Research Paper

Evaluation of four drug screening devices for detection of psychoactive drugs in pericardial fluid

Masae Iwai[1,2]*, Tadashi Ogawa[1,2], Tomohito Matsuo[1,2],
Katsutoshi Kubo[1,3], Fumio Kondo[1,4], Hiroshi Seno[1,2]

[1] Department of Legal Medicine, Aichi Medical University School of Medicine,
1−1 Yazakokarimata, Nagakute, Aichi 480−1195, Japan
[2] Poison Analysis Center, Aichi Medical University School of Medicine,
1−1 Yazakokarimata, Nagakute, Aichi 480−1195, Japan
[3] Department of Oral Pathology/Forensic Odontology, Aichi Gakuin University School of Dentistry,
1−100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464−8650, Japan
[4] Department of Biomedical Sciences, Chubu University College of Life and Health Sciences,
1200 Matsumoto-cho, Kasugai, Aichi 487−8501, Japan

Abstract We evaluated the applicability of four immunoassay-based drug screening devices, Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1, and DRIVEN-FLOW M8-Z, in the detection of psychoactive drugs in pericardial fluid as an alternative to urine. A total of 38 pericardial fluid samples from forensic autopsies were analyzed with the four drug screening devices. To confirm the results, the concentrations of psychoactive drugs in pericardial fluid samples were measured together with those in urine and blood samples by liquid chromatography-tandem mass spectrometry. Only IVeX-screen M-1 precisely detected psychoactive drugs without false positive results, whereas Triage® TOX Drug Screen and SIGNIFY™ ER showed several false positive results, and DRIVEN-FLOW M8-Z led to many false positive results. These results suggest that IVeX-screen M-1 is more useful than other screening devices for psychoactive drugs in pericardial fluid and that pericardial fluid is a valid alternative material when urine is not available.

Key words: drug screening devices, psychoactive drugs, pericardial fluid, liquid chromatography-tandem mass spectrometry

Introduction

The detection of possible drug consumption is a central aspect of forensic toxicological examinations[1)]. Devices used for urine drug screening can provide a rapid indication of the presence of analytes of interest during autopsy[2)], but these devices are associated with several drawbacks. For example, Triage® DOA frequently returns false positive results in the detection of amphetamines[3−5)]. These false positive results are particularly important in the context of forensic autopsies, as they can be exacerbated by the production of putrefactive amines, such as 2-phenethylamine, by saprogenic bacteria in moderately or heavily decomposed bodies.

In our previous report, 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, in the detection of amphetamines and methamphetamines in urine containing putrefactive amines. The results suggested that DRIVEN-FLOW M8-Z was 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[6)].

Another issue with the forensic detection of drug use is

* **Corresponding author**
Masae Iwai
Department of Legal Medicine, Aichi Medical University School of Medicine, 1−1 Yazakokarimata, Nagakute, Aichi 480−1195, Japan
E-mail: masaoshi@aichi-med-u.ac.jp
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that collection of urine is not always feasible. In these cases, blood, pericardial fluid, cerebrospinal fluid, vitreous humor, or other alternative fluids must be analyzed. In particular, pericardial fluid often can be readily obtained from the pericardial cavity. Typical volumes of pericardial fluid taken at the time of autopsy range from 5 to 35 mL[7]; this amount is sufficient for forensic drug testing. Because of the potential utility of this material in forensic analyses, in the present study we evaluated the applicability of four drug screening devices, Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1, and DRIVEN-FLOW M8-Z, to the detection of psychoactive drugs in pericardial fluid from autopsy samples. In this analysis, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed as a sensitive and quantitative tool to compare the performance of these immunoassay-based devices.

Materials and Methods

Chemicals and materials

Amphetamine was kindly provided by Dr. Kenji Hara (Fukuoka University). The following standard drugs were used: methamphetamine (Dainihonseiyaku, Osaka, Japan); amobarbital (NIPPON SHINYAKU, Kyoto, Japan); bromazepam and flunitrazepam (Eisai, Tokyo, Japan); brotizolam (Sumitomo Pharma, Tokyo, Japan); estazolam (Takeda Pharmaceuticals, Tokyo, Japan); phenobarbital, amitriptyline, nortriptyline, diazepam and triazolam (Fujifilm Wako Pure Chemical, Osaka, Japan); temazepam, zolpidem, diazepam-d_5, and phenobarbital-d_5 (Sigma-Aldrich, St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade methanol was obtained 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 system (Millipore, Molsheim, France).

