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DETECTION OF SOME ABUSE DRUGS IN HUMAN URINE

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Abstract:

Background: *Drug toxicology tests are most commonly performed on urine, since most drugs and their breakdown products are excreted in the urine at higher concentration.*

The aim of this work *is to evaluate the usefulness of using urine immunoassay kits for detection of some drugs of abuse, and study the correlation between the results obtained by EMIT and GC / MS techniques.*

Materials & Methods*: A total number of 449 inhabitant urine samples were collected from patients admitted to emergency hospital, Mansoura University. 449 urine samples were analyzed by EMIT and GC / MS for benzodiazepine, barbiturate, opiate and cannabinoid.*

Results *revealed that urine immunoassay kit is useful for rapid preliminary screening of abuse drug. GC / MS results confirm that 245 samples (54.56 %) are positive of the total number of samples. These positive samples by GC / MS were as follows; benzodiazepines; 159 ((clonazepam, oxazepam, temazepam), barbiturates; 58 (thiobarbiturate, butabarbital, seconal) and opiates; 28 (methadone metabolite)).*

Conclusions & Recommendations: *GC / MS analysis must be done for accurate identification and confirmation of EMIT results. Also, it is recommended as the most suitable technique for obtaining optimum analytical results.*

Keywords: *Urine samples, EMIT, GC/MS.*

INTRODUCTION

Drugs may cause a direct physiological and psychological change in the body. Drugs of abuse are any drug or substance which if taken by any route (oral-snuffing-injection) will lead to mood change, psychological disturbance and can affect brain functions and level of perception (Gold frank *et al*, 1990). They alter the cultural environment and cause considerable concern as they are used by large numbers of young people during their reproductive period (Jones, 1990). Surveying the most common drugs and substances of abuse in Egypt it were found to be opiates, benzodiazepines, barbiturates, alcohol, cannabis and CNS-stimulants (Abdel-Magid and Salem, 1995). Most drugs of abuse are detectable by

immunoassays, as far amphetamine, opiate, barbiturate, benzodiazepine, cocaine, P-C-P and cannabinoid. The concentrations of drugs are relatively high in urine, so it is the sample of choice for screening and identification of unknown drugs or poison. However, the metabolites of these drugs must be identified in addition or even exclusively, (Maurer, 1992). Immunoassay techniques such as enzyme multiplied immunoassay technique (EMIT) are commonly used for drug screening in part because they are quick and require a small amount of samples. Gas Chromatography / Mass Spectrum (GC / MS) analysis was performed for identification, quantification and confirmation of the obtained results.

The EMIT assay is a homogenous enzyme immunoassay technique used for the analysis of specific compounds in human urine. Once the urine sample has been identified as testing positive by a screening test, the specimen is retested with a more specific confirmatory test. The basic principle of confirming a positive drug test is to retest the same urine sample with different tests.

The aim of this work is to evaluate the usefulness of using urine immunoassay kits for detection of some drugs of abuse, and study the correlation between the results obtained by EMIT and GC / MS techniques.

MATERIALS AND METHODS

Samples

449 inhabitant urine samples were collected from patients admitted to poison unit at emergency hospital, Mansoura University (Nov 1999 to June 2005). Those patients were requested for drug screening; benzodiazepine, barbiturate, opiate and cannabinoid.

Reagents of EMIT

Four EMIT drug assay urine (d.a.u.) kits for benzodiazepine, Barbiturate, opiate and cannabinoid were purchased from Syva company and prepared according to the manufacturer manual (EMIT, 1984).

- EMIT d.a.u. assay composed of:
  - Reagent A; antibody to a particular drug/ substrate for the enzyme (G6PDH).
  - Reagent B; enzyme - labeled drug.
- EMIT drug assay buffer concentrate.

- EMIT calibrator 0 (negative) and calibrator A levels 1(cutoff) and 2 (high), these calibrators are used in the calibration of the EMIT d.a.u. (benzodiazepine, barbiturate, opiate) assays where the cutoffs are nearly the same; however the cannabinoid kit has separate calibrator.

All reagents were refrigerated at 2 – 8 ºC for storage and allowed to equilibrate for at least two hours at room temperature before use.

Technique of EMIT

Each sample was assayed by the four separate EMIT d.a.u. with the pipette diluter, 50 µl of the urine sample was added to 250 µl of buffer solution and mixed in a 2 ml disposable cup. 50 µl of reagent A and 250 µl of the buffer were added to the cup. After 30 sec. equilibration, 50 µl of reagent B and 250 µl of the buffer were added to the cup. The contents of the cup were immediately aspirated into the flow cell of the spectrophotometer. Absorbance readings were taken automatically at 15 and 95 sec. to calculate the absorbance difference (ΔA).

