Research Article

Detection of Amphetamines, Tramadol, Opiates and Trihexyphenidyl in Urine and Hair Samples at the Medico Legal Directorate in Baghdad Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry

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ABSTRACT

Substance abuse and dependence cause a significant burden to individuals and societies throughout the world including Iraq. Substance abuse analytics has become a commonly used tool in drug abuse problems. The forensic testing of hair for drugs of abuse is a recently acquired law enforcement tool that can be used to ascertain the truth about an individual's consumption of drugs because drugs can be detected for longer periods than in blood or urine or other biological samples. Consequently, there is a need for the continuous development of methods for the efficient determination of drugs of abuse and their metabolites in biological samples. Solid-phase extraction (SPE) has emerged as a variable solvent-free alternative to conventional liquid–liquid extraction procedures. Gas chromatography-Mass spectrometry (GC-MS) has played a fundamental role in determining how many components and in what proportion they exist in a mixture, molecular weight, elemental composition. This study was carried out on people from medico legal directorate and Ibn Rushed hospital in Baghdad, accused of abuse of abused substances to detect abused substances in their urine and hair samples by using solid phase extraction and gas chromatography-mass spectrometry technique first time in Iraq. From the results of this study, it found that the most abusers in Iraq are abusers of amphetamines and conclude that using hair samples and extraction method (enzyme hydrolysis and SPE) and the method of examination (GC-MS) with derivatization reagent (MSTFA), it was able to find various substances abused in hair even if disposed of in the urine.

Keywords: Iraq, Amphetamines, Tramadol, Opiates, Trihexyphenidyl, SPE, GC-MS.

INTRODUCTION

Substance abuse is the continued use of alcohol, illegal drugs, or the misuse of prescription or over-the-counter drugs with negative consequences. These consequences may involve problems at work, school, and home or in interpersonal relationships, problems with the law and physical risks that come with using drugs in dangerous situations. Addiction is defined as a chronic, relapsing brain disease that is characterized by compulsive drug seeking and use despite harmful consequence. [1], but recently Lewis has also argued the view that addiction should not be viewed as a brain disease.[2] Although abused drugs alter multiple brain pathways, they also appear to share some common effects a prevailing view is that the primary brain circuits relevant to drug addiction (equated to activation of neurochemical reward pathways) involve dopaminergic pathways, such as the mesolimbic dopamine system[3].

Hair is a filamentous biomaterial, each hair is a long, cylindrical structure that extends outward, past the epidermal surface, at the base of hair follicle is a small hair papilla, and a peg of connective tissue containing capillaries and nerves, the hair bulb consists of epithelial cells that surround the hair papilla. Hair composed of 65% to 95% proteins, 1 to 9% lipids, 0.1 to 5% pigments (melanin), and small amounts of trace elements, polysaccharides and water [4]. Drugs can enter the hair through 3 pathways: active or passive diffusion from the capillaries feeding the dermal papilla, diffusion from sweat and other secretions bathing the growing or mature hair fiber and external drug deposition from vapors or powders that diffuse into the mature hair fiber from air, water and cosmetic hair treatments[5]. the ability to detect past drug consumption is a unique feature of this matrix, as it provides researchers with a longer detection window (months to years), assuming hair grows approximately 1 cm per month, segmental analysis of hair strands allows the determination of the historic pattern of drug use, Additional advantages of testing hair include a non-invasive means of easily supervised sample collection, reduced risk of sample adulteration, easy sample storage and transportation, and reduced risk of exposure to biohazards. As such, hair analysis of illicit drugs and pharmaceuticals is currently employed to address a wide range of challenges including drug abuse history, workplace testing, post-mortem toxicology, therapeutic drug monitoring and DFA (drug facilitated assault) investigations [6, 7].

Solid phase extraction (SPE) can be a powerful method for sample preparation and today a laboratory cannot do without it, SPE have many advantages such as high recoveries of the analyte, concentration of highly purified extracts, ability to simultaneously extract analytes of wide polarity range, ease of automation, compatibility with instrumental analysis and reduction in organic solvent [8].

