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ORIGINAL ARTICLE

Evaluation of the Urine Drug Abuse Screening Tests

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SUMMARY

Background: **Substance use is an important public health problem and increasing all over the world. Different methods have been defined for drug abuse testing in medical laboratories. We aimed to compare two urine drug screening methods with liquid chromatography-tandem mass spectrometry (LC-MS/MS).**
Methods: **A total of 102 patients' urine samples were analyzed by test dip card and EMIT (enzyme multiplied immunoassay technique). Randomly selected samples (n = 51; 50%) were also analyzed by LC-MS/MS as the reference method.**
Results: **The drug results of all patients analyzed with the test card and EMIT were compatible. Nine of 51 samples (18%) were negative according to all methods. The sensitivity and specificity percentages of AMP, COC, MDMA, OPI/MOP, and THC using test card were 70/96, 100/100, 47/100, 50/100, and 80/85, respectively. Similarly, the sensitivity and specificity percentages of AMP, COC, MDMA, OPI/MOP, and THC using EMIT were 76/97, 100/100, 57/100, 56/100, and 76/91, respectively.**
Conclusions: **The performances of two immunochemical methods were similar for AMP, BZO, COC, MDMA, OPI/MOP, and THC whereas lower than LCMS/MS for AMP, MDMA, OPI/MOP, and THC. A sample that is positive according to any immunochemical method should be confirmed by definitive techniques such as LC-MS/MS.**
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INTRODUCTION

Substance abuse - in other words drug abuse - is the use of a drug by a person or others in harmful amounts. It may cause criminal or anti-social behaviors and long-term personality changes in individuals [1]. Therefore, substance use is an important public health problem and increasing all over the world. Worldwide, it was reported that there were about 210 million users (4.8% of the global population) aged 15 - 64 in 2009 and about 269 million users (5.3% of the global population) aged 15 - 64 in 2018 [2]. The most frequently used illicit drugs are as follows: cannabinoids (marijuana), cocaine, heroin, hallucinogens, and inhalants as well as the misuse of opioids, prescription pain relievers, tranquilizers

or sedatives, stimulants, and benzodiazepines [3]. Cannabinoids are the most often used drug and opioids are the most harmful [2]. Test requests for drug abuse may be due to medical, administrative, or forensic applications. It is important for medical laboratories conducting drug tests to be able to measure the most used and well-known substances [4]. Samples used for drug testing can be blood, urine, saliva, hair, or other body fluids. Since the substance and metabolites used in urine can persist for a longer time, the urine sample is the most suitable sample to be used in screening [5]. Different methods have been defined for urine drug testing (UDT) in medical laboratories: Qualitative detection using lateral flow chromatographic immunoassay (test dip card/strip) by the naked eye for visual interpretation or a strip reader, semi-quantitative or quantitative detection using some immunochemical methods (enzyme multiplied immunoassay technique, EMIT; cloned enzyme donor immunoassay, CEDIA; chemiluminescence immunoassay, CLIA; fluorescence polarization immunoassay, FPIA) on auto-analyzer and quantitative detection using chromatographic methods. In this case, specificity, simplicity, cost-effectivity, rapid turnaround time (TAT), and a broad range of tests will be among the determining factors that are important in the selection of methods for UDT [6]. Generally, immunochemical methods are used for drug screening, and gas chromatography-mass spectrometry (GC-MS) is used for confirmation as the reference method. Recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been used as an alternative to both screening and confirmatory methods for toxicology [7]. UDT is used to try to prevent drug use and monitor compliance in drug treatment programs [8].

The accuracy of the results of drug and stimulant analysis, which is requested to be investigated in alcohol and drug treatment centers (AMATEM), probation, and forensic cases, is very important for individuals. It is also important that the laboratories producing these results periodically monitor and are aware of the performance of the analytical method. As a result of this sense of responsibility, we desired to evaluate the performance of our laboratory's substance analysis methods by comparing them with the chromatographic method, which is accepted as the reference method. This comparison study is planned for routine performance measurement of our laboratory.