Identification method of drug abuse using GC / MS

Extraction of urine samples (Ghanem, 2005)

Acid extraction

- One ml of urine was acidified with 100 µl of 1N HCl, followed by extraction with 5 ml chloroform.
- Shake well for 10 seconds and centrifuge for 5 min. at 3500 rpm.
- Aspirate and discard upper layer and filter organic phase through Whatmann filter paper No 4 in glass tube.
- Evaporate the extract to dryness, reconstitute with 100 µl chloroform.

Base extraction

- An aliquot of 700 µl of urine sample were alkalized with100 µl of NaOH 5 M followed by extraction with 150 µl of butyl acetate.
- Vortex the mixture for 10 seconds, then centrifuge for 5 min. at 11000 rpm.
- Evaporate butyl acetate extract to dryness, reconstitute with 100 µl diethyl ether. Inject 1 µl of the extract injected into GC - MS (Hewlett Packard 6890 series) of ECD (Electron Captured Detector) as universal detector and examine in Wiley library.

- All chemicals used were of analytical grades.

Conditions of GC / MS (Maurer, 1992)

- Carrier gas (He)
- Capillary column; model No: HP19091Z-102, Hp-1 Methyl Siloxane, Length 25 m, diameter 200 µm, film thickness 0.33 µm.
- Flow rate 1.0 ml / min, Mode: split less,
- Thermal Aux 2 (MSD; Mass Spectrum Detector),
- Temp 280 ºC, Max temp 325 ºC, (Table 1)

Table I Conditions of GC / MS

Drug	Run Time	Average velocity of carrier Gas through column	Oven Temp.	Pressure of Carrier gas in back inlet
Benzodiazepine	14 min	41 cm/sec	110 ºC	22.2 psi
Barbiturate	5 min	41 cm/sec	110 ºC	22.2 psi
Cannabinoid	7 min	42 cm/sec	180 ºC	27.8 psi
Opiate	4 min	42cm/sec	200 ºC	29.3 psi

Standard spectra auto tune, (Fig. 1) was done daily and before injecting the samples using internal standard Pentafluorotetrabutylacetate (PFTBA), three peaks appeared (69, 219 and 502 ± 0.1) with peak width (0.55 – 65).

Statistical analysis for evaluation of drug screen examination by EMIT and GC/MS was carried out by specificity and sensitivity for each drug using the following equations, (Mustafa, *et al*., 1989):