Gas chromatography (GC) is a widely applied technique in many branches of science and technology GC has played a fundamental role in determining how many components

and in what proportion they exist in a mixture [9]. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule [10].

Amphetamine, tramadol, opiate and trihexyphenidyl determined in different biological samples including hair using different techniques for the detection.[11-14]

The aims of this research isto detect the amphetamines, tramadol, opiates and trihexyphenidyl in urine and hair samples using solid-phase extraction and gas chromatography-mass spectrometry technique.

Subjects and method

Fifty people from the medico legal directorate and Ibn Rushed hospital in Baghdad accused of abuse of abused substances and twenty people were not taking any drug for the last three months of the study served as control. The sampling site for hair is the back of the head, in the vertex posterior, cut close to the scalp as possible, the sample size taken is100 mg of hair. Once collected, the hair samples were wrapped in aluminum foil, enclosed in an envelope, and coded. Hair samples were washed twice for 5 min in dichloromethane. After drying, the hair samples were cut into small pieces of about 0.5 mm by hand scissors. The washing solutions were analysed by conventional gas chromatographic–mass spectrometric (GC/MS) procedures to exclude contamination. While urine samples (30 ml) were stored at -20◦C until used for detection of abused drugs.

The abused drugs detected in urine using acid hydrolysis method to remove glucuronide conjugates prior to extraction. [15] While in the hair used enzymatic digestion to extract the substances. [(16)] Extracted the samples by solid phase extraction method used SPE columns with multifunctional sorbent (benzene sulfonic acid + octyl C8), size 500mg/3ml/50pkg, column conditioning carried by added 2ml methanol, 2ml distilled water and add 2 mL 0.1 M K2HPO4 (pH 6.0), loading the samples (The flow rate should not exceed 1 to 2 mL/minute), column rinse with 2 mL acetic acid solution 0.5 N and 2ml methanol, the analyte eluted with 2ml mixture [propanol-2, dichloromethane, ammonia] and to derivatization 50 µL of derivatization reagent (15 µL N-Methyl-N-(trimethylslilyl) trifluoroacetamide (MSTFA) + 35 µL Toluene) the heat at 60°C for 15 minutes, After Acid hydrolysis for the 20ml of each urine samples extracted using Liquid-Liquid extraction(LLE) methodto compare with (SPE).[(17)] The identification was performed using the GC-MS device using split/splitless (S/SL) inlet at 250°C, 12psi, 1.2ml/min flow, capillary column (HP-5ms 30 m x 250 µm x 0.25 µm), the initial oven temperature is 80°C and the final temperature is 350°C and helium is attached to the carrier gas supply of the S/SL inlet.

RESULTS AND DISCUSSION

Results of this study showed that all the samples of urine of the control group showed absence of the abused substances. The samples of the hair of the control group also were be empty of the abused substances, just one sample of them showed presence of acetaminophen (Figure 1).The presence of acetaminophen in the hair sample indicate that we can use this method for monitoring prescribed and non-prescribed drugs used during treatment programs provides valuable information for the diagnosis and management of diseases. Saito (2008) used GC-MS and SPE method to hair analysis of acetaminophen in actual poisoning case. [18]

For amphetamines there were Seven out of fifty samples of the urine showed presence of amphetamine (Figure2) and nine other samples containing methamphetamine (Figure 3), meanwhile there were seventeen out of fifty of the hair samples containing amphetamine (Figure 2) and other twenty one containing methamphetamine (Figure3).This study have noticed that the number of people taking methamphetamine is more than people who take amphetamine which may contribute to the more euphoric and addictive properties of methamphetamine compared with amphetamine, methamphetamine released five times more dopamine than amphetamine. [19] The results of this study are in agreement with a study among Saudi patients in addiction treatment settings indicated that the most commonly abused substances were amphetamines (4–70.7%).[20]

