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Which Amphetamine-Type Stimulants Can Be Detected by Oral Fluid Immunoassays?

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Introduction: The use of oral fluid for monitoring drug consumption on roads has many advantages over conventional biological fluids; therefore, several immunoassays have been developed for this purpose. In this work, the ability of 3 commercial immunoassays to detect amphetamine-type stimulants (ATSs) in oral fluid was assessed. In addition, it was reviewed the main controlled ATSs available worldwide, as well as the oral fluid immunological screening tests that have been used for identifying ATSs in drivers.

Materials and Methods: The analytical specificity of amphetamine direct enzyme-linked immunosorbent assay (ELISA), methamphetamine direct ELISA (Immunalysis Corporation), and Oral-View saliva multidrug of abuse test (Alfa Scientific Designs) was evaluated using ATS-spiked oral fluid. Legislation and published articles that report the use of immunological screening tests to detect ATS consumption in conductors were reviewed, including the kit's technical information, project reports, police and drug databases.

Results and Discussion: Even at high concentrations, the tested assays were not able to detect methylphenidate, fenproporex, or diethylpropion, controlled ATSs legally marketed in many countries.

Conclusions: This evidences the need to develop new kits that enable one to control the misuse of prescription ATSs on roads through oral fluid immunoassays.

Key Words: amphetamine-type stimulants, oral fluid, immunoassays

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INTRODUCTION

Drug driving is one of the major contributors to road fatalities in the world. Over the past years, several studies[1−6] have shown a high prevalence in the consumption of psychoactive substances among drivers, especially amphetamine (AMP)-type stimulants (ATS), which are widely used by professional conductors to increase their alertness during long journeys.[7−11] According to the World Health Organization,[12] ATS can be defined as a group of substances that comprise stimulants from the AMP group, including AMP, methamphetamine (MET), and a range of structurally related compounds such as methcathinone, fenetylline, ephedrine (EPH), pseudoephedrine (PSE), methylphenidate (MPH), and methylenedioxymethamphetamine (MDMA) or 'ecstasy.'

The use of oral fluid for monitoring ATS consumption on roads has many advantages over conventional biological fluids (urine and blood),[13,14] including a good correlation with serum analytical data and impairment symptoms,[15−19] noninvasive collection, and difficulty to adulterate samples.[20]

Immunoassays are the most convenient techniques for initial oral fluid drug testing and therefore have been extensively employed in both research[1,4,16−18,21−25] and traffic police routine.[26−36]

Among the immunological techniques most widely used for the detection of ATS in oral fluid are the enzyme-linked immunosorbent assay (ELISA) and immunochromatography. Classical ELISA assays are laboratory tests based on the competition between the drug present in the sample and the same drug labeled with enzyme (added to the system), for the binding sites of antibodies immobilized in the wells of a plate.[37,38] On the other hand, immunochromatographic assays are on-site tests, consisting of collection pads attached to porous membrane strips, which are inserted into the subject's mouth. From the pad, oral fluid migrates by capillarity, thereby mobilizing a reservoir of colored antibodies that flow with oral fluid along the strip until lines with immobilized drugs are reached. In case some drug is present in the sample, at or above the kit cutoff concentration, the binding sites of the respective colored antibody will saturate and not attach to the immobilized drug in the strip, hence resulting in the absence of a colored band in the results window.[39]

Some issues of commercial ATS immunoassays, such as low sensitivity and lack of specificity of some tests, detecting over-the-counter ATSs (ephedrine, PSE, phenylephrine, etc.),

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and causing a large number of false positive results, have been extensively discussed.[22,25,40–42] However, nobody has evaluated if the oral fluid immunoassays that have been applied in drivers are suitable to detect the main controlled ATSs available in each region, including the prescription-only ATSs, such as diethylpropion (DIE), fenproporex (FEN), MPH, etc. In fact, the misuse of prescription ATSs is a growing health problem in a number of developed and developing countries, and in many regions, it is the primary source of ATS abuse.[43]

Considering that the nondetection of controlled ATSs legally marketed in countries can lead to erroneous estimates with respect to the consumption of ATSs by drivers, this study aimed to assess the ability of 3 commercial immunoassays to detect ATSs in oral fluid. In addition, the oral fluid immunological screening tests that have been used worldwide for identifying ATSs in drivers were reviewed, including the main controlled ATSs available in each country. These data were correlated and discussed by the authors.

MATERIALS AND METHODS

Review of Data

The latest published research studies available at ScienceDirect, PubMed, and Scirus databases that report the use of oral fluid screening immunoassays to detect ATS consumption in conductors and the legislation of countries officially employing oral fluid tests for the control of drugged drivers were reviewed.[1,4,16–19,21–36]

Leaflets and technical information provided by manufacturers of oral fluid kits were also been overviewed,[37,38,44–49] including project reports and articles[50,51] that had evaluated these kits. Based on the information provided by the manufacturers and the formula shown in the following subsection, ATS crossreactivity of the reviewed commercial kits was calculated.