Specificity = (true negative / true negative + false positive) x 100

Sensitivity = (true positive / true positive + false negative) x 100

HP5972 Standard Spectra of Auto Tune

Instrument: GC / MS Instrument # 1

C:\ HPCHEM\ 1 \ 5972 \ A TUNE.0

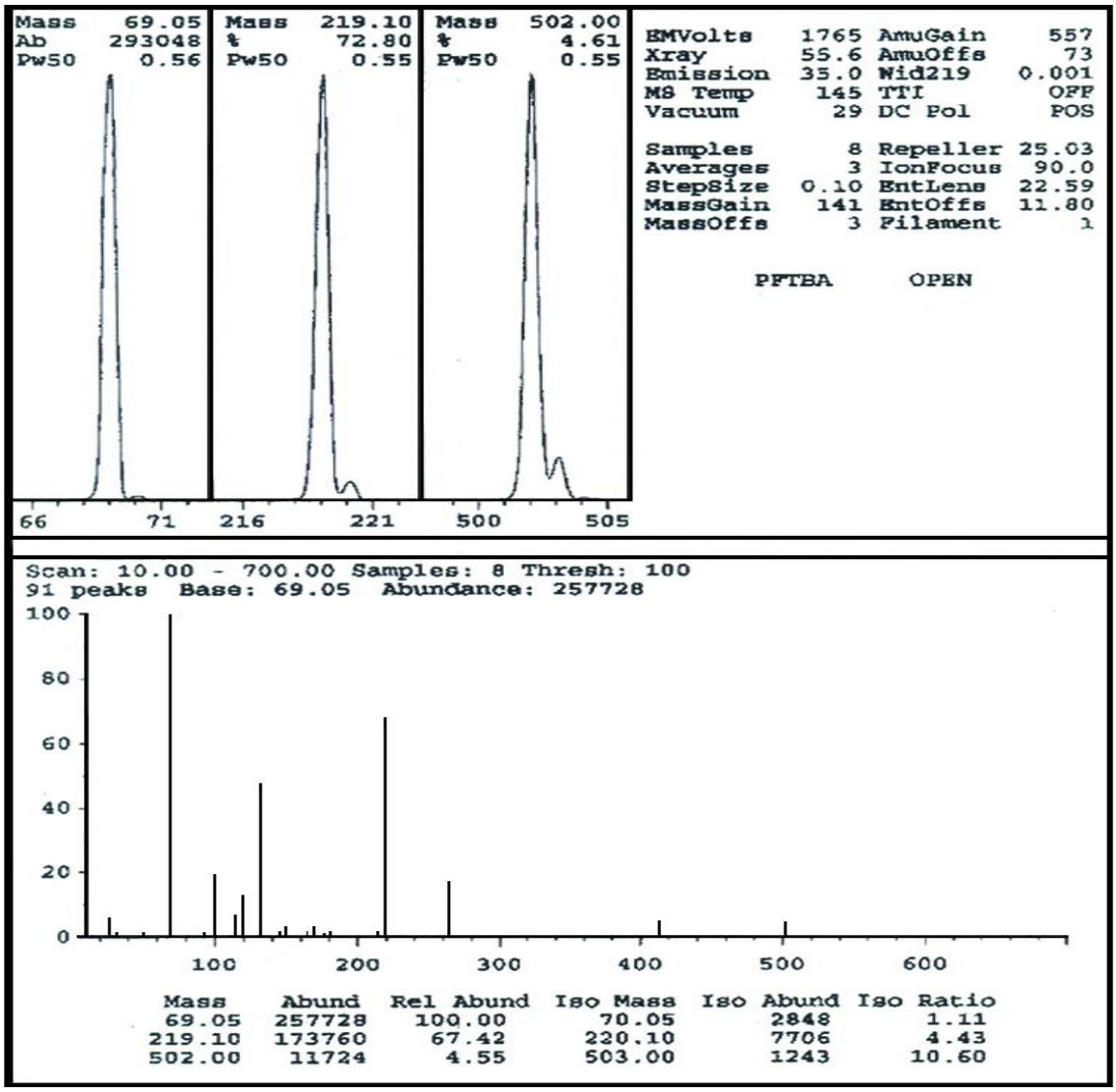

Fig (1) Standard Spectra of Auto Tune

RESULTS

The annual positive tests for each abused drug in (Mansoura area, Egypt) by EMIT system were; benzodiazepines (329), barbiturates (172), opiates (165), and cannabinoid (81). While, the positive tested samples by GC/MS (Table 2) are as follows; benzodiazepines (159), barbiturates (58) and opiates (28). The spectra and distinctive ion scanning (m / z) of the positive drugs; benzodiazepines, barbiturates and opiates by GC/MS analysis are illustrated in Figs (2a, 2b, 2c & 3a, 3b, 3c & 4).

The administered drugs and their metabolites were identified in urine samples through analysis of their respective GC/MS spectra as follows; for benzodiazepines (clonazepam: m/z 280, 314; oxazepam: m/z 268, 239; temazepam: m/z 271), for barbiturates (thiobarbiturate: m / z 42, 144; butobarbital: m / z 156, 141, 55;

Seconal: m / z 41, 168) and for opiates (methadone metabolite: m / z 276, 262, 105), (Table 3). Evaluation of drug screen examination by EMIT and GC / MS was carried out by specificity for each drug as follows; benzodiazepines (63.04 %), barbiturates (77.4 %) and opiates (75.4 %) and also by sensitivity for each drug as follows; benzodiazepines (48.3 %), barbiturates (17.3 %) and opiates (8.97 %), (Table 4).

Table 2 Comparison between results of positive drug tests by EMIT and GC / MS

Drug/ year	No of samples	Positive tests								Total No of Positive tests	
		Benzodiazepines		Barbiturates		Cannabinoid		Opiates			
		EMIT	GC/ MS	EMIT	GC/ MS	EMIT	GC/ MS	EMIT	GC/ MS	EMIT	GC/ MS
Nov1999	**16**	**12**	**3**	**2**	**2**	**3**	**-**	**2**	**2**	**19**	**7**
2000	**176**	**115**	**59**	**69**	**20**	**25**	**-**	**43**	**5**	**252**	**84**
2001	**197**	**185**	**94**	**76**	**35**	**22**	**-**	**117**	**16**	**400**	**145**
2002	**16**	**-**	**-**	**15**	**-**	**9**	**-**	**2**	**-**	**26**	**-**
2003	**3**	**-**	**-**	**-**	**1**	**2**	**-**	**1**	**-**	**3**	**1**
2004	**18**	**3**	**2**	**2**	**-**	**16**	**-**	**-**	**1**	**21**	**3**
April2005	**23**	**14**	**1**	**8**	**-**	**4**	**-**	**-**	**4**	**26**	**5**
Total	**449**	**329**	**159**	**172**	**58**	**81**	**-**	**165**	**28**	**747**	**245**

