A PRELIMINARY STUDY OF COLORIMETRIC NANOPARTICLE EVALUATION OF ADULTERATED SIMULATED URINE FOR AMPHETAMINE-TYPE STIMULANTS (ATS) ABUSE TESTING

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BY

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TABLE OF CONTENTS

CERTIFICATE ..... **i**

DECLARATION ..... **ii**

ACKNOWLEDGEMENT ..... **iii**

TABLE OF CONTENTS ..... **iv**

LIST ..... **vii**

LIST ..... **ix**

LIST OF ABBREVIATIONS ..... **x**

LIST OF APPENDICES ..... **xii**

ABSTRAK ..... **xiii**

ABSTRACT ..... **xv**

CHAPTER 1 ..... **1**

INTRODUCTION **1**

1.1 Research Background 1

1.2 Problem Statement 3

1.3 Objectives 3

1.3.1 General objective 3

1.3.2 Specific Objectives 3

1.4 Significance of the Study 4

CHAPTER 2 LITERATURE REVIEW **5**

2.1 Amphetamine-Type Substances (ATS) 5

2.2.1 Toxicity and-Sudden Death Related with Methamphetamine 7

2.3 Methamphetamine production 13

2.4 Narcotic Types and Addiction in Iraq 15

2.5 Adulterants 16

2.6 Drug Color Tests Screening in Urine 19

2.6.1 Dipstick color test 19

2.6.2 Immunoassay urine screening testing 21

2.6.3 Thin Layer chromatography (TLC) 24

2.7 Nanoparticle Techniques 29

CHAPTER 3 METHODOLOGY 37

3.1Chemicals, Reagents, Instrument, Apparatus 37

3.2 Laboratory Apparatus 39

3.3 Laboratory Instruments 41

3.4 Collection and Preparation of Adulterant Materials 42

3.5 Sample Preparation 43

3.5.1 Synthetic positive urine methamphetamine (METH)& amphetamine (AM) 43

3.5.2 Preparation of Reagent 43

3.5.1 Rationale for the mixture 47

3.5.2 Preparing the mixture 47

3.6.2 Procedure of Color UDSTs kits test 48

3.6.3 Procedure for Spot color assay in thin layer chromatography 48

3.6.4 Procedures for Nanoparticles Technique 50

CHAPTER 4 53

RESULTS AND DISCUSSION 53

4.1 Control Tests Using Color Strip and UDSTs Kits 53

4.2.1Adulterated Urine Sample Analysis Using Color Strip and UDSTs Kits. 54

4.3 TLC Analysis of Adulterated Urine Samples 58

4.4 Screening using Nanoparticles Reagent 61

CHAPTER 5 CONCLUSION AND FUTURE RECOMMENDATIONS 66

5.1 Conclusion 66

5.2 Future Recommendations 68

REFERENCE 70

APPENDICES 78

(APPENDIX A: METABOLISM OF AMPHETAMINE) 78

APPENDIX B: PREPARATION IODOPLATINATE 79

APPENDIX C: PREPARATION OF MARQUISE REAGENT 80

APPENDIX D: PREPARATION OF DRAGENDORFF REAGENT 81

LIST OF TABLES

		Page No.
Table 2.1	Quantities seized in 2023 in Iraq	16
Table 2.2	Solvent type and reagents & color	28
Table 2.3	Evolution Timeline of Nanoscience and Nanotechnology	34
Table 3.1	List of chemicals and reagents used in this study	36
Table 3.2	List of laboratory apparatus used in this study	38
Table 3.3	List of Instruments used in this study	40
Table 3.4	List of adulterant materials	41
Table 4.1	Color strip and UDST test observation for control samples	51
Table 4.2.1	Color strip and UDST test observation for adulterated urine samples	53
Table 4.2.2	Color strip and UDST test observation for adulterated urine samples containing methamphetamine & amphetamine	54
Table 4.3.1	RF for ATS compounds	56

Table 4.3.2	TLC results of methamphetamine detected in urine adulterated samples after acid base extraction	57
Table 4.4	Observation of nanoparticle color reaction in adulterated urine with (ATS) control & Negative control & pseudoephedrine –control &(ATS) compounds	59

LIST OF FIGURES

		Page No.
Figure 2.1	Structures of sympathomimetic amines	7
Figure 2.2	Chemical structures of the adulterants	18
Figure 2.3	Principle steps of thin layer chromatography Chemical	26
Figure 3.1	screening of urine test using UDSTs kit procedure	42
Figure 3.2	Preparation of GNPs	45
Scheme 3.1	Preparation of nanoparticles reagent	45
Figure 3.3	Examination of urine used color strips test	46
Figure 3.4	Examination of urine used color UDSTs Kit test	47
Figure 3.5	Steps of extraction	48
Figure 3.6	Steps of TLC Plate of Silica Gel (spot of extract) + (TLC system) + (reagent spray)	48
Figure 3.7	Calculate RF from the relation	49
Figure 3.8	Examination of urine used color nanoparticles reagent	50
Scheme 4.1	Complex of nanoparticle color detection in methamphetamine urine sample	61
Figure 4.1	Detection using nanotechnology that relies on DNA protein is more accurate than detection using immune test strips that rely on antibodies, and also detects false positive results	62

LIST OF ABBREVIATIONS

ATS	**Amphetamine Type Stimulants**
TLC	**Thin Layer Chromatography**
MDMA	**3,4-methylenedioxy-N-methamphetamine**
MA	**Amphetamine**
METH	**Methamphetamine**
MDA	**3,4-methylenedioxymethamphetamine Amphetamine**
UDSTs/UDS	**urine drug screening tests**
°C	**Degree Celsius**
Ag	**Antigen**
Ab	**Antibody**
PBP	**The salt base with pH of 8**
SG	**Specific gravity**
DNA	**Deoxyribonucleic acid**
HAuCl4	**Gold(III) chloride Trihydrate (<99.9% trace metals basis)**

AuNPs /GNPs.	**Gold nanoparticles**
RF	**Retardation Factor**
Aptamers	**Small, highly structured DNA/RNA molecules, having affinities for target molecules similar to the way antibodies work.**
G-quadruplexes	**noncanonical nucleic acid structures formed from stacked guanine tetrads.**
pH	**basic, or neutral.**
Mg	**Microliter**
g	**Grams**
µL	**Milligram**
Vitality test	**Dipstick test**

LIST OF APPENDICES

APPENDIX A	**METABOLISM OF AMPHETAMINE**
APPENDIX B	**PREPARATION OF IODOPLATINATE REAGENT**
APPENDIX C	**PREPARATION OF MARQUIS REAGENT**
APPENDIX D	**PREPARATION OF DRAGENDORFF REAGENT**