There were two samples of the fifty urine samples showed the presence of tramadol, meanwhile there were three out of fifty of the hair samples containing tramadol (Figure 4). Tramadol is a centrally acting synthetic analgesic with µ-opioid receptor agonist activity, it is a widely prescribed analgesic used in the treatment of moderate to severe pain and as an alternative to opiates, its efficacy and low incidence of side effects lead to its unnecessary prescribing in patients with mild pain.[21] Yilmaz (2015) determined tramadol and its metabolite in human urine by the SPE and GC-MS method [(22)] and Hadid (2003) determined tramadol in hair using SPE and GC-MS method.[23] Tramadol has been widely and freely used in Iraq over the last few years. It has a clear risk of causing dependency syndrome; tramadol abuse seems to be a growing problem in Iraq.[24]

Only one sample of the fifty urine samples showed the presence of methadone (Figure 5), meanwhile in the hair samples, there were two sample containing codeine and three other sample contains methadone (Figure5 and figure6).Methadone, a synthetic opioid analgesic, is used in the treatment for heroin dependence and chronic pain, Because of its long half-life, good enteral absorption, and low cost, it has become an important substance in the treatment for opiate addiction.[25] Codeine is a phenanthrene derivative extracted from opium or produced synthetically by the methylation of morphine, it

is the most commonly consumed opiate worldwide and is used for its analgesic, antitussive and anti-diarrheal properties.[26] Both morphine and codeine undergo extensive hepatic metabolism before renal elimination. The majority of morphine is eliminated in the urine as morphine 3-glucuronide or morphine 6-glucuronide. Codeine is mostly eliminated as codeine 6- glucuronide, and less than 10% as morphine or its glucuronides. Morphine and codeine are weak bases that under physiological conditions coexist in a protonated and a non-protonated form. The non-protonated forms of morphine and codeine may penetrate cellular membranes by passive diffusion. However, morphine, in contrast to codeine and some other opioids, has only limited membrane permeability by diffusion.[27]Genetic variations in activity of human cytochrome P450 2D6 vary rates of conversion codeine to morphine, up to 20% of Arabian are slow metabolizers. [28, 29]

A major difference in the analysis of different biological matrices is the relative concentration of parent drug and metabolite(s) expected, EDDP detected in urine more than in hair in contrast to methadone which is more detected in hair than its metabolites, but codeine is more likely to be detected in different biological matrices (urine, hair, saliva and sweat) more than its metabolites.[30]

Results of this study are in agreement with Cippitelli (2017) study who demonstrated the ability to detect opioids in hair in an exhumed body that has been buried for 1 year .[31]

There was one sample of the urine containing trihexyphenidyl, meanwhile there were four samples of the hair containing trihexyphenidyl (Figure 7). Trihexyphenidyl is a potent anticholinergic drug used in the treatment of Parkinsonism and in the control of drug-induced extrapyramidal side effects, the drug is of forensic toxicology interest because of its frequent abuse and reported overdose and fatal poisoning.[32] Mohammad Amin (2013) found that trihexyphenidyl abuse is a problem in Iraq and Kurdistan Region that needs to be addressed by the medical, legal and social authorities in the region and prisons are among the places that need to be looked at for this abuse. [33] The major target analyses for trihexyphenidyl in urine were found to be their cyclohexane-ring mono substituted hydroxyl metabolites.[14] Trihexyphenidyl analysis in hair may provide useful information about drug treatment and the history of usage, and that drugs can be detected in normally kept hair for at least 16 months after intake. [34]

Although in this study a larger volume of urine in the (LLE) method (20ml) were used, compared to the small size used in the (SPE) method (3ml) but it could not detect any kind of substance in the urine. SPE has several advantages over LLE such as low solvent consumption, enormous saving of time, increased extraction efficiency, decreased evaporation volumes, higher selectivity, cleaner extracts, greater reproducibility, of emulsion formation, and easier automation.[7,8,35, 36]

