Profiles of Urine Samples Taken from Ecstasy Users at Rave Parties: Analysis by Immunoassays, HPLC, and GC–MS

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Abstract

The abuse of the designer amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) is increasing throughout the world. They have become popular drugs, especially at all-night techno dance parties (Raves), and their detection is becoming an important issue. Presently, there are no MDMA- or MDA-specific immunoassays on the market, and detection of the designer amphetamines is dependent upon the use of commercially available amphetamine assays. The success of this approach has been difficult to assess because of the general unavailability of significant numbers of samples from known drug users. The objectives of the present study are to characterize the drug content of urine samples from admitted Ecstasy users by chromatographic methods and to assess the ability of the available amphetamine/methamphetamine immunoassays to detect methylenedioxyamphetamines.We found that, when analyzed by high-performance liquid chromatography with diode-array detection (HPLC–DAD), 64% of 70 urine samples (by gas chromatography–mass spectrometry [GC–MS]: 88% of 64 urine samples) obtained from Rave attendees contained MDMA and/or 3,4-methylenedioxyamphetamine (MDA) alone or in combination with amphetamine, methamphetamine, or other designer amphetamines such as 3,4-methylenedioxyethylamphetamine (MDEA). This suggests that the majority of the Ravers are multi-drug users. At the manufacturer's suggested cutoffs, the Abbott TDx Amphetamine/Methamphetamine II and the new Roche HS Amphetamine/MDMA assays demonstrated greater detection sensitivity for MDMA than the other amphetamine immunoassays tested (Abuscreen OnLine Hitachi AMPS, Abuscreen OnLine Integra AMPS, Abuscreen OnLine Integra AMPSX, CEDIA AMPS, and EMIT II AMPS). There is 100% agreement between each of the two immunoassays with the reference chromatographic methods, HPLC–DAD and GC–MS, for the detection of methylenedioxyamphetamines.

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Introduction

The amphetamine analogues of 3,4-methylenedioxyphenylalkylamines are a series of compounds referred to as designer amphetamines. As represented in Figure 1, these psychotropic drugs are ring-substituted derivatives chemically related to mescaline (1). They include 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butanamine (BDB), and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB).

MDMA is the most commonly known designer drug. After cannabis, it is the most abused illicit drug generally used at all-night techno dance parties (Raves) in Europe. It has been reported that 97% of the analyzed so-called Ecstasy preparations (pills, capsules, powders) contain a single active substance (2).

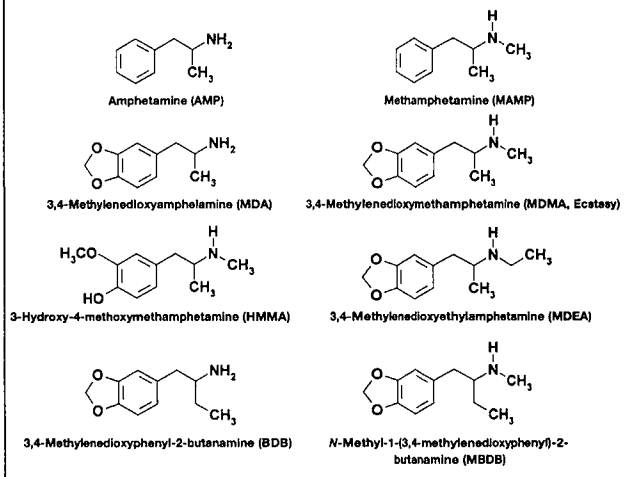

Figure 1. Structures of amphetamines and 3,4-methylenedioxyamphetamines.

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Of these specimens, 47.5% contained MDMA, 42.7% MDEA, 6.5% amphetamine (AMP), and 0.3% MDA and MBDB. These samples also include excipients for tableting and sometimes other pharmaceutical agents (e.g., caffeine).

The 3,4-methylenedioxyamphetamines (MDAMPS) are reported to enhance understanding, communicativeness, and empathy, almost without showing hallucinogenic effects (3,4). They are described as "entactogens", which is a new drug class different from the hallucinogenic phenylalkylamines (5). The mechanism of activity of MDMA is characterized by a high affinity at serotonin uptake sites. In animal experiments, MDMA has shown dose- and species-dependent neurotoxic effects on central serotonergic neurons in terms of degeneration of axon terminals (6-13).

Several animal and human studies have shown that MDMA is metabolized by demethylenation, *N*-demethylation, deamination, *O*-methylation, and *O*-conjugation to glucuronide and/or sulfate metabolites (14–21). The parent drug is mainly detected in urine, and the conjugates of 4-hydroxy-3-methoxymethamphetamine (HMMA) and 3,4-dihydroxymethamphetamine (HHMA) are the dominating metabolites. Minor metabolites are 4-hydroxy-3-methoxyamphetamine (HMA), 3-hydroxy-4-methoxymethamphetamine, 4-hydroxy-3-methoxyphenylacetone, 3,4-methylenedioxyphenylacetone, and 3,4-dihydroxyphenylacetone. Most of these compounds are also present in the blood, with HMMA glucuronide as the major conjugated metabolite and MDA as the major unconjugated metabolite (21). MDA is also a metabolite of MDEA (22).

Typically, MDMA and MDEA are sold in the racemic form. The enantiomers are different in respective to pharmacokinetics and pharmacodynamics (23–25). Several studies have evaluated the enantioselective metabolism and disposition of these compounds (26–32). As reported from mice and rat studies on racemic MDMA, *l*-MDMA and *d*-MDA were the predominant enantiomers in 24-h pooled urine. Although administration of racemic MDEA resulted in greater excretion of the *d*-enantiomer of MDEA, *d*-MDA was present in greater amounts than *l*-MDA in all of those samples except blood where the enantiomers were present in equal amounts. In another report, *l*-MDMA and *l*-MDA exceeded the respective *d*-enantiomers within the first 36-h postdose (33). Greater amounts of *l*-MDMA than *d*-MDMA were observed in bile, blood, liver, urine, and vitreous humor in samples collected at autopsy in a case of fatal poisoning (31).