Oxazepam C:\ DATABASE\ WILEY 275. L

Molecular Formula: C15H11ClN2O2

Molecular Weight: 286

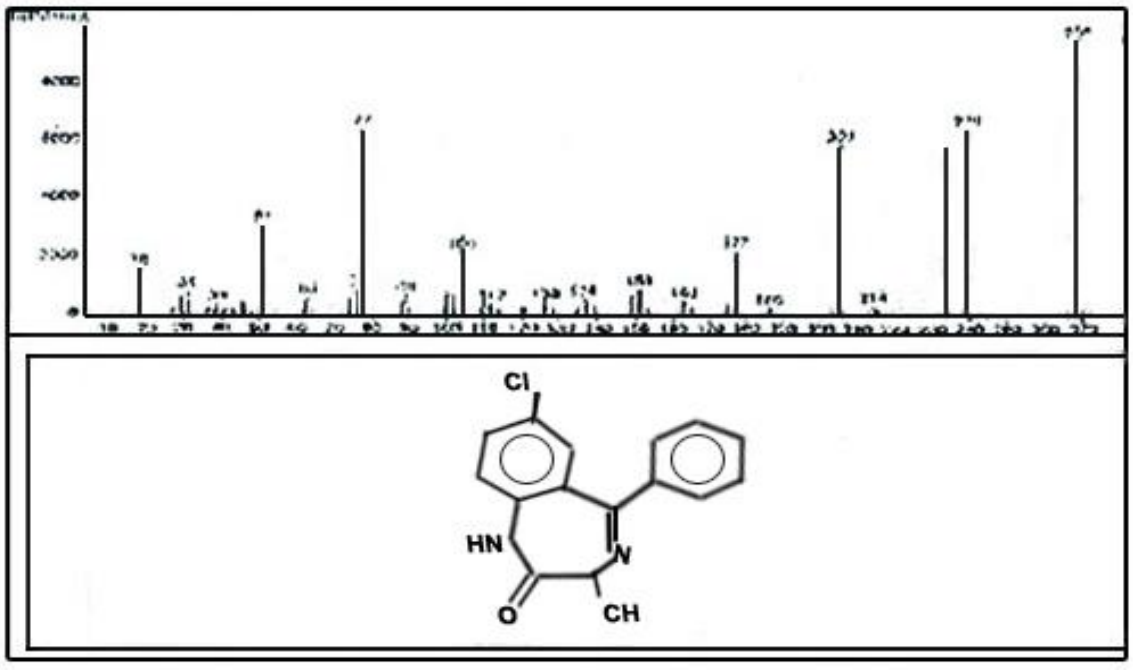

Fig (2a) Spectra and structure of Oxazepam

Temazepam C: \ DATABASE \ WILEY 275. L

Molecular Formula: C16H13ClN2O2

Molecular Weight: 300

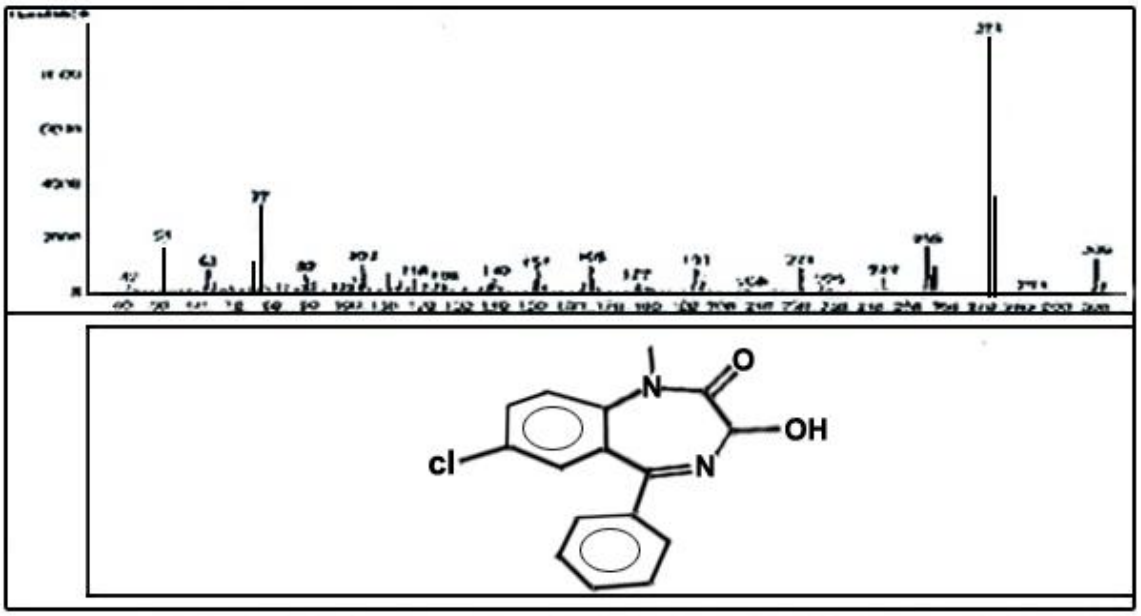