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ABSTRAK

Penyalahgunaan dadah adalah masalah yang serius dan meluas, dan ujian air kencing dianggap sebagai salah satu kaedah yang paling banyak digunakan untuk mengesan penyalahgunaan dadah, tetapi seperti yang dibuktikan oleh sejarah orang yang telah dirujuk ke makmal forensik, penggunaan serbuk pembersih, pangkalan dan mereka komponen, pil perancang, atau pil aspirin untuk mengubah keputusan analisis dadah, kerana dipercayai bahawa bahan-bahan ini akan mempengaruhi ujian saringan air kencing, tujuan kajian ini adalah untuk menentukan kesan bahan-bahan ini pada ujian makmal kolorimetrik biasa (UDSTs). dan menggunakan pengesanan kolorimetrik nanopartikel emas yang tidak diubah suai untuk mengesan keputusan negatif palsu.

Dalam kajian ini, jalur ujian saringan dadah (biasa dilakukan pada sampel air kencing menggunakan immunoassays) akan disaring menggunakan air kencing positif untuk perangsang jenis amphetamine (ATS) dan mengesahkan ketulenan sampel dan kehadiran ATS dalam sampel selepas penambahan tiruan menggunakan kaedah kromatografi lapisan nipis.

Satu kaedah telah digunakan untuk menyaring keputusan negatif palsu dengan usaha dan masa yang kurang daripada kaedah kromatografi lapisan nipis (TLC) menggunakan teknik nanopartikel emas yang tidak diubah suai, kesan sebelas jenis pemalsuan biasa telah dikaji. Asid asetik, asid sitrik, hidroksida, peluntur berasaskan hipoklorit, Pyridinium chorochromatic PCC, kalium dikromat, sebatian natrium klorida, ammonia dan nitrat, titisan mata dan detergen adalah sebatian yang

dibincangkan. Dalam empat ujian, telah diuji pada air kencing positif untuk ATS dengan menambah bahan penzina pada nisbah 2:1, ujian Dipstick air kencing dan ujian kehadiran dadah memberikan keputusan negatif untuk kes pemalsuan, dan ujian kolorimetrik ketiga dilakukan dalam air kencing menggunakan kaedah TLC, Reagen Marquis, iodoplatin, dan reagen Dragendoff. Plat silika telah disembur dengan reagen untuk penentuan maya amfetamin, methamphetamine, dan 3,4-methylenedioxy-N-methamphetamine (MDMA) pada suhu lebih tinggi daripada 20 °C akibatnya, ubat negatif palsu boleh dikesan dalam sampel air kencing. Tetapi kaedah ini memerlukan masa, usaha dan bahan, ujian keempat adalah menggunakan pengesan zarah nano yang diformulasikan untuk mengesan kehadiran perangsang jenis amphetamine, dengan menukar warna daripada merah kepada biru pada suhu 24 darjah Celsius, pengesan zarah nano adalah dapat mengesan kehadiran perangsang jenis amphetamine dalam kepekatan rendah dalam sampel air kencing, jika dibandingkan penggunaan kaedah pengesanan kolorimetrik TLC dengan kaedah pengesanan kolorimetrik nanopartikel, kami menyimpulkan bahawa yang kedua adalah unggul kerana ia memberikan hasil dalam masa yang lebih singkat dan memerlukan kurang usaha.

A PRELIMINARY STUDY OF COLORIMETRIC NANOPARTICLE EVALUATION OF ADULTERATED SIMULATED URINE FOR AMPHETAMINE-TYPE STIMULANTS (ATS) ABUSE TESTING

ABSTRACT

Drug abuse is a serious and widespread problem, and urine testing is considered one of the most used methods to detect drug abuse, but as evidenced by the history of people who have been referred to forensic laboratories, the use of cleaning powders, bases and their components, birth control pills, or aspirin pills to change Drug analysis results, because it is believed that these substances will affect the urine screening test, the purpose of this study is to determine the effect of these substances on common colorimetric laboratory tests (UDSTs) and to use colorimetric detection of unmodified gold nanoparticles to detect false negative results.

In this study, a drug screening test strip (commonly performed on urine samples using immunoassays) will be screened using urine positive for amphetamine-type stimulants (ATS) and verifying the authenticity of the sample and the presence of ATS in the sample after addition of counterfeits using thin layer chromatography method.

A method was used to screen false negative results with less effort and time than the thin layer chromatography (TLC) method using nanoparticle techniques of unmodified gold, the effect of eleven common types of adulteration was studied. Acetic acid, citric acid, hydroxide, hypochlorite based bleach, Pyridinium chorochromatic PCC, potassium dichromate, sodium chloride, ammonia and nitrate compounds, eye drops and detergents are the compounds discussed. In four tests, were tested on urine positive for ATS by adding adulterants at a 2:1 ratio, urine Dipstick testing and drug presence testing gave negative results for the adulteration cases, and a third colorimetric test was performed in the urine using TLC method, Marquis

reagent, iodoplatins, and Dragendoff reagent. A silica plate was sprayed with a reagent for the virtual determination of amphetamine, methamphetamine, and 3,4-methylenedioxy-N-methamphetamine (MDMA) at a temperature higher than 20 °C as a result, false negative medications can be detected in the urine sample. But this method requires time, effort and materials, the fourth test is to use a nanoparticle detector formulated to detect the presence of amphetamine-type stimulants, by changing the colour from red to blue at a temperature of 24 degrees Celsius, the nanoparticle detector was able to detect the presence of amphetamine-type stimulants in low concentrations in urine samples, when compared the use of the TLC colorimetric detection method with the nanoparticle colorimetric detection method, we conclude that the latter is superior because it gives results in less time and requires less effort.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Since world war I, chemists have been inventing stimulants of amphetamine type (ATS) (Rasmussen, 2008), when abused, they were prohibited and listed in the drug schedules (Lakhan & Kirchgessner, 2012), the objective of molecular redesign and modification is to circumvent scheduling restrictions, this was especially true for the class of "designer" “amphetamine” and “methamphetamine” compounds, especially 3,4-methylenedioxymethamphetamine (“MDMA”; "ecstasy"; "ADAM"; "XTC") and its N-dimethyl metabolite, 3,4-methylenedioxymethamphetamine amphetamine (MDA). Figure 2.1 shows that MDMA and MDA are two of the oldest so-called designer drugs. MDA is a derivative of amphetamine, whereas ecstasy is derived from methamphetamine. It is also essential to note that MDA is an MDMA metabolite (Rasmussen, 2008).