In addition, databases provided by drug regulatory agencies and specialized medicine information websites and books[52–74] were accessed for the purpose of establishing which pharmaceuticals containing controlled ATSs are currently licensed for use in each country. Information on illegal consumption of ATS, either prescription medicines or illicitly manufactured drugs, was majorly obtained from the UN reports,[43,75] the Brazilian Federal Police,[76] and recent articles.

Experimental Crossreactivity Evaluation

The crossreactivity (analytical specificity) of 3 commercial oral fluid immunoassays for ATS detection was evaluated: amphetamine direct ELISA and methamphetamine direct ELISA kits, purchased from Immunalysis Corporation (Pomona, CA), and Oral-View Saliva multidrug of abuse test, manufactured by Alfa Scientific Designs (Poway, CA) and kindly donated by Grimextur Brasil Diagnósticos (São Paulo, SP, Brazil).

Amphetamine and methamphetamine direct ELISA kits consist of classic ELISA assays, performed with buffered oral fluid collected with Quantisal device (Immunalysis Corporation).[37,38] On the other hand, Oral-View is an on-site immunochromatographic multianalyte assay designed to simultaneously detect up to 5 drug classes in the same cartridge—generally AMP, benzodiazepines, cocaine, morphine, and tetrahydrocannabinol.[39]

Methamphetamine direct ELISA, amphetamine direct ELISA, and Oral-View kits have 50-ng/mL cutoff concentrations, whereas *d*-MET is the target drug for the first kit and *d*-AMP is the target drug for the other kits.[37–39]

Amphetamine and methamphetamine Direct ELISA kits were originally purchased for using in our laboratory routine; therefore, there were several kits available to be evaluated. Thus, ELISA kits were tested for *d*,*l*-MET and *d*,*l*-amphetamine (*d*,*l*-AMP), purchased from Cerilliant (Round Rock, TX); *d*,*l*-FEN, *d*,*l*-DIE, and *d*,*l*-threo-methylphenidate (*d*,*l*-threo-MPH) hydrochlorides, kindly donated by Aché (Guarulhos, SP, Brazil) and Novartis (Resende, RJ, Brazil); *d*,*l*-3,4-methylenedioxymethamphetamine (*d*,*l*-MDMA), *d*,*l*-3,4-methylenedioxy-ethylamphetamine (*d*,*l*-MDEA), *d*,*l*-3,4-methylenedioxyphenyl-2-butanamine (*d*,*l*-MBDB), and *d*,*l*-3,4-methylenedioxyamphetamine (*d*,*l*-MDA), obtained from Lipomed (Arlesheim, Switzerland) as hydrochloride salts; *d*,*l*-synephrine (*d*,*l*-SYN), purchased from MP Biomedicals (São Paulo, SP, Brazil); *l*-phenylephrine (*l*-PHY) and *d*-PSE obtained from Sigma–Aldrich Corporation (São Paulo, SP, Brazil); and *l*-EPH from Merck (Darmstadt, Germany). The Oral-View was tested for *d*,*l*-AMP, *d*,*l*-DIE, *d*,*l*-FEN, and *d*,*l*-threo-MPD, due to the small number of kits available for testing, whereas these were donated to us.

Methanol stock solutions of all the mentioned ATSs were produced at 500 μg/mL, and a pool of blank oral fluid was prepared with collected fluids from 8 volunteers, directly into polypropylene tubes, which were mixed, centrifuged, and the pH was adjusted to 6.5 with HCl. The blank oral fluid and the methanol stock solutions were used to prepare 100,000 ng/mL of oral fluid solutions, which were subsequently diluted to prepare all the needed concentrations, resulting in a final methanol content ranging from 5% to 0.0025%. Direct ELISA kits were tested with ATS-spiked oral fluids at the concentrations of 50; 100; 150; 250; 500; 1,000; 10,000; 40,000; and 100,000 ng/mL, whereas Oral-View was assayed only with the concentrations of 100 and 500 ng/mL, due to the small number of kits available.

The Oral-View assay and Direct ELISA were performed strictly following the manufacturer's instructions[37–39] in duplicate for each concentration, employing ATS-spiked oral fluid in the neat form (for the first immunoassay), and diluted in Quantisal preservative buffer (1 mL of oral fluid in 3 mL of buffer), for the others. With regard to the Oral-View assay, the spiked oral fluid was slowly pipetted in the pad (~0.6 mL), thus keeping the opposite end of the device angled downward, until a pink color had begun to appear in the result window. In all the experiments, blank oral fluid was assayed as negative control.

The crossreactivity profile of the immunoassays was calculated according to the formula[77]:

$$\text{Crossreactivity}(\%) = \frac{\text{Apparent concentration of the target ATS}}{\text{ATS concentration of spiked oral fluid}} \times 100.$$

The crossreactivity provides an indication of how the assay responds to other ATSs in relation to the target ATS

response.[78] As for immunochromatographic assays (Oral-View), the apparent concentration is the kit cutoff concentration for the target ATS. For ELISA kits (amphetamine and methamphetamine direct ELISA), the apparent concentration is the equivalent target ATS concentration calculated based on the spiked sample response (sample absorbance divided by zero calibrator absorbance, multiplied by 100).

