MICROBIAL AND CHEMICAL ANALYSIS OF ILLICIT DRUG SAMPLES FROM DIFFERENT AREAS OF PAKISTAN

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A thesis submitted in partial fulfilment of the requirements for the

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بسم الله الرحمن الرحيم

Dedication

I dedicated all my work to my treasured father (Late) Ghazi Marjan Khattak who is role model for me and my lovely mother Shah Hayat.

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Zainab Khattak

Certificate

This thesis submitted by ***Zainab Khattak*** is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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List of Acronyms

%	Percentage
AIDS	Acquired immune deficiency syndrome
ATS	Amphetamine type stimulants
BC	Before Christ
BP	British pharmacopoeia
CFU	Colony forming unit
CNS	Central nervous system
EEG	Electroencephalography
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
Etc	Etcetera
FATA	Federally administrated tribal areas
GHB	Gammahydroxybutrate
Gm	Gram
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
i.e.	That is
IDUs	Injecting drug users
KPK	Khyber Pakhtoonkhwah
LA	Los Angeles
LSD	Lysergic acid diethylamide
MDA	3.4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxy-N-methylamphetamine
NaOH	Sodium hydroxide
NI	Not identified
NIDA	National Institute on Drug Abuse
NIH	National Institute of Health
NR	Not recommended
NY	New York
ODS	Octadecyl sulphonate
OH	Hydroxyl group

P2P	Phenyl-2-propanone
PCP	Phencyclidine
PMA	Paramethoxyamphetamine
PMMA	Paramethoxymethamphetamine
PPA	Phenylpropanolamine
Pvt	Private
*R*f	Retardation factor
SAMHSA	Substance Abuse and Mental Health Services Administration
SDA	Sabouraud Dextrose Agar
SLE	Spongiform leukoencephalopathy
SOI	Standard operating instructions
SOP	Standard operating procedure
Sp	Species
STD	Standard
TAVC	Total aerobic viable count
TB	Tuberculosis
TLC	Thin-layer chromatography
TSA	Trypton Soya Agar
UK	United Kingdom
UN	United Nations
UNDCP	United National International Drug Control Programme
UNIDCP	United Nations International Drug Control Program
UNODC	United Nations Office of Drug and Crime
US	United States
USA	United States of America
USCB	United States Census Bureau
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
-ve	Negative
VJA	Vogel-Johnson Agar
WHO	World Health Organisation

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ABSTRACT

A cross-sectional study was conducted at Drugs Control and Traditional Medicines Division (DCTMD), National Institute of Health Islamabad to inspect the microbial and chemical analysis of illicit drug samples from different areas of Pakistan i.e. Quetta, Karachi, Lahore and Islamabad. The drug samples were confiscated by anti-narcotics force of Pakistan and brought to DCTMD. Firstly, these drugs were microbially analysed and then chemical analysis was done. The microbial analysis was done by checking the bio burden and conduction of separated microbial tests in order to identify particular microorganisms present in the drug sample. Microbial analysis revealed the presence of various microbes i.e. gram –negative bacteria, fungus species, Streptococcus, Staphylococcus species. The medium used for this purpose was Trypton soya agar for total aerobic count, MacConkey agar for gram-negative bacteria, Sabouraud dextrose agar for fungus species and Vogel-Johnson agar for Streptococcus and Staphylococcus species. In certain drug samples S. aureus and Salmonella species were detected which was further confirmed by applying different biochemical test specific for these microbes. For S. aureus catalase and coagulase test were conducted and for Salmonella species TSI, indole and urease test were performed. The chemical analysis was conducted in order to know the different types of adulterants present in these samples. For this purpose different chromatographic techniques were employed e.g. Thin-layer chromatography (TLC) and High-performance liquid chromatography (HPLC). Colour tests were also conducted to identify the particular drug. Heroin, morphine, cocaine and acetic anhydride samples were used for this purpose. In our samples, heroin samples contained paracetamol, diazepam and dextromethorphan as adulterants while acetic anhydride samples were adulterated with HCl. The drug samples were also quantified by the High-performance liquid chromatography (HPLC) technique. Efforts should be made in the future to conduct large prospective studies in drug abusers of different age groups and different geographical areas of our country in order to achieve the better understanding of their effects on the health.

Introduction

Previously, and mainly in modern era, there has been an extensive examination that illegal drugs which are structurally varied set of chemical agents, possessing tremendously elevated ability for biological effects in humans and also in non target organisms. They contain other substances in addition to the purported active ingredient that can have serious adverse health consequences or even cause premature death (Daughton, 2011). These drugs possess diverse range of names which are frequently interchangeably, includes street drugs, designer drugs, club drugs, drugs of abuse, recreational drugs, clandestinely produced drugs, and hard and soft drugs (Daughton, 2011).

Due to inconsistent number of drug abusers that becomes sick or those bringing to the hospital having unusual drug problems, illicit drug adulteration brought the interest of health or drug services (Cole *et al.*, 2010). For the identification of adulterants and information of their unfavourable effects, an early warning system helps in enhancement of understanding of, public health reactions to illicit drug adulteration (Cole *et al.*, 2010).

The word ‘’illicit’’ is given to these drugs because of their way of production, circulation, their mode of getting and also their use. These drugs are utilised for non-medical reasons. On the basis of their origin and biological effects, these illicit drugs are illustrated in a variety of ways i.e. these may be naturally producing, semi artificial (chemical managements, such as analogs, of materials obtained from natural resources), or artificial (produced completely by laboratory production and management). The key groups are opiates, other central nervous system (CNS) depressants (sedative-hypnotics), CNS stimulants, hallucinogens, and cannabinoids (Daughton, 2011).

Benign substances i.e. sugars are the substances that will boost or imitate the effects of these drugs i.e. procaine in cocaine, or also are the substances that will assist in taking of illicit drugs such as caffeine in heroin, are normally used as adulterants in illicit drugs but these adulterants are characteristically altered with time. The reasons of these alterations are the accessibility of other materials, in addition to other substances that act as enhancers and also because of customer liking for a specific mixture of active component and adulterants. Because of the unclean or unsterile

process of making and production of these drugs, their unsatisfactory wrapping and unsuitable storage, by-products of the process, bacteria or other biological agents can also be added as adulterants in these drugs (Cole *et al.,* 2010).

These drugs are seldom employed and sold in market in their pure form (Lindholst *et al.,* 2008). In order to give an additional convenient amount these drugs are often combined with other substances (King, 1997). With the aim of making the drug to appear as that there is a large quantity of drug than is really present and also to increase the dealer's profits, the drugs e.g. diacetylmorphine (Heroin), amphetamine and cocaine are frequently combined or cut with a different types of materials, adulterants and/or diluents (Kaa and Kaempe, 1986; Kaa, 1994; Fucci and Giovanni, 1998). The increasing problem of drug abuse is a point of attention to government authorities and also to society (López-Artíguez *et al.*, 1995).

The problems produced in evaluating adulteration practice over time and also by country are due to the absence of standardised examination and extensive reporting. These methods mostly provide information about the adulterants present in these drugs but not the drug general composition and the quantity of these adulterants present and also unable to account the fraction of samples having no adulteration but aware public by the threats of these drugs to the public health (Cole *et al.,* 2010).

Detection of these hazardous adulterants is significant because they appear more poisonous than the drug itself e.g., cocaine contaminated with atropine or phenytoins (Katz *et al.,* 1993) are examples of precarious combinations sold on the European drug marketplace. Comprehensive information of these drugs provides information about their supply ways (Slooten *et al.,* 1975). Dissolution of these drugs in commercially obtainable acids, such as citric and tartaric acid increases the risk of tissue breakdown at the inoculation place and in this way they fasten bacterial expansion (Dancer *et al.,* 2002).

