İdrarda Madde Taranmasında CEDIA ve KIMS Yöntemlerinin Karşılaştırılması

Comparison of the CEDIA and KIMS Methods in Urine Drug Screen

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ÖZET

Amaç: Bağımlılık yapan maddelerin doğru ve güvenilir ölçümü birtakım yasal yükümlülüklerden ötürü dünya genelinde ciddi bir problemdir. Dolayısıyla bu çalışmada toplumlarda yaygın olarak kullanılan bağımlılık yapıcı maddeleri, yaygın kullanılan iki farklı teknikle ölçerek, çelişkili sonuçların gaz kromatografisi-kütle spektrometresi tekniğiyle doğrulanması yoluyla birbirlerine göre üstünlüklerinin ortaya çıkarılması amaçlanmaktadır.

Materyal-Metod: İdrar numuneleri madde kullanıcısı 83 hastadan prosedüre uygun olarak toplandı. Tüm örnekler eş zamanlı olarak her iki teknikle çalışıldı. Ölçümlerde elde edilen çelişkili sonuçlar gaz kromatografisi-kütle spektrometresi tekniği ile doğrulandı.

Bulgular: Amfetamin testi "Klonlanmış Donör Enzim İmmüne Ölçüm" ve "Mikropartiküllerin Kinetik Etkileşimi" teknikleriyle, sırasıyla bir ve üç numunede yanlış negatif olarak değerlendirildi. Benzodiazepin sonuçları için iki teknik genel olarak uyumluydu fakat mikropartiküllerin kinetik etkileşimi metoduyla bir numunede yanlış pozitif olarak raporlandı. Kokain testi açısından iki teknik arasında çelişkili sonuç tespit edilmedi. Kannabinoid sonuçları incelendiğinde bir örneğin klonlanmış donör enzim immüne ölçüm yöntemi ile yanlış pozitif, bir örneğin mikropartiküllerin kinetik etkileşimi yöntemi ile yanlış negatif olarak değerlendirildiği tespit edildi. Mikropartiküllerin kinetik etkileşimi metoduyla yanlış negatif olarak değerlendirilen bir örnek dışında iki teknikle elde edilen opiat test sonuçlarının tamamı birbiriyle uyumluydu.

Sonuç: Yukarıdaki sonuçlara göre, mikropartiküllerin kinetik etkileşimi tekniği genel olarak klonlanmış donör enzim immün ölçüm tekniği ile uyumlu idi. Ancak kromatografi sonuçları bakımından amfetamin testi (3,4 MDMA metabolite,ecstasy) için aynı durum söz konusu değildi. Gelecekte yapılacak olan çalışmalarda bu durumun göz önüne alınmasının faydalı olacağını düşünmekteyiz.

Anahtar Kelimeler: İdrarda madde taraması; metod karşılaştırma; kromatografi; klonlanmış donör enzim immüne ölçüm; mikropartiküllerin kinetik etkileşimi.

ABSTRACT

Objective: The accurate and reliable measurement of addictive substances is a very serious problem all over the world because it involves some legal liabilities. This study aims to measure the popular addictive substances using two common techniques and to reveal against each other their superior aspects using the gas chromatography/ mass spectrometry technique.

Methods: In accordance with the procedure, 83 urine samples were taken from the substance users. All samples were worked simultaneously by both techniques. The discordant results obtained were verified by the gas chromatography/ mass spectrometry technique.

Results: The amphetamine test was evaluated as false negative in 1 and 3 samples respectively by the techniques "Cloned Enzyme Donor Immunoassay" (CEDIA) and "Kinetic Interaction of Microparticles in Solution" (KIMS). Both techniques were overall compatible for benzodiazepine results but one sample was dedected as false positive by KIMS. No discordance was detected for cocaine in two techniques. When cannabinoid test results examined, one sample was evaluated as false positive by CEDIA, one sample was evaluated as false negative by KIMS. All of the results for opiate test, obtained by both techniques, were compatible with each other but there was only one sample that was evaluated as false negative by KIMS method.

Conclusions: According to above results the KIMS technique was overall compatible with CEDIA technique. However, it was not valid that particularly for the amphetamine/ecstasy test (3,4 MDMA metabolite) in terms of the chromatography results. We believe that it will be beneficial for future studiesto take the results into consideration.

Key words: Urine drug screen; method comparison; chromatography; cloned enzyme donor immunoassay; kinetic interaction of microparticles in solution.