Table I. Retention Times and Ions Monitored for all Amphetamines Tested by GC–MS

Compound	Retention time (min)	Ions monitored [*m/z*] (quantitation ion underlined)
Amphetamine	8.15	240, 118
Amphetamine-d_6	8.08	244, 123
Methamphetamine	10.46	254, 210, 118
Methamphetamine-d_9	10.34	261, 213
MDA	15.02	375, 240
MDA-d_5	14.99	380, 244
MDMA	16.42	254, 210, 389
MDMA-d_5	16.39	258, 213, 294
MDEA	16.75	162, 268, 240
MBDB	16.99	268, 176, 210
Epedrine	10.62	254, 210
Pseudoephedrine	11.73	254, 210
Phenylpropanolamine	9.17	240, 330
Phentermine	8.45	254, 91

Urine and blood are the most commonly studied biological matrices for MDMA, MDA, MDEA, and MBDB and are well documented in the literature. Determination of these designer drugs in other biological specimens such as saliva, sweat, and hair has been reported more recently (34). The parent drug is detected in higher concentrations than its metabolites in these matrices.

In urine, the MDAMPS can be measured by gas chromatography–mass spectrometry (GC–MS) using the same methods presently employed for the analysis of AMP and methamphetamine (MAMP). The enantiomers of MDA, MDMA, MDEA, AMP, and MAMP were reported to be determined simultaneously in human urine using liquid–liquid extraction followed by derivatization with trifluoroacetyl-*l*-propyl chloride (*l*-TPC) and analysis by GC–MS (33). High-performance liquid chromatography (HPLC) with electrochemical, UV, or diode-array detection (DAD) has been used for the detection of MDMA in biological specimens (14,19,32,35–37). GC–MS analysis is highly specific and is used for the confirmation of positive immunoassay results or a suspected diagnosis.

The abuse of these MDAMPS is increasing throughout the world, and their detection by screening methods is becoming a more important issue. There are currently no commercial immunoassays designed specifically for the detection of these substances, and their detection therefore depends on the relative cross-reactivities they exhibit in the AMP or MAMP screening method used. In general, the cross-reactivity of the commercially available AMP and MAMP assays toward many of these compounds is low, which suggests the possibility that some positive samples may go undetected. The potential for this has been difficult to assess because of the general unavailability of significant numbers of samples from known drug users.

The present study reports on the ability of a number of commercially available immunoassays to detect samples obtained from a population of people using MDAMPS at Raves. It also evaluates the new Abuscreen OnLine HS AMP/MDMA assay, which is specifically designed to increase the detection sensitivity for the use of MDMA. The sensitivities of the immunoassays are assessed relative to GC–MS and HPLC–DAD.

Experimental

Urine sampling

Seventy urine specimens (50–100 mL) were collected from anonymous Ecstasy users (self-declared in the interview prior to urine sampling) at two major Raves in Zurich (Switzerland) in December 1997 at the "Limmat House" and August 1998 at the "Red Fabric" with the permission of the Ethics Committee of the University of Bern. The time of collection was 1–8 h after consumption. The urines were kept frozen at –80°C until analysis.

Instrumentation and reagents

Immunoassays. The Abbott TDx AMP/MAMP II (TDx AMPS) reagents were purchased from Abbott Laboratories (Abbott Park, IL) and used on an Abbott Axsym analyzer. The Emit II AMP/MAMP assay (EMIT II AMPS) was purchased from Behring Diagnostics (San Jose, CA) and used on a Cobas Mira analyzer (Roche). The CEDIA DAU AMPS (Cedia AMPS) assay was purchased from Microgenics (Pleasanton, CA) and used on a Hitachi 917. Five different Roche Abuscreen OnLine (KIMS) formats were used. These were the standard OnLine reagents (AMPS) used on a Roche Hitachi 747; the standard Integra version reagents (AMPS) used on a Roche Integra 700; the standard OnLine Integra reagent using a high-sensitivity MDMA application (AMPSX) on the Integra 700 at both 500- and 1000-ng/mL cutoffs; and the recently developed OnLine HS AMP/MDMA assay with greater sensitivity for the designer AMP analogues using a Hitachi 917. All immunoassays were prepared and used according to the instructions provided by the manufacturer for the specified instrument (38–44). All immunoassays use a 1000-ng/mL cutoff with the exception of the OnLine HS AMP/MDMA assay (300-ng/mL cutoff) and the OnLine Integra AMPSX (500- and 1000-ng/mL cutoffs). CEDIA assays for drugs-of-abuse screening panel were used for identification of other drugs in the specimens. TDx AMPS, the CEDIA AMPS, and Abuscreen OnLine AMPS assays (Hitachi and Integra) were calibrated with *d*-AMP calibrator from the respective manufacturers. The EMIT II AMPS was calibrated with *d*-MAMP calibrator (Behring). The HS AMP/MDMA assay was calibrated with the Preciset *d,l*-MDMA Calibrators (Roche).

GC–MS analysis. GC–MS analysis was performed on a Hewlett-Packard (HP) 5890 GC interfaced with an HP 5970 MS. The GC column was a DB-5 (25 m × 0.2 mm, 0.33-µm film thickness). The GC column was operated at an initial temperature of 120°C for 2 min, programmed to 180°C at 5°C/min, and then to 250°C at 20°C/min with a final temperature hold for 4 min. Urine extractions were performed with chloroform under basic conditions. Heptafluorobutyric anhydride (HFBA) (Aldrich) was used to derivatize MDMA, MDEA, MDA, and other AMP-related compounds for GC–MS analysis. Deuterated internal standards (AMP-d_6, MAMP-d_9, MDA-d_5, and MDMA-d_5) were added to all calibrators, negative and positive controls, and samples. The calibration curve was spiked with all drugs of interest: AMP, MAMP, MDA, MDMA, MDEA, MBDB, EPH, pseudoephedrine, phenylpropanolamine (PPA), and phentermine. The retention times and ions monitored for the different analytes are shown in Table I. Identification of the individual drugs in the urine samples is based on having the proper ion ratios (within ± 20% of those of the standards) and the proper retention times (within ± 2% of those of the standards). At least two ions were monitored for each analyte (one ion ratio) and in some cases three ions (two ion ratios) were monitored. The limit of quantitation of AMP and MAMP was 50 ng/mL and 25 ng/mL for the other analytes mentioned here.