The abuse substances were detected in the twenty samples of the urine in comparison to fifty samples of the hair of the same individuals. (Figure 8).There are a number of reasons for the lack of these substances in the urine of people whose hair contains these abuse substances, One of the main reasons for this problem in Iraq is not to bring the accused to take urine samples at the same time as the arrest which leads to the disposal of the substances and unclean in the urine , The right time to find these substances in urine is within 48 hours also, diluting urine is a simple way to make an otherwise positive drug test result negative by reducing the concentration of the drug , in other countries, Federal guidelines recommend placing a toilet bluing agent in the toilet tank, if possible, so that the reservoir of water in the toilet bowl always remains blue, there should be no other source of water in the enclosure where urination takes place[37,38] and this is not done in Iraq. Hair analysis of drugs of abuse has been a subject of growing interest from a clinical, social and forensic perspective for years because of the broad time detection window after intake in comparison to urine and blood analysis. Additional advantages of testing hair include a non-invasive means of easily supervised sample collection, reduced risk of sample adulteration, easy sample storage and transportation, and reduced risk of exposure to biohazards. As such, hair analysis of illicit drugs and pharmaceuticals is currently employed to address a wide range of challenges including drug abuse history, workplace testing, post-mortem toxicology, therapeutic drug monitoring and drug facilitated assault (DFA) investigations Over the last few years, hair analysis has gained increasing attention and recognition for the retrospective investigation of drug abuse in a wide variety of contexts, shown by the large number of applications developed.[7, 38,39]

CONCLUSION

From the results of this study, it concluded that the most abusers in Iraq are abusers of amphetamine and using hair samples and extraction method (enzyme hydrolysis and SPE) and the method of examination (GC-MS) with derivatization reagent (MSTFA), it was able to find various substances used even if disposed of in the urine.

