Short Communication

Evaluation of five drug screening devices for testing of amphetamines and methamphetamines

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Abstract Triage® DOA is widely used for the on-site screening of drugs of abuse. However, it often provides false positive results for amphetamine due to interference by putrefactive amines, such as 2-phenethylamine, produced by saprogenic bacteria in moderately-to-heavily decomposed bodies. In the present study, we evaluated the performance of five drug screening devices: Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1, Status DS10 and DRIVEN-FLOW M8-Z. A total of 19 forensic autopsy urine samples, which were positive for amphetamines by Triage® DOA, were analyzed with the five drug screening devices and liquid chromatography tandem mass spectrometry. Only DRIVEN-FLOW M8-Z had no false positive or false negative results for methamphetamines. Triage® TOX Drug Screen and IVeX-screen M-1 each had one false positive result for methamphetamines. Other devices, including Triage® TOX Drug Screen, had multiple false positive and false negative results for amphetamines and methamphetamines. These results suggest that DRIVEN-FLOW M8-Z is more useful than other screening devices for screening of methamphetamines in the presence or absence of 2-phenethylamine, while none of the tested devices detected amphetamines precisely. It is necessary to develop platforms that can precisely detect both amphetamines and methamphetamines.

Key words: drug screening devices, amphetamine, methamphetamine, 2-phenethylamine, liquid chromatography tandem mass spectrometry

Introduction

The detection of drugs of abuse in biological samples is an important aspect of forensic toxicological examination[1]. Methods used for this purpose should be rapid, easy to handle and reliable[2] and should readily detect drugs or their metabolites from urine, the most commonly used matrix in forensic drug testing[2]. One platform for urine testing, the Triage® Drugs of Abuse (DOA) panel, is based on a competitive immunoassay that allows a qualitative determination of the presence of multiple drugs and is designed to provide simultaneous and discrete visual detection of seven drug classes in approximately ten minutes[3−5]. Although Triage® DOA has been widely used, this device frequently leads to false positive results in the detection of amphetamines. These false positive results are exacerbated by the production of putrefactive amines, such as 2-phenethylamine, by saprogenic bacteria in moderately or heavily decomposed bodies[3,6,7]. Therefore, additional technologies

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are needed in order to optimize forensic analyses, especially in regard to the detection of amphetamines.

Newer screening devices that utilize competitive immunoassay technology to detect drugs of abuse have been developed. In the present study we evaluated the performance of five such drug screening devices, Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1, Status DS10 and DRIVEN-FLOW M8-Z. A total of 19 urine samples from forensic autopsies that were positive for amphetamines according to Triage® DOA were analyzed with the five drug screening devices. The presence or absence of methamphetamine and amphetamine were confirmed by liquid chromatography tandem mass spectrometry (LC-MS/MS). We then compared the sensitivities and specificities of the five screening devices in the detection of amphetamines and methamphetamines.

Materials and Methods

Chemicals and materials

Amphetamine was kindly donated by Dr. Kenji Hara (Fukuoka University). Methamphetamine was purchased from Dainihonseiyaku (Osaka, Japan). 2-Phenethylamine was purchased from Fujifilm Wako Pure Chemical (Osaka, Japan). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Fujifilm Wako Pure Chemical (Osaka, Japan). Other common chemicals used were of the highest purity commercially available. Laboratory distilled water was purified using a Direct-Q UV 3 (Millipore, Molsheim, France).

Urine samples

Human urine samples were obtained from autopsy cadavers at Aichi Medical University from 2016 to 2021. Samples were collected in 5- or 15-mL tubes and stored at −80°C until analysis.

Devices and their principles of detection

Triage® DOA (Alere San Diego, CA, USA), Triage® TOX Drug Screen (Alere San Diego), SIGNIFY™ ER (Innovacon, CA, USA), IVeX-screen M-1 (Biodesign, Tokyo, Japan), Status DS10 (LifeSign, Skillman, NJ) and DRIVEN-FLOW M8-Z (Alfa Scientific Designs, CA, USA) were assessed in this study. All five devices are based on a competitive immunoassay and give qualitative responses to the presence or absence of drugs in urine. Cutoff values of amphetamines and methamphetamines for these devices are shown in Table 1.