For this purpose, we aimed to compare two urine drug-screening methods, the one step multi-drug screen test dip card® and enzyme multiplied immunoassay technique-EMIT® used in our laboratory with liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.

MATERIALS AND METHODS

Patients and samples

Our study included 102 outpatient cases over the age of 18 who applied to Diyarbakır Selahaddin Eyyubi State Hospital, Department of Psychiatry in June 2020 (n = 102, age: 29 (18 - 56); male: n = 99, age: 30 (18 - 56) female: n = 3, age: 40 (25 - 51). Ages are given as median and minimum - maximum). At least 30 mL of urine sample was collected from each patient. The temperature of each urine sample was evaluated within four minutes and the urine integrity tests were performed to identify any substitution, adulteration, or dilution by patients to produce false-negative test results. Then, each patient's urine sample was aliquoted into the plastic tubes (10 mL) and analyzed by the test dip card and immunochemical automated method as double-blind for screening. Among them, a total of 51 (50%) samples were randomly selected and stored at -20°C for three months until analyzed by LC-MS/MS. No preservative was used in the urine samples.

The personal information of each subject was recorded (age, gender, and medication history for any disease). Written consent was routinely obtained from all our patients. Our study was approved by the Research and Ethics Committee of the Medical Faculty of Katip Çelebi University (approval number: 0488 date: 24/11/2022) and followed the ethical standards of the Declaration of Helsinki.

Reagents, materials, and instrumentation

The urine integrity tests: pH, nitrite, and specific gravity were measured by the Cobas u411 urine analyzer (Roche Diagnostics, Mannheim, Germany) using reflectance photometry, reflectance photometry, and refractometry, respectively. Creatinine was measured using the Advia 1800 chemistry analyzer (Siemens Healthcare Diagnostics, NY, USA) by a kinetic Jaffé colorimetric method [9,10].

A twelve-drug panel (Table 1) was analyzed by a one-step multi-drug screen test dip card® (Healgen Scientific, TX, USA) using a solid-phase qualitative immunochromatographic assay based on competitive binding. Test cards were performed according to the package insert, with two people reading each sample's results. The card had one region as quality control (C) and another region as drug test (T) for each parameter. If two lines appeared as red color on C and red or pink colors on T, it meant a negative test result. If one red line appeared on C, it meant a positive test result. If the control line failed to appear, it meant an invalid test. One sample analysis was about 5 minutes.

A nine-drug panel was analyzed by the Advia 1800 chemistry analyzer (Siemens Healthcare Diagnostics, NY, USA) using EMIT® method. The EMIT® kits were obtained from Siemens Healthcare Diagnostics' original assays along with a five-point calibration pack for amphetamines (AMP), barbiturates (BAR), benzodiazepines (BZO), cocaine and metabolites (COC), ecstasy

(MDMA), tetrahydrocannabinol (THC), and with a four-point calibration pack for opiates/morphine (OPI/MOP). The synthetic cannabinoids' kits with a two-point calibration pack were obtained from ARK Diagnostics JWH-018 assay for group I, UR-144 assay for group II, and AB-PINACA assay for group III. All parameters' results were evaluated as positive or negative based on the manufacturer's cutoffs (Table 1). One sample analysis was about 13 minutes. The intra- and inter-assay CVs were < 5% in internal quality control studies (both negative and positive levels) for all parameters. The external quality assessment (EQA) was suitable for each parameter in June 2021 (One World Accuracy EQA Programme, Vancouver, Canada).

We used a chromatographic method by Sciex 5500 QTRAP LC-MS/MS (Sciex Applied Biosystems, MA, USA) for broad-spectrum urine drug screening as a reference method (Table 1). LC-MS/MS was validated according to CLSI rules. The fast toxicological screening kit (code: SC9005U) was purchased from Sciex. Each internal standard was added up to each sample for each test. The chemical solvents were purchased from EUREKA (Eureka Institute for Translational Medicine, Syracuse, Italy). All working procedures were performed according to the manufacturer's instructions. The separation columns were a drug screen and quant analytical column (5 µm 60 Å) and a PFP Propyl column [50X2.1 mm (P/N 9169552]. Any drug's lower limit of quantification (LLOQ) was 5 ng/mL. The coefficient of variation (CV) for the accepted LLOQ was < 5%. The quality-control procedure suggested by the manufacturer was also followed for each parameter. One sample analysis was about 12 minutes after one and a half hours of sample preparation.