Fig (2b) Spectra and Structure of Temazepam

Clonazepam C: \ DATABASE \ WILEY 275. L

Molecular Formula: C15H10ClN3O3

Molecular Weight: 315

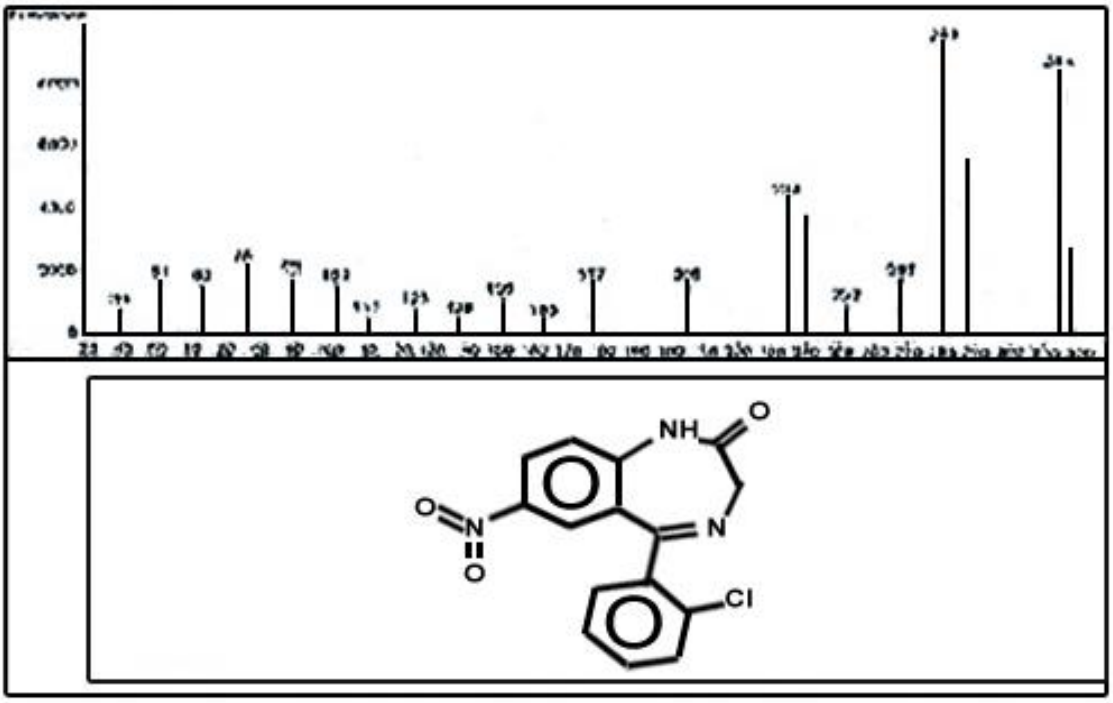

Fig (2c) Spectra and Structure of Clonazepam

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Seconal C: \ DATABASE \ WILEY 275.L