LC-MS/MS analysis

Human urine sample (100 μL) was mixed with 100 μL methanol and 200 μL acetonitrile. The mixture was vortexed for 60 s and centrifuged at 15,000 g for 10 min, and the supernatant was transferred to another tube, followed by addition of 100 μL of 0.1% TFA in acetonitrile, and evaporated with a centrifugal evaporator (CVE-200D; Tokyo Rikakikai, Tokyo, Japan). The residue was reconstituted in 100 μL methanol and centrifuged at 15,000 g for 1 min. A 5-μL aliquot of supernatant was used for the analysis by LC-MS/MS. The samples containing high concentrations of target compounds were analyzed after dilution as needed.

LC-MS/MS analysis was performed using a Nexera X2 liquid chromatograph coupled to an LCMS-8040 mass spectrometer (Shimadzu, Kyoto, Japan). For separation, a Kinetex column (2.1 mm I.D.×100 mm, particle size 2.6 μm; Phenomenex, Cheshire, UK) was used. The column temperature was maintained at 40°C. The gradient system used for separation included mobile phase A (a solution of 0.1% formic acid in 10 mM ammonium formate in water) and mobile phase B (a solution of 0.1% formic acid in 10 mM ammonium formate in methanol). The flow rate was 0.5 mL/min. The elution gradient involved a linear increase from 5% B to 95% B over 3.0 min, followed by constant 95% B for 1.5 min. The mobile phase was then returned to 5% B over 0.01 min and maintained at 5% B for 3.0 min to equilibrate the column for the next sample. The desolvation

Table 1. Cutoff values of amphetamines and methamphetamines for five drug screening devices

Drug name	Abbreviation used on the test	Cutoff values (ng/mL)				
		Triage® TOX Drug Screen	SIGNIFY™ ER	IVeX-screen M-1	Status DS10	DRIVEN-FLOW M8-Z
Amphetamines	AMP	1000	1000	—	1000	—
Methamphetamines	MET, mAMP or METH	1000	—	500	1000	500

Table 2. Summary of results for five drug screening devices and LC-MS/MS

Case	Triage® TOX Drug Screen		SIGNIFY™ ER	IVeX-screen M-1	Status DS10		DRIVEN-FLOW M8-Z	LC-MS/MS*		
	AMP	mAMP	AMP	METH	AMP	MET	METH	2-phenethylamine	Methamphetamine	Amphetamine
1	+	+	+	+	+	+	+	−	+ (2.89)	+ (0.84)
2	−	+	−	+	−	−	−	−	−	−
3	+	−	−	−	−	−	−	+ (0.35)	−	−
4	−	−	+	−	+	−	−	+ (45.8)	−	−
5	−	−	−	−	−	−	−	+ (0.016)	−	−
6	−	−	−	−	+	−	−	+ (2.14)	−	−
7	+	−	+	−	+	+	−	+ (77.6)	−	−
8	+	−	+	−	+	−	−	+ (38.7)	−	−
9	−	−	+	−	+	−	−	+ (0.44)	−	−
10	+	−	−	−	−	−	−	+ (0.004)	−	−
11	−	−	+	−	+	+	−	+ (13.6)	−	−
12	−	−	+	−	+	−	−	+ (3.02)	−	−
13	−	+	+	+	−	+	+	−	+ (10.5)	+ (1.68)
14	+	+	+	+	+	+	+	+ (0.005)	+ (151)	+ (4.70)
15	+	−	+	−	+	−	−	+ (4.39)	−	−
16	+	−	+	−	−	+	−	+ (71.7)	−	−
17	−	−	+	−	−	+	−	+ (0.51)	−	−
18	+	−	+	−	−	+	−	+ (20.4)	−	−
19	−	−	−	−	−	−	−	+ (1.50)	−	−

*Numbers in parentheses indicate the found concentrations (μg/mL).

line temperature and heat block temperature were 250°C and 400°C, respectively. Electrospray ionization was applied in the positive mode. Quantification was performed by selected reaction monitoring (SRM) using the peak area. The SRM transitions were *m/z* 122→105 for 2-phenethylamine, *m/z* 136→91 for amphetamine and *m/z* 150→91 for methamphetamine.