Drug use refers to the consumption of any illegal substance, these substances are ingested, inhaled, smoked, or injected by those who use them. methamphetamine (MA) is a powerful and addictive synthetic stimulant of the central nervous system., furthermore, as part of an investigation or court proceeding, legal or forensic drug testing may be conducted to provide evidence of a crime, drug testing identifies the use or abuse of illicit substances, the most prevalent use of drug testing is as a pre-employment requirement in the workplace. employees may also be subjected to random or periodic drug screenings, in addition, drug testing may be required for military personnel along with athletic evaluation (Kwon, 2018).

Adulteration is the manipulation of a urine specimen to alter the results of a drug test, by introducing substances into the body or adding them to a urine sample with chemical adulterants to produce a false negative test result urine drug testing plays a crucial role in monitoring both legal and illegal drug use for medico legal and clinical purposes, is one of the major challenges of urine drug testing. this issue is exacerbated by the number of readily available chemicals that can be used to falsify urine samples "(ALIM, 2021)".

In many countries, urine drug tests (UDS) are typically performed on urine samples using immunoassays, since the basis of immunoassay technology in drug detection is the interaction between antigen and antibody, one of the most important causes of false negative and positive results is interference with the formation of the Ag-Ab complex by factors. Specifically, the optimal pH range for antigen-antibody complex formation is between 6.5 and 7. At pH 5.0 or 9.5, the equilibrium constant for the Ag-Ab reaction is one hundred times smaller than between 6.5 and 7.0. On either side of the cap, the interaction between antibody and antigen is strongly inhibited, extreme PH values cause significant conformational changes in the antibody molecule, which may destroy its ability to bind to the antigen, therefore, a PH greater than or less than 9.5 or 5, respectively (Amitava , 2019), the reaction between Ag and Ab may not form, resulting in a false negative result, the ionic strength of the urine environment is a further crucial factor that can affect the Ag-Ab interaction. if the environment is so altered that antigen or antibody conformational changes occur, adding sodium chloride to the urine environment, for instance, may produce a false-negative result. (Amitava , 2019)

In recent years, nanoparticles based on mass spectrometry have been utilized extensively in chemical and bimolecular analyses of food safety, environmental pollutants, disease biomarkers, and illegal drugs, among others, nanotechnology is

required to simplify the pre-treatment of samples for the analysis of drugs and drugs of abuse due to the long-standing issues and complex experimental procedures that accompany traditional drug testing and abuse methods. “(Wu, 2018)”.

Previous studies focused on the effect of adulterants on the results of positive urine samples but did not discuss new methods for drug detection in samples that have been tampered with, this study focuses on the application of this method to false-negative urine test results for the detection of ATS in adulterated urine.

1.2 Problem Statement

False-negative results in positive urine testing ATS using UDSTs are common due to adulterous substances, the basis of immunoassay technology in drug detection relies on the interaction of antigen and antibody, one of the most important reasons for producing false negative and positive results is associated with factors that may interfere with forming the Ag-Ab complex., such as pH therefore, if the substances added to the urine environment are able to change a pH and the ionic strength of the urine environment.

1.3 Objectives

1.3.1 General objective

The overall aim of this research is to study the adulterated simulated urine test using existing colorimetric USTDs tests and colorimetric detection of unmodified gold nanoparticles tests.

1.3.2 Specific Objectives

1. To determine the effected of adulterants to simulated urine samples containing ATS when examined using UDTS & DIPSTICK Test.

2. Used thin layer chromatography method for detection of ATS in simulated adulterated urine samples, and it was used to ensure that the sample was not damaged adulterated, and to confirm the ability of the colorimetric detector for unmodified gold nanoparticles to detect ATS in adulterated urine samples by comparing the results.

3. To conduct a colorimetric test using nanoparticle complex (use of aptamers and the salt-induced aggregation of unmodified gold nanoparticles) for detection of ATS in simulated adulterated urine samples.

1.4 Significance of the Study

The study allows to understand the composition and effected of adulterous substances on the urine sample and the type of effects resulting from the addition of these compounds.

This study also provides some information about adultery substance that give false negative drug test results in addition, due to the problems mentioned previously, research interest is in developing and test the nanoparticle matrices to detect ATS compounds in simulated adulterated urine containing ATS results

This method is potentially accurate and may overcome the adulteration effect in urine sample, it may have low cost and saves time and effort providing a faster and productive service in forensic laboratory operation

CHAPTER 2
LITERATURE REVIEW

2.1 Amphetamine-Type Substances (ATS)

In 1887, the chemist Edelino isolated the plant's primary alkaloid, amphetamine "(phenylisopropylamine or methylphenylethylamine"), from the Ephedra vulgaris plant. In the same year, Ngai was successful in producing it. two years later, Merck introduced amphetamine as a treatment for the common cold, an antiamphetamine for asthma treatment. "In 1938, Boehringer/Ingelheim introduced the same preparation in Germany under the name Bencedrines. During World War II", (Rasmussen, 2008) chemists Timler, Dubke, and Hochschild developed methamphetamine as a "endurance pill" in numerous countries due to its performance-enhancing and appetite-suppressing properties. In the final days of the war in Germany, attempts were made to increase the military's effectiveness by combining methamphetamine with cocaine and morphine derivatives.

Temmler-Werke sold methamphetamine under the brand name Pervitin as a central sweetener, and ecstasy is fully functional according to this definition, the box's label reads, "Reactive depression, melancholy and mild depression, narcolepsy, lethargy, sleep intoxication, and withdrawal therapy (Hartogsohn, 2017)." Hypotension and circulatory collapse (such as after treatment for malaria). Post-herpetic Parkinsonism (combination therapy) Between deviant behaviour and scopolamine. Paralysing stimuli"Epilepsy of Jacksonian origin." (Whitley, 2010)

By avoiding fatigue, there is a performance-enhancing effect that results in enhanced performance, well-being, and concentration. Since 1941, addiction has been suspected. due to the extended period of recovery, psychosis has also been observed during long-

term use of amphetamine (Kramer, 2015). Global use and production have been restricted since the 1960s. In 1986, amphetamine products were placed under the DCT (BTMG), and two years later, the vast majority of indications were eliminated.

Only a few amphetamine derivatives, including methylphenidate (Ritalin) and phenethylline (Caption), are legal and available (in some countries) only with a prescription for anaesthesia (Rasmussen, 2015).