RESULTS AND DISCUSSION

Oral Fluid Immunoassays for Amphetamine-Type Stimulant Detection in Drivers

The oral fluid immunoassays that have been used for detecting ATS consumption by drivers mostly consist of immunochromatographic tests, produced by the US, German, and British companies (Table 1).

According to the data in Table 1, the oral fluid immunoassays that have been applied worldwide to identify ATS consumption in conductors are directed to the detection of illicitly produced ATSs, mainly AMP, MET, and MDMA[37,38,44–50]. Prescription-only ATSs such as MPH and phentermine (PHT) generally do not crossreact or poorly react with the specific AMP and MET kits used in drug-driving detection. Information about DIE and FEN crossreactivity was not found in the evaluated kits, and most assays employ only *d*-AMP or *d*-MET antibodies, which means that they will detect these target drugs much more efficiently than AMP or MET levo isomers and racemates. Indeed, data from the manufacturers of the kits report crossreactivity inferior to 10% for *l*-AMP or *l*-MET and about 50% for *d*,*l*-AMP.

Many research studies and pilot tests around Europe, Oceania, and North America have used oral fluid immunoassays to detect ATS consumption on roads (Table 1). In some countries, however, they are currently being employed in traffic police routine, to detect drugged drivers.

Since 2007,[31,32] the Portuguese legislation regulates the use of oral fluid screening tests by traffic agents, setting the types and models of immunoassays approved in the country. At present,[33–36] 5 immunochromatographic assays are granted to be used by traffic agents in Portugal (Table 1). In the case of a positive result, the driver is taken to a public health establishment to collect blood for confirmatory tests.[31]

In Australia, roadside drug testing has been routinely and randomly conducted by the police enforcement of several states, thus employing on-site oral fluid immunoassays.[26,30] In the State of Victoria, for example, this has been occurring since 2004,[26,30] with the use of 2 consecutive immunochromatographic assays (Table 1) to detect delta-9-tetrahydrocannabinol (THC), MET, and MDMA in oral fluid samples of drivers.[27] In the case of positive results, oral fluid or blood sample can be used for laboratory confirmation.[28,29]

Experimental Crossreactivity Evaluation

Table 2 shows the results of the crossreactivity tests performed with 3 commercial immunoassays specific for the AMP or MET molecules and the comparison with the manufacturers' technical information. Despite not being reported by international studies (Table 1), the Oral-View Saliva Multidrug of Abuse Test was evaluated experimentally by the authors because it is regularly imported by a national company; it is therefore easily available in Brazil.

In general, the experimental results have corroborated the information regarding manufacturers. The tested immunoassays have shown crossreactivity with the racemates (*d*,*l*-AMP or *d*,*l*-MET) of 50% or near this value, and none of the kits have crossreacted with FEN, DIE, and MPH, either in high concentrations.

Controlled Amphetamine-Type Stimulants Used Around the World

ATS ranks as the second most commonly used drug in the world (*Cannabis* is the first), whereas in 2008, between 13.7 and 52.9 million people aged from 15 to 64 have used ATSs.[43]

Oceania, East and South-East Asia, North America, and West and Central Europe are the regions with the highest prevalence rates of ATS use.[43] However, different ATSs pose different problems for different world regions. In Oceania, illicitly manufactured MET is the most consumed ATS, followed by AMP and MDMA; in Asia and North America, illegal MET and MDMA are more prevalent; in Europe, illicit AMP and MDMA (with a few exceptions such as the Czech Republic and Slovakia, where MET abuse is higher); in the Middle East, illegal AMP; in Africa, illicit MET and ATS containing medicines are the major problem; and in South America, the consumption of ATS mainly occurs through diverted pharmaceuticals (anorectics and psychostimulants).[2,30,43,79–81]

According to the United Nations,[43] the global number of people using ATS has been growing, including the problem of misusing prescribed ATS obtained through the black market and used for nonmedical purposes. Diversion of ATS medicines from licit domestic distribution channels is the major source used to supply illicit markets,[75] and it comprises the use of falsified prescriptions, supplying of substances by pharmacies without the required prescriptions, obtained from persons to whom they were prescribed by physicians, smuggling from other countries, theft in pharmacies, wholesalers, or factories, and illegal trading by means of websites.[75,82,83]

Prescription ATS used as appetite suppressants such as FEN, DIE, PHT, clobenzorex (CLO), benzphetamine (BEZ), and phendimetrazine (PHD) or to treat narcolepsy and attention deficit hyperactivity disorder, such as AMP, MET, and MPH, are under international control since the UN Convention on Psychotropic Substances of 1971,[84] which were part of schedules II and IV of that convention. ATSs were officially recognized to be liable to abuse and the object of illicit traffic, thus requiring rigorous measures to restrict the use of such substances to legitimate medical and scientific purposes.[85] Despite that, misuse and diversion of ATS medicines remain major problems worldwide.[75,86] In the United States, the problem is well documented, thereby making the abuse of prescription medicines more prevalent than the abuse of cocaine, heroin, or MET.[75]