Biological samples

Pericardial fluid, urine and blood samples were obtained from autopsy cadavers at Aichi Medical University from 2021 to 2023. Samples were collected in 5- or 15-mL tubes and stored at −80°C until analysis. Additionally, pericardial fluid samples were assessed without any pretreatment.

Devices and their principles of detection

Triage® TOX Drug Screen (Alere San Diego, CA, USA), SIGNIFY™ ER (Innovacon, CA, USA), IVeX-screen M-1

Table 1. Cutoff values of psychoactive drugs for four drug screening devices

Drug classification	Drug name	Abbreviation*1	Cutoff values (ng/mL)			
			Triage® TOX Drug Screen	SIGNIFY™ ER	IVeX-screen M-1	DRIVEN-FLOW M8-Z
Barbiturates	Phenobarbital	BAR	230	100	150	2200
	Amobarbital	BAR	250	300	200	2000
Sedative-hypnotics	Bromazepam	BZO/BZD	750	1562	600	500
	Brotizolam	BZO/BZD	—*2	—*2	＞10000	—*2
	Estazolam	BZO/BZD	400	2500	400	—*2
	Flunitrazepam	BZO/BZD	200	390	400	—*2
	Lorazepam	BZO/BZD	200	1562	2000	550
	Midazolam	BZO/BZD	200	12500	10000	—*2
	Temazepam	BZO/BZD	200	98	—*2	—*2
	Triazolam	BZO/BZD	100	2500	600	300
	Zolpidem	ZOL	—*2	—*2	50	50
Tricyclic antidepressants	Amitriptyline	TCA	600	1500	300	1000
	Nortriptyline	TCA	900	1000	1000	1000
Stimulants	Amphetamine	AMP	500	1000	100000	85000
	Methamphetamine	mAMP/METH/MET	500	—*2	500	500

*1BAR; barbiturates, BZO/BZD; benzodiazepines, ZOL; zolpidem, TCA; tricyclic antidepressants, AMP; amphetamines, mAMP/METH/MET; methamphetamines.
*2Not available.

(Biodesign, Tokyo, Japan) and DRIVEN-FLOW M8-Z (Alfa Scientific Designs, CA, USA) were assessed in this study. All four devices are based on a competitive immunoassay and give qualitative responses to the presence or absence of drugs, and they are recommended to be used for urine only by their manufacturers. Cutoff values that are used by the devices to determine positive results are shown in Table 1.

LC-MS/MS analysis

Samples of human pericardial fluid, urine, or blood (100 μL) were mixed with 100 μL methanol and 200 μL acetonitrile after addition of 40 μL internal standard solution (0.5 μg/mL diazepam-d_5 and 25 μg/mL phenobarbital-d_5). 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% trifluoroacetic acid in acetonitrile. The solvent was removed 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 subjected to analysis by LC-MS/MS. Samples containing high concentrations of target compounds were analyzed after dilution as needed.

LC-MS/MS analyses were 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 line temperature and heat block temperature were 250°C and 400°C, respectively.

Electrospray ionization was applied in the negative mode for phenobarbital and amobarbital and positive mode for other compounds. Quantification was performed by selected reaction monitoring (SRM) using the peak area. The SRM transitions were *m/z* 231→42 for phenobarbital, *m/z* 225→42 for amobarbital, *m/z* 136→91 for amphetamine, *m/z* 150→91 for methamphetamine, *m/z* 278→91 for amitriptyline, *m/z* 264→233 for nortriptyline, *m/z* 316→209 for bromazepam, *m/z* 393→314 for brotizolam, *m/z* 285→193 for diazepam, *m/z* 295→267 for estazolam, *m/z* 314→268 for flunitrazepam, *m/z* 301→255 for temazepam, *m/z* 343→308 for triazolam, *m/z* 308→235 for zolpidem, *m/z* 290→154 for diazepam-d_5 and *m/z* 236→42 for phenobarbital-d_5.

Ethics approval

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

Results

A total of 38 pericardial fluid samples with matched urine and blood samples were obtained during forensic autopsies. The presence of psychoactive drugs was first evaluated in urine by Triage® TOX Drug Screen or SIGNIFY™ ER. Nine urine samples (cases 1–9) tested positive for the presence of at least one psychoactive drug (Table 2). The concentrations of the drugs in urine and blood samples were also determined by LC-MS/MS. The concentrations of drugs that were present in these samples were determined to be within the ranges of 2.0 to 20,300 ng/mL in urine samples (Table 2) and 0.50 to 3230 ng/mL in blood samples (Table 3). Because of low sample volumes, four urine and three blood samples were not analyzed by LC-MS/MS.