Molecular Formula: C12H18N2O3

Molecular Weight: 238

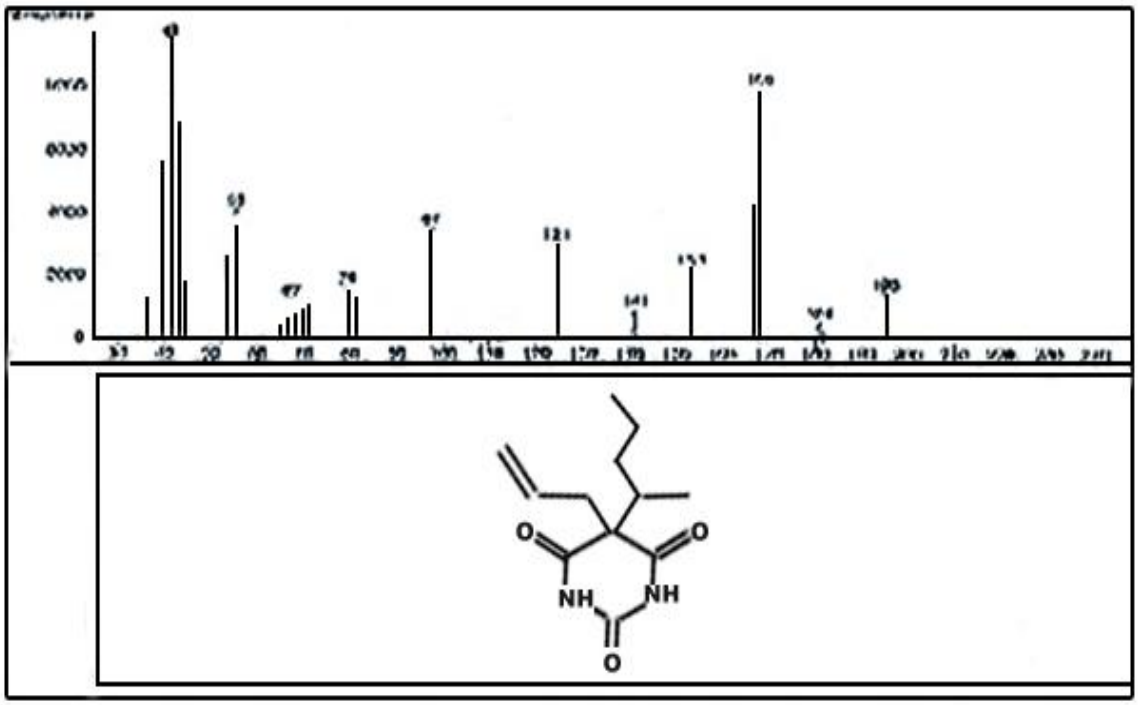

Fig (3a) Spectra and Structure of Seconal

Butobarbital

C:\ DATABASE \ WILEY275.L

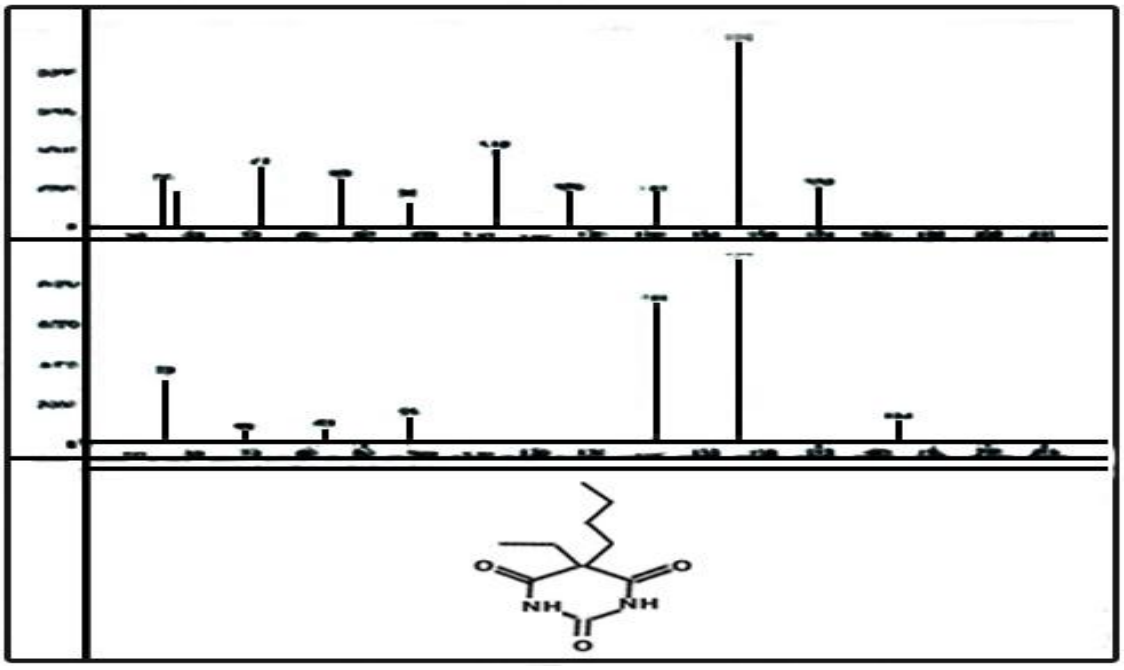

Fig (3b) Sectra and Structure of Butobarbital

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Thiobarbitone C:\ DATABASE \ WILEY 275.L