Table 3 shows that among the countries assessed, the United States is the one with the largest number of ATS registered for medical purposes, followed by Mexico and

TABLE 1. Oral Fluid Screening Immunoassays Used Around the World for the Detection of ATS in Drivers

Screening Test	Assay Type	Target Drug (Cutoff in ng/mL)	Crossreactivity (%)* Calculated Based on the Information Provided by the Manufacturer[37,38,44–51]	Country/Year of Kit Use
Cozart DDS/RapiScan (Cozart Biosciences Ltd, United Kingdom)	IMMU	AMP (45)	AMP test: AMP (100); MDA (45); MDMA (0.9); MDEA (0.1); MBDB (0.1); MET and *d*-EPH (<0.04)	Victoria State, Australia/used since 2004[26–29]; Finland/2001[21]; Queensland State-Austrália/2006–2007[4]; Portugal/approved since 2008[34]; Denmark/2000[21]
		MET (50)	MET test: MET (100); MBDB (50); MDMA (17); MDEA (2); ANF, MDA and *d*-EPH (<0.05)	
Cozart microplate EIA (Cozart Biosciences Ltd)	ELISA	*d*-AMP (45)†	AMP test: *d*-AMP (100); MDA (188–216); MET (0.9–1.7); MDMA (1.0–1.8); MDEA (0.1–0.2); MBDB (0.1–0.2); *d*-EPH, *l*-EPH, *d*-PSE, and *l*-PSE (<0.03); MPD (<0.002)	Denmark/2002–2004[1]; Denmark/2000[22]
Immunalysis direct ELISA kits (Immunalysis Corporation)	ELISA	*d*-AMP (50)	AMP test: *d,l*-MDA (178); *d*-AMP (100); *l*-AMP (10); *d*-PSE (1–4); *l*-EPH (0.4–1); *d*-MET (<0.1); *l*-MET (<0.02); *d,l*-MET, *d,l*-MDMA, and *d,l*-MDEA (not detected)	United States/2007[23]
		d-MET (50)	MET test: *d*-MET (100); *d,l*-MDMA (78–98); *d,l*-MDEA (6); *l*-MET (2–3); *d*-PSE (1–4); *l*-EPH (1–2); *d,l*-AMP, *d*-AMP, *l*-AMP, and *d,l*-MDA (<1)	
Dräger drug check (Dräger Safety AG & Co. KGaA, Germany)	IMMU	*d*-AMP (50)	AMP test: *d*-AMP (100); *d,l*-AMP (50); *d,l*-MDA (50); PHT (17); *l*-AMP (5); *d,l*-MDEA (0.5); *d,l*-MDMA (0.2); *d,l*-MET (0.05); EPH and MPH (<0.05).	Germany/2001[19]; Portugal/approved since 2008[33]
		d-MET (50)	MET test: *d*-MET (100); *d,l*-MET (100); *d,l*-MDMA (50); *d,l*-MDEA (1); *d,l*-AMP (0.2); *l*-AMP (0.2); PSE (0.1); *d,l*-MDA (0.05); EPH and MPH (<0.05)	
Drager drug test 5000 (Dräger Safety AG & Co. KGaA)	IMMU	*d*-AMP (50)	AMP test: *d*-AMP (100); *d,l*-MDA (25); PHT, MPH, *d*-EPH, *d*-PSE, and *l*-PSE (<0.05)	Portugal/approved since 2009[36] Belgium/2009[24]
		d-MET (35)	MET test: *d*-MET (100); MBDB (87); MDMA (44); MDEA (5.8); MDA (0.5); *d*-EPH (0.07); PHT, MPH, *d*-PSE, and *l*-PSE (<0.05)	Spain/2004–2005[25]
Drugwipe (Securetec Detektions-Systeme AG, Germany)	IMMU	*d*-AMP (50–200)		Victoria State-Australia/used since 2004[26–29]
		d-MET/MDMA (25–100)		Finland/2001[21] Finland/2004–2005[18] Belgium/1999–2000[16] Portugal/approved since 2008[35] Belgium/2009[24]
Oratec III (Branan Medical Corporation, United States)	IMMU	*d*-AMP (25)	AMP test: *d*-AMP (100); *d,l*-AMP and MDA (62); MDA; MDEA (25); PHT (25); *l*-AMP (3.2); *d,l*-MET, *l*-MET, *d*-MET, and PHY (<0.25)	Portugal/approved since 2008[33]
		d-MET/MDMA (25)	MET/MDMA test: *d*- MET and MDMA (100); *d,l*-MET (83); MDEA (8.3); *l*-MET (5); *d,l*-EPH and *l*-EPH (2.5); *d*-PSE and PHY (0.5), *d,l*-AMP, *l*-AMP, *d*-AMP, and PHT (<0.25)	
RapidSTAT (Mavand Solutions, Germany)	IMMU	*d*-AMP (25) MET (25) MDMA (50)		Belgium/2009[24]
Toxiquick (Biomar Systems, Germany)	IMMU	AMP (500) MET (500)		Germany/2000–2002[17]
Varian Oralab test (Varian, United States)	IMMU	*d*-AMP (50) *d*-MET (50)		Spain/2004–2005[25]

*Crossreactivity (percentage) was calculated as follows: (apparent concentration of the target ATS/ATS concentration of spiked oral fluid) × 100. For immunochromatographic tests, the apparent concentration was the kit cutoff concentration; for ELISA, it was the equivalent target drug concentration calculated from the spiked sample response (absorbance).