The analyzed urine drug tests with calibrators and cutoff concentrations are listed in Table 1.

Statistical analysis

The data were analyzed by IBM SPSS© Statistics (version 22.0) program (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to check the deviation from the normal distribution. The results were expressed as mean ± standard deviation (SD) for normal distribution or as the 1st and 3rd quartiles (Q1 - Q3) and median for the non-normal distribution. For categorical variables, the chi-squared test was used, and the results were stated as numbers and percentages. The sensitivity, specificity, and accuracy were calculated for the urine drug tests in two immunoassays when compared to LC-MS/MS. Sensitivity = True positives / (True positives + False negatives), Specificity = True negatives / (True negatives + False positives), Accuracy = (True positives + True negatives) / (Total positives + Total negatives).

RESULTS

A total of 102 urine samples were collected for this study. The temperatures of the samples were between 33 - 37°C. The urine integrity tests were performed, and all results were in acceptable ranges [9,10] as seen in Table 2.

All samples (n = 102) were analyzed: Twenty-two (21%) specimens were completely negative according to both immunochemical methods. Twenty-six (25%) and twenty-two (23%) specimens were completely negative according to the test card and EMIT®, respectively. The positive and negative test numbers are presented in Table 3. All the patients who were positive for AMP (n = 35) were also positive for mAMP in the test card. AMP (n = 33), BZO (n = 1), COC (n = 2), MDMA (n = 15), OPI/MOP (n = 11), BAR (n = 0), K2 (n = 0) tests that were positive in EMIT® were also positive in the test card. Conversely, except for two specimen patients who were positive for THC (n = 59) in the test card were also positive in EMIT®.

Among all samples, the randomly selected 50% of samples (n = 51) were also screened by LC-MS/MS and summarized in Table 4. A total of 9 (18%) patients' results were negative according to all methods. There was no positive BZO result in all patients according to LC-MS/MS, but only one patient who used Alprazolam 1 mg/day and Olanzapine 5 mg/day, Sertraline 50 mg/day had a positive BZO result according to both test card and EMIT®. The drugs' sensitivity, specificity, and accuracy were calculated for both the test card and EMIT® when compared to LC-MS/MS and presented in Table 5. The sensitivity and accuracy for BZO, K2, and MTD were not given because there were not enough positive results.

DISCUSSION

UDT is generally applied for probation, forensic toxicology, and clinical cases because of its simplicity and cost-effectiveness. It is traditionally performed by immunochemical methods as a drug panel. A sample that has a positive result in UDT by any screening method is generally confirmed by definitive techniques such as LC-MS/MS [11]. In this study, we evaluated two immunochemical methods compared with LC-MS/MS for UDT.

The sensitivity, specificity, and accuracy of amphetamines, cocaine and metabolites, ecstasy, tetrahydrocannabinol, and opiates/morphine were high for both immunochemical methods when compared to LC-MS/MS (see Table 5). This finding was consistent with previous studies [8,12-14]. The sensitivity and accuracy for BZO, K2, and MTD were not interpreted because there were not enough positive results.

True-negative results occur when there is no drug in the urine sample or because the drug is rapidly metabolized, and its metabolites cannot be detected. We found nine

Table 1. The analyzed urine drug tests with calibrators and cutoff concentrations.