INTRODUCTION

It is estimated that the worldwide, there are between 119 million and 224 million cannabis users, 14.3 million and 52.5 million amphetamine-type stimulants users and 26.4 million and 36 million opioid users. (1). It was reported in recent years that there was an increase in substance addiction in all over the world and our country (1,2). This caused an increase in laboratory numbers conducting these tests and an increase in the number of tests. In addition, considering some legal obligations related with these tests, the performance of the test method becomes more important.

Today, urine is a significant biologic sample for the detection of substance addiction (2). The use of the gas chromatography/ mass spectrometry (GC/MS) technique is regarded the as gold standard in the measurement of addictive agents like cannabinoid, opiate and cocaine in urine (3). However, this technique is difficult to apply, requires educated personnel, takes long time and is very expensive. Therefore, immunoassay techniques come to the forefront as an initial test as they are intended for screening, take less time, are faster and cheaper (4). These immunoassay techniques are worked as semi quantitative or qualitative and it is recommended to use the test results of these techniques as an initial test and to verify the positive results with the GC/MS which is an appropriate method (5, 6). Therefore, it is important that there should be low rate of false negativity and false positivity of the immunoassay technique which is preferred to reduce the cost and loss of time. This would enable correct detection of the substance users and lower the cost of the test as it would reduce the number of positive samples to be verified with the GC/MS (4, 7). In recent years, the Cloned Enzyme Donor Immunoassay (CEDIA) and Kinetic Interaction of Microparticles in Solution (KIMS) methods are frequently used in screening the levels of the addictive substances in urine, however, there are very few detailed studies that compare the accuracy performance of the methods in the measurement of substance addiction (7,8,11).

In this paper it is planned to study the performance and compatibility of the CEDIA and KIMS methods which are the most popular methods in determining the urinary levels of the most frequently abused addictive substances amphetamine, benzodiazepine, opiate, cocaine and cannabinoid.

MATERIALS and METHODS

A total of 83 urine samples were selected randomly among the samples received from the substance users for examination in the laboratory. Tests were performed in the Cobas Integra 400 (Roche Diagnostics, Mannheim, Germany) analyzer with the KIMS (Roche Diagnostics, Mannheim, Germany) method while Roche Hitachi Modular P800 (Diamond Diagnostics, Holliston, USA) systems was used to study with the CEDIA (ThermoFischer Scientific, Fremont, USA) method. Cut-off levels were determined for both methods as cannabinoid 50, amphetamine 1000, benzodiazepine 300, opiate 300 and cocaine 300 ng/mL. The Roche Preciset DAT Plus II calibrators were used in the opiate test for the KIMS method, while Roche Preciset DAT Plus I calibrators were used for the other tests. For the CEDIA method, Thermoscientific (Microgenics) THC multi level calibrator was used in the cannabinoid test and Thermoscientific (Microgenics) multidrug calibrator clinical cut-off was used for other tests. All kits, calibrators and controls were used in line with the recommendations of the manufacturer company. All samples were worked simultaneously by both methods. Contradictory results were verified by the GC/MS system (Shimadzu, Kyoto,Japan and using kits Eureka Lab Dvision, Chiaravalle, Italy). Ethyl alcohol, amphetamine, benzodiazepine, cannabinoid, cocain and opiate tests were analyzed in all levels of the MAS DOA TOTAL (Microgenics, USA) 6-level liquid assayed drugs of abuse controls were analyzed by both techniques and compared with the expected results. In addition, two levels, low and high, were selected to calculate the intraassay and interassay repeatability of both methods as previously described by Aydın (9). Sample validity tests of all samples (creatinine, pH, density and nitrite) were studied and the incompatible samples were excluded from the study (10). With approval from the Firat University Ethics Committee, Elaziğ, Turkey, the study was carried out. SPSS Software 21 was used in statistical analysis.

RESULTS

Table 1 showes the contradictory results obtained from 83 urine samples that were selected randomly from the samples taken from the substance users for examination and studied simultaneously by two methods. Patient comparison for ethyl alcohol couldn't be done as the patient number was not sufficient. According to the 1000 ng/mL cut-off in the amphetamine test, 13,33% (n:10) of total 75 samples were compatible positive and 81,33% (n:61) were compatible negative. As shown in the Tabe 1, 5,33% of the samples (n:4) had contradictory results. According to the verification analysis with GC/MS, KIMS had false negative evaluation of 3 samples (4%) and CEDIA had false negative evaluation of 1 sample (1,33 %). Three samples evaluated as false negative by KIMS included 3-4 MDMA metabolites in the GC/MS verification and one sample evaluated as false negative by CEDIA had methamphetamines. According to 300 ng/ml cut-off for benzodiazepine, 29,33% of total 75 samples (n:22) had compatible positive results in both methods and 69,33% (n:52) of total samples had compatible negative results. As shown in Table 1,1 sample (1,33%) had contradictory results. In verification analysis by GC/MS, haloperidol was found in this sample and evaluated as false positive for KIMS method. 31,32% of total 83 samples (n:26) according to the 50 ng/ml cut-off for the cannabinoid test had compatible results in both methods and 66,26% (n:55) of them had compatible negative results while 2 samples (2,4%) had contradictory results. According to the verification analysis by GC/MS, 1 sample was evaluated as false negative by KIMS and 1 sample was evaluated as false positive by