†Technical information about the MET kit was not found on the internet and not sent by the manufacturer, upon request. AMP, amphetamine; ELISA, enzyme linked immunosorbent assay; EPH, ephedrine; IMMU, immunochromatographic assay; MBDB, 3,4-methylenedioxyphenyl-2-butanamine); MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylene-dioxymethamphetamine; MET methamphetamine; MPH, methylphenidate; PHT, phentermine; PHY, phenylephrine; PSE, pseudoephedrine.

TABLE 2. EXP and MAN[37–39] Analytical Specificity of Immunalysis Amphetamine (AMP ELISA) and Methamphetamine (MET ELISA) Direct ELISA kits and Oral-View

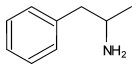
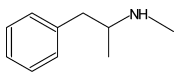
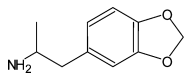
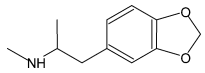
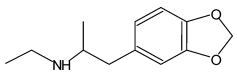
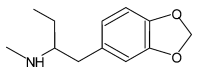
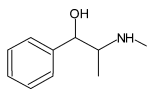
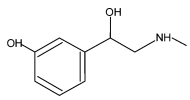
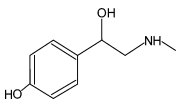
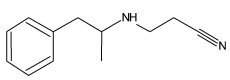
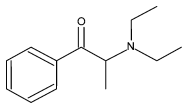
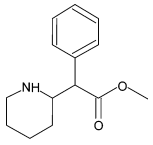
		Crossreactivity (%)*					
		AMP ELISA		MET ELISA		Oral-View	
ATS		EXP	MAN	EXP	MAN	EXP	MAN
d-AMP		†	100		<1		100
d,l-AMP		37		0.14	<1	50	50
l-AMP			10		<1		
d-MET			<0.1		100		
d,l-MET		0.05	n.d.	51			
l-MET			<0.02		2–3		0.1
d,l-MDA		347	178	0.17	<1		50
d,l-MDMA		0.2	n.d.	95	78–98		<5
d,l-MDEA		0.2	n.d.	28	6		<0.5
d,l-MBDB		0.2		19			<0.05
d-PSE		<0.007	1–4	0.6	1–4		
l-EPH		<0.006	0.4–1	0.9	1–2		
l-PHY		<0.006		<0.02			
d,l-SYN		<0.007		<0.02			
d,l-FEN		<0.01		<0.02		<10	

TABLE 2. (*continued*) EXP and MAN[37–39] Analytical Specificity of Immunalysis Amphetamine (AMP ELISA) and Methamphetamine (MET ELISA) Direct ELISA kits and Oral-View

		Crossreactivity (%)*					
		AMP ELISA		MET ELISA		Oral-View	
ATS		EXP	MAN	EXP	MAN	EXP	MAN
d,l-DIE		<0.006		<0.02		<10	
d,l-Threo-MPH		<0.006		<0.02		<10	

*Crossreactivity (percentage) was calculated as follows: (apparent concentration of the target ATS/ATS concentration of spiked oral fluid) x 100. For the immunochromatographic assay Oral-View, the apparent concentration was kit cutoff concentration; for Immunalysis ELISA, it was the equivalent target drug concentration calculated from the spiked sample response (absorbance).

†Not tested.

AMP, amphetamine; ATS, amphetamine-type stimulant; DIE, diethylpropion; EPH, ephedrine; EXP, experimental; FEN, fenproporex; MAN, manufacturer; MBDB, 3,4-methylenedioxyphenyl-2-butanamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylene-dioxymethamphetamine; MET, methamphetamine; MPH, methylphenidate; PHY, phenylephrine; PSE, pseudoephedrine; SYN, synephrine.

Chile. In 2008, the United States was the country with the highest per capita consumption of appetite suppressants, accounting for 58% of the global consumption, and 75% of worldwide use of MPH.[75] When compared with Europe, the Americas (North, Central, and South) have much more approved ATS medicines, a tendency already observed by the United Nations.[75]

MPH is currently marketed in all the countries studied, whereas DIE is commercialized in 29% of them, PHT in 21%, and FEN and AMP in 12% (Table 3). According to the International Narcotics Control Board,[75] MPH is the most used stimulant to treat narcolepsy and attention deficit hyperactivity disorder in the world, whereas PHT, FEN, and DIE are the most frequently used amphetamine-based anorectics. Over the past years, the consumption of anorectics has increased in some countries, such as Australia, Chile, Switzerland, United Kingdom, and United States, and the highest per capita average rates of MPH consumption have been observed in Iceland, United States, Canada, Norway, Israel, Netherlands, and Switzerland.[75]

Nowadays, AMP and MET are available for therapeutic use in a few countries, such as United States, Canada, and Chile (Table 3); the worldwide misuse of AMP and MET is therefore mainly associated with illegal production sources (clandestine laboratories).[43] In fact, AMP and MET can be virtually produced anywhere at a relatively low cost.[43]

Unlike the countries where the use of illegally produced ATS, such as AMP, MET, or methylenedioxy ring−substituted ATS, is a matter of concern, the major problem in countries such as Brazil is the consumption of prescription ATSs, specially DIE and FEN.