There is a difference between contamination and adulteration. Contamination is an unintentional procedure while the adulteration is generally intentional process and it includes the accumulation of different materials to the drug for a specific reason. It also augments the drug effect or mimics the substance from another one. Finally the

main objective of this adulteration is to enhance the economic profit at the least probable rate. That is why the drug traders at small- and medium-level enhance their profit by adulteration process with cheap substances such as lactose and glucose (Dom~nguez-Vilches *et al.,* 1991).

Drug contaminants are divided into two main groups abiotic and biotic (Dom~nguez-Vilch *et al.,* 1991). Abiotic contaminants contain distant chemical materials which are present in drugs mainly these are heavy metals such as lead, magnesium, and aluminium along with atmospheric contaminants which are harmful to health and wellbeing (Garcia-Ferrer, 1986). Biotic contaminants contain bacterial and fungal spores that are expand by air due to their small microscopic size. These become part of the drug at the time of drug production and adulteration step (Dom~nguez-Vilch *et al.,* 1991).

Pharmacological mediators can also be employed as adulterants in these street drugs such as cocaine and heroin in order to increase or reduce the drug effects. In the Philadelphia Medical Examiner's Office during April 21, 2006 and August 8, 2006, xylazine (a veterinary tranquillizer) and fentanyl (an artificial opioid) was identified in the drug samples obtained from seven different cases (Wong *et al.,* 2008).

The presence of adulterants in recreational heroin, amphetamine, and cocaine specimens has reduced considerably but this indicates the occurrence, diversity and amount of adulterants and diluents that have altered over time (Andreasen *et al.,* 2009).

In the spring of 1995, a novel type of heroin that was contaminated with scopolamine emerged in New York City and was distributed to other East Coast cities. This heroin produced harsh anti cholinergic toxicity in heroin abusers who were frequently presented to the emergency departments of hospitals in huge ratio (Hamilton *et al.,* 2000).

Until 2009 in cocaine specimens phenacetin has been the more plentiful adulterant in view of rate of emergence and proportion while in 2010 the veterinary antihelminthic drug levamisole has been the prominent additive identified in cocaine specimens

having proportions 1.5-4.1% but this was the low quantity. In 2005, the average quantity of the heroin was 26.6%, but in 2006 the rate was decreased having constant proportion since then (15.8–17.4%). Paracetmol and caffeine were more prominent additives identified in heroin specimens (Schneider and Meys, 2011).

Bacterial diseases are a general threat related with illegal drug employment and amongst inoculating drug abusers; these can produce diseases related to bacteria, fungus and virus specifically (Brazier *et al.,* 2002; McLauchlin *et al.,* 2002; Brett *et al.,* 2005; Cole *et al.,* 2010). Among the drugs addict's, intradermal insertion is most general (Graham *et al.,* 1999) that produces unfavourable health consequences due to microbial residence (Dancer *et al.,* 2002).

Illicit drugs insertion produced several microbial diseases (Horsburgh *et al.,* 1989; Brettle, 1992; Peat *et al.,* 2000). Detailed examination indicated that during April and August 2000 over 108 cases with no less than 43 deaths were documented (Christie, 2000; Anon, 2000; Bellis *et al.,* 2001). In producing illnesses, *C. novyi* type A (Anon, 2000; Ryan *et al.,* 2001; McGuigan *et al.,* 2002), other endospore-forming bacteria including *C. perfringens* (McGuigan *et al.,* 2002) and *B. cereus* (Dancer *et al.,* 2002) are important. As heroin was the cause of illness, particularly with *C. novyi*, researches were made in order to develop techniques for the scrutiny of the microbial flora of heroin. But the data containing micro flora of heroin is little (Tuazon *et al.,* 1974; Shamsuddin *et al.,* 1982; Moustoukas *et al.,* 1983; Alvarez *et al.,* 1990). Heroin has been accounted to possessed noticeable antimicrobial characteristics (Tuazon *et al.,* 1980).

In 2000, in United Kingdom (UK) and Ireland, a report showed the remarkable raise in rate of morbidity and causalities amongst illicit inoculating drug abusers and in them *Clostridium novyi* was recognized as the probable cause of the severe infectivity, though illnesses due to *C. botulinum* and *Bacillus cereus* were also accounted (Mc Lauchlin *et al.,* 2002). In heroin abusers candidiasis has been accounted to happen (Calandra *et al.,* 1985), so illicit drugs that are sold in streets are verified to be extremely infected (Luna *et al.,* 1986). This provoked us to decide the normal fungal range of the three main general kinds of illegal street-sold drugs (brown and white heroin and cocaine). The employment of these drugs as markers of

the level of adulteration and the toxicological ability of the diverse taxa has been encountered (Dom~nguez-Vilches *et al.,* 1991).

From unclean inoculating stuff and apparatus partaking, drug abusers are already at danger of illness (Hughes, 2000). Among abusers, *Bacillus cereus* has already been concerned in severe diseases (Weller *et al.,* 1979; Hinchliffe and Thornton, 1987). Amongst illicit drug users (IDUs) through 2000, in the duration of the researches, *B. cereus, C. botulinum* and *C. Perfringens* were secluded from both heroin or drug inoculation tools (Dancer *et al.,* 2002).

Federal Bureau of Investigation declared that in United States (US) in 1990 there were about 5% of mortalities incidents that was related to the street drugs (Drug-Related Crime - Factsheet - Drug Facts, 2008) while Mexican government stated approximately 90% of the deaths cases related to these drugs (Carl, 2009). According to Substance Abuse and Mental Health Services Administration (SAMHSA) in last some months' ecstasy users increased from 555,000 in 2008 to 760,000 in 2009. Novel information indicated the use of these drugs increased from 2009 to 8.7% amongst 12 years age children and elder aged peoples, 8% in 2008 (SAMHSA, 2010). United States National Health Survey stated that in 2009 there were approximately 2.2 million populations that employed street drugs (SAMHSA, 2010). About 17,000 mortalities were linked to these illicit drugs in 2000, though the link of mortalities and drug abuse cannot be simply elucidated (Mokdad *et al.,* 2004). The factors of mortalities related to these drugs are overdosing; AIDS, suicides but the main cause of mortality amongst users is the cardiac syndrome. Usually the evidences related to these cardiac problems are subjective (Brust, 2002). The United Nations (UN) states that 200 million populations around 4.8% of the whole population of the world having age between 15-64 years get the illegal drugs per year and amongst 25 million that have been categorize as problem seekers (0.6%)(UNODC, 2007).

Heroin (diamorphine) is originated from morphine. In 1995, an approximate world opium production was 4157 tonnes, with the mainstream (2561 tonnes) being formed in South-East Asia (Myanmar, Burma, Thailand, China, Vietnam and Cambodia (Cole, 2003).

Studies propose that brown heroin is suitable for smouldering use while white heroin is apposite for inoculation (Strang *et al.*, 1997). Heroin chemical composition differs with diacetylmorphine ratio which is in the form of salt or free base, and also differs with the quantities and uniqueness of diluents and adulterants that have been added. Different studies suggest that brown heroin is made up of about 35–45% of diacetylmorphine hydrochloride content (Kaa and Bent, 1986; Fuente *et al.*, 1996) while white heroin is more pure having about 85–95% of diacetylmorphine hydrochloride ratio (Huizer, 1992; Fuente *et al.*, 1996). Pure heroin accessibility is increasing so it is adulterated with more drugs (e.g. paracetamol, quinine, strychnine or other poisons) or other essences such as sugars (glucose, lactose, sucrose and mannitol) and yet starch or powdered milk (Kaa and Bent, 1986; Fuente *et al.*, 1996; Risser *et al.*, 2000).