Molecular Formula: C4H4N2O2S

Molecular Weight: 144

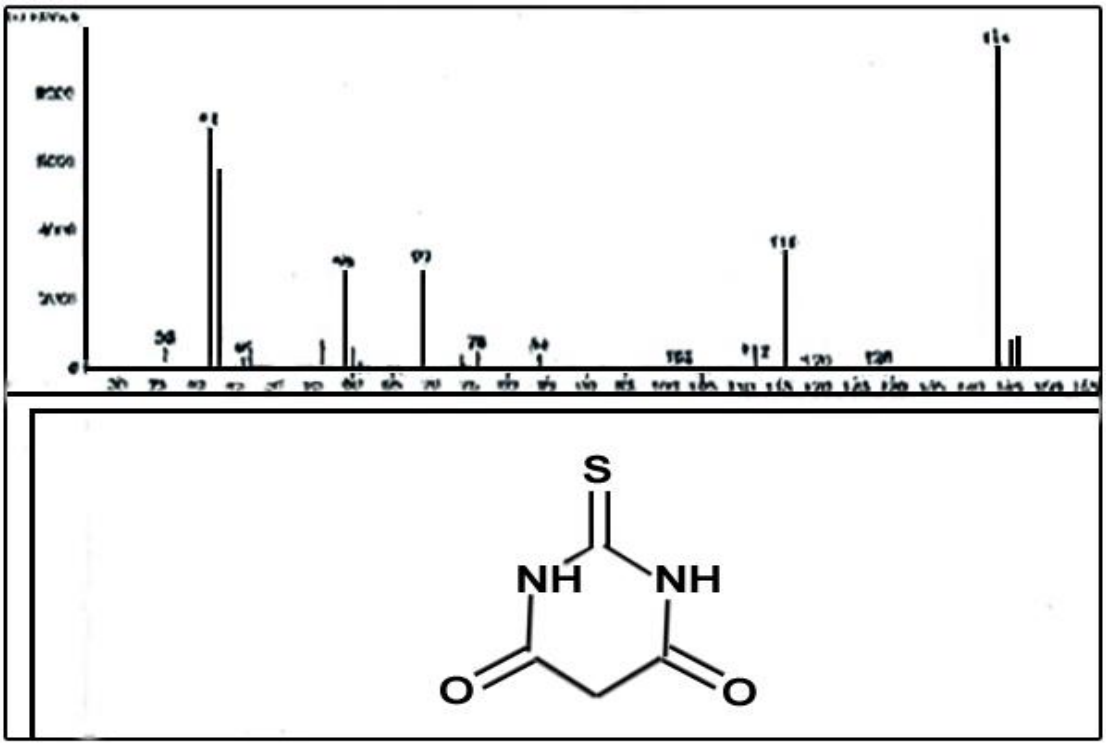

Fig (3c) Spectra and Structure of Thiobarbitone

Methadone Metabolite C: \ DATABASE \ WILEY 275.L

Molecular Formula: C20H23N

Molecular Weight: 277.183

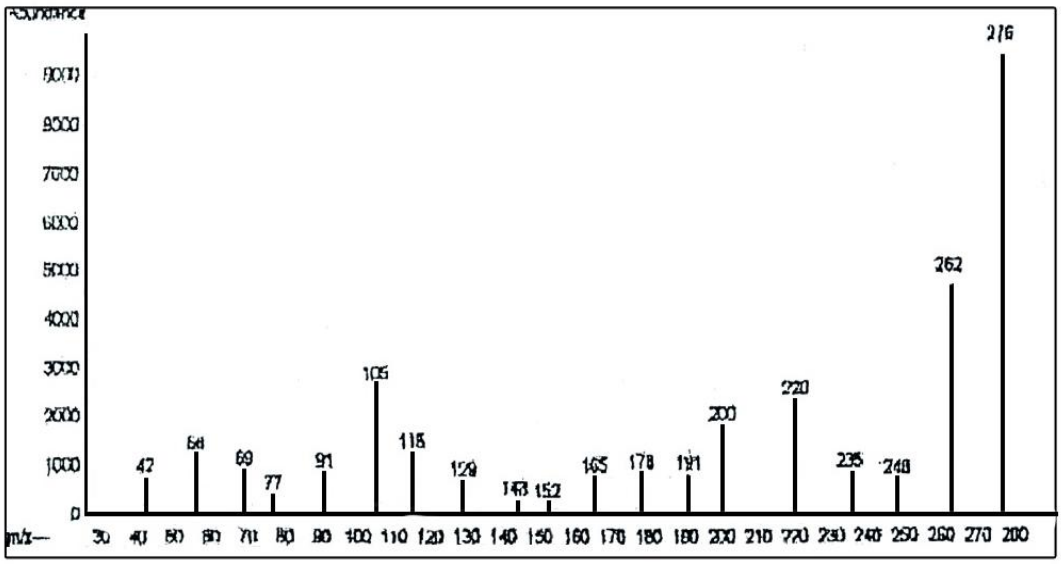

Fig (4) Spectra of Methadone Metabolite

Table 3GC/ MS ion scanning

Group	Compounds	Selective fragment ions amu (m/z)
Benzodiazepines	Clonazepam Oxazepam temazepam	280, 314 268, 239 271
Barbiturates	Thiobarbiurate Butobarbital Seconal	42, 144 156, 141, 55 41, 168
Opiates	Methadone metabolite	276, 262, 105

Table 4 GC/ Specificity and Sensitivity for each drug detected by GC/ MS Analysis

Drug	Sensitivity	Specificity
Benzodiazepines	48.3 %	63.04 %
Barbiturates	17.3 %	77.4 %
Opiates	8.97 %	75.4 %

DISCUSSION

Drug screening is preferably preformed in the urine (Frazer, 1992 and Walls *et al.,* 1997), where the concentrations of drugs are relatively high and the time of detection is long, so that this choice is for screening and identification of unknown drugs or poison. However, the detection of drugs or their metabolic products in the urine indicate only the possibility, but not the certainty that those drugs were active at the time of accident. GC / MS is today the method of choice for systematic toxicological analysis in clinical and forensic toxicology. The screening can be performed using mass chromatography followed by a library search. Results of the present work revealed that urine immunoassay kit is useful for rapid preliminary screening of abuse drug. The qualitative examination by EMIT system of the collected samples indicates that positive tests are as follows; benzodiazepines (329), barbiturates (172), cannabinoid (81) and opiates (165), in the 499 samples for each test, (Table 2). Moreover, EMIT use is generally limited for identification of drug abuse as a whole.

In spite of the immunoassay rapidity, it must be confirmed by GC / MS analysis, especially for samples withdrawn from patients ingesting low dose of these drugs or those with high potency in small doses. The positive tested samples by GC / MS are as follows;