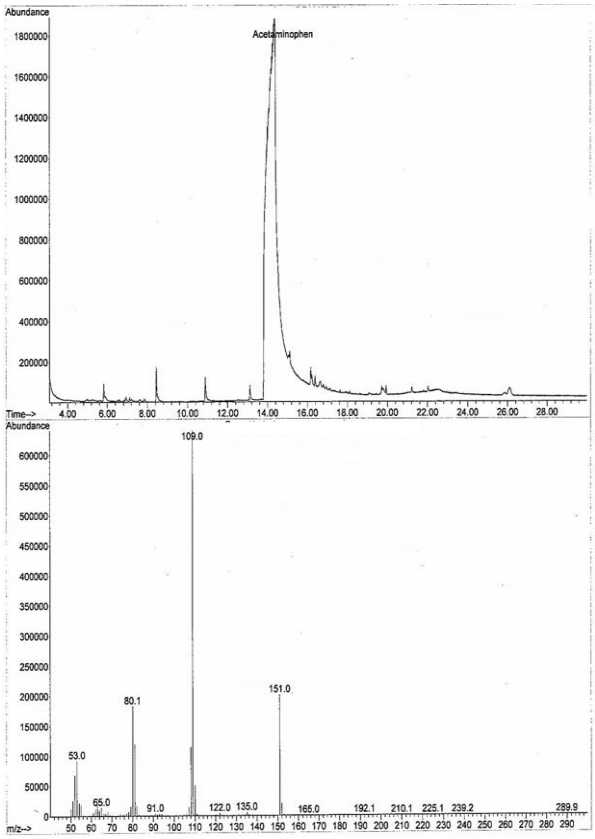

Figure 1: Acetaminophen in one of the samples of the hair of the control group

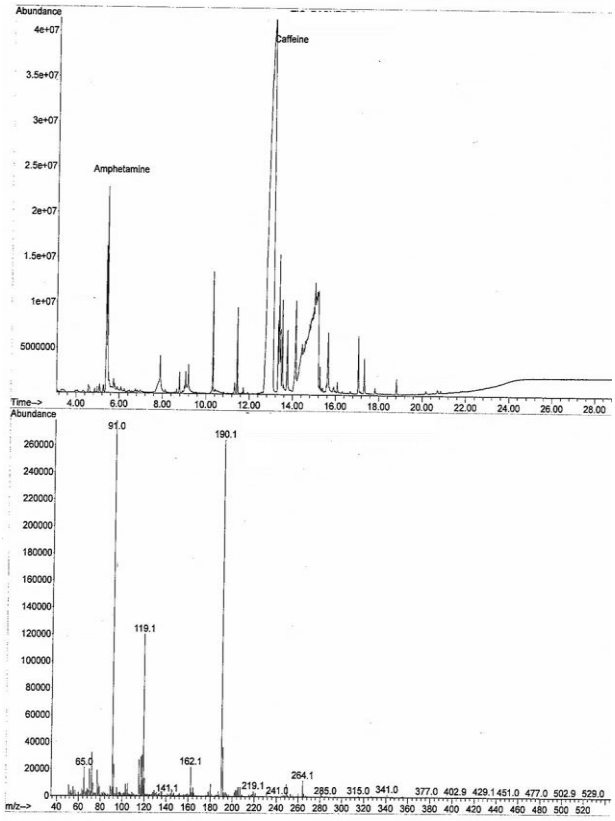

Figure 2: Amphetamine in the urine and hair samples

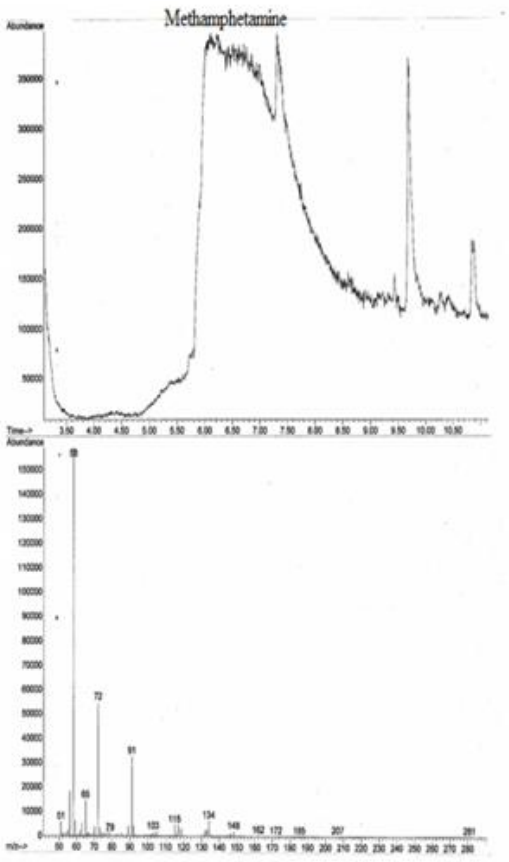

Figure 3: Methamphetamine in the urine and hair samples