The Brazilian Federal Police has only few records of seizures of illegal AMP and MET, and the registers of clandestine laboratories are restricted to MDMA manufacture.[76] Then, AMP and MET are not common in the Brazilian illicit market. In fact, studies analyzing urine and oral fluid of Brazilian workers—including truck drivers—have shown no positive samples for MET,[7,92,93] whereas the positive AMP results have majorly resulted from FEN consumption, because FEN is metabolized to AMP.[7] Other than as expected, the number of Federal Police seizures of medicines containing FEN and DIE have been increasing over the years, whereas it has been observed in 2009 that there were increases of 61% and 183%, respectively, when compared with that in 2008. There are several national studies showing a high prevalence in the consumption of medicines containing DIE and FEN[94,95] especially by professional truck drivers[96–98] aiming to avoid fatigue and therefore being able to keep on driving for longer periods. In general, these pharmaceuticals are irrationally prescribed and used by Brazilians,[94,95] whereas there are many reports of illegal sales at gas stations, tire repair shops, restaurants, snack bars, markets, and even at the commercial trucking companies.[96–98] Also, there are reports about anorectics smuggled from Paraguay,[97] probably FEN, once it is the only anorectic legally marketed in that country (Table 3).

Important Issues

In Brazil, the national traffic code[99] states that driving on public roads with alcohol blood concentration ≥0.6 g/L, or under the influence of any other psychoactive substance, is considered to be a crime subject to arrest, fine, and suspension of the driver's license. The law also establishes that every driver stopped by traffic patrol on the suspicion of driving under the influence of drugs will be subjected to clinical examinations and alcohol and drug tests to verify their condition. However, the only apparatus available for roadside

TABLE 3. Some Prescription ATS Marketed Worldwide[20,52,55–77,87–91*]

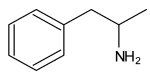
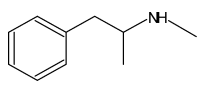
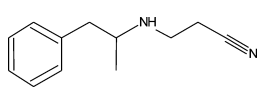
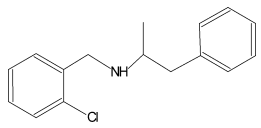
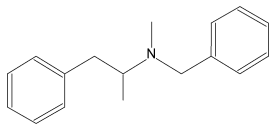
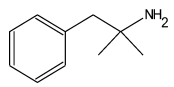
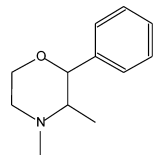
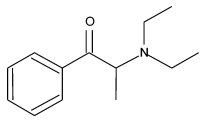
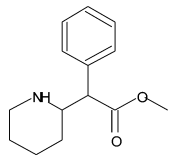
ATS	Molecular Structure	Biotransformed to AMP or MET	BRA	ARG	URY	PRY	COL	VEN	CHL	PER	MEX	USA
AMP			X	X	X	X	X	X	√	X	X	√
MET		AMP	X	X	X	X	X	X	X	X	X	√
FEN		AMP	√	X	X	√	X	X	√	X	X	X
CLO		AMP	X	X	X	X	X	X	X	X	√	X
BEZ		MET, AMP	X	X	X	X	X	X	X	X	X	√
PHT		UNB	X	X	X	X	X	√	X	X	√	√
PHD		UNB	X	X	X	X	X	X	X	X	X	√
DIE		UNB	√	X	X	X	X	X	√	X	√	√
MPH		UNB	√	√	√	√	√	√	√	√	√	√

TABLE 3. (*continued*) Some Prescription ATS Marketed Worldwide[20,52,55–77,87–91*]

ATS	CAN	POR	SPA	ITA	FRA	GER	GBR	NLD	BEL	DNK	AUT	FIN	NOR	AUS
AMP	√	X	X	X	X	X	X	X	X	X	X	X	X	X
MET	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CLO	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BEZ	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PHT	X	X	X	X	X	X	X	X	X	X	√	X	X	√
PHD	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DIE	X	X	X	X	X	√	X	X	X	√	X	X	X	√
MPH	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*Ethylamphetamine, amfetaminil, mefenorex, prenylamine, fenethylline, mesocarb, fencamfamine, dimethylamphetamine, furfenorex, famprofazone, fencamine, fenfluramine, and aminorex are not currently marketed in the countries surveyed.