Chemically, amphetamine-type stimulants are a group of "designer" amphetamine and methamphetamine compounds, including “3,4-methylenedioxymethamphetamine (“MDMA” "ADAM" "XTC")” and its dimethyl metabolite “N,3,4-methylenedioxymethamphetamine (MDA)”. (Figure2:1) demonstrates that “amphetamine”, “methylamphetamine (MA)”, “MDMA”, and “MDA” are among the oldest so-called designer drugs. “MDMA”, or “MDMA”, is a “methamphetamine derivative”. Meanwhile, “MDA” is an “amphetamine derivative”. additionally, it is important to note that “MDA is an MDMA metabolite (Moore, 2020)”. The structure contains “dextro and levo isomers” and contains a single analytic center.Reduces the sedative effects of drugs and causes agitation and insomnia. In 1935, Smith, Klein, and French were granted a patent for a mixture of racemic “amphetamine”. 1976 saw the approval of a product by ‘Smith, Klein”, and French by the “(US Food and Drug Administration)”. “amphetamine” is synonymous with “(“alpha-methylbenzenethylamine, beta-aminopropylbenzene’, “beta-phenylisopropylamine”, and “deoxynorphedrine”. “ADHD” and other disorders of the central nervous system, such as narcolepsy, are treated with amphetamine (Heal DJ, 2013)). ADHD is caused by a “complex interaction” of “genetic and non-genetic” factors, which results in complex mental disorders in children and adolescents. it is a disorder characterised by

substantial “heterogeneity” in treatment outcomes, clinical symptoms, and aetiology. (Kornum BR, 2017))”

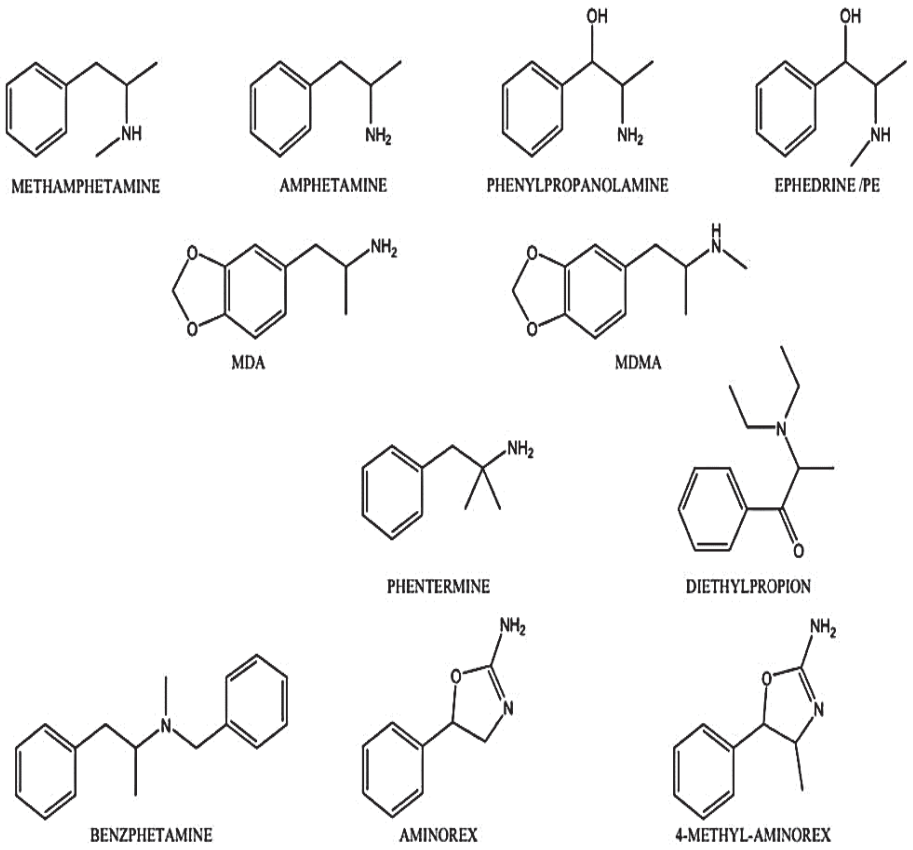

Figure 2.1: Structure of sympathomimetic amines (Barger, 2010).

2.2.1 Toxicity and-Sudden Death Related with Methamphetamine

Users experience euphoria, increased libido, improved efficiency, increased vitality, and decreased anxiety immediately after taking the drug (Homer, 2008). "Methamphetamine abuse is also associated with a significant number of negative issues and obstacles for humans, “this toxicity is classified as acute, altering cognitive and behavioral functions and causing neurological damage. “METH” users suffer from tachycardia, agitation, aggression, high blood pressure, and hyperthermia, impaired

control, euphoric inhibition, and psychomotor arousal are associated with “METH” abuse (Meredith, 2005)”.

The body temperature rises and may reach above 41 degrees Celsius when taking large doses of drugs, high temperatures above 41 degrees Celsius are life-threatening, including irregular heartbeats, kidney and liver failure, heart attacks, “cerebrovascular hemorrhage, strokes, and seizures (Pérez, 2008)

‘Amphetamine” itself may be more closely associated with vasculitis (Matick H, 1983)(JF)”. “Vasculitis may not correlate with “CT” findings, the dramatic increase in illicit “amphetamine”: use in the “United States” may lead to an increase in devastating vascular complications, many of which resemble those associated with cocaine intoxication. the occurrence of stroke in young patients is a devastating event, while stroke in this group represents only a small proportion of the total, the associated morbidity and mortality rates are significant (Kaku DA, 1990)found that heart disorders, substance abuse, diabetes, and high blood pressure (in that order) are associated with the highest risk of stroke in young adults, over a 10-year period, study’s in greater detail the role of substance abusers as a risk factor for the development of stroke in populations aged 15 to 45. It was found that 21 cases of stroke were associated with amphetamine, it is possible to take other drugs associated with stroke, but urine toxicity analysis only revealed amphetamine in users.

(Bacigaluppi, 2010), high blood pressure may be present in about 50% of cases, these strokes are either hemorrhagic or ischemic.

Post-mortem analyzes demonstrated conclusive evidence of decreased levels of tyrosine hydroxylase (TH), dopamine (DA) and DAT in the nucleus accumbency and caudate putamen in frequent METH users, lipid peroxidation, "4-hydroxynonenal" and "malondialdehyde", which are located in the caudate and frontal cortex of frequent

METH addict users. Finally(Appendix A), studies report differences in the levels of the antioxidant compounds "CuZn" Superoxyde Dismutase "(CuZn-SOD)", and "Glutathione", and the urinary tract among perpetrators of "METH" abusers. (Davidson, 2001)

2.2.2 Drug abuse and criminal behavior

Methamphetamine use and psychopathy work together to promote violent, economic, and drug crime. Abnormalities of cortical functional connectivity are seen in both methamphetamine-abusing individuals (Hoffman, "Psychopathy and corticostriatal connectivity: The link to criminal behavior in methamphetamine dependence." Frontiers in psychiatry , 2020).