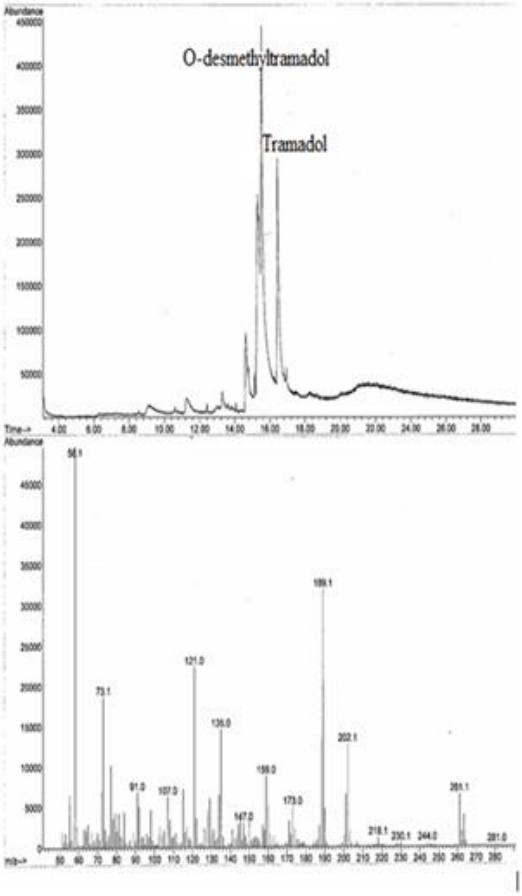

Figure 4: Tramadol in the urine and hair samples

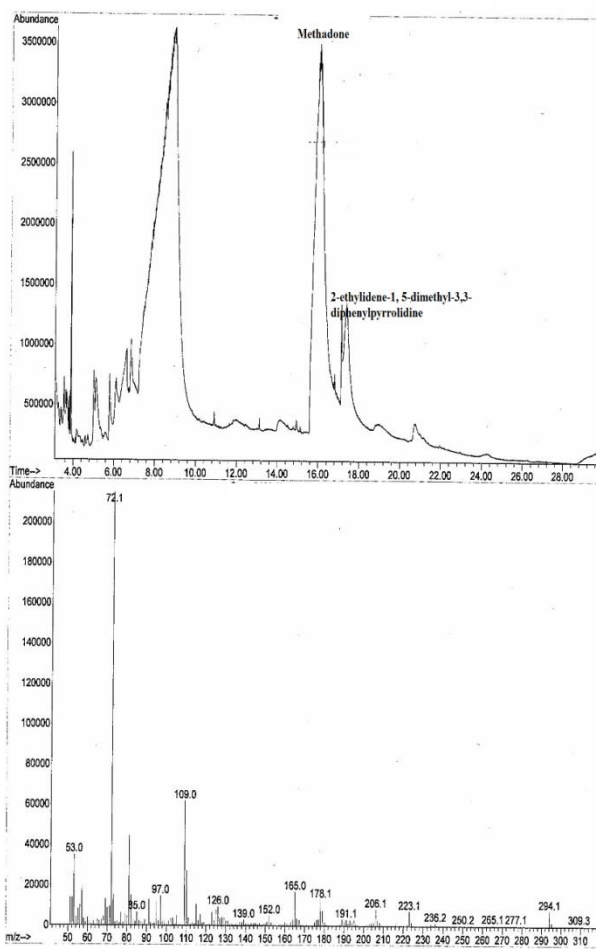

Figure 5: Methadone in the urine and hair samples

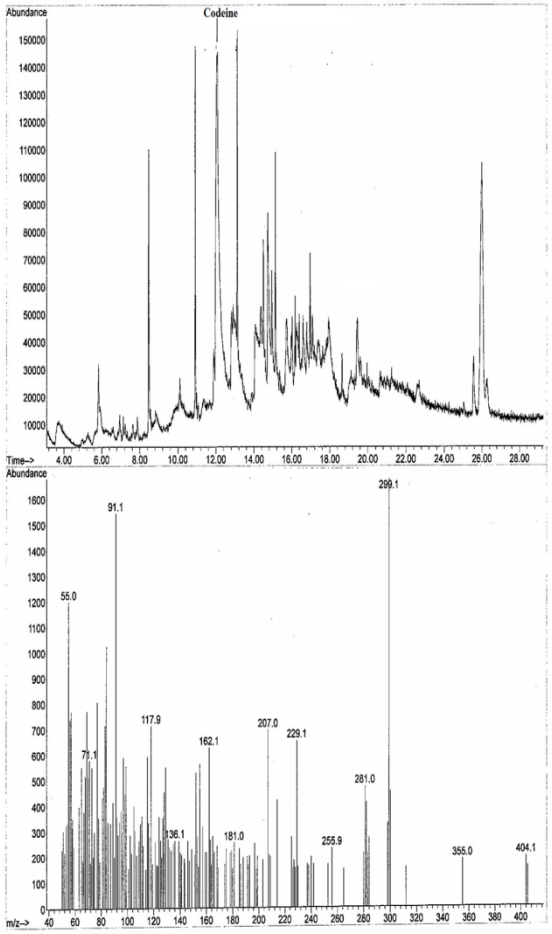

Figure 6: Codeine in the hair samples

