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Original Article

Screening of common drugs of abuse using rapid GC/MS/MS method and immunoassay: Application in an Egyptian hospital

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ABSTRACT

Good and effective management of emergency unit (EU)-admitted patients is substantial. Qualitative screening is the first step used for clinical intervention protocol. Although immunoassay is reliable and reproducible for detection of opioids, it often does not detect other substances such as tramadol, oxycodone, buprenorphine, or substances that can lead to false negative or positive reports. The present study evaluated the results of commonly used immunoassay test with the GC/MS/MS protocol developed in our lab. The protocol included screening for common substances abused in the country. Thirty-two samples from patients admitted to EU were screened using both techniques, immunoassay and GC/MS/MS. The screening results were tested for false positives and false negatives. Immunoassay screening showed that tramadol was present in 14 samples, tetrahydrocannabinol (THC) in 14, phenobarbital in 1, and benzodiazepines in 3 samples. There were 5 negative samples. GC/MS/MS showed the presence of tramadol in 26 samples, THC in 19, opiates in 5 and phenobarbital in 4 samples. One sample out of 32 was recorded as positive for tramadol by immunoassay technique while lidocaine and paracetamol were confirmed by GC/MS/MS without detection of tramadol. GC/MS/MS showed that immunoassay screening missed out drug detection particularly tramadol and THC in 12% of test samples. This shows that immunoassay is not recommended for testing drugs such as tramadol and THC. GC/MS/MS represents a reliable and reproducible technique with a rapid and comprehensive protocol applied. It detects most common drugs abused with high accuracy in short time. Thus it is better to recommend the application of the presented protocol in EU to avoid misleading results.

KEYWORDS: benzodiazepine, drug abuse, Egypt, false positive, GC/MS/MS, immunoassay, THC, tramadol, urine screening.

ABBREVIATIONS

THC	:	tetrahydrocannabinol
THCA	:	Tetrahydrocannabinol carboxylic acid
THCA-d3	:	Tetrahydrocannabinol carboxylic acid-d3
THC-COOH-d3	:	Tetrahydrocannabinol carboxylic acid-d3

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GC/MS/MS	:	Gas chromatography hyphenated with triple stage mass detector
MSTFA	:	N-Methyl-N-trimethylsilyltrifluoro acetamide
ISTD	:	Internal standard
TBME	:	*tert*-butyl methyl ether
MRM	:	Multiple Reaction Monitoring

1. INTRODUCTION

Drug abuse is a major health and strategic issue in Egypt. It affects the economic and social integrity. Substance abuse in a lifetime was recorded to range between 6-14% in Egyptian population aged 15-46 years [1]. A qualitative drug screen is used to detect the presence of a drug in the body. A blood or urine sample may be used. However, urine is the preferred specimen for broad qualitative screening [2]. It is easy to collect and provides relatively good specificity and sensitivity. Urine screen for drugs is performed in cases of emergency, crimes, substance use disorder treatment, monitoring drivers, workplace employee monitoring, and in schools [3]. Current methods of drug analysis include chemical tests, immunoassay, chromatography, and mass spectrometry. Immunoassay, chemical tests and chromatography are the major chosen techniques for qualitative screening [4]. Classes of drugs that are commonly assayed by qualitative screen usually include alcohols, amphetamines, benzodiazepines, cocaine and metabolites, methadone, opiates, phencyclidines, tetrahydrocannabinol (THC), and sometimes tricyclic antidepressant drugs [5]. Egypt has criminalized the abuse of drugs listed under schedule I or II such as tramadol HCl, benzodiazepines, or THC and other synthetic cannabinoids, and hence these drugs should be included in routine toxicology screening. Most if not all drug testing laboratories depend on immunoassay as a screening technique followed by chromatographic confirmation through mass spectrometric analysis. Although immunoassay is reliable and reproducible for detection of morphine, codeine, and heroin, it often does not detect other opioids such as tramadol, hydrocodone, oxycodone, methadone, buprenorphine, and fentanyl [6] or other synthetic cannabinoids or cathinones. In addition immunoassay lacks sensitivity and specificity that can lead to false reports either negative or positive [7]. The present study evaluated the results of commonly used immunoassay test kits and a GC/MS/MS protocol developed in our lab. The protocol included screening for common substances abused among the country. The screening results were tested for false positives and false negatives.

2. MATERIALS AND METHODS

2.1. Reagents

Multi-Drug rapid test panel (Accurate CO., USA) contains anti-drug mouse monoclonal antibody and corresponding drug-protein conjugates. The control line contains goat anti-rabbit IgG polyclonal antibodies and rabbit IgG. The panel includes tetrahydrocannabinol (THC), tramadol, phenobarbital, benzodiazepine, cocaine, buprenorphine, and opioids.