Immunoassay (qualitative)	Immunoassay (semi-quantitative)	Chromatography (quantitative)
Dip test cards with calibrators and cutoffs (ng/mL)	EMIT® tests with calibrators and cutoffs (ng/mL)	LC-MS/MS tests with calibrators and cutoffs (ng/mL)
AMP (D-Amphetamine: 500)	AMP (D-Amphetamine: 500)	AMP (D-Amphetamine: 500)
BZO (Oxazepam: 300)	BZO (Diazepam: 300)	BZO (Alprazolam: 300, bromazepam: 300, chlordemethyldiazepam: 300, clobazam: 300, clonazepam: 300, diazepam: 300, flunitrazepam: 300, flurazepam: 300, lorazepam: 300, midazolam: 300, nitrazepam: 300, nordiazepam: 300, oxazepam: 300, triazolam: 300)
COC (Benzoylecgonine: 150)	COC (Benzoylecgonine: 150)	COC (Benzoylecgonine: 150, cocaine: 150, cocaethylene: 150, propylbenzoylecgonine: 150)
Ecstasy (MDMA: 500)	Ecstasy (MDMA: 500)	Ecstasy (MBDB: 500, MDA: 500, MDEA: 500, MDMA: 500)
OPI/MOP (Morphine: 2,000)	OPI/MOP (Morphine: 2,000)	OPI/MOP (6-O-monoacetlymorphine: 10, heroin (diacetylmorphine): 2,000, codeine: 2,000, dihydrocodeine: 2,000 ethylmorphine: 2,000, morphine: 2,000, norcodeine: 2,000, normorphine: 2,000, oxycodone: 2,000)
THC (Δ^9-THC: 50)	THC (Δ^9-THC: 50)	THC (Δ^9-THC: 50, THC-COOH: 50)
K2 (JWH-073/JWH-018: 25)	K2-1 (JWH-018: 20) K2-2 (UR-144: 10) K2-3 (AB-PINACA: 10)	K2 (APINACA (AKB-48): 10, AM-2201: 20, JWH 018-5 OH pentyl: 20, JWH 073-4 OH butyl: 20, JWH 073-N butanoic: 20, JWH-015: 20, JWH-018: 20, JWH-018 N-pentanoic: 20, JWH-019: 20, JWH-073: 20, JWH-081: 20, JWH-122: 20, JWH-200: 20, JWH-203: 20, JWH-250: 20, PB-22: 10, RCS-4: 10, WIN 55-212 1: 10, XLR-11: 10)
BAR (Secobarbital: 300)	BAR (Secobarbital: 300)	NA
mAMP (D-Methamphetamine: 500)	NA	mAMP (D-Methamphetamine: 500)
MTD (Methadone: 300)	NA	MTD (MTD: 0, EDDP: 0)
PCP (Phencyclidine: 25)	NA	NA
TCA (Nortriptyline: 1000)	NA	NA
NA	NA	Ephedrines (Ephedrine: 0, norpseudoephedrine: 0, pseudoephedrine: 0), gabapentin: 0, ketamine: 0, naloxone: 0, naltrexone: 0, pregabalin: 0, tramadol: 50, buprenorphine: 5, norbuprenorphine: 5, pethidine: 0

EMIT - Enzyme multiplied immunoassay technique, LC-MS/MS - liquid chromatography-tandem mass spectrometry, AMP - D-Amphetamine, BZO - Benzodiazepines, COC - Cocaine and metabolites, EDDP - 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, MDMA - D,L-3,4-Methylenedioxymetamphetamine, OPI/MOP - Opiates/Morphine, THC - Tetrahydrocannabinol, K2 - Synthetic Cannabinoids K2-1 – Synthetic Cannabinoids group I, K2-2 - Synthetic Cannabinoids group II, K2-3 - Synthetic Cannabinoid group III, BAR - Barbiturates, mAMP - D-Methamphetamine, MTD - Methadone, PCP - Phencyclidine, TCA - Tricyclic antidepressant, NA - Non-analyzed.

samples with no positive results detected chromatographically, which were also found to be negative by immunochemical methods. This finding shows us that the specificity of the immunochemical method is high [15]. When the amphetamine results were examined one by one, one sample was measured as 24,000 ng/mL by LC-MS/MS and measured as 541 ng/mL by EMIT. However, the same sample was also positive for amphetamine in EMIT. Similarly, in the test card, amphetamine and methamphetamine were also positive except for ecstasy. False positive results for amphetamine or methamphetamine are due to the similar molecular structure of am-

Table 2. The urine integrity tests results.