CEDIA. According to 300 ng/ml cut-off for the opiate test, 28% (n:21) of 75 samples had compatible positive in both methods and 70,66% (n:53) had compatible negative. As shown in Table 1, morphine was detected in the GC/MS verification of 1 sample (1,33%) which had contradictory result.

MAS DOA TOTAL (Microgenics USA) 6-levels liquid assayed drugs of abuse controls were studied in both devices and the results were compared with the values specified by the manufacturing company. It was determined that the results obtained both methods for amphetamine, cocain, opiate, ethyl alcohol tests were compatible with the expected results.

However, the results of the KIMS method in the cannabinoid test were found to be more compatible with the expected results. Recovery results are shown in Table 2. It was determined that the reading with the high level drug abuse controls for the benzodiazepine test provided very low recovery in both methods. In addition, the intraassay and interassay repeatability, accuracy and recovery of both methods were calculated by taking the values specified by the manufacturer into consideration. It was observed that the intraassay and interassay CV values in the KIMS technique varied respectively between 0,76% –10,81% and 1,46% –14,93%. In CEDIA technique, it was observed that the intraassay and interassay CV values varied respectively between 1,12% –5,77% and 3,76% –32,78% (Table 3 and 4).

DISCUSSION

According to our study, it was seen that the CEDIA method provided more reliable results when the results of both methods were compared with the GC/MS results and 6-levels liquid assayed drugs of abuse controls results with respect to the amphetamine test. This is because reagent of KIMS method is only specific to amfetamin while reagent of CEDIA method a reagent that can detect both amphetamin and ekstazy metabolite. Beck et al.(8) reported that the CEDIA method was more sensitive. Lekskulchai et al.(4) determined that the 3,4 MDMA metabolite under a certain concentration was not evaluated as positive by the KIMS method. When evaluated with respect to the benzodiazepine tests, the CEDIA technique was remarkable with respect to the GC/MS results, while the recovery of the high level drug abuse control was very low in both methods (Table 3). These results were compatible with the previous studies (11,7,12). Haloperidol was determined in one sample which was considered as false positive with the KIMS method and it was seen that this substance was not stated in the cross reaction list of the manufacturer. There was no superiority of both methods against each other in according to the GC/MS results for the cannabinoid test. However, the results of the KIMS method were more compatible with the expected values of the 6-levels liquid assayed drugs of abuse controls. The cannabinoid results of 4th level of drug abuse liquid assay control (38 ng/mL), were evaluated as positive with the CEDIA method. One sample, was evaluated as 0 ng/mL with CEDIA, was reported as negative near the cut-off by KIMS method (49 ng/ml). The same sample was reported as negative with GC/MS. In this sample, it was not determined whether or

Table 1. Contradictory results obtained by two methods and comparison of these results with the GC/MS results**.**

Test name	Amphetamine n:75		Cannabinoid n:83		Opiate n:75		Benzodiazepine n:75		Cocaine n:75	
Number of contradictory samples	4		2		1		1		0	
Method	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS
False negative	1	3	-	1	-	1	-	-	-	-
False positive	-	-	1	-	-	-	-	1	-	-

Table 2. Comparisons of the results of both methods according to the expected values of the MAS DOA TOTAL 6-levels liquid assayed drugs of abuse controls