Temgesic and Bungesic are the most accepted dealer names of buprenorphine which are figures of parentral dosage. In Tehran's illegal drugs black marketplace these are most significant illegal opioid drugs and are currently amongst the extensively battered one by opioid users. So in Tehran imitating in these criminal dose forms has amplified (Soltaninejad *et al.*, 2007).

Over the years in the incidence of contaminated cocaine powder, there has been a statistically important rising tendency mostly during 1999 to 2007. The adulterated cocain have more accounted unfavourable outcomes than unadulterated one and among the adulterants to be added in cocain, phenacetin, hydroxyzine and diltiazem produce more harmful effects. The effects produced by them are mostly cardiac and hallucinatory but are not understood visibly. These effects are probably due to numerous reasons which include relations of adulterants with cocaine and the way of entrance (Brunt *et al.*, 2009).

In Vienna in 1999 the study related to the worth of the detained street heroin revealed that pureness of street heroin and the number of disasters and deaths related to the heroin have connection between them. But according to the time-series investigation, no statistically important connection was present among the rate of death related to heroin and the diacetylmorphine ratio of street heroin samples that was sequestered in Vienna in 1999. A widespread belief could not be validated that explain the relation

between the deaths produce by heroin via alterations in the transparency of street heroin, although the outcomes of this belief do not exclude a link among the transparency of heroin and causalities related to it (Risser *et al.,* 2007). Inadequate assessment is done by the drug trader and consumer concerning the value, purity and production of each drug that they acquire or consume (Reuter and Caulkins, 2004).

The alcohol and tobacco consumption creates the potent dangerous results in addition to the health troubles, violence, and unlawful approach, domestic and socio-economic nuisances (AHMAC, 2006). Utilisation of illicit drugs and bacterial diseases are connected and these are more common among injecting drug abusers. *Bacillus* and *Clostridium* species are common infections (Cole *et al.,* 2010).

In Pakistan the farming of illegal opium, it's making, trafficking and misuse has been present since long time. In different areas of Pakistan, cannabis develops wildly and in north areas of the country it grows as a cultivated plant. In current period, poppy development was limited to three kinds of organizationally varied localities; Khyber PakhtoonKhawah (KPK) is strategically especially significant one (UNODC, 2010).

As these drugs are subsequently harmful, so the entire world struggled to generate laws in order to control them. In UN seminars they accepted various universal rules that pointed to drug that have to be controlled (UNODC, 2008).

From different studies and few sample reading this thesis will focus on confiscated drugs and their microbial and chemical analysis (Katherine *et al.,* 2011).

Aim and Objectives

The main aim of our study was to analyse qualitatively and quantitatively various illicit drugs using various chromatographic techniques and to analyse them for the presence of certain microbes collected from various regions of Pakistan.

Our objectives are:

1. Qualitative and quantitative analysis of illicit drugs confiscated by various drug law enforcing agencies from various cities of Pakistan i.e. Karachi, Lahore, Islamabad and Quetta.

2. Microbiologically analysis of illicit drugs for the presence of various micro-organisms i.e. *E. Coli, Staphylococcus aureus, Salmonella* etc. using WHO, 2011, guidelines.

Broad medicinal literature re-evaluation showed that illicit drugs are precarious for several causes, and among them some possess high person's threat for both ischemic and hemorrhagic strokes (Esse *et al.*, 2011). After marijuana, opioids are the next generally tainted drug amongst persons having age range between the ages of 16 to 25 year. It is then pursued by cocaine, medicated tranquilizers, ecstasy, and recommended stimulants (SAMHSA, 2010). In two large cities of US i.e. New York (NY) and Los Angeles (LA) (USCB, 2010), there is large local drug marketplaces for both illicit and recommended drugs (Lankenau *et al.*, 2012).

In LA, smoking tar heroin is frequent (Lankenau *et al.*, 2012). Contaminants are supplemented to mass, dilute, boost or increase the drugs impacts. Some of the other contaminants are added to the drugs by the process of forming, making or storing practices. The examples are alkaloids, microorganisms or other biological and contagious agents. Risks and deaths related to the contaminated drugs are because of the toxins, bad production practices, bad storing and wrapping, and also linked to other substances effects that are sold as the illegal drugs e.g. paramethoxymethamphetamine (PMMA) and/or paramethoxyamphetamine (PMA) which are sold as ecstasy (Cole *et al.,* 2010). Some adulterants are undamaging ''diluting agents'' such as sugars e.g. glucose, mannose, saccharose or starch. In cocaine samples, phenacetin, hydroxyzine, diltiazem, levamisole and caffeine were frequently identified adulterants while in heroin samples quinine, lidocaine, phenobar-bital, methaqualone strychnine, scopolamine were detected (Schneider and Meys, 2011).

Other materials that were present in minor amount in heroin samples were seldom detected. So the knowledge related to drug clarity, occurrence and quantity of adulterants and diluents is important for policy makers, drug and addiction conduction centres and doctors. It is also helpful about the drug distribution routes and sample reporting. In comparison to cocaine, heroin samples are more stable in adulteration ratio and their composition. Cocaine and heroin utilization is dangerous due to unpredicted and/or potentially unsafe adulterants. So more investigation will be needed for inspection and supervision of recent styles, to warn the drug abusers and health care labours. Further investigation will also be necessary to prevent the health worker for discovery of novel adulterants or contaminants and also for scientists and

policy creators for improved perceptive of local and global drug marketplaces (Schneider and Meys, 2011).

Mainly in developed countries heroin utilization shows high level of casualty and death, mostly in youngsters (EMCDDA, 1999; Sporer, 1999; NIDA, 2000; Hall and Zador, 2000). In Vienna morphine is the frequent illicit drug involve in drug-link deaths. It was believed that in the early 90s, raise in drug-link mortalities was because of improved worth heroin at a lesser cost (Risser *et al.,* 2007). In Iran death amongst drug abusers is a significant trouble (Iravani *et al.,* 2010). In Iran in latest years, acidic and basic drugs possess pharmacological properties on CNS are employed as adulterants in pure heroin. These are benzodiazepines, barbiturates, antihistamines, caffeine and opium alkaloids and their acetylated derivatives. This contaminated heroin is called "crack" that emerges as a white, creamy, or brown powder. This heroin is different from white crystalline cocaine that is recognized as crack in other countries (Akhgari *et al.,* 2011).

In 1999, United Nations Office on drugs and Crime (UNODC) claimed that the annual occurrence of opiates utilization was 2.8% in 15-64 year olds in Iranian populace (Akhgari *et al.,* 2011). In 2008, because of its geological and position on drug trafficking ways, Iran guide all the countries by reporting 23% of all heroin seizures in the world (UNODC, 2010). In 2003, in Scotland there were an approximate 51,582 people with opiate and/or benzodiazepine drug trouble (Hay *et al.,* 2005). The frequent psychoactive materials can be separated into depressants (e.g. alcohol, sedatives/hypnotics, volatile solvents), stimulants (e.g. nicotine, cocaine, amphetamines, ecstasy), opioids (e.g. morphine and heroin), and hallucinogens (e.g. PCP, LSD, cannabis) (WHO, 2005).

The foremost maker of the illegal drug traffic in the world is the Afghanistan, though; the drug injurious effects are not restricted to the only state that produced it. In the world the approximate figure of heroin consumers is highest in Asia having 5,917,000 people, followed by Africa having 1,763,000 people, then America having 1,672,000, then Europe having 2,982,000 and then last in Oceania having 69,000 persons. The sum of world heroin abusers is to 12,376,000 with a utilization of 375 tons of pure heroin yearly (Dutta and Ehsan, 2012).