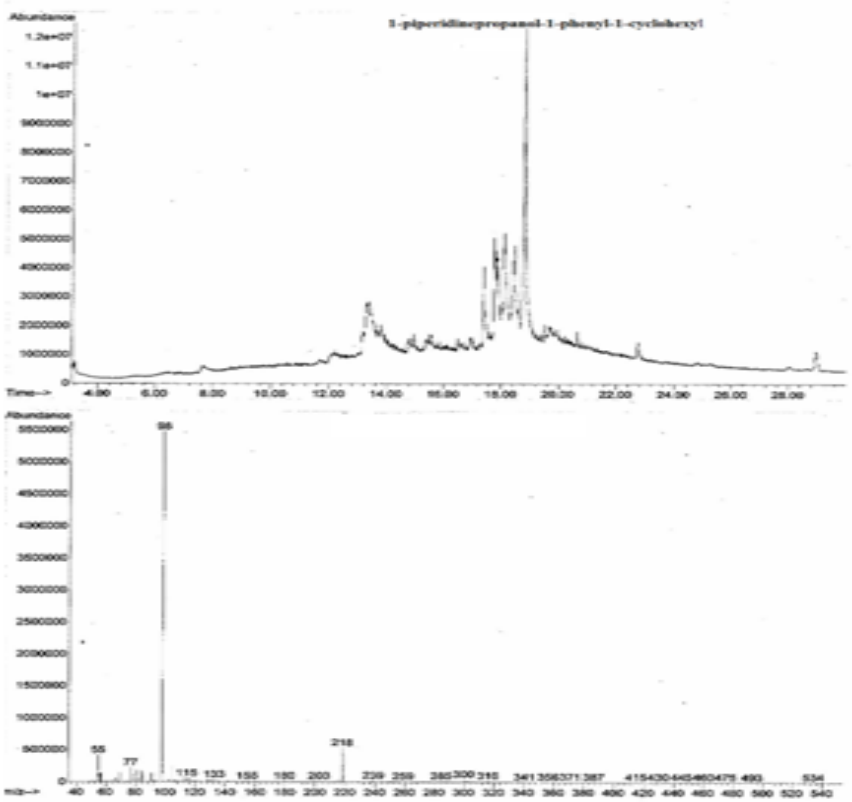

Figure 7: Trihexyphenidyl in the urine and hair samples

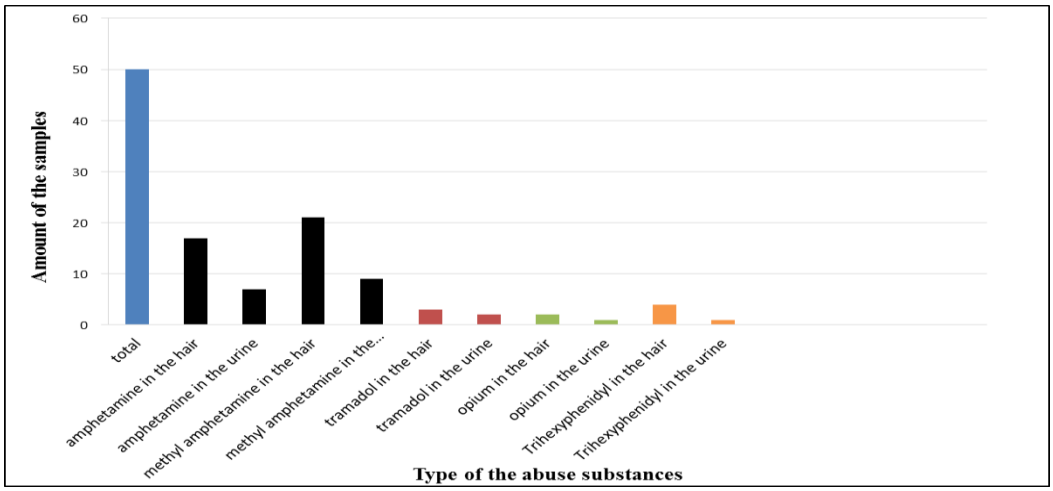

Figure 8: Amount of the samples and type of the abused substances in the urine and the hair

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