HPLC–DAD analysis. The HPLC system consisted of an HP 1090M liquid chromatograph with an HP 1040M DAD and an HP HPLC Chemstation. The separation was performed gradiently at 40°C on a 150 × 4.6-mm internal diameter column with a 20 × 4-mm internal diameter precolumn packed with 3-µm Spherisorb C-18 ODS-1. The mobile phase was (A) water containing 8.5 g H_3PO_4 (85%) and 280 µL hexylamine and (B) a mixture of 702 mL acetonitrile and 91.6 mL water containing 8.5 g H_3PO_4 (85%) and 280 µL hexylamine per liter. The gradient program was as follows: 0–12 min, 0–15% B; 12–15 min, 15% B; 15–20 min, 15–35% B; 20–25 min, 35–36% B; 25–28 min, 36% B; 28–32 min, 36–50% B; 32–35 min, 50% B; 35–45 min, 50–0% B; 45–75 min, 0% B. The flow rate was 150 µL/min; the injection volume was 1 µL. Peak identification was performed by DAD at 198–300 nm and by library match; quantitation was performed at 198 nm by measuring the peak areas versus internal standard. Sample preparation was conducted as follows: the unhydrolyzed urine specimens were extracted by solid-phase extraction (SPE) according to a method published previously (19) with the exception that instead of MAMP mescaline was used as internal standard. Pseudoephedrine, PPA, and phentermine were not measured.

REMEDi™ HPLC analysis. The Rapid Emergency Drug Identification System (REMEDi, Bio-RAD Laboratories) is an automated drug-profiling system consisting of multicolumn HPLC with fast-scanning spectrophotometric detection. It allows a broad screening of more than 500 drugs and was used according to the manufacturer's instructions and a method published previously (45).

Results and Discussion

Immunoassay screening and evaluation of the sensitivity of commercial amphetamine assays for MDMA

Urine samples from Ecstasy users were tested by the commercial AMPS immunoassays at respective manufacturers' mandated cutoffs. Most were run at a 1000-ng/mL cutoff with the exception of the Abuscreen OnLine HS AMP/MDMA assay (300-ng/mL cutoff). Integra AMPSX was evaluated at both a 1000- and 500-ng/mL cutoffs using the same set of parameters and calibrators.

In general, as shown in Tables II–IV, the immunoassays exhibit a good sensitivity for MDMA containing drugs as compared to the chromatographic methods, HPLC–DAD and GC–MS. In each case, the immunoassay positive screening rate was calculated based on the screened positives versus total positives confirmed by the GC–MS or HPLC–DAD reference method at a 300-ng/mL cutoff. Some samples could not be tested by all the assays because of the limited sample volume. The positive-screening sensitivity of these immunoassays for MDAMPS (see Table IV) was in the following descending order: Abuscreen OnLine HS AMP/MDMA (300-ng/mL cutoff) > TDx AMPS (1000-ng/mL cutoff) > OnLine Integra AMPSX (500-ng/mL cutoff) > OnLine Hitachi AMPS (1000-ng/mL cutoff) > OnLine AMPSX (1000-ng/mL cutoff) ~ CEDIA AMPS (1000-ng/mL cutoff) > EMIT II AMPS (1000-ng/mL cutoff) > OnLine Integra AMPS (1000-ng/mL cutoff). The corresponding rates were 100, 98, 96, 92, 87, 87, 86, and 84%. The 13 samples (nos. 7, 16, 17, 27, 33, 34, 54, 57, 58, 62, 64, 65, 70) analyzed positive by the reference methods (GC–MS and/or quantitative HPLC–DAD) and negative by at least one immunoassay using a 300-ng/mL cutoff are noted in Table V.

Table II. Detectability of Ecstasy Use with Instrumental Immunoassays, HPLC, and GC–MS*

Sample no.	Abuscreen OnLine AMPS[†,‡] Integra 700	Abuscreen OnLine AMPSX[†] Integra 700	Abuscreen OnLine AMPS[†] Hitachi 747	Abuscreen OnLine HS AMP/MDMA[§] Hitachi 917	EMIT II AMPS[†] Cobas Mira	TDx AMPS[†] Axsym	Cedia AMPS[†] Hitachi 917	Cedia Other Hitachi 917	HPLC Qual. HPLC, REMEDi	HPLC Quant. HPLC–DAD (ng/mL)	GC–MS (ng/mL)
1	pos	pos	pos	pos (>AMAX)	pos	pos	pos	BD	MDA, MDMA	457 MDA, 23955 MDMA	689 MDA, 27310 MDMA
2	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP	16303 AMP	34150 AMP
3	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, caffeine	12577 AMP	21030 AMP
4	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP	3504 AMP	7912 AMP, 190 MDMA
5	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	MDMA, phenobarbital, isopropamide	10652 MDMA	157 MDA, 14190 MDMA
6	pos	pos	pos	pos (>AMAX)	pos	pos	pos	OP	AMP, EPH, MDMA, meperidine metab.	24300 AMP, 1430 EPH, 44800 MDMA	62490 AMP, 100 PPA, 4414 EPH, 976 MDA, 72200 MDMA
7	neg	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	MDMA	4720 MDMA	96 MDA, 8944 MDMA
8	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDA, MDMA, verapamil metab.	1991 AMP, 411 MDA, 18822 MDMA	2562 AMP, 486 MDA, 19300 MDMA
9	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDMA, quinine	193 AMP, 897 MDMA	840 AMP, 256 EPH, 2562 MDMA
10	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, EPH, COC	2397 EPH, 39218 AMP	168200 AMP, 415 PPA, 6051 EPH
11	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA, isopropamide	5850 AMP, 6720 MDA, 73000 MDMA	7928 AMP, 72 PPA, 7671 MDA, 74020 MDMA
12	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP	5945 AMP	8255 AMP, 112 PPA, 71 MDMA
13	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA	5471 AMP, 782 MDMA	11220 AMP, 72 PPA, 2870 MDMA
14	neg	neg	neg	neg	neg	neg	neg	neg	dihydroergotamine	neg	neg
15	pos	pos	pos	pos (>AMAX)	n.a.	pos	pos	neg	MDA, MDMA	980 MDA, 28672 MDMA	n.a.
16	pos	pos	pos	pos (>AMAX)	neg	pos	pos	neg	AMP, bupivacaine	9500 AMP	11240 AMP, 79 PPA
17	neg	neg	pos	pos (>AMAX)	pos	pos	neg	neg	neg	neg	715 AMP
18	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDA, MDMA	5704 AMP, 5743 MDMA, 3843 MDEA	11340 AMP, 88 PPA, 1037 MDA, 21240 MDMA, 4755 MDEA
19	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA, verapamil metab., dihydroergot.	830 AMP, 4500 MDMA, 200 MDEA	430 AMP, 80 MDA, 3537 MDMA, 179 MDEA
20	pos	pos	pos	n.a.	pos	pos	pos	neg	AMP	3837 AMP	5088 AMP