Data analysis

Sensitivity means ability to detect the presence of a target compound and was calculated as follows: Sensitivity=true positives/(true positives+false negatives). Specificity implies ability to detect the absence of a target compound and was calculated as follows: Specificity=true negatives/(true negatives+false positives).

Ethics approval

All experiments were approved by the Ethics Committee of Aichi Medical University (approval no. 2020-172).

Results

A total of 19 urine samples that were positive for amphetamines according to Triage® DOA were obtained from forensic autopsies. These samples were subjected to analysis with five other drug screening devices, Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1, Status DS10 and DRIVEN-FLOW M8-Z, and they were also analyzed by LC-MS/MS (Table 2). LC-MS/MS analysis revealed that three samples (cases 1, 13 and 14) contained both amphetamine and methamphetamine, and 16 samples contained 2-phenethylamine. A representative LC-MS/MS analysis of a urine sample (case 14) shows peaks with the same retention times (3.38 min, 3.47 min and 2.69 min) as those of standard amphetamine, methamphetamine and 2-phenethylamine (Fig. 1a). Diagnostic fragment ions and their ion abundance ratios were also fully consistent with those of standard amphetamine, methamphetamine and 2-phenethylamine, thus confirming the presence of these compounds in this urine sample (Fig. 1b).

The Triage® TOX Drug Screen system returned seven false positive results for amphetamines, one false positive result for methamphetamines, and false negative result for amphetamines. The SIGNIFY™ ER system led to ten false positive results for amphetamines. The IVeX-screen M-1