Drugs contribute to crime, which typically consists of assault or other forms of crime, rape, robbery, financial coercion, and actual abuse of drug-affected individuals (Beyer, 2002), which aims to impair the behavior, vision, or dynamic limitations of the drug user. In addition, these contraindications may involve obtaining unwanted benefits from the patient, with or without his or her consent.

Criminal behavior is associated with methamphetamine use and psychopathy; However, it affects maladaptive and impulsive decision-making processes. Antisocial diagnoses are more common in people with substance use disorders. Psychopathy affects only about 1% of the population and is associated with early onset of substance abuse and development of polysubstance dependence. (Corrado RR, 2004), "Poor behavioral control, novelty seeking, impulsivity, and risk-taking are phenotypes" (London ED, 2015) (Boles S, 2008)which may contribute to increased criminal activity in both groups. MA users also show significant categorical and dimensional features for antisocial characteristics and psychopathy, including anger, aggression, and interpersonal violence (Beyer, 2002)

Data published by the drug abuse monitoring program for detainees in prisons confirmed that an average of 67% of crime perpetrators used drugs and had positive urine test results (amphetamine, cocaine, marijuana) and methamphetamine. (The Program, 2003) At the time of arrest, opioids or phencyclidine (PCP), a total of 23.4% of sites tested positive for several substances including barbiturates, benzodiazepines, methadone, and ibuprofen, a percentage rising to 70.3%. (Program, Annual Report.) "There is evidence of disproportionate drug use among detainees" (National Institute of Justice, Washington, DC, 2008), according to ADAM II data, which included ten sites across the USA (Chicago (), revealed that most detainees held everywhere tested positive for at least one banned drug, ranging from 49% to 87% (White, 2000).

In previous years, the most common narcotic substance was marijuana, followed by cocaine, then amphetamine. During criminal investigations conducted after the crime occurred, criminals who were sentenced by court for the crimes they committed admitted that they were under the influence of drugs, and in statistics issued by the Bureau of Justice Statistics, " (Statistics, 1997). A) "(Statistics from the Survey of Inmates in Correctional Facilities in Washington State)," that (22%) of those convicted in criminal cases are drug users (theft, burglary, and car theft), with a rate of 40%. In addition, 27% of those serving prison sentences for burglary in state and federal prisons reported that 30% to 32% admitted in investigations that they committed their crimes to buy drugs and carried out their crimes with the aim of obtaining money. (Statistics, Inmate Survey of Federal Correctional Facilities). "(1999a-1999b:)" "US department of Justice, Bureau of Justice Statistics, and Federal Bureau of Investigation, "(Washington, DCIn 2010)",

Studies have been published with statistical numbers on perpetrators of crimes who use drugs. There are certain categories of drugs that are most closely linked to the commission of crimes, most often violent crimes and theft crimes with the aim of obtaining money. It has been shown that there is a direct relationship between drug abuse and criminal activity. If the comparison is made with the years when drug use decreased, crimes were found to be relatively lower than they are now, so there is a direct relationship between the rate of drug use and the rate of crime. Reportedly 50% of it is due to drug use for money or violence, and drug use and crime have been closely linked to Goldstein's three-part framework over the years (Goldstein, 1998).

Goldstein's concept includes three categories: drug violence, coercive economic crime, and institutional violence. According to McCone, Kilmer, and Reuter, "Although Goldstein's classification is still frequently used as a model for understanding the various links between drug use and crime, not without criticism (MacCoun, 2003)law enforcement often proposes the hypothesis that Areas where methamphetamine is endemic will have higher rates of burglaries when all counts of criminal activity are investigated. In a greater number of crimes than other criminals. Furthermore, clinical studies indicate that stimulant drugs, such as methamphetamine, can increase Possibilities of hostile and aggressive behavior in humans, due to the proven fact that methamphetamine users are more likely to commit aggressive behavior and commit robbery-related crimes to justify their drug use (Provincial, 2006; Biehl, 1997). That MA users may endanger public safety due to their nervousness and paranoia, which may lead them to retaliate violently when in contact with others, especially with medical or law enforcement officials (DeLeon, 2000).

It was discovered that “47% of participants who reported using MDMA were violent.” Violent crimes: 24% reported that their participation in violent crimes was directly attributable to their use of MDMA.

California is one of the states with a high production of methamphetamine. Local authorities in California counties formed specially trained teams to combat drugs in terms of their production, distribution and combat their trade in order to save the community, enforce the law and prevent crime and violence associated with MA (Blankstein, 2001). “News reports helped expose the drug trade and the violence and crime associated with it” (Press, 2012), and news agencies revealed that “methamphetamine” dealers threw hand grenades and opened fire in broad daylight after the organized crime leader was arrested in “Mexico.” (Grillo, 2012): Reports on drug rehabilitation revealed that more than “50% are methamphetamine users” in California” (Grillo, 2012):

Methamphetamine use was associated with violent criminal behavior in a study by PB (2009), which showed that the likelihood of committing a homicide was approximately nine times higher even after controlling for demographic characteristics and use of other drugs such as alcohol, heroin, and crack. / Cocaine. is greater for people who use methamphetamine compared to those who do not use it. According to (Sommers I, 2006), (Tyner E., 2008), the association between methamphetamine use and aggression is neither reliable nor unidirectional in terms of direct causality; Instead, it seems to depend on a combination of individual and environmental factors. Methamphetamine use offers a number of ways to encourage violence motivation, such as inhibiting signals that normally control behavior, increasing arousal, and interfering.

As a result, methamphetamine-related violence can be the result of the interaction between the person, the drug, and the circumstances.