Pericardial fluid samples were then subjected to analysis with four drug screening devices: Triage® TOX Drug Screen, SIGNIFY™ ER, IVeX-screen M-1 and DRIVEN-FLOW M8-Z; these samples were also analyzed by LC-MS/MS. LC-MS/MS analysis revealed that 9 samples (cases 1, 2, 3, 6, 7, 8, 9, 11, 14 and 16) contained at least one type of psychoactive drug at a concentration between 9.8 and 3270 ng/mL (Table 4). A representative LC-MS/MS analysis of a pericardial fluid sample (case 7) shows peaks with the same retention times as those of standard amitriptyline (6.25 min) and nortriptyline (6.30 min) (Fig. 1a). Diagnostic fragment ions and their ion abundance ratios were also fully consistent with those of standard amitriptyline and nortriptyline, thus confirming the presence of these compounds in this pericardial fluid sample (Fig. 1b).

The results from the application of the four drug screening devices to pericardial fluid samples are summarized in Table 4. The Triage® TOX Drug Screen device returned

Table 2. Summary of results for drug screening devices and LC-MS/MS in urine samples

Case	drug screening device	Positive test result	LC-MS/MS (concentration, ng/mL)
1	Triage® TOX Drug Screen Drug Screen	mAMP, BZO, BAR	Phenobarbital (4630), Methamphetamine (1140), Amphetamine (247), Estazolam (37.3)
2	Triage® TOX Drug Screen	AMP, mAMP, BZO	Methamphetamine (1520), Amphetamine (317), Bromazepam (150),
3	SIGNIFY™ ER	BAR	Phenobarbital (6250)
4	Triage® TOX Drug Screen	mAMP, BZO	Methamphetamine (13260), Amphetamine (970), Temazepam (55.3), Diazepam (7.0)
5	Triage® TOX Drug Screen	BZO	Lorazepam (4.0)
6	Triage® TOX Drug Screen	BAR	Phenobarbital (1030)
7	Triage® TOX Drug Screen	BZO, TCA	Amitriptyline (513), Nortriptyline (200), Triazolam (1.2), Flunitrazepam (0.89)
8	Triage® TOX Drug Screen	BAR	Amobarbital (380)
9	Triage® TOX Drug Screen	AMP, mAMP, BZO	Methamphetamine (20300), Amphetamine (2680), Brotizolam (7.4), Flunitrazepam (5.2)
10	Triage® TOX Drug Screen	—	—
11	SIGNIFY™ ER	—	NT*
12	SIGNIFY™ ER	—	NT*
13	Triage® TOX Drug Screen	—	NT*
14	Triage® TOX Drug Screen	—	NT*
15	Triage® TOX Drug Screen	—	—
16	Triage® TOX Drug Screen		Zolpidem (0.20)
17–28	Triage® TOX Drug Screen or IVeX-screen M-1	—	—
29–38	Triage® TOX Drug Screen or SIGNIFYTM ER	—	—

*Not analyzed due to insufficient sample quantity.

Table 3. Quantification of psychoactive drugs in blood samples by LC-MS/MS

Case	Psychoactive drug (concentration, ng/mL)
1	Phenobarbital (3230), Estazolam (117), Methamphetamine (28.0), Amphetamine (5.1), Flunitrazepam (13.3)
2	Bromazepam (280), Methamphetamine (40.4), Nitrazepam (23.5), Amphetamine (17.5)
3	Phenobarbital (2610)
4	Methamphetamine (23.0), Temazepam (3.1), Amphetamine (3.0)
5	Lorazepam (3.7)
6	Phenobarbital (1750)
7	Amitriptyline (986), Nortriptyline (221), Flunitrazepam (6.9), Clonazepam (4.8), Triazolam (2.9)
8	Amobarbital (670)
9	Methamphetamine (190), Amphetamine (38.3), Flunitrazepam (19.1)
10	—
11	Midazolam (3.4)
12	NT*
13	NT*
14	NT*
15	—
16	Zolpidem (0.50)
17–28	—
29–38	—

*Not analyzed due to insufficient sample quantity.