For stimulating anxiety, rebellion and violence illegal drug farming and manufacture in Afghanistan is one of the main aspects (Dutta and Ehsan, 2012). In reality this area appeared to be identified as 'the Golden Crescent' and it substituted 'the Golden Triangle' of Laos, Myanmar and Thailand as the chief manufacturer of opium (Ahmed, 2008). Amongst the 'Golden Crescent' states earlier than 1979, this area was certainly not the significant opium manufacturer. Yearly among Iran, Pakistan and Afghanistan, Iran manufacture approximately about 600 tons of opium, Pakistan 500 tons, whereas Afghanistan about formed 300 tons. From Pakistan the heroin directed to Iran, China, North America, Africa and South East Asia and then to Oceania mainly Australia (Dutta and Ehsan, 2012).

Illicit drugs are not essentially unlawful. Many among them are legal i.e. they are employed in medical pharmaceuticals possessing precious beneficial applications e.g. morphine and oxycodone. These possess several names and among these names term "designer" drug attained popularity in the 1980s after the introduction of 3- 4-methylenedioxymethamphetamine (MDMA, ecstasy) into the black market. In the 1920s the remarkable first designer drugs launched was dibenzoylmorphine and acetylpropionylmorphine (Daughton, 2011). The production of illicit drugs mainly methamphetamine produces widespread environmental harm with irreparable harm to infrastructures, such as buildings (USEPA, 2009b).

Mass of the alleged drug are occasionally produced by adulteration e.g., noscapine is present about 60% in heroin and phenacetin is about 50% in cocaine. These impurities formed the products of production or manufacturing like precursors, intermediates, by-products, normal contaminations like natural product alkaloids, products of degradation like oxidation during storage, and pharmacologically active adulterants like many licit drugs and other chemicals, obtained illegally, such as levamisole, xylazine, lidocaine, phenacetin, hydroxyzine, and diltiazem. Some possess significant toxic effect (Daughton, 2011).

In the epidemiology of tuberculosis (TB) in industrial and developing countries, illegal and inoculation drug abuse are vital aspects (Van *et al.,* 2005; Van *et al.,* 2006; Mattos *et al.,* 2006; Vries and van, 2006; Ngoc *et al.,* 2007; Story *et al.,* 2007).

Several in vitro studies explained the injurious effects of these illicit drugs on the immune system of the body (Friedman *et al.,* 2003), with the straight destruction of the cell-mediated immune response by opiates (Wei *et al.,* 2003), but it lacks its clinical implications (Kapadia *et al.,* 2005). Several epidemiological factors impose drug use, e.g. tobacco utilization, homelessness, alcohol misuse, and imprisonment that grant extra threat of TB (Drobniewski *et al.,* 2005; Altet-Gomez *et al.,* 2005; Niveau, 2006).

In England cluster examination demonstrated the epidemics of drug-resistant TB between drug abusers (Story *et al.,* 2007) and multidrug-resistant TB in Thailand (Punnotok *et al.,* 2000), Argentina (Palmero *et al.,* 2006), Latvia (Morozova *et al.,* 2003), and Portugal (Hannan *et al.,* 2001). In the US, at a methadone-treatment service a TB epidemic took place (Conover *et al.,* 2001). In the manufacture, transport and utilization of opioids, mostly heroin the international raise continues (Gossop and Grant, 1990; Childress, 1994; UNDCP, 1997). In various states of South East Asia drug inoculation is extensive. In these states the former opium smoking substitutes the heroin smoking which is then substitute by heroin inoculation (Stimson *et al.,* 1996). With the use of heroin in injection the risks of HIV and hepatitis spread are extensive (Kumar and Nath, 2012).

2.1 History

Drug addiction record starts many thousands of years ago. The growing and production of opium in the clay tablets of the Sumerians, begins 7,000 years BC (Zacken, 1988). Hippocrates, the Greek Physician, was the pioneer to explain the medical utilization of opium (Malik and Sarfaraz, 2011). In Shanghai in the 1920s the first heroin smoking event was initiated, which was then transferred to Eastern Asia and to the United States over the next decade (Strang *et al.,* 1997). In Hong Kong in the 1950s the form of heroin smoking “Chasing the dragon” or “Chinese blowing” was produced and it was given to the breathing of heroin smoke after heating the drug on aluminium foil over a fire (Brenneisen and Hasler, 2002). Later, in the late 1970s and early 1980s, this activity of smoking was seen in different regions of the Europe

like the Netherlands and today it is attaining fame between heroin abusers looking for to resists the threats of parenteral drug direction (Kriegstein *et al.,* 1999).

2.2 Pakistan

In Pakistan the deterioration of drug abuse state is liable if it is measured against the background of the sharply increased poppy farming and manufacture of heroin in Afghanistan. In Pakistan there has conventionally been a development of two narcotics plants opium poppy and the other one is cannabis (Malik and Sarfaraz, 2011). In the federally administrated tribal areas (FATA), the Khyber Agency (area having border with Nangarhar province in Afghanistan) has produced the opium mass about 1.2 percent of the region cultured in Afghanistan over the past three years. In Pakistan there is a danger of increased production if continued struggles are made to deter farmers from farming poppy and also to obliterate opium harvest before they are yielded (UNODC, 2010).

In Afghanistan, narcotics manufacture and its refining have key affect on Pakistan (Malik and Sarfaraz, 2011). UNODC's Afghanistan Opium Survey 2007 claimed about 70% of Afghanistan's opium was produce in five regions alongside the boundary with Pakistan, these includes Kandahar, Nirmoz, Nangarhar, Badakhshan and Helmand. For trafficking, Pakistan geological state gives a constructive passage (UNODCP, 2002). From Pakistan attractive information claimed that main metabolites of cannabis were present in cow milk which had browsed upon naturally producing cannabis plants (Altunkaya *et al.*, 1991; Sharma *et al.,* 2012). The children that nourished on such milk exhibited cannabis metabolites in the urine which suggested its inactive utilization via milk (Kalant, 1972; Sharma *et al.,* 2012).

For opiates formed in Afghanistan and also for manufacture and trafficking of imitation drugs like amphetamine type stimulants (ATS) and benzodiazepines are at comparatively small levels, Pakistan proves a chief transportation country. From different countries ecstasy which is the generally ordinary state of ATS, is smuggled into Pakistan. On the other side, in Pakistan benzodiazepines are either produced illegally or for importation. So, for enhancement in partaking real time information on

drug trafficking, collaboration among regional and national law enforcement agencies is important (UNODC, 2008).

2.3 Impurity profiling

Basically it helps in yield detection from fresh illegal laboratories and also aid in the checking of ordinary processes that are employed for drug production. Consecutively, it may offer information which is supportive to the continuation of other intelligence meeting tools, i.e. precursor-monitoring programmes. In the end, it is also supportive for illegally produced drugs that should be distinguished from those that are abstracted from licit sources. In impurity profiling, UNODC laboratory possess extensive record of participation. The chemical profiles, “signatures” or “impurity profiles’’, helps in the recognition and quantification of main elements in the sample and it also have some more analytes to detect the minor components (UNODC, 2005).

2.4 Adulteration

Adulterants are chief materials that are likely accessible and among them most frequently are being caffeine, procaine, paracetamol, sugars (Cole *et al.,* 2011), sucrose, lactose, dextrose, mannitol, caffeine etc (Cole *et al.,* 2010). At minimum dose, they possess least influence on abusers' physical condition. In abusers, that inoculate the drugs, other adulterants possess strong health problems but the amount accounted like strychnine in heroin, are not critical (Cole *et al.,* 2011). The causes for adulteration are complicated and are not easy enough to understand because of following reasons like enhancement of drug effects, alterations in customer styles, novel drug making methods or local/ geographical restrictions (Cunningham *et al.,* 2010). Addition of pharmacologically active compounds alters the drug impact in an unexpectable way (Schneider and Franc, 2011).