AMP, d,l-amphetamine or d-amphetamine; ARG, Argentina; AUS, Australia; AUT, Austria; BEL, Belgium; BEZ, benzphetamine; BRA, Brazil; CAN, Canada; CHL, Chile; CLO, clobenzorex; COL, Colombia; DIE, Diethylpropion; DNK, Denmark; FEN, fenproporex; FIN, Finland; FRA, France; GBR, United Kingdom; GER, Germany; ITA, Italy; MET, d-methamphetamine; MEX, Mexico; MPH, methylphenidate; NLD, Netherlands; NOR, Norway; PHD, phendimetrazine; PHT, phentermine; PRY, Paraguay; PER, Peru; POR, Portugal; SPA, Spain; UNB, not biotransformed to AMP or MET; URY, Uruguay; USA, United States of America; VEN, Venezuela; X, not marketed; √, marketed.

police enforcement are breath analyzers to evaluate alcohol consumption. The Brazilian officers do not dispose of any on-site screening kit to attest drug consumption preliminarily, either oral fluid collection devices to take biological samples representative of the driver's condition at the moment of the police approach. In the case of suspected impairment due to drugs other than alcohol, the conductor must be taken to the nearest forensic unit to collect blood and urine samples, which may take >1 hour. Thus, despite legal provisions, Brazil has not yet regulated on-site tests or oral fluid collection devices to be employed by roadside police enforcement.

As already pointed out, several nationwide studies have demonstrated a high prevalence of ATS consumption among Brazilian truck drivers. By means of questionnaires, these surveys have shown a self-reported prevalence of anorectics use (at least once) of 11%,[8] 65%,[98] 66%,[96] and 97%.[97] Yet, some studies employing oral fluid and urine samples to determine ATS consumption of truckers through laboratory tests have shown a prevalence ranging from 0.7% to 4.8%.[7,92] The great lack of concurrence between self-reported ATS use and positive laboratory tests suggests that the tests used in the laboratories have not been adequate to detect the abuse of appetite suppressants by Brazilian truck drivers. The studies mentioned have employed immunoassays or gas chromatography−mass spectrometry with selected-ion monitoring mode directed only to AMP and MET *m/z* fragments, to screen positive samples.

An awareness of the possible misuse of the available assays by Brazilian researchers has set us working on tools to assist the police in the identification of drugged drivers; our research group has been aided by the government to study and evaluate the oral fluid immunoassays used worldwide for ATS detection. Our results showed that most countries have been employing only AMP and MET oral fluid immunoassays to test drivers (Table 1). Besides, these kits are very specific for the detection of the AMP and MET molecules, respectively, and do not crossreact with FEN, DIE, and MPH, not even in very high concentrations (Tables 1 and 2). Although structurally related to AMP and MET molecules, some differences in the chemical structures have resulted in FEN, DIE, and MPH being undetected by the available AMP or MET kits. As DIE and FEN are the most abused ATS on Brazilian roads, an initial screening performed in that country with specific AMP and MET immunoassays would produce a large number of negative results for ATS consumption.

The only appetite suppressant that seems to crossreact with some AMP and MET oral fluid immunoassays is the PHT, due to its high molecular similarity with AMP; yet, its crossreactivity is in general low (<0.05% to 25% as seen in Table 1). This goes against the fact that PHT is the most frequently consumed amphetamine-based anorectic in the world,[75] whereas its detectability was expected to be much better.

Considering that most roadside studies (or routine police applications) employ immunoassays as preliminary drug screening tests and that only positive results in these assays are subsequently submitted to confirmatory techniques, the specificity of the antibodies will determine which drugs can be detected in the evaluated population.

According to Walsh,[100] the immunological screening assays have been designed over the years for the purpose of being increasingly specific and no longer crossreact with other similar compounds; this can be one of the explanations for the lack of concurrence between the number of positive laboratory tests (66% decrease) and self-reported drug use (30% increase) also observed in the United States between 1988 and 2004. Another explanation provided by Walsh is that immunoassays could not be tested for the right drugs. Indeed, most of the current AMP or MET kits (Tables 1 and 2) probably will not detect the US prescription ATS (Table 3), such as MPH, PHD, DIE, and BEZ, either newly appearing drugs such as FEN. Notwithstanding that FEN pills have not been approved for marketing in the United States, over the past years, FEN imported from Brazil has been detected in US residents.[101−103]

Despite the United States having alleged the problem of medicine diversion,[75,86] a recent pilot study[23] conduced to evaluate procedures that would be used in the American national roadside surveys has determined the consumption of psychostimulants among drivers using the same oral fluid AMP and MET ELISA kits, and an additional specific MPH ELISA kit, tested by the authors in "Experimental Cross-reactivity Evaluation." This means that the use of DIE by

drivers could not be detected in the study, whereas the same has probably occurred for PHD and BEZ (Table 3).