Table 4. Summary of results for four drug screening devices and LC-MS/MS in pericardial fluid samples

Case	Triage® TOX Drug Screen	SIGNIFY™ ER	IVeX-screen M-1	DRIVEN-FLOW M8-Z	LC-MS/MS (concentration, ng/mL)
1	BAR	BAR	BAR	TCA*2, ZOL*2	Phenobarbital (744), Methamphetamine (7.0), Amphetamine (0.95) Estazolam (35.8)
2	—	BZO*3, TCA*2	—	TCA*2, ZOL*2	Bromazepam (24.1), Methamphetamine (8.9), Amphetamine (2.5)
3	BAR	BAR	BAR	BAR, ZOL*2	Phenobarbital (3270)
4	AMP*1	—	—	ZOL*2	Methamphetamine (6.3), Amphetamine (0.60)
5	—	—	—	ZOL*2	—
6	BAR	BAR	BAR	ZOL*2	Phenobarbital (505)
7	TCA*3	TCA*3	TCA	TCA*3	Amitriptyline (488), Nortriptyline (33.1)
8	BAR*3	—	BAR*3	ZOL*2	Amobarbital (116)
9	BZO*1	AMP*3	METH*3	MET	Methamphetamine (73.2), Amphetamine (9.8)
10		TCA*2	—	TCA*2, ZOL*2	—
11	BZO*3	—	—	ZOL*2	Midazolam (1.6)
12	—	TCA*2	—	TCA*2, ZOL*2	—
13	BZO*2	—	—	ZOL*2	—
14	BZO*3	—	—	TCA*2, ZOL*2	Midazolam (38.7)
15	*1	—	—	ZOL*2	—
16	—	—	—	ZOL*3	Zolpidem (0.928)
17–28	—		—	ZOL*2	—
29–38	—			TCA*2, ZOL*2	—

*1Undeterminable. *2False positive.
*3Positive even though the sample drug concentrations determined by LC-MS/MS analyses were under the cutoff values.

three ambiguous results (cases 4, 9 and 15) for unknown reasons, and it returned one false positive result for benzodiazepines (case 13). The SIGNIFY™ ER device produced three false positive results for tricyclic antidepressants. The IVeX-screen M-1 device returned no false positive results. The DRIVEN-FLOW M8-Z device returned false positive results for zolpidem or tricyclic antidepressants in 35 of the 38 cases.

Eleven samples (cases 2, 7, 8, 9, 11, 14 and 16) tested positive for psychoactive drugs according to drug screening devices, even though the sample drug concentrations determined by LC-MS/MS were under the immunoassay cutoff values that are considered to indicate positive results (Table 4). Therefore, to investigate this potential discrepancy, blank pericardial fluid samples spiked with psychoactive drugs at the concentrations found by LC-MS/MS analyses were subjected to analyses with the drug screening devices. Using a blank sample spiked with the concentration of TCA found in case 7 returned a positive result using Triage® TOX Drug Screen; the concentration of BZO from case 14 returned a positive result using Triage® TOX Drug Screen; and the concentration of ZOL from case 16 returned a positive result using DRIVEN-FLOW M8-Z. Other samples returned negative results for the spiked psychoactive drugs.

Discussion

In the present study, we investigated four drug testing devices with respect to their performance in detecting psychoactive drugs in forensic pericardial fluid samples. One of these devices, IVeX-screen M-1, was found to be more useful than the other three screening devices. This device accurately detected phenobarbital (cases 1, 3 and 6), amitriptyline (case 7), amobarbital (case 8) and methamphetamine (case 9), and returned no false positive results. On the contrary, the Triage® TOX Drug Screen and SIGNIFY™ ER devices returned several false positive results, and the DRIVEN-FLOW M8-Z device returned many false positive results. To our knowledge, the only information concerning the applicability of drug testing device to the detection of psychoactive drugs in pericardial fluid has been reported by Tominaga et al[5]. This report showed that Triage® TOX Drug Screen returned a few false positive results as ours and recommended the combined usage of another device to minimize misinterpretation prior to instru-