A study demonstrated that the heroin have been ‘cut to 6 to 7 times before reaching to the dealer’ (Richter and Rosenberg, 1968) along with its adulterants. UK, USA, Canada and Australia have done the similar kind of less injurious contamination actions like sugars or caffeine (Coomber, 1997c, 1997d; Coomber and Maher, 2006). From adulterations the drug trader produces the main beneficial trade (Strang and

King, 1996; Coomber, 1997c; Coomber and Maher, 2006) and these adulterants have been prepared in the hidden laboratories (Behrman, 2008). Illicit drugs contamination possesses strong substantial effect by the complex connections of deliver, order, and control of these drugs (Cole *et al.,* 2011).

In the investigated cocaine powder between 1999 and 2007 there was a raise in adulteration rate and this enhanced rate was linked with comparatively extra severe effects like cardiac and hallucinatory effects with cocaine employment. These complex effects are produced because of numerous aspects which include relations of adulterants with cocaine and the way of entrance (Brunt *et al.,* 2009). Some adulterants are advertized for just a little time duration and in nature their addition in the product appears to be merely opportunistic (Andreasen *et al.,* 2009). With the time these are changed and these changes occur because of following reasons like accessibility of other compounds, addition of compounds as enrichments and because of consumer priority for a specific combination of dynamic elements and adulterants (Cole *et al.,* 2010).

2.4.1 Heroin adulteration

Unclean preparation and injection of street drugs produces bacterial, fungal and viral diseases (Brazier *et al.,* 2002; McLauchlin *et al.,* 2002; Brett *et al.,* 2005). Benign compound or compounds that will augment the heroin are usually employed as heroin adulterants, than with the yield that will produce severe health complications or even casualty. Usually adulterations are taken at production time and recognized adulterants for heroin are caffeine, sugars and paracetamol (Cole *et al.,* 2010). In the UK heroin possess different street names like ‘dope’, ‘junk’ and ‘smack’ and it is employed to denote diamorphine. More efficient way of drug administration is the inoculation but fear of constricting needle, syringe-borne diseases like HIV and hepatitis, raise in the transparency of the accessible heroin, all these factors increase the rate of smoking and inhaling the drug. This in turn produces ecstasy, sleepiness, sluggish and low breathing, damp skin, vomiting, seizures, unconsciousness and probable death (Cole, 2003).

Past 1800, in the dried out latex of *P. somniferum* morphine was first discovered. Among about 110 species of *Papaver*, just two produces morphine in considerable

ratios, these are *P. Somniferum* L. and *P. setigerum* DC. *P. somniferum* is employed to give the dehydrated latex for heroin making. The latex possesses 10–20 wt% alkaloids and the remains contain sugars, proteins, lipids, gums and water. Opium give morphine (4–21 weight %), codeine (0.7–3 weight %) and thebaine (0.2–1 wt %) (Cole, 2003).

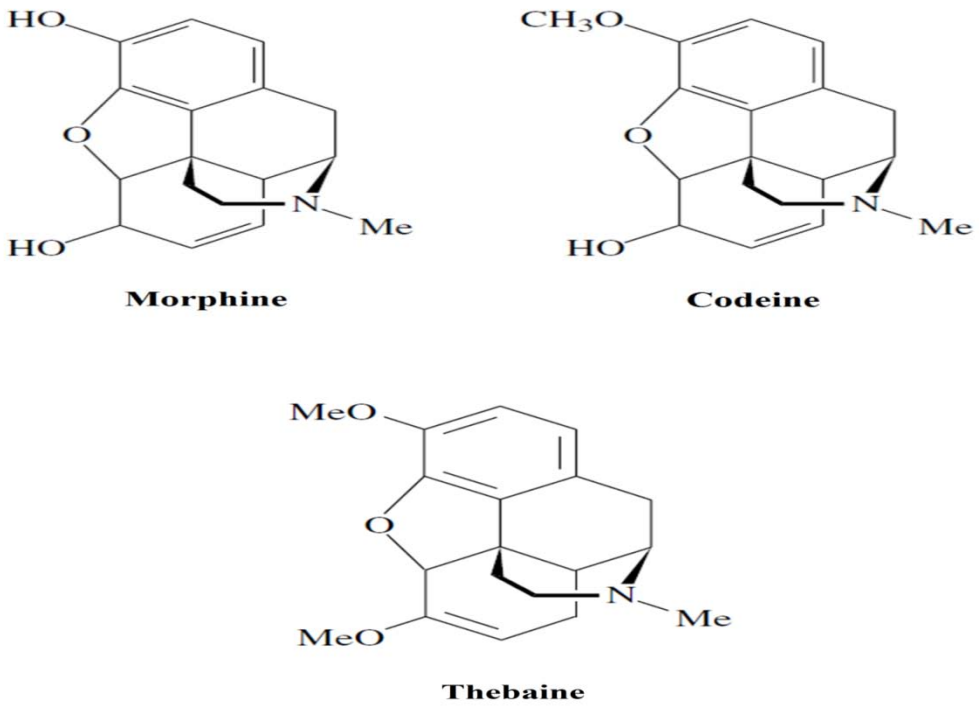

Figure 2.1: Chemical structures of morphine, codeine and thebaine

Caffeine was employed as adulterants in heroin due to its stimulatory effect. It also possesses synergistic potential with heroin because it makes administration of diacetylmorphine better in heroin base smoking. Paracetamol possess analgesic effect. Griseofulvin, which is also used as an adulterant in heroin, is an antifungal drug and is employed to cure ringworm skin infectivity and nails in animals and humans. Heroin hydrochloride (HCL) is frequently present as a white powder, while heroin base is typically light brown in color. Lives of drug users are made in danger by the unpredicted adulterants and astonishing drug purities (Andreasen *et al.,* 2009).

2.4.2 Amphetamine adulteration

Illegal amphetamine is contaminated or broadly diluted prior to attaining the customer. It is usually adulterated with caffeine and creatine. Similar to amphetamine, caffeine possess stimulatory impact while creatine is easily accessible because it is authorized substance that is extensively employed to increase sporty performance (Andreasen *et al.,* 2009).

2.4.3 Cocaine adulteration

Adulterants employed in cocaine are phenacetin, caffeine, creatine, procaine, paracetamol, benzocaine, phenazone, ephedrine, mirtazapine, and ketamine. Phenacetin possesses pain-relieving ability. The employment of some other adulterants cannot be elaborated due to their pharmacological property (Andreasen *et al.,* 2009). Different adulterants and diluents are widely added to heroin, amphetamine, and cocaine. Drug transparency has considerably reduced with time so in upcoming time they were more contaminated and/or diluted (Andreasen *et al.,* 2009). Among inoculating drug abusers (mainly heroin and cocaine inoculators) bacterial illnesses ascribed to illegal drug contaminations were most frequent. Because of poor or unclean production, making methods, inadequate wrapping and unsuitable storage, by-products, bacteria or other biological means can also contaminate illegal drugs (Cole *et al.,* 2010).

2.5 Heroin

Heroin (diacetylmorphine) is a semi synthetic narcotic that was first synthesized in 1874. It was originally marketed as a safer, non addictive substitute to morphine (Habal, 2009). Heroin, diacetylmorphine, is largest and extremely addictive, misused and quickly effecting opiates. It exists in different forms that differ from one another in manifestation (powder or course granules), colour (white, off-white, brown, pink, red) and chemical composition. Street samples possess several kinds of alkaloidal part and among them five alkaloids i.e. morphine, codeine, thebaine, papaverine and noscapine make main mass of alkaloidal portion. Presently, four universal regions, Southeast Asia, Southwest Asia (including Pakistan and Afghanistan), Mexico and

South America are considered as places of opium and heroin production (Subhan *et al.*, 2009).