* Samples obtained at Rave "Future Scope", Limmat House, Zurich, Switzerland, December 6, 1997.
[†] Cutoff, 1000 ng/mL.
[‡] Abbreviations: >AMAX, > 600 ng/mL (highest calibrator); n.a., not analyzed; AMP(S), amphetamine(s); BD, benzodiazepines; CANN, cannabinoids; COC, cocaine; EPH, ephedrine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; METH, methadone; OP, opiates; and PPA, phenylpropanolamine.
[§] Cutoff, 300 ng/mL.

Table II (continued). Detectability of Ecstasy Use with Instrumental Immunoassays, HPLC, and GC–MS*

Sample no.	Abuscreen OnLine AMPS†,‡ Integra 700	Abuscreen OnLine AMPSX† Integra 700	Abuscreen OnLine AMPS† Hitachi 747	Abuscreen OnLine HS AMP/MDMA§ Hitachi 917	EMIT II AMPS† Cobas Mira	TDx AMPS† Axsym	Cedia AMPS† Hitachi 917	Cedia Other Hitachi 917	HPLC Qual. HPLC, REMEDi	HPLC Quant. HPLC–DAD (ng/mL)	GC–MS (ng/mL)
21	neg	neg	neg	neg	neg	neg	neg	neg	caffeine, quinine	neg	neg
22	pos	pos	pos	pos (>AMAX)	pos	pos	pos	BD, METH, CANN, OP	AMP, MDMA, methadone metab., morphine, codeine	2661 AMP, 4650 MDMA	5929 AMP, 11430 MDMA
23	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, EPH, MDA, MDMA, verapamil	730 AMP, 690 EPH, 200 MDA, 24600 MDMA	751 AMP, 73 PPA, 917 EPH, A264 MDA, 20140 MDMA
24	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDA, MDMA	54490 AMP, 3900 MDA, 173000 MDMA	52990 AMP, 148 PPA, 2233 MDA, 140400 MDMA
25	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA, buflomedil	15400 AMP, 670 MDA, 55000 MDMA	14870 AMP, 86 PPA, 38520 MDMA
26	pos	pos	pos	n.a.	pos	pos	pos	BD	MDA, MDMA, verapamil	2700 AMP, 10908 MDMA	n.a.
27	neg	neg	pos	pos (>AMAX)	neg	pos	pos	neg	AMP, MDMA	3098 MDMA	2384 MDMA
28	neg	neg	neg	n.a.	neg	neg	neg	neg	caffeine	neg	n.a.
29	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA	15980 AMP, 7060 MDMA	n.a.
30	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDMA, phenobarbital	65000 AMP, 500 MDA, 66000 MDMA	64720 AMP, 124 PPA, 243 MDA, 34840 MDMA
31	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA, diphenhydramine	2881 AMP, 1372 MDMA	8037 AMP, 67 PPA, 84 MDA, 9275 MDMA
32	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA, codeine	6260 AMP, 3600 MDMA	6154 AMP, 56 PPA, 2456 MDMA
33	neg	neg	neg	pos	neg	pos	neg	neg	MDMA	816 MDMA	n.a.
34	neg	neg	neg	pos	neg	pos	neg	neg	AMP, MDA, MDMA, verapamil metab.	887 MDMA	n.a.
35	neg	neg	neg	neg	neg	neg	neg	neg	AMP	neg	108 AMP
36	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP	2120 AMP	1730 AMP
37	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA, buflomedil	7156 AMP, 17403 MDMA	13590 AMP, 90 PPA, 426 MDA, 34860 MDMA
38	pos	pos	pos	pos (>AMAX)	pos	pos	pos	neg	AMP, MDA, MDMA	568 AMP, 434 MDMA	696 AMP, 2947 MDMA
39	pos	pos	pos	pos (>AMAX)	pos	pos	pos	CANN	AMP, isopropamide, methylphenidate	2594 AMP, 541 MDMA	2541 AMP, 59 MDA, 4879 MDMA
40	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	279 MDA

* Samples obtained at Rave "Future Scope", Limmat House, Zurich, Switzerland, December 6, 1997.
† Cutoff, 1000 ng/mL.
‡ Abbreviations: >AMAX, > 600 ng/mL (highest calibrator); n.a., not analyzed; AMP(S), amphetamine(s); BD, benzodiazepines; CANN, cannabinoids; COC, cocaine; EPH, ephedrine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; METH, methadone; OP, opiates; and PPA, phenylpropanolamine.
§ Cutoff, 300 ng/mL.

