSYM 94.6% and Cedia 94.6%. Samples screened negative by all 5 IA are considered as true negatives as well as the negative samples by GC/MS. Concordance is calculated as the ratio of the number of true positive plus true negative samples divided by the total number of samples. Although negative samples after IA screening have not been analyzed further with GC/MS, it is very unlikely that all 5 IA failed to detect the analyte (false negative). We feel justified to treat those samples screened negative by all IA as true negatives.

V. *THC Discordant Results by IA and GC/MS*

The THC discordant results between IA and GC/MS are presented in Table 6. GC/MS positive samples are defined as those with THC $\geqq$ 15 ng/mL. The concentrations of the discordant samples are all very close to the cutoff value (12.9 to 33.4 ng/mL). All the reagents are acceptable for the initial screening of THC in urine samples.

Table 4. A/MA and MDA/MDMA abuse pattern

A/MA	MDA/MDMA	% Positives Samples*
+	+	2.1
–	+	17.8
+	–	6.2
–	–	73.0

*: Total number of samples analyzed is 146.

Table 5. Comparison of THC results between GC/MS and various Immunoassays

IA Test*	IA Status	GC/MS Status		Concordance**
		+	–	
1	+	12	2	97.3%
	–	0	60	
2	+	8	0	94.6%
	–	4	62	
3	+	10	1	95.9%
	–	2	61	
4	+	9	1	94.6%
	–	3	61	
5	+	8	0	94.6%
	–	4	62	

*: Number represents different reagents
1. DRI (Beckman Synchron System) (DRI)
2. Syva Emit d.a.u. (Emit)
3. Roche Abuscreen Online (Online)
4. Abbott AxSYM (AxSYM)
5. Microgenics Cedia (Cedia)
**Concordance = (True positive + True Negative) / All Samples

Table 6. THC discordant samples tested by GC/MS and IA

GC/MS Status	GC/MS (ng/mL)	IA Status				
		1*	2*	3*	4*	5*
+	33.4	+	+	+	+	–
–	14.3	+	–	–	+	–
+	21.9	+	–	–	–	–
–	14.7	+	–	–	–	–
+	22.6	+	–	+	+	+
+	15.0	+	–	–	–	–
–	12.9	–	–	+	–	–
+	19.0	+	–	+	–	–

*: Number represents different reagents as in Table 5.

Table 7. Percent cross reactivity of amphetamine analogs with various immunoassays

Analyte \ Reagents	1*	2*	3*	4*	5*	6*	7*
(+)–Amphetamine	102	132	98	190	122	92	252
(–)–Amphetamine	47	33	6	9	2	48	5
(+)–Methamphetamine	246	99	135	117	80	135	279
(–)–Methamphetamine	69	16	15	6	5	4	43
(+/–)–MDA	10	100	10	82	37	84	>100
(+/–)–MDMA	13	49	9	58	50	101	>100
(+/–)–MBDB	11	62	9	76	45	7	>100
(+/–)–MDEA	23	25	13	8	20	62	>100
(+/–)–Brompheniramine	0	8	0	0	0	0	0
(+/–)–Chlorpheniramine	0	0	0	0	0	0	0
(–)–Ephedrine	7	3	0	1	0	0	0
(+)–Pseudoephedrine	3	1	0	1	0	0	0
(–)–Pseudoephedrine	1	2	0	0	0	0	0
Fenfluramine	5	2	1	15	10	9	100
Ibuprofen	0	0	0	0	0	0	0
Ketamine	0	0	0	0	0	0	0
Phentermine	19	94	5	4	0	9	0
(+/–)–Phenylephedrine	9	1	0	0	0	0	0
–Phenylephedrine	0	0	0	0	0	0	0
Diethylpropion	0	1	0	0	0	0	0
Diphenhydramine	0	1	0	0	0	0	0

*: Number represents different reagents as in Table 2.

Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003

VI. *Cross Reactivity of the Amphetamines with Different Immunoassays*

Different amphetamine analogs were screened with 7 different amphetamine immunoassays. Cross reactivities are calculated as the percent ratio of the observed values to the expected values. Results are shown in Table 7. Emit-P and Emit II reagents show low cross reactivity with MDA/MDMA. The remaining five IA reagents all possess significant cross reactivity.

VII. Drugs of Abuse Pattern in Pub Urine Samples

The pattern of drugs of abuse in the Pub 1 samples is shown in Table 8. DRI reagents were used for the initial screening of the samples. The amphetamines are the most important drugs of abuse with positive rate of 26.1%. Only one opiate positive sample was detected, while cannabinoids (6.2%) and cocaine metabolites (2.1%) were also detected. This is in contrast to the samples from the general public (relatively high positive rate for opiates and no THC and cocaine metabolites[22,23]).

CONCLUSION

Seven amphetamine immunoassays were evaluated for the initial screening of MDMA in urine samples. Most of the immunoassays are acceptable for the purpose except Syva Emit II Plus Monoclonal Amphetamine/ Methamphetamine Assay, which shows high false negative rate and Syva Emit d.a.u. Amphetamine Class Assay, which shows high false positive rate. The high false negative rate of Emit II is caused by the low cross reactivity to MDA and MDMA. The high false positive rate of Emit-P is caused by the low cutoff concentration employed.

Five different THC immunoassays were evaluated. They are all suitable for the initial screening of THC in urine samples.

MDMA is the most popular drugs of abuse in the pub. Marijuana and cocaine (the two most popular drugs of abuse worldwide) can also be detected in the urine samples collected from pub patrons.

Table 8. Sample Patterns of Drugs of abuse from pub source

Drugs	Positives (%)	
	IA	GC/MS*
Amphetamine	26.1	26.1
Opiates	4.1	0.7
Benzodiazepine	6.2	ND**
THC	7.5	6.2
Cocaine metabolite	2.1	2.1

*: Total number of samples analyzed is 146.
GC/MS positive samples for opiates and cocaine metabolites are defined as follows:
Opiates: Morphine ≧ 300 ng/mL or Codeine ≧ 300 ng/mL.
Cocaine metabolite: Benzoylecgonine ≧ 150 ng/mL.
**: ND: not done

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