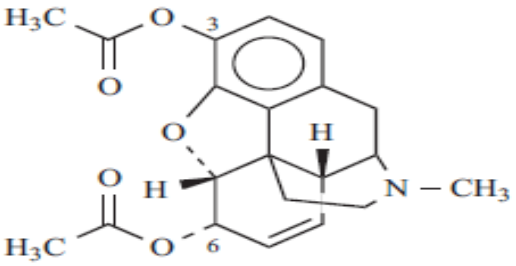

Figure 2.2: Molecule structure of heroin

In numerous European states for example Switzerland, the U.K., Spain, and Germany, and in Canada, heroin-assisted management is presently functional or is under deliberation (Fischer *et al.*, 2002). The opium alkaloids and their acetylated derivates reveal the employment of illegal heroin because they are absent in pharmaceutically uncontaminated heroin formulations (Staub *et al.*, 2001; Brenneisen *et al.*, 2002). By hydrolysis, heroin is quickly breakdown into 6-acetylmorphine and the morphine which is eventually conjugated to glucuronides. Its quantity turns down after its entering into body (Rook *et al.*, 2006).

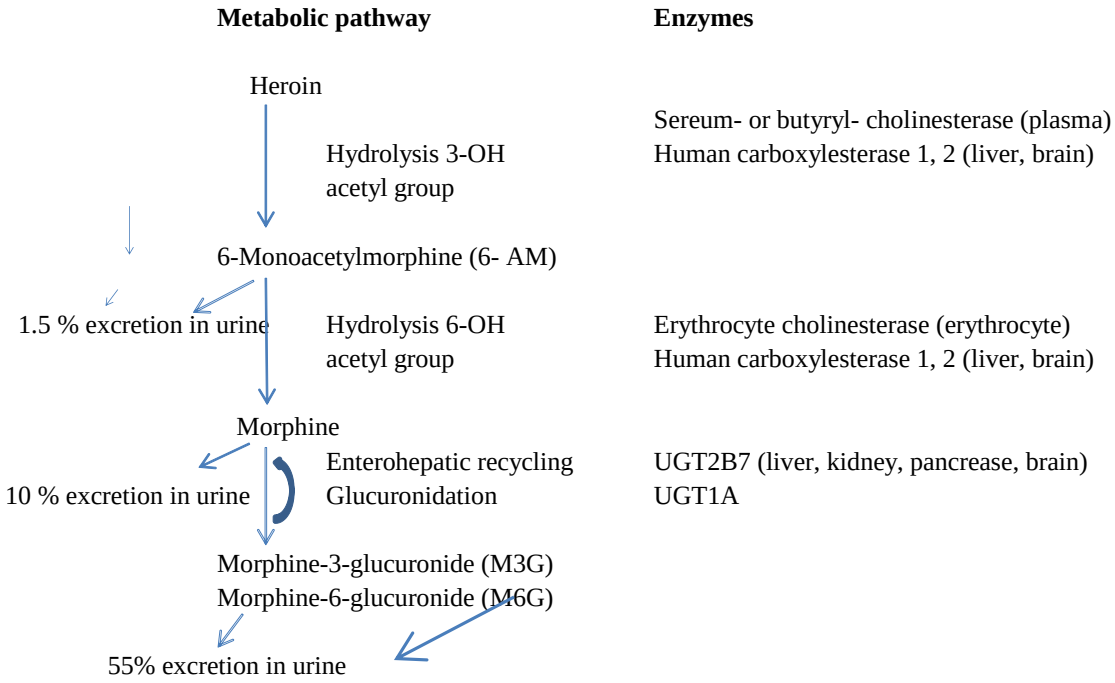

Figure 2.3: Heroin metabolism

In the process of hydrolysis and glucuronidation of the heroin metabolite morphine liver enzymes perform significant function (Rook *et al.,* 2006). Purest heroin is white powder and have bitter taste. It is sold as black, tarry matter and the adulterations and additives which are present in it also limit its absorption via mucous membranes. It produces hypoxia, respiratory dejection, lessen secondary vascular confrontation (that results in gentle hypotension), gentle vasodilation of the cutaneous blood vessels (that results in reddening) and motivates histamine discharge (that results in pruritus). Furthermore, it also restrain baroreceptor response that result in bradycardia, hypotension, reduces gastric motility that restrain acetylcholine impact on the small intestine, reduces the colonic propulsive emission, results in gastric-emptying time that is long-lasting by to the extent that 12 hours and constipation. It also reaches the placenta and the blood-brain barrier of the fetus that produce narcotic reliance in the fetus (Habal, 2009).

In the United States, heroin was given the first place then to codeine and methadone. Due to adulterations, heroin appears in various colours from white to dark brown. It is mostly adulterated with cocain, amphetamines, quinine, quinidine, chloroquine, phenobarbital, lidocaine, benzocaine, tetracaine, caffeine, methaqualone, fentanyl and other opiates and among them prominent adulterants were paracetamol and caffeine (Ghanem *et al.,* 2008).

Diacetylmorphine, a part of street heroin, have approximately absolute degradation (up to 99%), with major thermal breakdown products i.e. 6-monoacetylmorphine, N, 6-diacetylnormorphine, N, 3, 6-triacetylnormorphine, 1, 10-diacetoxy-phenanthrene and N-acetylnormorphine. The second main frequent drug heroin constitutes about 25% of all seizures while combined cannabis and hashish form about 65% of all seizures. Opium contain about 35 alkaloids, among them most significant are morphine, codeine, narcotine, papaverine and thebaine but their quantities are differ and depends upon the soil, weather, developing circumstances and period of latex harvesting (Zelkowicz *et al.,* 2005).

Heroin is morphine derived semi-synthetic lipophilic drug having approximate short half-life of 2 and 5 minutes but have long-lasting pharmacodynamic potential of numerous hours. It normally performs its function by its constant agonistic

metabolites 6-monoacetylmorphine, morphine and morphine-6-glucuronide (Rook *et al.,* 2005). Mostly poppy cultivation and heroin production occurs in Asia like Afghanistan, Pakistan, Myanmar and Lao People's Democratic Republic and to minor level in Latin America like Mexico and Columbia. Ethanol, diethyl ether, concentrated hydrogen chloride, activated charcoal, sodium carbonate, ammonium chloride and acetic anhydride are usually employed in heroin making. After completion of process heroin is not 100% pure, and brown heroin have 70% diamorphine content when it is transparent (Cole *et al.,* 2010)

2.6 Other drugs

Stroke related drugs are cocaine, amphetamines, Ecstasy, heroin/opiates, lysergic acid diethylamide (LSD), cannabis/ marijuana, tobacco and ethanol (Esse *et al.*, 2011). In numerous states extensively misused materials are opiates (Mattoo *et al.,* 1997; Peters and Reid, 1998; Hall and Darke, 1998; Hulse *et al.*, 1999; Zhou and Li, 1999), cannabinoids (Tang and Wong, 1996), amphetamines (Peters *et al.*, 1997; Robson and Bruce, 1997; McKetin and Mattick, 1998) and cocaine (Torrens *et al.,* 1991; Dunn and Laranjeira, 1999; Iravani *et al.,* 2010). Due to their progressing extensive accessibility and their capability to persuade casualty, opioids are still the chief illegal drugs of attention to forensic toxicology (Drummer, 2005).