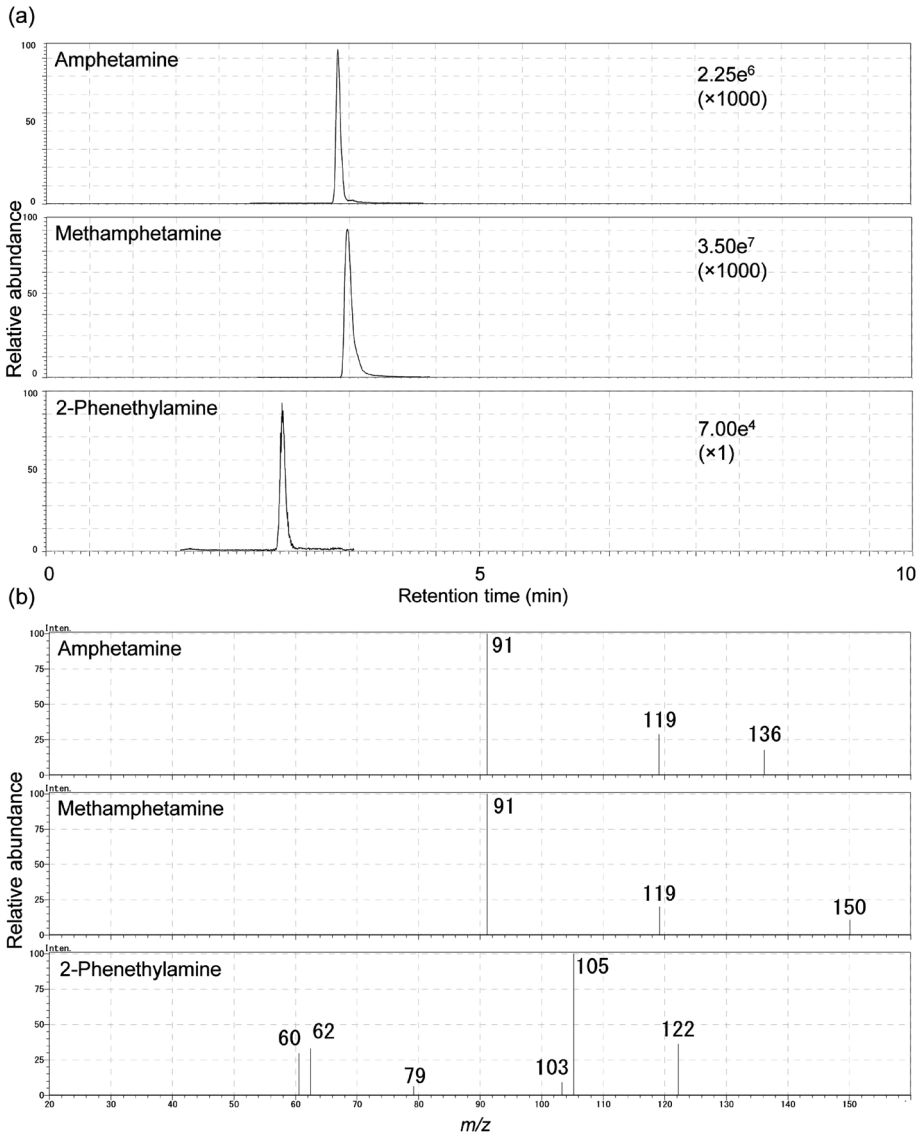

Fig. 1. Representative LC-MS/MS analysis of an extract of urine sample (case 14).
(a) Selective ion monitoring liquid chromatograms show the same retention times (3.38 min, 3.47 min and 2.69 min) as those of standard amphetamine, methamphetamine and 2-phenethylamine. Amphetamine and methamphetamine were analyzed after 1000 fold dilution. The SRM transitions were *m/z* 136→91 for amphetamine, *m/z* 150→91 for methamphetamine and *m/z* 122→105 for 2-phenethylamine. (b) Diagnostic fragment ions with ion abundance ratios of the noted fractions from the urine sample shown here were fully consistent with those of standard amphetamine, methamphetamine and 2-phenethylamine, thus confirming the presence of these compounds in this urine sample.

system led to one false positive result for methamphetamines. The Status DS10 system returned eight false positive results for amphetamines, five false positive results for methamphetamines and one false negative result for amphetamines. The DRIVEN-FLOW M8-Z system returned no false positive or false negative results for methamphetamines. In case 2, the Triage® TOX Drug Screen and IVeX-screen M-1 systems for methamphetamines showed false positive results even though 2-phenethylamine was not present in urine. In case 1, the results of Triage® TOX Drug Screen and Status DS10 were accurately positive even in the presence of low concentration of amphetamine (0.84 μg/mL) near the cutoff value. However, in case 13, these two devices showed false negative results in the presence of high concentration of amphetamine (1.68 μg/mL). Clear relationship between false positive results and concentration of 2-phenethylamine in urine was not found although false positive results tended to appear with the concentration of 2-phenethylamine. Another putrefactive amines such as tyramine and tryptamine might affect the results. These discordant results remain unresolved and further investigations to clarify the reasons are

Table 3. Sensitivity (Sens) and specificity (Spec) of the five drug screening devices

Drug name	Triage® TOX Drug Screen		SIGNIFY™ ER		IVeX-screen M-1		Status DS10		DRIVEN-FLOW M8-Z	
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
Amphetamines	0.67	0.56	1.0	0.38	−	−	0.67	0.50	−	−
Methamphetamines	1.0	0.94	−	−	1.0	0.94	1.0	0.69	1.0	1.0

necessary.