Table III. Detectability of Ecstasy Use with Instrumental Immunoassays, HPLC, and GC–MS II*

Sample no.	Abuscreen OnLine AMPS[†,‡] Integra 700	Abuscreen OnLine AMPSX[†] Integra 700	Abuscreen OnLine AMPS[†] Hitachi 747	Abuscreen OnLine HS AMP/MDMA[§] Hitachi 917	EMIT II AMPS[†] Cobas Mira	TDx AMPS[†] Axsym	Cedia AMPS[†] Hitachi 917	Cedia Other Hitachi 917	HPLC Qual. HPLC, REMEDi	HPLC Quant. HPLC–DAD (ng/mL)	GC–MS (ng/mL)
41	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	COC, CANN	AMP, MDA, MDMA, benzoylecgonine	820 AMP, 4537 MDMA	1544 AMP, 249 MDA, 7099 MDMA
42	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	COC	AMP, MDA, MDMA, COC, benzoylecgonine	11700 AMP, 6790 MDA, 15650 MDMA, 55700 MDEA	20300 AMP, 3553 MDA, 17410 MDMA, 50990 MDEA
43	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	COC, CANN	MDA, MDMA, benzoylecgonine, caffeine	4063 AMP, 1287 MDA, 6287 MDMA, 11750 MDEA	6813 AMP, 90 PPA, 2407 MDA, 10190 MDMA, 18730 MDEA
44	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA, quinine	760 AMP, 1460 MDMA	674 AMP, 1377 MDMA
45	neg	neg	n.a.	neg	neg	neg	neg	neg	caffeine	neg	neg
46	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	neg	AMP, MDA, MDMA, caffeine, quinine, verapamil metab	5250 AMP, 318 MDA, 26179 MDMA	10990 AMP, 111 PPA, 1085 MDA, 29320 MDMA
47	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	MDA, MDMA	135 MDA, 12208 MDMA	548 MDA, 18240 MDMA
48	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	neg	MDMA, quinidine	3342 AMP	3711 AMP
49	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA	2084 AMP, 2090 MDMA	2586 AMP, 198 MDA, 2597 MDMA
50	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA	13368 AMP, 710 MDA, 26925 MDMA	42670 AMP, 68 PPA, 1437 MDA, 57980 MDMA
51	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN, LSD	AMP, MDA, MDMA	1291 AMP, 2403 MDMA	2946 AMP, 64 PPA, 136 MDA, 5303 MDMA
52	neg	neg	n.a.	neg	neg	neg	neg	COC	MDA, COC, benzoylecgonine	neg	132 MDA
53	neg	neg	n.a.	neg	neg	neg	neg	COC	COC, benzoylecgonine	neg	neg
54	neg	neg	n.a.	pos	neg	neg	neg	CANN	AMP	neg	404 AMP
55	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDMA	34148 AMP, 589 MDA, 8466 MDMA, 11840 MDEA	171100 AMP, 199 PPA, 1385 MDA, 18520 MDMA, 25290 MDEA
56	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MDA, MDMA	16334 AMP, 880 MDA, 53548 MDMA	77870 AMP, 1868 MDA, 109900 MDMA
57	neg	neg	n.a.	pos (>AMAX)	neg	pos	neg	COC, CANN	MDA	159 MDA	142 AMP, 424 MDA
58	pos	pos	n.a.	pos (>AMAX)	pos	neg	pos	CANN, LSD	AMP, MDA, MDMA, caffeine	2781 AMP, 1923 MDMA	555 AMP, 439 MDA, 4417 MDMA

* Samples obtained at Rave "Street Parade", Red Fabric, Zurich, Switzerland, August, 8, 1998.
[†] Cutoff, 1000ng/mL.
[‡] Abbreviations: >AMAX, > 600 ng/mL (highest calibrator); n.a., not analyzed; AMP(S), amphetamine(s); BD, benzodiazepines; CANN, cannabinoids; COC, cocaine; EPH, ephedrine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; METH, methadone; OP, opiates; and PPA, phenylpropanolamine.
[§] Cutoff, 300 ng/mL.

Table III (continued). Detectability of Ecstasy Use with Instrumental Immunoassays, HPLC, and GC–MS II*

Sample no.	Abuscreen OnLine AMPS†,‡ Integra 700	Abuscreen OnLine AMPSX† Integra 700	Abuscreen OnLine AMPS† Hitachi 747	Abuscreen OnLine HS AMP/MDMA§ Hitachi 917	EMIT II AMPS† Cobas Mira	TDx AMPS† Axsym	Cedia AMPS† Hitachi 917	Cedia Other Hitachi 917	HPLC Qual. HPLC, REMEDi	HPLC Quant. HPLC–DAD (ng/mL)	GC–MS (ng/mL)
59	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	neg	MDA, MDMA	532 MDA, 38621 MDMA	1062 MDA, 137000 MDMA
60	neg	neg	n.a.	neg	neg	neg	neg	neg	neg.	neg	neg
61	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	MDA, MDMA, hydrocortisol, quinidine	7434 MAMP, 561 MDA, 30391 MDMA	211 AMP, 15090 MAMP, 1148 MDA, 83490 MDMA
62	neg	neg	n.a.	pos	neg	pos	neg	COC, CANN	MDA, MDMA, COC, benzoylecgonine, caffeine	780 AMP, 1700 MDA	482 AMP, 615 MDA
63	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	AMP, MAMP	9952 AMP	27140 AMP, 395 MAMP, 116 PPA
64	pos	pos	n.a.	pos (>AMAX)	neg	pos	neg	CANN	AMP, carbamazepine metab.	1200 AMP	969 AMP
65	pos	pos	n.a.	pos (>AMAX)	pos	pos	neg	CANN	AMP, MDA, MDMA	740 AMP, 1200 MDMA	398 AMP, 1277 MDMA
66	pos	pos	n.a.	pos (>AMAX)	pos	pos	pos	CANN	MDMA	6336 MDMA	279 MDA, 10980 MDMA
67	neg	neg	n.a.	neg	neg	neg	neg	neg	neg	neg	neg
68	neg	neg	n.a.	neg	neg	neg	neg	CANN	caffeine	neg	neg
69	neg	neg	n.a.	neg	neg	neg	neg	neg	neg	neg	neg
70	neg	neg	n.a.	pos (>AMAX)	neg	pos	neg	neg	AMP, MDA, MDMA, caffeine	111 MDMA	195 AMP, 545 MDMA