Methyltestosterone (internal standard), THC-COOH-d3 (internal standard) and THC-COOH were purchased from Sigma Co. (St. Louis, MO), while codeine, morphine, benzoylecognine, hydromorphine tramadol, phenobarbital, buprenorphine, norbuprenorphine, and benzodiazepam were purchased from Cerilient Co. (USA). β-glucuronidase enzyme from *E. coli* (K12) was obtained from Roche Corp., USA. N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Machery-Nagel, Germany.

2.2. Samples

Thirty-two urine samples were collected from patients admitted at the ER unit in an Egyptian hospital (Cairo, Egypt) during the period from December 2018 till February 2019. Samples were collected in plastic containers in the presence of a guardian to prevent any sample adulteration, and preserved at 2-8 °C until preparation. Verbal consent was taken from all participants. The protocol was approved by the Ethical Committee.

2.3. Screening of urine samples

Samples were screened at first using an immunoassay multi-drug panel with the cut-off values listed in Table 1. Two lines in control and test area indicate negative result while one colored line in control but no line at test area indicates positive result for a specific drug.

Table 1. Cut-off value for the multi-drug panel used.

Drug name	Cut-off level (ng/ml)
THC	25
Tramadol	100
Phenobarbital	200
Benzodiazepine	150
Cocaine	100 z
Buprenorphine	5
Opioids (calculated as morphine base)	100

THC: Tetrahydrocannabinol.

2.4. GC/MS/MS analysis

2.4.1. Sample preparation

Urine samples (2.5 ml) were first hydrolyzed by β-glucuronidase enzyme from *E. coli* (K12) (30 µl) for 60 min at 55 °C with 50 µl of THC-COOH-d3 (1.0 µg/ml) and 25 ul of methyltestosterone (10 ug/ml) as the internal standard (ISTD), followed by addition of 1 ml of phosphate buffer (1.0 M). The mixture was extracted by liquid-liquid extraction using *tert*-butyl methyl ether (TBME):chloroform (70:30, v/v) at pH 9.5. The organic layer was transferred carefully to a dry clean glass tube and evaporated under oxygen-free nitrogen, derivatized with 50 µl of MSTFA:NH4I:ethanethiol (640:1:2, v/w/v) and heated at 80 °C for 60 min.

2.4.2. Instrumentation

One µl of the derivatized sample was injected into the GC/MS/MS system that consisted of an Agilent (Agilent Technologies, USA) TQD 7000C mass detector directly interfaced to an HP 7890A GC equipped with a 17-m HP cross-linked methylsilicone Ultra-1 column (0.20-mm i.d., 0.11 um film thickness). The oven temperature program was set at 185 °C (hold 0.5 min), increased at 6 °C/min to 230 °C (0 min), then to 250 °C at 12 °C/min, changed to 20 °C/min till 280 °C (0.5 min), then increased at 25 °C/min to the final temperature of 310 °C (hold 2 min). The electron energy was set at 70 eV, and the ion source temperature was set at 280 °C. The injection volume was 1 µl in the split mode at a split ratio of 1:10. Injector temperature was 280 °C. The analysis was done at a constant flow mode of 0.8 ml/min. Detection and confirmation was performed in the selected ion monitoring mode. The monitored transitions are shown in Table 2.

3. RESULTS

3.1. Sample demographics

Thirty-two patients were admitted to the EU for toxicology screening; 15.6% were females (80% with the age range 14-37 years), while 84.3% were males (81% with the age range 16-76 years, median 34 years), Figure 1. About 25% of them were admitted for car accidents.

3.2. Immunoassay screening of samples

Initial screening with immunoassay dip test revealed that 28 samples were positive for at least one of the test drugs. Tramadol was present in 14 samples, THC was positive in 14 samples, opioids in 5 samples, phenobarbital in 1 sample, and benzodiazepines in 3 samples. There were four negative samples as shown in Table 3.

3.3. GC/MS/MS screening of urine samples

Screening using GC/MS/MS recorded positive drug appearance in 31 samples. It showed presence of THC in 19 samples, opiates in 5 and phenobarbital in 4 samples. Tramadol was reported positive in 26 samples. There was only 1 negative sample. Results are displayed in Table 3.

Table 2. MRM and retention times of the monitored drugs.