The urine integrity tests (n = 102)	Results *
pH (acceptable range: 3 - 11)	5 (5 - 6)
Specific gravity (acceptable range: 1,003 - 1,035)	1,025 (1,020 - 1,025)
Creatinine * (acceptable range: 20 - 200 mg/dL)	116 ± 84
Nitrite (cutoff: 500 mg/L)	negative

*** If the results had normal distribution, expressed as mean ± standard deviation (SD), if not as median and 1st - 3rd quartiles (Q1 - Q3).**

Table 3. The urine drug test results in two immunochemical methods.

	One step multi-drug screen test dip card® (n = 102)		EMIT® (n = 102)	
Drug Groups *	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)
AMP	67, 66	35, 34	69, 68	33, 32
BZO	100, 98	2, 2	101, 99	1, 1
COC	100, 98	2, 2	100, 98	2, 2
ECSTASY	84, 82	18, 18	87, 85	15, 15
OPI/MOP	90, 88	12, 12	91, 89	11, 11
THC	41, 40	61, 60	37, 36	65, 64
K2 **	102, 100	0, 0	102, 100	0, 0
BAR	102, 100	0, 0	102, 100	0, 0
mAMP	66, 65	36, 35	NA	
MTD	101, 99	1, 1	NA	
PCP	102, 100	0, 0	NA	
TCA	102, 100	0, 0	NA	

EMIT - Enzyme multiplied immunoassay technique, AMP - Amphetamine, BZO - Benzodiazepines, COC - Cocaine and metabolites, OPI/MOP - Opiates/Morphine, THC - Tetrahydrocannabinol, K2 - Synthetic Cannabinoids, BAR - Barbiturate, mAMP - Methamphetamine, MTD - Methadone, PCP - Phencyclidine, TCA - Tricyclic antidepressant, NA - Non-analyzed.
*** See Table 1 for cutoff concentrations of the drugs.**
**** NA - Non-analyzed.**

phetamine, methamphetamine, ecstasy, and their derivatives. Antibodies recognized by the analyte in the immunochemical methods can also give positive results in other tests due to structural similarity. In this case, if the immunochemical methods are used for the drug, it may be necessary to give this information in the analysis report and inform the clinician about it. The samples with positive results for amphetamine or methamphetamine according to LC-MS/MS are also positive for ecstasy according to EMIT and test card [15,16]. The Healthcare Implementation Communique (SUT) in Turkey says to check for amphetamine positivity first and then to do an ecstasy analysis, which seems to confirm this result [17].

One patient had a negative result for amphetamine, methamphetamine, and ecstasy in chromatography, but amphetamine was 444 ng/mL in EMIT. Although the threshold value has not been exceeded, it is known that a substance similar to the molecular structure of the amphetamine used by the person may cause this, since the bonding relationship between the structure of the analyte and the antibody in the reagent is essential for immunochemical methods. This situation suggests false positivity in immunochemical methods. In this case, it becomes important to send each positive sample and forensic negative sample for confirmation analysis according to the results determined by immunochemical methods [6,16]. Amphetamine could not be detected ac-

Table 4. The urine drug test results in two immunochemical methods and LC-MS/MS.

	One step multi-drug screen test dip card® (n = 51)		EMIT® (n = 51)		LC-MS/MS (n = 51)	
Drug Groups *	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)
AMP	28, 55	23, 45	30, 59	21, 41	34, 67	17, 33
BZO	50, 98	1, 2	50, 98	1, 2	51, 100	0, 0
COC	49, 96	2, 4	49, 96	2, 4	49, 96	2, 4
ECSTASY	34, 67	17, 33	37, 72	14, 28	43, 84	8, 16
OPI/MOP	41, 80	10, 20	42, 82	9, 18	46, 90	5, 10
THC	26, 51	25, 49	22, 43	29, 57	27, 53	24, 47
K2	51, 100	0, 0	51, 100	0, 0	50, 98	1, 2
mAMP	27, 53	24, 47	NA		32, 63	19, 37
MTD	50, 98	1, 2	NA		51, 100	0, 0
Ephedrines	NA		NA		39, 76	12, 24
Gabapentin	NA		NA		49, 96	2, 4
Pregabalin	NA		NA		48, 94	3, 6
Norbuprenorphine	NA		NA		50, 98	1, 2
All test negative	16, 31		12, 24		12, 24	
Total results	16, 31	35, 69	12, 24	39, 76	12, 24	39, 76