The lack of detection of the most common prescription ATSs also occurs in urine samples. In an earlier study performed in the United States,[9] truck drivers have been submitted to field sobriety tests conducted by drug recognition expert officers, in which urine samples were taken and screened for drugs of abuse with the enzyme multiplied immunoassay technique. Fifty percent of the drivers who have been arrested for driving under the influence of central nervous system stimulants—according to the evaluation of drug recognition expert officers—had positive urine results for these compounds. The authors have concluded that this has probably occurred due to very early drug consumption, low immunoassay sensitivity, or due to the presence of stimulants undetected by the kit, hence suggesting that the screening methodology was inappropriate. In fact, the study has casually revealed a variety of ATS in the urine of truckers, ranging from illicit to over-the-counter, including AMP, MET, PHT, phenmetrazine (metabolite of PHD), EPH, and PSE. In a research study conducted in Brazil,[7] employing urine immunological kits to detect ATS use among truck drivers, the authors have also realized that the AMP and MET fluorescence polarization immunoassay used in the screening phase was not appropriate to detect the appetite suppressants abused by truckers. According to their results, FEN has been identified in the confirmation step by using GC−MS in 52.7% of the positive AMP samples, thus showing that AMP was indeed a metabolite of the ingested FEN.

Although recent publications report the use of ATSs by Australian drivers as related to illicitly manufactured drugs such as MET, AMP, and MDMA,[4,104] previous studies have shown that prescription appetite suppressants such as DIE and PHT were also widely used among conductors, especially by truck drivers.[11] Through a questionnaire administered to truck drivers in Western Australia, Mabbott and Hartley have found a prevalence of 20.8% in the consumption of prescription and/or illicit ATS, whereas AMP and MET have been the most abused ones, followed by prescription DIE and PHT (illegally obtained). Through the analysis of blood samples, an investigation of the incidence of drugs in drivers killed in road accidents in Australia between 1990 and 1999[105] has found PHT to be the third most prevalent central nervous system stimulant. In a later study, cases of fatally injured truck drivers that occurred in Victoria between 1999 and 2007 have been reviewed, and 13.1% of these were found to be positive for stimulants, including PHT. As DIE and PHT are still marketed in Australia (Table 1), and the current employed oral fluid (and probably urine) immunoassays are very specific for the AMP and MET molecules, the consumption of such anorectics by drivers is probably underestimated in more recent studies.

In evaluating a survey conducted with car drivers in a Danish rural area, Behrensdorff and Steentoft[22] have discussed the limitations of the 2 oral fluid screening immunoassays employed, thus highlighting that some frequently used medicines in Denmark could not be detected by these kits. As shown in Table 3, DIE is an anorectic currently marketed in Denmark, and the kits used in the study probably have not crossreacted with this substance (Table 1).

The oral fluid immunoassays that have been used for identifying ATS in drivers (Table 1) restrict not only the detection of prescription ATS but also the clandestinely manufactured ones. According to the UN Office on Drugs and Crime,[106] illicit AMPs are typically encountered as *d,l*-AMP, for which oral fluid immunoassays (Tables 1 and 2) have shown low to regular crossreactivity (37%−62%). The immunological kits usually employ only antibodies for *d*-AMP and therefore are not ideal for the detection of illegal AMP. The same does not occur with MET, because it is frequently seen in the illicit market, either as the dextro enantiomer or as the racemic mixture[79,106]; besides, both crossreact well with some available kits (Tables 1 and 2).

The issue of *d,l*-AMP low crossreactivity of oral fluid immunoassays also hinders the indirect detection of FEN, once it is marketed as racemate[107] and metabolizes to *d,l*-AMP.[23,108,109] The same will not occur with BEZ and CLO, as they metabolize to *d*-AMP.[23,88,110]

In summary, it is very important to make a previous study of what ATS are being legally and illegally sold in a country, before selecting the immunoassays that will be employed to evaluate the ATS consumption by drivers of that country. By choosing only highly specific AMP and MET kits, the real problem of ATS misuse by drivers could be underestimated.

CONCLUSIONS AND PERSPECTIVES

Although structurally related to the molecules of AMP and MET, ATS as DIE, FEN, CLO, BEZ, PHD, PHT, and MPH are generally not detected by the available AMP and MET oral fluid immunoassays. DIE, FEN, and MPH are the most used prescription ATS in several countries and, to the knowledge of the authors, few companies have developed specific immunoassays for the detection of MPH in oral fluid, and none has produced such tests for FEN and DIE. Thus, this evidences the necessity of developing new ATS immunoassays to enable the detection of prescription stimulant misuse on roads through oral fluid tests. Considering the structural variability of the ATS, ideal immunoassays designed to drug driving control must consist of a pool of mono or polyclonal antibodies directed to each different ATS molecule.

We therefore agree with some recent studies[41,42,111,112], as regards the fact that despite being a promising technique, the oral fluid drug detection immunoassays still need to be more developed before they can be massively introduced on worldwide police routine. In addition to problems such as low sensitivity and the large number of false-positive results already extensively discussed for some ATS immunoassays,[22,25,40−42] we consider a major cause of concern as the absence of kits that detect all the controlled ATS legally marketed in countries worldwide; it follows that the nondetection of prescription ATS can lead to erroneous estimates of the stimulants consumed by drivers. Indeed, the International Narcotics Control Board[75] has recommended that authorities give special attention to the problem of prescription drugs abuse, and whenever it is possible, to include the monitoring of abuse and misuse of controlled medicines in their national surveys.

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