2.3 Methamphetamine production

In the period 1970 to 1979, “phenyl-2-propanone” “(P2P)”, an organic compound also known as “phenyl acetone” or benzyl methyl” “ketone”, was the most popular precursor for methamphetamine (Kaewnam, 2011), this substance is inexpensive and widely available in unrestricted quantities used to methamphetamine production ,in 1980, “pseudoephedrine” and “ephedrine” were used to produce meth using readily available chemicals and equipment, in November 1989, “ephedrine”, “pseudoephedrine”, and “phenylpropanolamine” were classified as listed precursor compounds and placed under surveillance due to their structural similarity to methamphetamine, however, over-the-counter (Badoud, 2010) “ephedrine” and “pseudoephedrine” products, tablets, capsules, and other finished products are exempt, and in 1996 additional precursors and laboratory apparatus used in the illicit production of methamphetamine were placed under federal control,

Illicit methamphetamine production necessitates the use of toxic, volatile chemicals in mixtures known to cause fires and explosions, this explains the increased use of ephedrine and pseudoephedrine, prior to 1980, a typical method for producing methamphetamine involved combining “(P2P)” “phenyl-2-propanone” with: N-methyl formamide”, an industrial solvent, in a vial immersed in an oil reservoir heated to a specific temperature, and then undergoing a series of chemical reactions. (Kaewnam, 2011)

Produces methamphetamine crystals, variations of "stove" recipes have been developed over time to replace items whose sale has been restricted under DEA regulations, the water "mixes phosphorus, acetone and Freon" with ephedrine, illegal pseudoephedrine

and other ingredients to produce methamphetamine, these substances are either highly toxic, highly flammable, or both, the mixture is then combined with a solvent such as gasoline and heated, to form crystals, the one-pot method of manufacturing methamphetamine was used in, (Nishida, 2006) using this method, drug addicts combine common household chemicals with pseudoephedrine tablets in a makeshift container, usually an empty 2-liter soda bottle, to produce small amounts of methamphetamine. Pseudoephedrine crystals and tablets are combined with a solvent, the resulting solution is then filtered and heated to a high temperature, and a low temperature is used to separate and extract the inactive component from the tablet, pseudoephedrine does not exist in purified powder form; Therefore, it must be extracted from the pharmaceutical tablets that contain it. Each packet of pseudoephedrine tablets yields less than "3 grams per packet" for this purpose.

The production and manufacturing of methamphetamine are volatile and uncontrolled processes, and the majority of those involved in methamphetamine production either use the drug themselves or sell it illegally to make huge profits (Kaewnam, 2011) (Nishida, 2006).

Almost all of the compounds used in the separation of ephedrine or pseudoephedrine and the production of methamphetamine are toxic and dangerous. Pure pseudoephedrine is then combined with "red phosphorus" and "dihydric acid". The "red phosphorus" is filtered out (and later reused), the remaining acid is neutralized with a hydroxide solution, the liquid methamphetamine is purged and a methamphetamine-related substance is added. Hydrogen chloride gas is cycled through liquid meth to produce a crystalline hydrochloride salt, which is then poured through a filter cloth, where the remaining meth is dried.

2.4 Narcotic Types and Addiction in Iraq

Many economic, social, and psychological factors have contributed to the spread of substance abuse in Iraq. Post 2003, particularly among young people, or for reasons associated with a lack of religious faith, there was an increase in domestic violence and a decline in self-confidence. youth and adolescence are a crucial age group that, for various reasons and causes, causes them to live a preposterous lifestyle, law and security are not provide inadequate deterrence or official punishment (Gallahue, 2010), inspection and supervision are inadequate, and it is difficult to control international land and river borders. modifying the substance penal code is regarded as one of the most crucial factors.

Before 2003, the law administered the death penalty to those who traffic in drugs, the second reason is the closure of liquor establishments in the majority of Iraqi cities. In Iraq, various drugs and psychotropic substances have proliferated (Gallahue, 2010). Iraq is one of the countries in “Western Asia”, and narcotics are widely abused in the nations on its eastern border. Also of concern is alcohol abuse in northern nations “(World Health Organization, 2016)”, prescription drugs, such as cough syrup, Somadril, benzodiazepines, Benzhexol, stimulants, and other medicines, are on the list of abused drugs in Iraq, with a current abuse rate of 5.2% and 2.1%, respectively.

There were 7,500 defendants in three categories in 2020: drug users, drug dealers, and drug dealers, there were 12,800 defendants in 2021, including consumers, dealers, and dealers, number of defendants, including consumers, dealers, and traffickers, reached 16,800 in 2022.In July of 2023, there were over 10,000 defendants, including drug users (https://ultrairaq.ultrasawt.com/, 2023) (https://www.alhurra.com/iraq, 2023), traffickers, and dealers, the types of drugs spread in Iraq that come from "neighboring countries" (crystal): methamphetamine spread in southern Iraq, the cities of (Basra and Maysan), and its source (Iran); (hashish), cannabis resin spread in southern Iraq and its

source (Iran and Afghanistan); and pills (Captagone, zero) in western Iraq, amphetamine(table 2.1) , which originates in Syria and Lebanon, is prevalent, as are all types of narcotics and psychotropic substances in the capital, Baghdad (https://ultrairaq.ultrasawt.com/, 2023)

Table 2.1: Quantities seized in 2023 in Iraq. (https://ultrairaq.ultrasawt.com/, 2023)

NO	**Amounts**	**Types**	**Entrance port**
1-	500 kilograms	Drugs originating	(Iran)
2-	450 kilograms	Captagon pills	(Iran)
3-	50 kilograms	Opium	(Iran)
4-	11 kilograms	Crystal and Captagon	(Syria)

2.5 Adulterants

This practice involves manipulating the urine sample in various ways, including adding external chemicals to produce a false negative result "(Dasgupta, 2019,)" or taking birth control pills and sometimes vinegar and some cleaning powders or aspirin.

Urine drug testing is required to monitor legal and illegal drug use for medical, legal, and clinical purposes, one of the main challenges for those working in this field is the adulteration of the sample using unmanufactured or manufactured materials (medical or chemical) that anyone can obtain "(ALIM, 2021)". The procedures followed in the laboratories concerned with these tests, the "forensic toxicology laboratories", are the procedures for examining the safety of samples, which often helps in detecting adulteration of urine samples, which are often replaced or diluted by adding water or diluting with intravenous solutions, but the challenges facing safety tests to confirm the

safety the sample is the addition of oxidizing chemicals, these materials destroy the analyses involved in the examination and this leads to it being undetectable "(Dasgupta, 2019,)".

Although forensic considerations differ from those of forensic chemistry, which are affected by the presence of adulterate in both fields, they can present interpretive difficulties of which the analyst must be aware, this study highlights the common theme of adulterate and the difficulties they can cause during forensic investigation (Clarke's Analytical Forensic Toxicology) by adding adulterate to the sample.