EMIT - Enzyme multiplied immunoassay technique, LC-MS/MS - liquid chromatography-tandem mass spectrometry, AMP - Amphetamine, BZO - Benzodiazepines, COC - Cocaine and metabolites, OPI/MOP - Opiates/Morphine, THC - Tetrahydrocannabinol, K2 - Synthetic Cannabinoids, mAMP - Methamphetamine, MTD - Methadone, NA - Non-analyzed.
*** See Table 1 for each group classification and cutoff concentrations of the drugs.**
**** Barbiturates, tricyclic antidepressants, and phencyclidine are not listed because not analyzed in LC-MS/MS.**

Table 5. The calculations of sensitivity, specificity, and accuracy for the urine drug tests in two immunochemical methods when compared to LC-MS/MS.

	One step multi-drug screen test dip card® (n = 51)			EMIT® (n = 51)		
Drug Groups *	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
AMP	70	96	84	76	97	88
BZO	**	100	**	**	100	**
COC	100	100	100	100	100	100
ECSTASY	47	100	82	57	100	88
OPI/MOP	50	100	90	56	100	92
THC	80	85	82	76	91	82
K2	**	98	**	**	98	**
mAMP	75	96	86		NA	
MTD	**	100	**		NA	
NEGATIVE	-	75	-	-	75	-

EMIT - Enzyme multiplied immunoassay technique, LC-MS/MS - liquid chromatography-tandem mass spectrometry, AMP - Amphetamine, BZO - Benzodiazepines, COC - Cocaine and metabolites, OPI/MOP - Opiates/Morphine, THC - Tetrahydrocannabinol, K2 - Synthetic Cannabinoids, mAMP - Methamphetamine, MTD - Methadone, NA - Non-analyzed, Sensitivity = true positives/(true positives + false negatives), Specificity = true negatives/(true negatives + false positives), Accuracy = (true positives + true negatives)/total.
*** See Table 1 for each group classification and cutoff concentrations of the drugs.**
**** The sensitivity and accuracy are not calculated because there are not enough positive results.**
***** Barbiturates, tricyclic antidepressants, and phencyclidine are not listed because not analyzed in LC-MS/MS.**

cording to EMIT and test card for another patient. It is thought-provoking that the high amphetamine level such as 3,215 ng/mL was detected chromatographically but not by immunochemical methods.

We did not calculate the accuracy for benzodiazepines because there was no positive sample according to LC-MS/MS. Only one patient used Alprazolam 1 mg/day with a positive result for benzodiazepines according to two immunochemical methods. The alprazolam value of the patient was only 45 ng/mL in LC-MS/MS, and it was below the threshold value. If the patient did not take another benzodiazepine derivative, the benzodiazepine result, which is > 900 ng/mL in EMIT and positive in the test card, may have been caused by alprazolam usage. If this sample had been studied in LC-MS/MS, the patient's result would have been negative. As we have seen in our previous experiments, some immunochemical methods can result in higher benzodiazepine results [18]. A similar situation may have occurred in this example. When the LC-MS/MS method is accepted as the gold standard method, it can be interpreted that the immunochemical methods may give false positive results for BZD. On the other hand, the positive results of BZD were obtained by measuring the same sample with both test card and EMIT. Therefore, it is also necessary to exclude the presence of an incorrect measurement by LC-MS/MS, a technical error during sample injection into the device, an inability to prepare the sample, a pipetting error, or a device injection error that would result in working with a smaller volume of sample than necessary. The technical, operational, or productional problems of internal standards that were injected with the sample may also affect the drug analysis. This shows the importance of internal standard analysis [19].