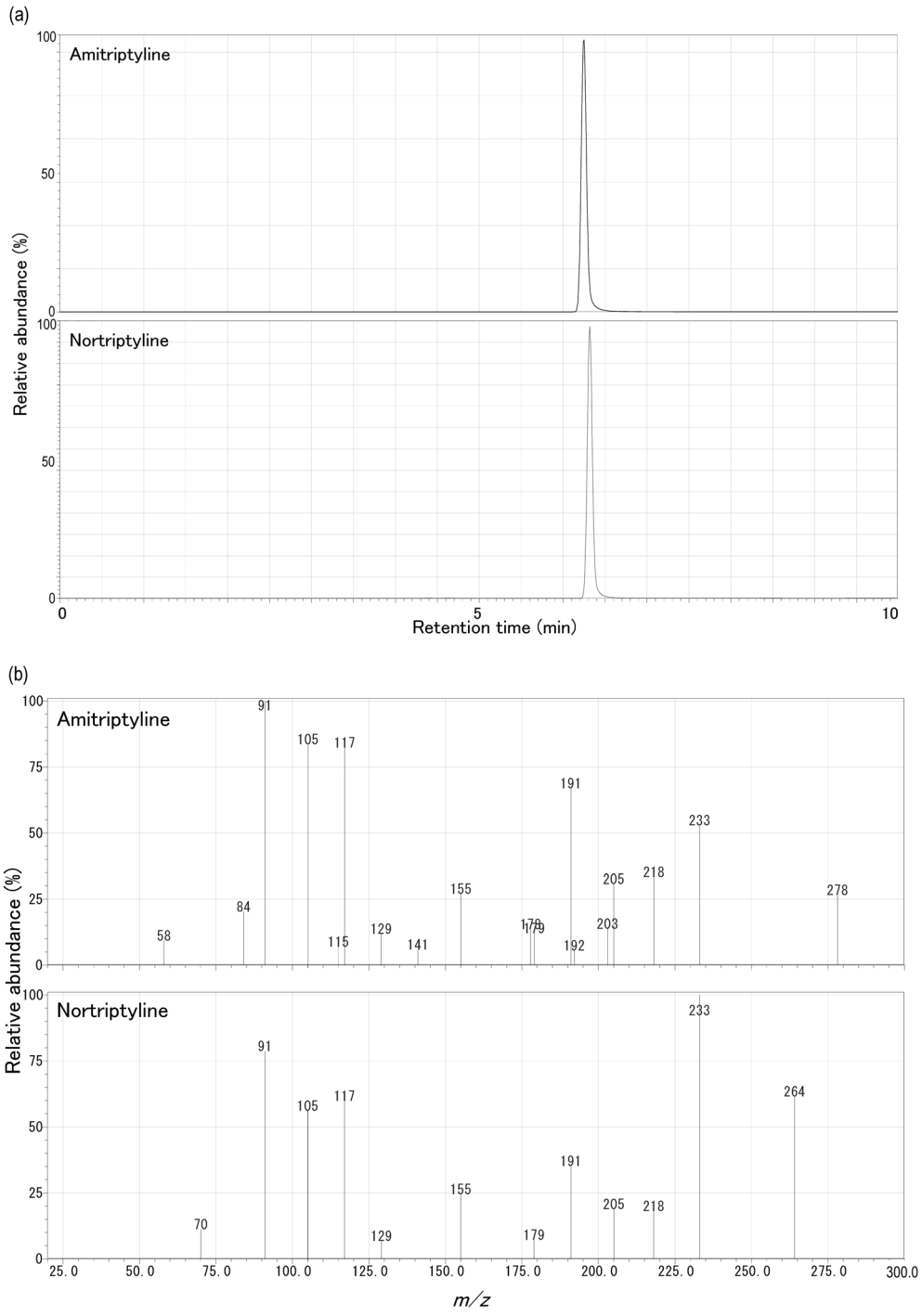

Fig. 1. Representative LC-MS/MS analysis of a pericardial fluid sample (case 7).

(a) Selective ion monitoring liquid chromatograms show the same retention times (6.25 min and 6.30 min) as those of standard amitriptyline and nortriptyline. (b) Diagnostic fragment ions with ion abundance ratios of the noted fractions from the pericardial fluid sample were fully consistent with those of standard amitriptyline and nortriptyline, thus confirming the presence of these compounds in this pericardial fluid sample.

mental analysis.

The principal advantage of the use of pericardial fluid is that pericardial fluid is easily obtained from the pericardial cavity, and the usual volume of pericardial fluid taken at the time of the autopsy is enough for forensic drug testing[7]. As the pericardial fluid is found in a closed compartment, contamination by microorganisms is less likely as compared to blood and cerebrospinal fluid, and pericardial fluid is thus relatively stable in the postmortem period[8]. In the present study, however, we found that drug concentrations were

lower in the pericardial fluid than in the urine. Specifically, the concentrations of methamphetamine and amphetamine in pericardial fluid were considerably lower than those in urine samples. We conclude, then, that pericardial fluid is an important alternative material when urine is not available, but it is unsuitable for detecting methamphetamine and amphetamine because of low concentrations compared to urine.