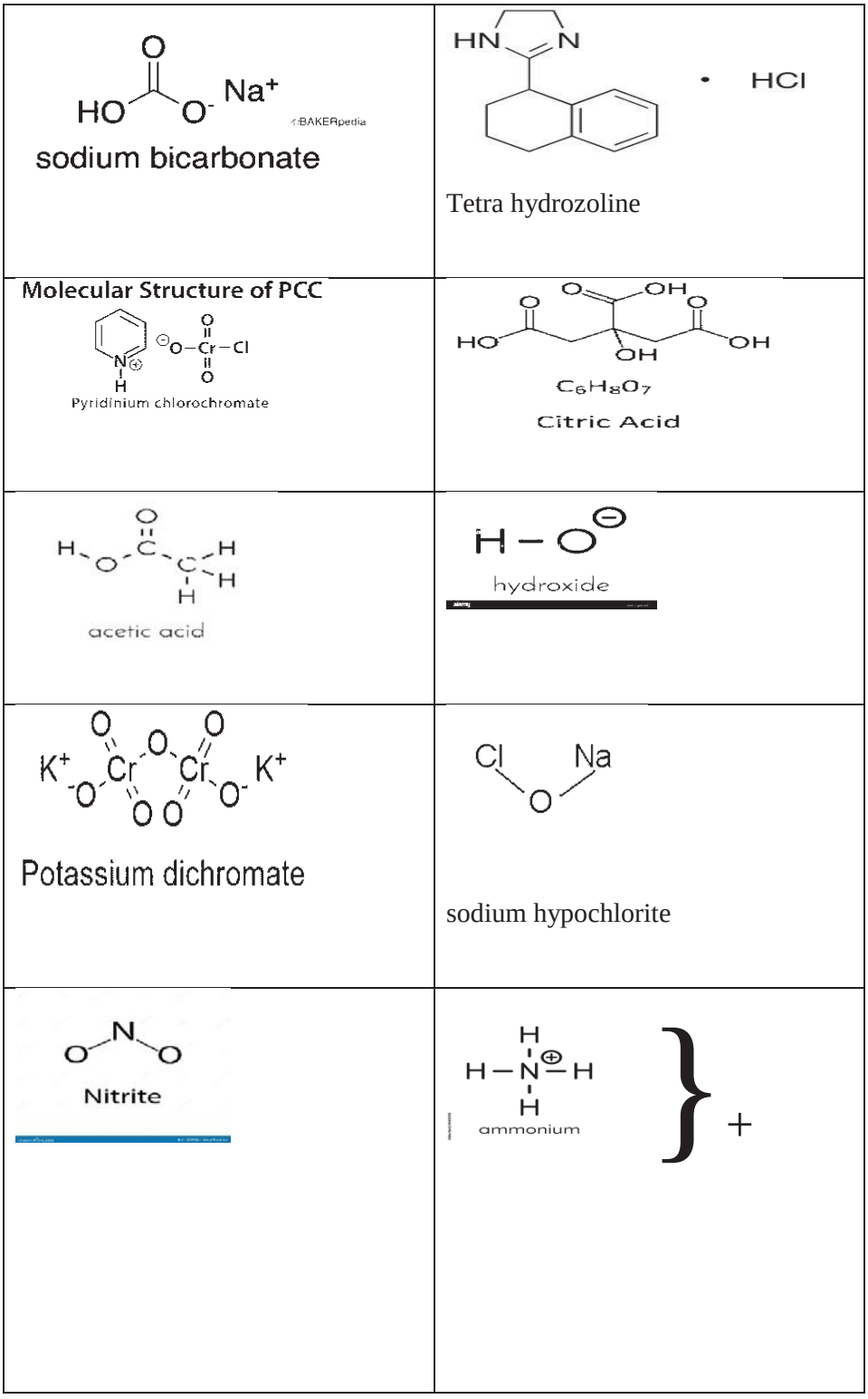

Figure 2.2 Chemical structures of the adulterants.

(Studied in this thesis).

2.6 Drug Color Tests Screening in Urine

In clinical toxicology, color tests are considered conclusive, particularly in emergency situations and when the patient is being treated, clinical symptoms are considered indicators and can identify symptoms of poisoning; therefore, the doctor must quickly identify the substance in question to begin processing (Markway, 2011) , therefore, the colorimetric assay is the conclusive diagnostic test for the condition, colorimetric assays are used for screening because they can indicate the class of a compound much more rapidly than immunoassays and chromatographic techniques such as "gas chromatography and high-performance liquid chromatography (Van Wijk, 2019), biological fluids such as stomach contents, urine, etc. undergo color tests, they are used to assign unidentified elements to a specific class of compounds, color tests of particular interest "(organophosphate pesticides)"; sodium dithionate test for parquet and Daquan pesticides; are one of the first tools toxicologists and drug analysts use to identify drugs and putative toxins, some criminologists find it difficult to identify illegal drugs due to their inaccessibility in a unique structure in the evaluation sample shipped from the measurable science laboratory ,there are numerous issues with the conventional drug testing strategy, including cost effectiveness, instrument limitations, and applicability (Markway, 2011).

2.6.1 Dipstick color test

Parisian chemist Jules Mominy created the first test strips "in 1850 by impregnating merino wool with elemental tin chloride (tin chloride), placing a drop of urine on it (Underwood, 1999), and heating it with a candle, the strip turned black immediately if the urine contained sugar".

“George Oliver”, an English physiologist, marketed his urine test papers “in 1883, reagent papers became commercially available from the chemical company Helfenberg AG just after 1900”. in “1904, it was discovered that Benzedrine could be used to detect the presence of blood”, and in “1920, the Viennese chemist Fritz Weigel published his method for spot analysis”. (Underwood, 1999)

Urine diagnosis made significant strides in the “1930s as reliability improved and the test became easier to administer”, the first urine test strips manufactured on an industrial scale and sold commercially were produced “in 1950. Boehringer Mannheim Roche introduced the first COMPOR test strips in 1964. Although the appearance of test strips has remained relatively unchanged since 1960, they now incorporate a number of innovations new impregnation techniques” (Clarke, 2012), more stable color indicators, and a steady improvement in color gradation have contributed to the widespread acceptance of urine test strips as a diagnostic tool for pathological conditions in clinical and general practice, the number of available parameters has steadily increased over the past several decades. “(Winn, Cavusi, Novick, Partin, & Peters, 2007)” urine test strips are comprised of a 5 mm wide strip of plastic or paper, for paper strips, the reactants are absorbed directly onto the paper “(Strasinger & Di Lorenzo Schaub, 2008)”. Plastic strips contain pads impregnated with chemicals that react with compounds in urine to produce a distinct color, whereas for plastic strips, the reactants are absorbed onto the pad.