2.6.1 Cannabis

It is perhaps the most extensive and frequently abused drug world-wide (Kumar and Nath, 2012). The United Nations International Drug Control Programme (UNDCP) estimates about 141 million cannabis abusers (UNDCP, 1997). It is more common in young people and greater in males than in females (Kumar and Nath, 2012). Usually it is supposed to be recreational and nontoxic drug in a few countries (Grotenhermen, 2007) but in other countries it is considered as a drug of misuse and its employment is severely banned (Raharjo and Verpoorte, 2004; Grotenhermen, 2007). After combined with other materials, it is usually smoked or ingested (Sharma *et al.*, 2012).

Amusingly, via pyrolysis smoking of cannabis produces about 2000 substances (Appendino *et al.,* 2011; Sharma *et al.,* 2012), characterized by dissimilar classes of chemicals that includes nitrogenous compounds, amino acids, hydrocarbons, sugar, terpenes and simple fatty acids (Sharma *et al.,* 2012).

It produces different behavioural and physiological changes like condition of ecstasy, recreation, changed time observation, absence of attentiveness, weakened knowledge, recall and temper alterations such as panic and suspicious responses (Sharma *et al.,* 2012), quick changed heart rate and diastolic blood pressure, conjunctival stain, dehydrated mouth and throat, amplified hunger, vasodilatation and reduced respiratory rate (Sharma *et al.,* 2012). It also has an effect on the immune and endocrine system and its misuse is linked to damaged lung and EEG changes (Ashton, 2003; Sharma *et al.,* 2012). Mostly rural areas are the better place for their cultivation but in latest years the approximate cultivated area has been a contentious topic (Peltzer *et al.,* 2010).

2.6.2 Cocaine

It is widely used drug (UNODC, 2007) and cocaine hydrochloride intranasal intake is the frequent administration way amongst powder users (Van *et al.,* 2008). Adulterants used in cocaine are caffeine, lidocaine, benzocaine, diltiazem, procaine, phenacetin (Kenyon *et al.,* 2005; Staack *et al.,* 2007; Brunt *et al.,* 2009), hydroxyzine, levamisole (Fucci, 2007; Behrman, 2008), atropine (Brunt *et al.,* 2009).

Cocaine have two chemical types one is water soluble powdered hydrochloride salt and other is the lipid soluble cocaine alkaloid which is a free base. It stops the reuptaking of neurotransmitter like dopamine; norepinephrine, serotonin and acetylcholine at presynaptic nerve endings thus enhance their amounts for motivation of sympathetic nerves. It also has its effect on calcium channels in smooth muscle cells in vascular walls. It causes cardio embolism, a familiar cocaine-related stroke, infective endocarditis (Esse *et al.,* 2011), ischemia and infarctions in a few hours after intake (Brunt *et al.,* 2009).

2.6.3 Amphetamines

World War II is the beginning time for prevalent amphetamine when it was accessible to military to brawl exhaustion and develop confidence. Amphetamines possess chemical resemblance to the natural neurotransmitters epinephrine and dopamine. By obstructing the pre-synaptic reuptaking of the catecholamines dopamine, norephinephrine, and serotonin, it allows them to stay in the synapse to generate and

drench the postsynaptic receptors. It also causes myocardial infarction, cardiomyopathy, kidney, liver and respiratory malfunction, stroke, amnesia, uncertainty and lots of psychiatric signs (Esse *et al.,* 2011).

They are central nervous system stimulants, nasal decongestant, a hunger suppressant, or to fight exhaustion but possess limited medicinal use (Seidi *et al.,* 2011). They enhance self-assurance, insomnia, attentiveness, competitiveness, and violence (Chung *et al.,* 2008). Its consumption also produces cases of causalities (Nishida *et al.,* 2002).

2.6.4 Methamphetamine

Methamphetamine is an increasing worldwide dilemma due to difficulty in its management as it can be prepared from chemicals that are present in daily goods that start from cleaning liquids to medicines (Mcketin, 2007).

Its global making lies at 290 tons per year having selling value of about US$28 billion (UNODC, 2007). Pseudoephedrine and ephedrine are the more frequent chemicals that are employed as decongestants in different cold-and-flu medicines (McKetin *et al.,* 2011). Other comprises phenylpropanolamine (PPA) and phenyl-2-propanone (P2P or phenylacetone) (Reed, 2009). Precursor regulations or 'vital chemicals' are significant in the production procedure (McKetin *et al.,* 2011) because they avoid the distraction of chemicals from their genuine uses into secret drug production (McKetin *et al.,* 2011).

2.6.5 Amphetamine-type stimulants (ATS)

Amphetamine-type stimulants (ATS) are also turns out to be a universal dilemma because of their constant demand. They are also manufactured in concealed laboratories with lack of management and any type of quality control on its making which in turn give contaminated and poor value products that are detrimental to health (Khajeamiri *et al.,* 2011).

2.6.6 Ecstasy

Ecstasy is an imprecise name for various designer drugs that are employed by youth. Ecstasy, derivatives of amphetamine, is 3, 4-methylenedioxymeth-amphetamine, or MDMA, N-methyl-3-4-methylenedioxyamphetamine or MDA (sometimes called "Eve"), or 3.4-methylenedioxyamphetamine (MDA) (Esse *et al.*, 2011). It enhances the discharge of, and stops the reabsorption of, serotonin and norephinephrine having smaller impact on dopamine (Esse *et al.*, 2011).

2.6.7 Alcohol

It is most extensively employed and misused drug. It is present in all over the world but in separated regions or in some countries having firm religious ban (Kumar and Nath, 2012).

2.6.8 Hashish and Marijuana

Hashish is a cannabis preparation that had its place in Egyptian mummies. The extensively employed street drugs in the world are the marijuana, hashish, bhang and ganja. The plant *Cannabis sativa* (Indian hemp) and a few of its type is the source of these psychoactive drugs production. Leaves, stems and desiccated flower buds of the plant produce marijuana (Sharma *et al.*, 2012) while blossoming buds of the hemp plant produce the resin hashish (Hazekamp and Grotenhermen, 2010). In the United States, marijuana is the large frequently employed recreational drug and about 15 states employed it for medicinal purposes (State Medical Marijuana Laws 2010).

2.7 Other associated illicit drugs

2.7.1 Ketamine

In humans that are mostly in surgery and animals ketamine is employed as potent sedative and is an arylcycloalkylamine. Its administration produces feelings of ecstasy, dissociative psychedelic condition and when taken at large dosage, produces delusions. It is present in a tablet, powder or liquid form and is normally engulfed, inhaled or inoculated (Cole *et al.*, 2010).

2.7.2 Gammahydroxybutrate (GHB)

Gammahydroxybutrate, liquid ecstasy or liquid E is a liquid, and produces condition of ecstatic, sleep and cause tranquillizing, analgesic effects (Cole *et al.,* 2010).

2.7.3 Lysergic acid diethylamide (LSD)

Lysergic acid diethylamide, or LSD, is a strong hallucinogen (Esse *et al.,* 2011) and semi-synthetic psychedelic drug (Cole *et al.,* 2010). It possesses vasospasm of cerebral arteries (Esse *et al.,* 2011). It is a white powder and marketed as a liquid absorbed into tiny tablets. Among all in the illicit drugs, it is regarded to be least adulterated (Cole *et al.,* 2010).

2.8 Microbial Infections

Bacillus and *Clostridium* species are the main frequently recognized bacterial contaminants (Cole *et al.,* 2011). Disorder of "toxic, spongiform leukoencephalopathy" (SLE) is uncommon but tremendously serious and mostly lethal neurological problem of heroin smoking (Büttner *et al.,* 2000; Brenneisen and Hasler, 2002). Its first incidence was accounted in 1982 from the Netherlands having about 25% of death rate. In fact, street heroin, its adulterant and by-products all robustly enhances the pyrolysis rate (Zelkowicz *et al.,* 2005).