Calculated sensitivities and specificities are shown in Table 3. The Triage® TOX Drug Screen for methamphetamines, SIGNIFY™ ER for amphetamines, IVeX-screen M-1 for methamphetamines, Status DS10 for methamphetamines and DRIVEN-FLOW M8-Z for methamphetamines showed high sensitivities (1.0), whereas the Triage® TOX Drug Screen for amphetamines and Status DS10 for amphetamines provided decreased sensitivities (0.67). The DRIVEN-FLOW M8-Z for methamphetamines showed high specificity (1.0). The Triage® TOX Drug Screen for methamphetamines and IVeX-screen M-1 for methamphetamines revealed slightly decreased specificities (0.94). The Triage® TOX Drug Screen for amphetamines and Status DS10 for amphetamines and methamphetamines had moderate specificities (0.50−0.69). The SIGNIFY™ ER for amphetamines showed the worst specificity (0.38).

Discussion

In the present study, we compared five drug testing platforms with regard to their performance in detecting amphetamines and methamphetamines in forensic urine samples. One of these platforms, DRIVEN-FLOW M8-Z, was found to be more useful than the other four screening devices, especially in cases when 2-phenethylamine was present. This device was sufficiently sensitive, in that it identified the three samples that were shown by LC-MS/MS to be positive for methamphetamines and thus resulted in no false negative results. DRIVEN-FLOW M8-Z has a further advantage in that visual determination is possible for up to two hours after a urine sample is applied to the test strip.

Immunoassays, as analyzed in the present study, represent the primary screening method for the detection of amphetamines and methamphetamines. Different commercial drug screening devices use either monoclonal or polyclonal antibodies against amphetamine and methamphetamine[6]. The antibodies bind to antigenic determinants (epitopes) on antigens, including the amino group of amphetamine, which is a primary amine, or the amino group of methamphetamine, which is a secondary amine[6]. Because the antibodies recognize relatively common chemical structures, amphetamine and methamphetamine immunoassays are especially prone to false positive results compared to assays for other drugs of abuse. Specifically, in this case, there tends to be a broad range of cross-reactivity of the antibodies to compounds having structural similarity to phenethylamines[6]. Accordingly, Triage® DOA, Triage® TOX Drug Screen, SIGNIFY™ ER and Status DS10 had unacceptably high rates of false positive results. Differences in cross-reactivities of the antibodies likely explain the differential specificities of these devices, and a focus on the further development of monoclonal antibodies may yield systems with even higher specificities.

The relatively high overall rate of errors, including both false positives and false negatives, was noteworthy. While DRIVEN-FLOW M8-Z caused no false positive results and the use of Triage® TOX Drug Screen and IVeX-screen M-1 led to only a single false positive result for methamphetamines, other devices, including the commonly used Triage® TOX Drug Screen for amphetamines, led to multiple false positive and false negative results. These errors would have important influences on forensic analyses. Fortunately, LC-MS/MS is available as a powerful technique for the identification and quantification of target compounds. In the present study, we were able to confidently confirm the presence or absence of amphetamine, methamphetamine and 2-phenethylamine with LC-MS/MS. These results suggest that positive results using the screening devices should be considered preliminary and should always be confirmed by LC-MS/MS.

Among the five drug screening devices tested in the present study, only Triage® TOX Drug Screen, which is used in conjunction with a portable Alere Triage® Meter fluorescence spectrometer, does not require the visual interpretation of colored lines[7−9]. The results of the other four devices are determined visually[10,11]. In a visual determina-

tion, the operator must read the results at a fixed time after application and may hesitate about a judgment when the sample drug concentrations are near the immunoassay cut-off values[9,11]. Therefore, combining the automated reading feature of the Triage® TOX Drug Screen with the accuracy of the DRIVEN-FLOW M8-Z system would be expected to lead to an optimized detection tool.

In conclusion, among the five drug screening devices tested in the present study, DRIVEN-FLOW M8-Z was found to be the most useful for the screening of methamphetamines in urine and was accurate even when 2-phenethylamine was present. None of the tested devices was perfectly accurate in the detecting of amphetamines, suggesting a need within the forensic drug screening field. The Triage® TOX Drug Screen system, which is an instrument-read test, also has important advantages in the screening of methamphetamines. It is necessary to develop platforms that can precisely detect both amphetamines and methamphetamines.

Conflict of Interest

The authors declare no conflicts of interest.

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