It is a thin plastic stick that contains chemical strips, the dipstick is dipped in the cup urine. the lines change color if specific substances are present or if their concentrations are above average. Dipstick tests include acidity PH, glucose, protein, ketones, bilirubin, micro albumin, leukocytes, nitrite, urobilinogen, ascorbate, creatinine, calcium, and red blood cells, among others.

With the exception of medical situations, strips are used to test for drugs in urine to prevent sample adulteration temperature, weight measurement, a redox assay, and pH are all included in these protocols. while it has been demonstrated that these sample validity tests are effective at detecting the presence of some common adulterants, they cannot identify the majority of them; however, the difference in colors in the test indicates the presence of certain common pathological conditions "(Cone, 2009)", therefore, this test is considered inconclusive for detecting urine adulteration, as noted in this study.

2.6.2 Immunoassay urine screening testing

During the "period 1995-2017, a wide range of international assessments were developed to provide quantitative, semi-quantitative or qualitative disclosure of analyses", for the effective quality of biopharmaceuticals, food, and the environment, accurate early detection of analyses is important and essential in all bioanalytical settings, effectively diagnosing patients' health, monitoring them, and following up on their treatment are essential diagnostic procedures. Immunoassay analysis of food toxins was conducted in 2007. (Immunoassays Food Toxicants Analysis, 2007)

Bioanalytical platforms, "complementary technologies", and "immunoassay systems" have facilitated the emergence of numerous novel forms. Prior to several decades ago, the "majority of immunoassays were based on radioimmunoassay "(RIA)" and "immunosorbent assay (LIA)". "Enzymatic immunosorbent assay "(ELISA)", as a result, "RIA" and ELISA" kits for a vast array of analyses are now commercially available, and "antibodies against these analyses have been produced, due to the extraordinarily high sensitivity, specificity, accuracy, and throughput of ELISA (Actor, 2012Enzyme.), it has served as the gold standard for a vast number of analyses".

“ELISA technology” has undergone “significant advancements over the past two decades”, automated assays are equipped with automated workstations, and the technology, features, and “cost-effectiveness of micro titer plate (MTP) readers have also advanced significantly”. “ (Integrated Review Immunology and Microbiology , 2012).

“Bioengineering techniques” have contributed microfluidics and biosensors to immunoassay system development, “based on the identification of bimolecular interactions”, it has become the global standard for “the rapid development of detection analysis” and examination of immune components. (Integrated Review Immunology and Microbiology , 2012)

The advent of microfluidics has prompted the search for new platforms and formats that enable rapid screening with a minimal volume of reagents, such as the optimizer ELISA from Siloam Biosciences, which converts a conventional “96-well MTP-based ELISA into an ELISA. ELISA with microfluidic technology”, this hybrid system's analysis time is only a few minutes, and it employs fewer steps and a microfluidic protocol. numerous “lab-on-a-chip techniques” and formats have been developed in order to create numerous microfluidics.

Although immunoassay has numerous benefits, it also has a number of limitations. These include: the extensive time necessary to develop immunological reagents for new analyses. multiple residue analysis is inadequate because immunoassays can typically detect only two to five relevant compounds, the assays provide limited information, and very few immunoassays have official status (Immunoassays , 2014)

Using immunoassay, urine has been tested for drugs, however, immunoassays are only used for initial screening causes the possibility of false-positive and false-negative test results due to the presence of interfering substances other than the illicit drug of interest.

In the design of such immunoassays, a competitive immunoassay employing a single drug- or metabolism-specific antibody is typically utilized. Common forms of immunoassay used in drug abuse testing include EMIT (Multiple Enzyme Immunoassay Technology), CEDIA (Clonogenic Donor Immunoassay), chemical Immunoassay (CLIA), KIMS "(Kinetic Reaction of Micro particles in Solution), etc. Other than issues with reactivity, benzodiazepine immunoassays are insufficiently sensitive to monitor benzodiazepines in urine. (Clinical Challenges in Therapeutic Drug Monitoring , 2016)

The most prevalent colorimetric test yields immediate results within a few minutes. Its purpose is to detect the presence of drugs in a sample, but the result is either negative (none) or positive (present), and the device is unable to provide an immediate, definitively positive drug analysis result, this test employs an immunoassay system.

A urine test is used for drug detection as evidence. Immune chromatography strips for urine drug screening tests (UDSTs) are standard and appropriate for monitoring drug use, but they are also highly susceptible to domestic adulterants, which can significantly alter test results.

Enzyme-linked immunosorbent assays (EIA), which use antibodies to detect drug metabolites in urine, are one of the many indications for UDSTs, and one of the mechanisms underlying UDSTs. The UDS test is useful as a quick screening tool for assessing medication adherence. In addition, providers "use UDSTs in patients suspected of drug toxicity and overdose in illicit drug use (Mosack, 2010), however, the purpose of "UDSTs is to be a screening tool; a confirmatory test is required for a definitive answer, of which the gold standard is gas mass spectrometry (GC-MS)". GC-MS can determine the drug's concentration, whereas UDSTs can only detect its presence or absence. Due to cost and time constraints, forensic experts typically employ UDSTs

to guide their decision-making. If the results are negative, it is impossible to determine whether they are negative due to the presence of adulterants or due to the absence of illegal drugs.

2.6.3 Thin Layer chromatography (TLC)

In “1977, chromatography and capillary electrophoresis were proposed for modelling the fundamental mechanisms of drug action (1999)” (Kaliszan et al., 2011). Since then, “QSRR”” has evolved into a powerful theoretical tool with numerous applications: predicting the retention time of analytics” “(Komsta et al., 2010)”, in thin layer chromatography as a tool for determining lipophilicity - in a previous comparative study of several techniques employing a typical solute array (1010), the mechanism of molecular separation was investigated (Materials, 1950). analysis and comparison of stationary phases “(Values, 1962)’. TLC method is good for separation with fats and is called lipophilic

Lipid liking method is a physical and chemical description of a molecule's ability to cross biological membranes, thus, “lipid binding determines the absorption, distribution, and excretion of drugs and other xenobiotic in the body, moreover, the compound's ability to undergo complexation with blood proteins and bind to receptors at the site of drug action is affected by its binding to lipids, therefore, the pharmacodynamics and pharmacokinetic properties of drugs or poisons are closely related to the substance's binding to lipids” “(Kubik et al., 2016)”.

TLC is a method that is frequently used for the separation and determination of drugs; it applies equally to drugs in their pure state, those obtained from formulations, illegally generated substances, and biological samples with responsive detectors yet fails to provide comparable sensitivity when substances are present in inadequate