One of the disadvantage of immunoassays, including those investigated in the present study, is that antibodies tend to be variable in terms of their potentials for cross-reactivity[4]. This cross-reactivity can lead to inconsistent results or false positive signals. In our previous study, we found that the DRIVEN-FLOW M8-Z device was particularly useful for the detection of methamphetamines in forensic urine samples[6]. One advantage of this device when screening urine samples was the lack of false positive signals even in the presence of the putrefactive amine 2-phenethylamine. However, in the present study, this device led to many false positive results for zolpidem and tricyclic antidepressants. False positive results are relatively common with immunoassays, as antibodies tend to be variable in terms of their potentials for cross-reactivity. In this case, we hypothesize that antibodies employed by the DRIVEN-FLOW M8-Z device may cross-react with substances commonly found in the pericardial fluid, thus limiting its usefulness in this context.

We also investigated the potential discrepancy that eleven samples returned positive results for TCA, BZO and ZOL, even though the sample drug concentrations determined by LC-MS/MS were under the immunoassay cutoff values. As a result of test spiked with psychoactive drugs at the concentrations found by LC-MS/MS analyses, three samples returned positive results and other samples returned negative results. These discordant results remain unresolved, and further studies incorporating LC-MS/MS analyses will be performed to clarify the reasons for these discrepancies and to accurately evaluate the performance of the drug screening devices.

We used LC-MS/MS analyses to evaluate the results from the drug screening devices. LC-MS/MS has been widely used in recent bioanalytical work, since it is a powerful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry[9]. In the present study, we found that LC-MS/MS was more accurate and sensitive than were the immunoassay-based devices for the detection of psychoactive drugs. Thus, we recommend that positive results obtained using the screening devices should be confirmed by LC-MS/MS.

It should be noted that the concentrations of the drugs in the blood samples seem to be higher than those in the pericardial fluid, except for a couple of drugs such as phenobarbital in case 3. Because drug concentrations in pericardial fluid can be thought to be influenced from, or almost the same with those of blood from anatomical perspective, pericardial fluid thus has the potential to be very useful alternative when blood is not available.

In conclusion, pericardial fluid is an important alternative material for forensic analyses when urine is not available. Among the four drug screening devices tested for this application, we found IVeX-screen M-1 to be the most useful, as it accurately detected the highest number of psychoactive drugs without returning false positive results. Despite the accuracy of this device, it is strongly recommended LC-MS/MS methods should be used to confirm any positive results achieved with immunoassay-based screening devices.

Conflict of Interest

The authors declare no conflicts of interest.

References

1) Harper L, Powell J, Pijl EM: An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services. *Harm Reduct J* 14: 52, 2017.
2) Towler S, Concheiro M, Pearring S, Rodda LN: Evaluation and applicability of Alere iCup DX 14 for rapid postmortem urine drug screening at autopsy. *J Forensic Sci* 66: 375−382, 2021.
3) Contreras MT, Hernández AF, González M, González S, Ventura R, et al: Application of pericardial fluid to the analysis of morphine (heroin) and cocaine in forensic toxicology. *Forensic Sci Int* 164: 168−171, 2006.
4) Maharjan AS, Johnson-Davis KL: Issues of interferences with immunoassays used for screening of drugs of abuse in urine, in Dasgupta A (ed): *Critical issues in alcohol and drugs of abuse testing*. p. 129, Elsevier, Amsterdam, 2019.
5) Tominaga M, Michiue T, Maeda H: Evaluation of the on-site immunoassay drug-screening device Triage-TOX in routine forensic autopsy. *Legal Med* 17: 499−502, 2015.

6) Iwai M, Ogawa T, Matsuo T, Kondo F, Kubo K, et al: Evaluation of five drug screening devices for testing of amphetamines and methamphetamines. *Med Mass Spectom* 6: 64−69, 2022.
7) Ferreira E, Corte Real F, Pinho E Melo T, Margalho C: A novel bioanalytical method for the determination of opioids in blood and pericardial fluid. *J Anal Toxicol* 44: 754−768, 2020.
8) Contreras MT, González M, González S, Ventura R, Valverde JL, et al: Validation of a procedure for the gas chromatography-mass spectrometry analysis of cocaine and metabolites in pericardial fluids. *J Anal Toxicol* 31: 75−80, 2007.
9) D'Ovidio C, Locatelli M, Perrucci M, Ciriolo L, Furton KG, et al: LC-MS/MS application in pharmacotoxicological field: current state and new applications. *Molecules* 28: 2127, 2023.