* Samples obtained at Rave "Street Parade", Red Fabric, Zurich, Switzerland, August, 8, 1998.
† Cutoff, 1000 ng/mL.
‡ Abbreviations: >AMAX, > 600 ng/mL (highest calibrator); n.a., not analyzed; AMP(S), amphetamine(s); BD, benzodiazepines; CANN, cannabinoids; COC, cocaine; EPH, ephedrine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; METH, methadone; OP, opiates; and PPA, phenylpropanolamine.
§ Cutoff, 300 ng/mL.

Table IV. Positive Screening Rate* of the Commercial AMPS Immunoassays for AMPS and MDAMPS (MDMA, MDA, MDEA)

	Abuscreen OnLine AMPS Integra 700	Abuscreen OnLine AMPSX Integra 700	Abuscreen OnLine AMPSX Integra 700	Abuscreen OnLine AMPS Hitachi 747	Abuscreen OnLine HS AMP/MDMA Hitachi 917	EMIT II AMPS Cobas Mira	TDx AMPS Axsym	Cedia AMPS Hitachi 917
Cutoff (ng/mL)	1000	1000	500	1000	300	1000	1000	1000
Positive screening rate for amphetamines and methylenedioxyamphetamines	49 / 58	50 / 58	55 / 58	33 / 35	56 / 56	48 / 57	56 / 58	48 / 58
Positive screening rate for methylenedioxyamphetamines	38 / 45	39 / 45	43 / 45	24 / 26	44 / 44	38 / 44	44 / 45	39 / 45

* The positive screening rate is expressed as the number of samples that screened positives divided by the total positives confirmed by the reference methods (HPLC–DAD or GC–MS at the 300-ng/mL cutoff for AMPS and MDAMPS).

One major cause for the variation in the positive-screening sensitivity is the difference in cross-reactivity to MDMA and other MDAMPS in these assays. As summarized in Table VI, the information in the literature (46) and the respective manufacturer's inserts (38–44) indicate a large difference in cross-reactivities to these substances. The cross-reactivity to MDMA ranged from 97 to 0.2%. The cross-reactivity to MDA ranged from 148 to 1.9%. The sensitivity of most immunoassays to MDA is less than 40% except for TDx AMPS (148%). Moreover, both Abuscreen OnLine HS AMP/MDMA and TDx AMPS have demonstrated higher cross-reactivity with MBDB, which is less neurotoxic than MDMA and increasingly abused (46). In addition, the cross-reactivity to other AMP-like medications such as EPH and PPA seems not to increase significantly as the cutoff of an assay decreases.

Higher detection sensitivity for MDAMPS is available with the TDx AMPS and Abuscreen OnLine HS AMP/MDMA assays. This is demonstrated by the results obtained with the 10 samples (nos. 1, 5, 7, 15, 27, 33, 34, 47, 59, 66) containing only MDMA and/or MDA when analyzed by the reference methods (GC–MS or quantitative HPLC–DAD) and using a 300-ng/mL cutoff. As shown in Table VII, the positive-screening rate for MDMA/MDA

Table V. Discrepant Samples Tested by Instrumental Immunoassays, HPLC, and GC–MS using a 300-ng/mL Cutoff

	Abuscreen OnLine				EMIT II	TDx	Cedia	HPLC	
Sample no.	AMPS*,† Integra 700	AMPSX* Integra 700	AMPS* Hitachi 747	HS AMP/MDMA‡ Hitachi 917	AMPS* Cobas Mira	AMPS* Axsym	AMPS* Hitachi 917	Quant. HPLC–DAD (ng/mL)	GC–MS (ng/mL)
7	neg	pos	pos	pos (>AMAX)	pos	pos	pos	4720 MDMA	96 MDA, 8944 MDMA
16	pos	pos	pos	pos (>AMAX)	neg	pos	pos	9500 AMP	11240 AMP, 79 PPA
17	neg	neg	pos	pos (>AMAX)	pos	pos	neg	neg	715 AMP
27	neg	neg	pos	pos (>AMAX)	neg	pos	pos	3098 MDMA	2384 MDMA
33	neg	neg	neg	pos	neg	pos	neg	816 MDMA	n.a.
34	neg	neg	neg	pos (>AMAX)	neg	pos	neg	887 MDMA	n.a.
54	neg	neg	n.a.	pos	neg	neg	neg	neg	404 AMP
57	neg	neg	n.a.	pos (>AMAX)	neg	pos	neg	159 MDA	142 AMP, 424 MDA
58	pos	pos	n.a.	pos (>AMAX)	pos	neg	pos	2781 AMP, 1923 MDMA	555 AMP, 439 MDA, 4417 MDMA
62	neg	neg	n.a.	pos (>AMAX)	neg	pos	neg	780 AMP, 1700 MDA	482 AMP, 615 MDA
64	pos	pos	n.a.	pos (>AMAX)	neg	pos	neg	1200 AMP	969 AMP
65	pos	pos	n.a.	pos (>AMAX)	pos	pos	neg	740 AMP, 1200 MDMA	398 AMP, 1277 MDMA
70	neg	neg	n.a.	pos (>AMAX)	neg	pos	neg	111 MDMA	195 AMP, 545 MDMA

* Cutoff, 1000 ng/mL.
† Abbreviations: >AMAX, > 600 ng/mL (highest calibrator); n.a., not analyzed; AMP(S), amphetamine(s); BD, benzodiazepines; CANN, cannabinoids; COC, cocaine; EPH, ephedrine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; METH, methadone; OP, opiates; and PPA, phenylpropanolamine.
‡ Cutoff, 300 ng/mL.