benzodiazepines (159), barbiturates (58) and opiates (28), in the 499 samples for each test, (Table 2). It must be remembered that negative results of some drugs as cannabinoid using GC / MS analysis doesn't mean that those drugs weren't ingested, only they weren't detected due to their concentrations were below the sensitivity range of the assay used, the sampling time wasn't optimal or their clearance rate in urine is rapid. GC / MS distinctive ions of each category individually detected from the mass spectra of the corresponding drugs and their metabolites detected in urine samples as follows; for benzodiazepines (clonazepam: 280, 314; oxazepam: 268, 239; temazepam: 271), for barbiturates (thiobarbiturate: 42, 144; butobarbital: 156, 141, 55; seconal:41, 168) and for opiates (methadone metabolite: 276, 262, 105), (Table 3). All chromatographic peaks have to be identified because any of them may represent a potential poison. Positive signals and the identity of any of those drugs and/or their metabolites can be confirmed by visual or computerized comparison of the peaks underlying full mass spectra with reference spectra, (Pfleger, *et al.,* 1992). Since GC / MS has been reported to be more specific than EMIT for benzodiazepines (63.04 %), barbiturates (77.4 %) and opiates (75.4 %) while, it is approximately equally in sensitivity (48.3 %) with EMIT for benzodiazepines and less sensitive than EMIT for barbiturates (17.3 %) and opiates (8.97 %), (Table 4). So, we used it as a reference test for evaluation of drug screen examination.

Moreover, on the basis of the previous results, GC / MS proved to be of higher specific in recognition of certain drugs, where 245 tests/samples were positive representing 54.56% of the total positive samples (449) and 32.79 % of the total positive tests (747) by EMIT. Thus GC / MS analysis help to solve the false positive results obtained by EMIT.

CONCLUSIONS

In conclusion, this work revealed that EMIT is a rapid preliminary detection test for drug screening in the urine. The data obtained revealed the failure of EMIT to identify the specific drug or its metabolite as do GC / MS. Hence, in spite of the rapidity of EMIT, GC / MS analysis must be done for accurate identification and confirmation of EMIT results. It is recommended to extend the use of EMIT for drugs screening in urine when a rapid diagnosis is needed. Also, GC / MS is recommended as the most suitable technique for obtaining optimum analytical results.

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