The most common prescribed or abused benzodiazepines are alprazolam, oxazepam, triazolam, midazolam, diazepam, and nordiazepam. Benzodiazepine derivatives such as temazepam and flurazepam are less common. Benzodiazepine analysis may have different results compared to available immunochemical methods. The calibrator used for the benzodiazepine analysis in the immunochemical methods cannot recognize the benzodiazepines in some systems, depending on the benzodiazepine type in the sample. In some cases, benzodiazepine level can be measured much higher than it is. Therefore, it should be recommended that benzodiazepine results be submitted for confirmation analysis, especially if the patient objects to the results [20].

The samples with ecstasy levels higher than the threshold value by the immunochemical method were also detected by LC-MS/MS as positive. The same samples had positive results in the test card, too. So, we can say that the test card and EMIT are safe in terms of false negatives. Of course, more samples are needed to express this outcome clearly. The presence of methamphetamine and/or amphetamine positivity in addition to ecstasy in the analysis of ecstasy-containing samples by EMIT and test card is associated with cross-reactions occurring in antibody tests due to the structural similarity of amphetamine, methamphetamine, MDMA, and MDA substances. Similarly, although MDMA is not found in a chromatographically analyzed sample, MDMA positive in EMIT and test card can be interpreted as the antibodies making MDMA positive due to the structural similarity of amphetamine and methamphetamine [21].

The samples that were opiate positive by chromatography were also positive by EMIT and test card. Although there is no difference between these three methods in terms of accurate detection in samples containing high levels of opiates, it would be appropriate to determine the immunoassay methods' accuracy by studying samples with opiate levels close to the threshold limit by LC-MS/MS [22].

In our randomly selected samples, there are very few samples with cocaine and benzoylecgonine positivity, but when the current positivity is evaluated, it is seen that the methods can give consistent results [12,14].

The high THC values in LC-MS/MS remain at values such as 181 and 182 ng/mL in EMIT. This may be because the linearity of the immunoassay method is up to this point and EMIT method cannot read at higher concentrations. In addition, immunochemical methods cannot quantify low levels of the drugs. Therefore, it is not possible to monitor the patient's compliance with the treatment and supervision in regular check-ups at certain intervals such as probation patients followed up by sampling at intervals of two or three weeks. On the other hand, all analytes in the sample can be quantified in a method such as LC-MS/MS, and it may be possible to monitor adherence to treatment based on clinical evidence [12,23]

In our study, methadone was not evaluated in LC-MS/MS. Hence, no comment can be made for methadone positivity in the test card. However, negative amphetamine and methamphetamine levels in LC-MS/MS and EMIT and being positive on the test card suggest the possibility of similar false positivity for methadone [10].

A wide panel of substances and drugs can be scanned quickly with confirmation methods. LC-MS/MS is more attractive because of its ability to avoid the need for manual extraction in broad-spectrum substance analysis compared to other confirmation methods. It is also suitable for analyzing many samples with a low sample volume and gives low false positive and false negative results. LC-MS/MS can be used directly in scanning, but it is an expensive method, which is a deterrent, unfortunately. Considering all these, a sample that is positive according to any immunochemical method should be confirmed by definitive techniques such as LC-MS/MS [6,24,25]

The limitations of our study were as follows: Our sample size was small because of cost. Although patients' drug usage was questioned, there may be missing or incorrect declarations, and this may have affected the interpretation of the cross-reactivity. The last one, there

were non-common tests that could not be measured by all three methods such as methamphetamine, some synthetics, phencyclidine, barbiturate, and methadone.

CONCLUSION

The performances of the two immunochemical methods were similar for amphetamine, benzodiazepines, cocaine and metabolites, ecstasy, opiates and morphine, and tetrahydrocannabinol but lower than LC-MS/MS for amphetamine, ecstasy, opiates and morphine, and tetrahydrocannabinol. A sample that is positive according to any immunochemical method should be confirmed by definitive techniques such as LC-MS/MS.

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Declaration of Interest:
The authors have no conflict of interest to declare.

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