Table VI. Cross-Reactivities* of Commercial AMPS Immunoassays for the Detection of MDAMPS

Compound	Abuscreen OnLine AMPSX Integra and AMPSX 500† (MDMA sensitive)	Abuscreen OnLine AMPS Hitachi 500†	Abuscreen OnLine AMPS Hitachi 1000†	Abuscreen OnLine HS AMP/MDMA 300†	TDx AMPS 1000†	Cedia AMPS 1000†	EMIT II AMPS 1000†
MDMA	79	36	0.2	90	97	69	16
MDA	40	36	35	22	148	1.9	33
MDEA	n.a.‡	n.a.	n.a.	11	43	24	n.a.
MBDB	n.a.	n.a.	n.a.	64	(+)	n.a.	(low)
BDB	n.a.	n.a.	n.a.	4	(+)	n.a.	(low)
l-EPH	< 0.1	< 0.1	< 0.2	0.3	< 0.3	0.4	0.5
l-PPA	1.1	1.5	1	0.6	< 0.1	0.3	0.3

* Values (%) according to literature (46) and respective manufacturer's package inserts (38–44).
† Cutoff (ng/mL).
‡ Not analyzed.

was in the following descending order: Abuscreen OnLine HS AMP/MDMA ~ TDx AMPS > CEDIA AMPS > Abuscreen OnLine AMPS (Hitachi) > Abuscreen OnLine AMPSX (Integra) > EMIT II AMPS > Abuscreen OnLine AMPS (Integra). Two specimens (nos. 40, 52) containing MDA concentrations below 300 ng/mL by GC–MS were negative by all immunoassays.

Table VII. Positive Screening Rate for MDMA and MDA Using Commercial AMPS Immunoassays at Respective Cutoffs

Immunoassay	No. of samples tested positive by immunoassay/reference methods	Rate (%)*
Abuscreen OnLine AMPS (Integra)	6/10	60
Abuscreen OnLine AMPSX (Integra)	7/10	70
Abuscreen OnLine AMPS (Hitachi)	5/7	71
Abuscreen OnLine HS AMP/MDMA	10/10	100
EMIT II AMPS	6/9	67
TDx AMPS	10/10	100
Cedia AMPS	8/10	80

* Calculated by the number of samples tested positive by immunoassay versus those tested positive for MDMA and/or MDA with the reference methods (GC–MS or quantitative HPLC–DAD) at a 300-ng/mL cutoff.

Table VIII. Distribution of Positive Samples Containing Multiple AMP Analogues and Other Drugs of Abuse at Rave Parties

Compound	Number of samples tested positive for...*	Number of samples also tested positive for...*						
		AMP	MAMP	MDMA	MDA	MDEA	PPA, EPH	Other†
AMP	47		2	33	24	5	21	28
MAMP	2	2		1	1	0	1	2
MDMA	40	33	1		28	5	18	26
MDA	32	24	1	28		5	13	23
MDEA	5	5	0	5	5		3	4
PPA, EPH	22	21	1	18	13	3		13
Other†	37	28	2	26	23	4	13	

* Results according to GC–MS analysis (64 samples analyzed).
† Benzodiazepines, cannabis, cocaine, LSD, methadone, and/or opiates (Cedia DAU).

Table IX. Correlation of HPLC with GC–MS*

Method	Cutoff (ng/mL)	Correlation (%)			
		Positive samples		Negative samples	
		Yes	No	Yes	No
GC–MS	300	100	0	100	0
HPLC–DAD	300	75	25	100	0
REMEDi	–	62	38	82	18
GC–MS	100	100	0	100	0
HPLC–DAD	100	64	36	100	0
REMEDi	–	61	39	100	0

* Related to the detection of amphetamines (AMP, MAMP) and MDAMPS (MDA, MDMA, MDEA) in 64 samples.

The OnLine assays at different cutoffs exhibit a large range of cross-reactivities to MAMP and its structurally related analogues, MDMA, MDEA, and MBDB (Table VI). At the 1000-ng/mL cutoff for the Hitachi application, the cross-reactivity with MAMP is less than 2%, whereas at the 500-ng/mL cutoff, the cross-reactivity is significantly increased to 80%, which is similar to that of AMP. This is partly attributable to the use of a different set of instrument parameters. This phenomenon contributes to the increase in detected positive specimens at the lower cutoffs.

Lowering the cutoffs of the existing commercial AMP assays below 1000 ng/mL will increase the detectability for AMP and its analogues significantly. However, the number of positive results from those samples containing high concentrations of AMP-related medications such as *l*-MAMP and the β-hydroxyphenylethylamines would also increase. Therefore, assays for MDAMPS should be designed and assessed for maximum positive screening rate (sensitivity) and for minimum cross-reactivity with other medications such as EPH, pseudoephedrine, phentermine, and tyramine. These medications are usually taken at high doses. Pragmatically, an AMP or MAMP assay should be developed with high cross-reactivity to MDMA and/or other MDAMPS instead of developing MDAMPS-specific assays. By following this design strategy for the selection of antibodies, the potential false positives derived from AMP-related medications would be decreased or eliminated.

Drug-testing profile at Rave parties

AMP alone or in combination with MDMA, MDEA, or MBDB was present in the urine of most Ecstasy users as indicated from this comprehensive analysis of Rave samples by the chromatographic methods. As shown in Tables II, III, V, and VIII, of 64 specimens analyzed by GC–MS, 56 (88%) contained AMPS (AMP, MAMP) and/or MDAMPS (MDA, MDMA, MDEA). Thirty-five urine samples (55%) were tested positive for both AMPS and MDAMPS. AMP was detected in 47 (73%), MDMA in 40 (63%), and MDA in 32 samples (50%). Five samples (8%) contained MDEA, and two samples (3%) MAMP. Nine samples (14%) contained only MDMA and/or MDA. Eight samples (13%) were negative for AMP-like substances.