Compound	Precursor ion (m/z)	Product ion (m/z)	Dwell time (ms)	Collision energy (V)	Retention time (min)
Tramadol	335.1	259.3	40	8	2.572
Phenobarbital	261.2	147	35	20	2.573
Benzoylecgonine	361.1	82	72	11	4.678
Benzodiazepam	283	238.1	75	24	6.019
Codeine	371.1	234.3	15	22	6.35
Hydromorphine	429	287.1	17	20	6.94
Morphine	429.1	287.1	17	20	7.12
THCA-d3 (IS)	374.1	292.1	50	15	9.576
Methyltestosterone (IS)	446.3	169.1	50	30	9.584
THCA	371.1	289.1	50	15	9.59
Norbuprenorphine	524.4	317	50	27	12.587
Buprenorphine	554.3	295.2	50	25	13.144

THC: Tetrahydrocannbinol, THCA: Tetrahydrocannabinol carboxylic acid, THCA-d3: deuterated tertrahydrocannabinol carboxylic acid, IS: Internal Standard.

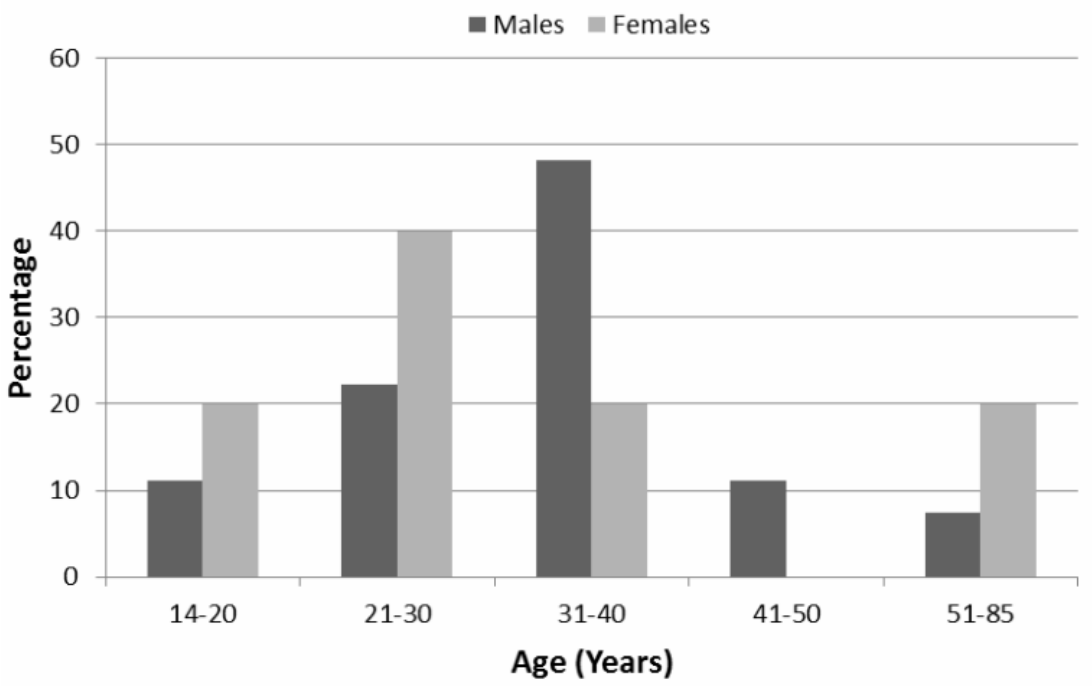

Figure 1. Age and sex distribution of the test samples. (Urine samples were taken from 32 patients admitted to the emergency unit).

Table 3. Screening results of urine samples.

Drug	Immunoassay cases count	GC/MS/MS cases count
THC	14	19
Opioids	5	5
Benzodiazepine	3	1
Tramadol	14	26
Barbiturate	1	4
Negative	4	1
Buprenorphine	0	0
Cocaine	0	0

Urine samples were taken from 32 patients admitted to the emergency unit, THC: Tetrahydrocannabinol.

Table 4. False positives and false negatives of immunoassay test result.

Drug	% false positive	% false negative	Concentration range of sample (ng/ml)
THC	0	15.6	0.8 - 14.7
Opioids	0	0	-
Benzodiazepine	6.25	0	-
Tramadol	3.125	40.6	4.3 - 14.3
Barbiturate	3.125	12.5	41.2 - 57.7

Urine samples were taken from 32 patients admitted to the emergency unit, THC: Tetrahydrocannabinol; % False positive (FP) = number of FP/total number (TP + TN), % False negative (FN) = number of FN/total number (TP + TN).

3.4. Selectivity

One sample out of 32 was recorded as positive for tramadol by immunoassay technique while GC/MS/MS showed the presence of lidocaine and paracetamol as interfering agents without detection of tramadol. Immunoassay screening missed out THC detection in 15.6% of the tested samples, tramadol in 40%, and phenobarbital in 12.5% that were confirmed by GC/MS/MS. Quantitation of drugs was performed, and 20% of false results were of drug concentration above the cut-off limit. Table 4 shows an overview of negative and positive results.