Amphetamines	CEDIA		KIMS		Benzodiazepines	CEDIA		KIMS	
Concentrations ng/ml	Detected values ng/ml	Recovery %	Detected values ng/ml	Recovery %	Concentrations ng/ml	Detected values ng/ml	Recovery %	Detected values ng/ml	Recovery %
0	0	0	0	0	0	0	0	0	0
375	366	97,6	325	86,66	150	114	76	112	74,66
625	668	106,88	472	75,52	225	214	95,11	184	81,77
750	802	106,9	605	80,66	250	210	84	193	77,2
1250	1489	119,12	1099	87,92	375	366	97,6	325	86,66
2000	2184	109,2	1875	93,75	1000	592	**59,2**	675	**67,5**
Cannabinoids	CEDIA		KIMS		Cocaine	CEDIA		KIMS	
0	0	0	0	0	0	0	0	0	0
19	30,2	**158,94**	10,60	55,79	112	126	112,5	90	80,35
31	42,70	137,74	26,90	86,77	188	218	115,95	162	86,17
38	53,00	139,47	28,60	75,26	225	246	109,33	191	84,88
62	80,20	129,35	55,30	89,19	375	380	101,33	302	80,53
150	91,30	**60,86**	121,90	81,27	500	555	111	401	80,2
Opiates	CEDIA		KIMS		Ethyl Alcohol	CEDIA		KIMS	
0	0	0	0	0	0	1	0	0,4	0
225	214	95,11	189	84	15	16	106,66	13,4	111,94
375	358	95,46	330	88	25	27	108	23,8	105,04
750	699	93,2	682	90,93	40	40	100	34,8	114,94
1500	1251	83,4	1276	85,06	70	71	101,42	62,5	112
2500	2397	95,88	2145	85,8	300	295	98,33	260,9	114,98

Table 3. Imprecision and accuracy of the CEDIA and KIMS drug-screening immunoassays. (intraassay coefficient of variation,CV)

	Amphetamines				Cannabinoids				Benzodiazepines			
	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS
Target mean	1250		750		62		38		375		225	
Mean value, ng/ml	1354,45	1279	808,8	693	76,98	58.0	56,4	39,3	345	313	217,15	201
CV, %	2,87	5,23	1,78	10,81	5,1	6.24	2,88	7,76	5,77	1,35	3,54	5,64
Recovery, %	108,35	102,32	107,84	92,4	124,16	93,54	148,42	103,42	92	83,46	96,51	89,33
n	20	20	20	20	20	20	20	20	20	20	20	20
	Opiates				Cocaine				Ethyl Alcohol			
	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS
Target mean	375		225		375		225		70		40	
Mean value, ng/ml	343,8	372	234,7	219	376,7	318	248,65	202	61,5	63,5	35,75	35,9
CV, %	2,61	1,91	3,26	4,41	2,38	1,32	3,31	1,44	1,12	0,76	1,54	1,02
Recovery, %	91,68	99,2	104,31	97,33	100,45	84,8	110,51	89,77	87,85	90,71	89,37	89,75
n	20	20	20	20	20	20	20	20	20	20	20	20

Table 4. Imprecision and accuracy of the CEDIA and KIMS drug-screening immunoassays (interassay coefficient of variation,CV)

	Amphetamines				Cannabinoids				Benzodiazepines			
	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS
Target mean	1250		750		62		38		375		225	
Mean value, ng/ml	1319,30	1187,40	750,90	697,67	69,44	62,70	48,74	38,27	310,40	330,20	195,52	218,67
CV, %	3,76	5,88	6,72	8,91	19,23	5,91	18,55	14,93	30,39	6,08	32,78	7,33
Recovery, %	105,54	94,99	100,12	93,02	112,00	101,13	128,26	100,71	82,77	88,05	86,90	97,19
n	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00
	Opiates				Cocaine				Ethyl Alcohol			
	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS	CEDIA	KIMS
Target mean	375		225		375		225		70		40	
Mean value, ng/ml	366,64	349,27	222,40	205,07	317,10	315,00	192,16	199,27	61,53	60,69	34,33	33,61
CV, %	8,98	5,55	12,53	5,44	22,46	2,38	29,76	2,68	12,11	1,46	11,58	2,48
Recovery, %	97,77	93,14	98,84	91,14	84,56	84,00	85,40	88,56	87,90	86,70	85,83	84,03
n	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00	15,00

not there is any agent providing a cross-reaction. When considered with respect to the cocain results, both the GC/MS results and the results of 6-levels liquid assayed drugs of abuse controls had compatible values obtained by the two methods. These results were in accordance with the results of the study of Schwettmann et al (7). From the opiate results, the one sample, in which morphine metabolite was detected by GC/MS method, was reported as false negative with the KIMS method. Looking at the CV values, the KIMS method overall appeared more distinguished. However, it should not be ignored different devices were used in this study. Creatinine, pH, density and nitrite levels were analyzed in order to determine if any adulterant was included in the samples that could affect the results.

Our study had some limitations. Different model and ages of the devices could have affected the CVs. Another limitation is that no patient comparison could be made for ethyl alcohol due to the insufficient number of samples.

Despite these limitations, both methods were able to measure addictive substances. However, it was noted that the CEDIA method was more distinguished in detecting the 3,4 MDMA metabolite. We believe that these two methods would reveal the substance addiction and could be prefered in regions with limited resources. However, we recommend that when doubtful results are obtained GC/MS verification should be made for proper decision making by judicial authorities.

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