A broad range of AMP analogues has also been detected on the Rave scene. Using REMEDi and GC–MS, licit compounds such as caffeine, quinine, dihydroergotamine, verapamil, EPH, PPA, etc. were detected in 31 (44%) of the 70 samples. These substances may have been added to the Ecstasy pills as

adulterants or diluants or originated from drinks and medications. Other classes of abused drugs were detected in 37 of 70 samples (53%) using the CEDIA DAU assays and REMEDi. Thirty-one (44%) were positive for cannabis, seven (10%) were positive for cocaine, and three (4%) were positive for benzodiazepines. Opiates, LSD, and methadone were detected in two (3%), two (3%), and one sample (1%), respectively, above the CEDIA DAU cutoff levels. It appears that the majority of the Ravers are multi-drug users, with cannabis as the dominating co-consumed drug.

In this study, MDMA and MDA were considered as evidence of Ecstasy use. As reported (19), the MDMA concentration in urine climbs after at least 4 h postadministration. Peak concentration of MDMA in urine is usually reached at 21.5 h. Because the samples used in this study were collected randomly between 1 and 8 h after administration, the absence of MDMA in some samples may partly represent samples collected within 2 h of administration. HMMA (as glucuronide) is reported to be the major urinary metabolite of MDMA present in much higher concentration than MDA and HMA. MDA and HMA are formed upon further metabolism. The peak excretion period for HMMA is from 5 to 21.5 h (19). The AMPS immunoassays are not designed to detect this type of ring-opened metabolite, and the cross-reactivity to these compounds is low. Screening and confirmation for HMMA may offer improved detection rate. Further characterization of the HMMA content in these samples by chromatographic methods will help to answer these MDMA abuse questions in Ravers.

The positive urine specimens in this study generally exhibit concentration ratios of MDA to MDMA of less than 0.15. Only two samples (nos. 42, 43) had values greater than 0.2. The ratio of MDA to MDMA in human urine has been reported to be indicative of either MDA abuse or MDA as the *N*-demethylation metabolite of MDMA (47). A ratio lower than 0.15, which is the metabolic ratio of MDA to MDMA in humans, suggests a higher probability of MDMA abuse (MDA absence in original preparation). In contrast, when the ratio is greater than 0.15, there is a higher probability of MDA abuse in addition to MDMA abuse. The low ratio of MDA to MDMA suggests that MDMA was taken in the scene.

Most specimens have been found to contain high to extremely high concentrations of AMP and MDAMPS. The mean concentrations of AMP, MDMA, MDA, and MDEA were 9.8 µg/mL (0.19–65 µg/mL, n = 43, HPLC quantitation), 19.2 µg/mL (0.11–173 µg/mL, n = 43), 1.4 µg/mL (0.14–6.8 µg/mL, n = 19), and 16.7 µg/mL (0.20–56 µg/mL, n = 5), respectively. This suggests that these drugs were administered at high doses. In addition, within the limited collection period (1–8 h), concentrations of parent drugs such as MDMA, MDEA, or MBDB would be higher than their respective metabolites in urine.

Correlation of reference methods: HPLC versus GC–MS

Chromatographic confirmation assays with MS, DAD, or fast-scanning UV detection (GC–MS, LC–MS, HPLC–DAD, REMEDi) are necessary to verify AMPS-positive immunoassay results and identify the drugs present. In general, the present study demonstrates a good correlation between GC–MS and quantitative HPLC–DAD analysis related to the detection of amphetamines (AMP, MAMP) and MDAMPS (MDA, MDMA, MDEA). At a cutoff of 300 ng/mL, a correlation was observed in 75 and 100% of positive and negative samples, respectively, whereas the correlation was 64 and 100% at the 100-ng/mL cutoff (Table IX). The two chromatographic reference methods exhibited mainly some discrepancies in the detection of drugs such as MDA and AMP at lower concentrations. For example, 16 samples negative for MDA and/or AMP by HPLC–DAD were positive for these compounds when analyzed by GC–MS using a 100 ng/mL cutoff (Tables II and III). Retention times and ions used for GC–MS identification and quantitation are shown in Table I. At the 300-ng/mL cutoff, only six samples were positive for MDA and/or AMP by GC–MS. This could be due to either the differences in the extraction procedures and internal standards used by the two methods or the inability to detect 100 ng/mL of AMP or MDA by the HPLC–DAD method because of the limited detector sensitivity. With the qualitative HPLC REMEDi system, which is less often used for forensic than for clinical toxicology, the correlation rates were 60 and 61% for positive samples and 73 and 88% for negative samples, respectively (Table IX). Nevertheless, HPLC has the potential as an alternative method to GC–MS for the detection of AMPS and MDAMPS.

Conclusions

This report describes a comprehensive analysis of samples collected from participants at Rave parties in Zurich, Switzerland. By the combination of immunoassays and chromatographic methods, it was found that AMPS (AMP, MAMP) and/or their 3,4-methylenedioxy analogues (MDA, MDMA, and MDEA) were present in 89% of the samples. The majority of these samples (82%) contained MDMA and/or MDA. About one-half of the samples (53%) contained other classes of abused drugs, suggesting that a high percentage of Ravers are multi-drug users. The evaluation of a number of commercially available AMPS immunoassays demonstrated a generally good effectiveness for the detection of Ecstasy users. At the manufacturer's suggested cutoff, the Abuscreen OnLine HS AMP/MDMA and TDx AMPS assays have demonstrated higher detection sensitivity than the other commercial AMPS immunoassays tested (Abuscreen OnLine Hitachi AMPS, Abuscreen OnLine Integra AMPS, Abuscreen OnLine Integra AMPSX, CEDIA AMPS, and EMIT II AMPS). These two immunoassays were in total agreement using these samples and demonstrated the best correlation to the reference chromatographic methods, GC–MS and HPLC–DAD. This study also suggests that HPLC has the potential as an alternative method to GC–MS for the confirmation of methylenedioxyamphetamine-type drugs.

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Note: As this report goes to print, CEDIA has recently launched a new amphetamine assay, which has different cross-reactivity to the designer amphetamines.

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