4. DISCUSSION

Drug abuse is an alarming and growing problem in Egypt raising a major health and societal concern. Nationally, epidemiological data on drug abuse are scarce. The WHO reported that the prevalence of drug abuse was 0.8% in Egypt [8]. An estimate of 3% of the population was reported with tramadol dependence in 2016. Tramadol accounted for about 68% of drugs abused by patients in 2017 [9]. In a recent Egyptian study, the prevalence of drug abuse was reported to reach 14% [1]. Admission to EUs is often associated with drugs of abuse [10] where nearly

half of the visits were for substance-related incidents. Besides, 30% of fatal car accidents were found to be linked to some drug presence [11]. In Egypt, a study showed that about 21% of drivers abused different drugs. 53.5% of them abused tramadol, while 30% abused cannabis [12]. In another study, cannabis was reported to be abused by 46% of drivers [13]. The study of Mageid [14] indicated that drivers in Egypt abuse drugs in order to increase their working hours and hence wages. Therefore, toxicological screening in road traffic accidents is a necessary step for good management and rescue of patient. Patients mostly under-report drug use in fear of legal actions, thus misleading the health care providers at the EU.

The current study showed that females represent around 15% of drug abusers admitted to the hospital. The study of Hamdi *et al*. [1] stated that 1.1% females abuse a substance in their lifetime, while the study of El-Awady [15] recorded females to be 8% drug abusers. On the other hand, Fawzi [16] described the use of tramadol by 22% females.

The results of the present investigation demonstrated that tramadol was abused by 78% of selected patients admitted to EU, followed by THC (59.4%) and opiates (16%). Aglan and Adawi [17] showed that 53.3% of cab drivers involved in non-fatal car accidents were positive for drug abuse screening. 91% of them were positive for THC, 60% used tramadol, 34% were positive for barbiturates and 28% for opiates. Benzodiazepines were 25% and methamphetamine was 21% of the samples. An Iranian report showed the prevalence of opioids in drivers involved in fatal car accidents followed by cannabis [18]. In another study in Australia cannabis was the most prevalent (46.7%) followed by benzodiazepines (15.6%) and opioids (11%) among drivers involved in car accidents [19]. In Egypt, tramadol is abused mainly due to its sexual-enhancing effects [16, 20].

It has been indicated that emergency clinical management do not depend on the result of drug screen since it is not very accurate [21]. An interesting finding in the current study was false recording of 12.5% of test samples as negative while they contained tramadol, THC, phenobarbital or mixture of two of them, 20% of which was above the cut-off concentration of the immunoassay test kit. Immunoassay technique is a primary step in drug analysis. It depends on competitive binding of antibodies and ligands with the analyte being more specific to antibody. Nowadays, the companies provide low cut off concentrations with very high specificity that can be relied upon in drug testing. However, some drugs are hardly detected such as tramadol. A study has evaluated on site screening kits and found some kits unresponsive to oxazepam, morphine, secobarbital, and phencyclidine with varying proportions [22]. In 2012, two tramadol seizure cases who were urine-positive for phencyclidine were reported [23]. Furthermore, many reports on false positive screen result for many drugs such as amphetamines, benzodiazepines, opioids have been recorded after the use of other drugs such as tricyclic antidepressants or analgesics (For review see [24]). False positive reports can be detrimental to a patient's life due to health and legal consequences. Besides, it increases the health care cost. Published reports describe false positive urine screen for phencyclidine associated with certain commonly used medications, such as tramadol, dextromethorphan, or diphenhydramine. Marchei *et al*., Brahm *et al*. and Rengarajan *et al*. [25-27] reported that false positive urine screens for phencyclidine were associated with the presence of tramadol, dextromethorphan, or some benzodiazepines. Ranitidine and chlorpromazine are associated with false opioid and amphetamine results [28].

False negative results are reported for many reasons. One reason could be the adulteration of sample or urine tampering [29] or that the assay is not specific for a certain drug [30] or the drug content is below the immunoassay cut-off level [31]. The latter underlies most of the present false negative results of immunoassay screening of samples. A clinician has to take these observations into consideration when interpreting lab drug test results and correlate the result with the clinical picture. He may ask for confirmation even if the result is negative if such drugs are suspected.

5. CONCLUSION

Emergency cases especially in traffic accidents need a comprehensive toxicology screening that is rapid and reliable. The protocol developed in the present study takes a short time for analysis with 100% sensitivity and specificity which makes it reliable in EUs. The present investigation recommends implementation of this protocol in EU of major hospitals in Egypt for confirmatory analysis. In addition, emergency clinicians should note the present observations and correlate the immunoassay results with the symptoms manifested as further analysis may be required for negative samples. However, a limitation to application of the current protocol could be the cost and instrumentation of analysis.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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