With the application of illegal injection of drug use, various viral and bacterial contaminations are related (Horsburgh *et al.,* 1989; Brettle, 1992; Peat *et al.,* 2000). *Clostridium botulinum* (Martin *et al.,* 1999; Werner *et al.,* 2000; Jensenius *et al.,* 2000), *C. tetani* (Talan and Moran, 1998) and *Bacillus anthracis* (Ringertz *et al.,* 2000) which are endosporeforming bacteria (Martin et al., 1999; Werner *et al.,* 2000; Jensenius *et al.,* 2000) are progressively more known as a group of agents that infects injecting drug users (IDUs) (Mclauchlin *et al.,* 2002). *C. novyi* type A produced more severe infections (Anon, 2000; Anon, 2000; Ryan *et al.,* 2001; McGuigan *et al.,* 2002), though few other endospore-forming bacteria like *C. perfringens* (McGuigan *et al.,* 2002) and *B. cereus* (Dancer *et al.,* 2002) were also linked with diseases (McGuigan *et al.,* 2002). Among aerobic endospore-forming bacteria *Bacillus* species

and *Paenibacillus macerans* were more leading, *B. cereus* was most frequent (Mclauchlin *et al.*, 2002) while *B. Licheniformis*, *Staphylococcus* spp, *S. Warneri*, S. *Epidermidis* and three species of *Clostridium* i.e. *C. perfringens, C. sordellii and C. tertium*, *B. subtilis* and *B. Pumilus*, *Aspergillus* species were major bacteria (Mclauchlin *et al.*, 2002).

From Norway, the first anthrax incident with injecting drug users was illustrated in 2000 (Ringertz *et al.*, 2000). While in Germany among injecting drug abuser, anthrax incident was recognized in June 2012 (Holzmann *et al.*, 2012). In 2000, wound botulism was also accounted in IDUs and in the UK these signify the first identification of this situation (Athwal *et al.*, 2000; Khan *et al.*, 2000; Hood *et al.*, 2000; Mulleague *et al.*, 2001).

In heroin abusers, candidiasis has been accounted to take place (Calandra *et al.*, 1985), mainly among of the Iranian brown heroin diversity consumers (DomÍnguez-Vilches *et al.*, 1991). The infections produced by these drugs are results of reductions in immunological state of the person which in turn produces opportunistic infections of bacteria or fungus (Orangio *et al.*, 1985). White heroin was found to be less adulterated then brown one. During the period of production and preparation in packets of drug powder contamination with fungal conidia occurs. Air and the substances which are used for drug adulteration, are the sources of fungal contamination (DomÍnguez-Vilches *et al.*, 1991).

Between the consistent drug addicts, skin 'popping 'is familiar (Graham *et al.*, 1999). This way of drug abuse takes place when the drugs are straightly inoculated into tissues in spite of the vessels. This act produces severe health effects if the object is microbiologically contaminated because tissue is the ideal culture medium for bacterial growth (Dancer *et al.*, 2002). They are also at a great risk of infection by equipment sharing (Hughes, 2000). When the commercially accessible acids like citric or tartaric acid are dissolved into these drugs, breakdown of tissues at the inoculation spot is enhanced and therefore may increase bacterial growth (Dancer *et al.*, 2002).

Molecular typing approaches explained the definite microbiological relation among the patient and his inoculated heroin. Perhaps the first information was associated with *B. cereus* (Dancer *et al.*, 2002). Heroin related deaths can be secondary to the

effect of other drugs and adulterations because when they are employed in combination with heroin, usually acceptable dosage of heroin may be poisonous or lethal. In Iran a death of drug trafficker due to peritonitis and bowel obstruction was caused by two big sachets that leaks in small intestine (Akhgari *et al.,* 2011). These drugs are the reasons of several severe health and social troubles that includes HIV, hepatitis C diseases, suicide and drug overdo (Roy *et al.,* 2008). Several drug toxins may be the reason of causality in abusers or sufferers who employed these illicit materials. Moreover, ethanol is recognized to increase the poisonous of these substances (Akhgari *et al.,* 2011).

3.1. Bio burden (Detection of Microorganisms)

3.1.1 Materials

i. Media and chemicals

Vogel-Johnson agar (Oxoid), Sabouraud dextrose agar (Oxoid), Tryptone soya agar (Oxoid), Mac-Conkey agar (Oxoid), Distilled water, Normal saline.

ii. Instruments and Equipments

Petri dishes, Test tubes, Test tubes rack, Autoclave, Pipettes, Cotton plugs, Marker, Laminar flow hood, Incubator, Conical flask, Lamp.

iii. Collection of Drug Samples

100 different illicit drugs samples were collected from various regions of Pakistan and transported to the NIH laboratory.

3.1.2 Procedure

3.1.2(1) Preparation of Media and isolation of pathogenic microorganisms

Drug samples were subjected to total aerobic viable count (TAVC) by pour plate method and for presence or absence of bacteria and fungi. Mac-Conkey, Sabouraud dextrose, Tryptone soya and Vogel-Johnson agar media were prepared according to the instruction of the manufacturer. Media were sterilized at 15 pound pressure, at 121˚C temperature for 15 minutes. Sterilized test tubes were taken according to the samples and were marked. 9 ml of normal saline were pipetted out in these tubes. After marking the tubes, 1g of samples were then dissolved in respective tubes. These tubes were incubated in an incubator at 35 for 30 minutes for dissolution. Petri plates were sterilized and marked. 1 ml of the samples was poured in each respective plate. Then 10-15 ml of sterilized Tryptone soya media was poured and allowed to solidify. Some more petri plates were sterilized and marked according to the samples. For the detection of gram –ve, fungus, *Streptococcus* and *Staphylococcus* species, these plates were divided into three groups and marked them by the name of the media i.e. Mac-Conkey, Sabouraud dextrose and Vogel-Johnson agar medium. 1ml of each sample were poured according to the marking of the plates and then poured 10-15ml of the sterilized medium in their respective petri plates. Plates were rotated to mix the sample in the melted agar and then leaved them for solidification. After

solidification of the media, these plates were incubated at 35°C for 72 hrs except Sabouraud dextrose agar. Sabouraud dextrose agar plates were incubated at 20-25˚C for 5-7 days. After the completion of incubation period, total aerobic microorganisms were counted on Tryptone soya agar plate. Growth of *streptococcus* and *staphylococcus* species were observed on Vogel-Johnson agar, gram –ve bacteria growth were observed on Mac-Conkey agar and fungus and molds on Sabouraud dextrose agar medium. All this procedure was performed in the laminar flow hood.

3.2 Biochemical test:

In certain samples due to the presence of black colonies with yellow zone (indicated *S. aureus*) and black shiny colonies (indicated *Salmonella* species) different biochemical tests were conducted for their confirmations (Cheesbrough, 2000).

(1) *Staphylococcus aureus*

Biochemical test	Reagent	Reaction
Catalase test	3% Hydrogen peroxide	Breakdown of hydrogen peroxide to oxygen and water
Coagulase test	Distilled water, plasma	Conversion of fibrinogen to fibrin

(2) *Salmonella* species

Biochemical test	Reagent	Reaction
Triple sugar iron (TSI)	TSI agar	Acid production
Triple sugar iron (TSI)	TSI agar	Gas production
Triple sugar iron (TSI)	TSI agar	H2S production
Urease test	Christensen's urea broth	Yellow colour
Indole test	Trypton water, Kovac's reagent	Yellow ring

3.3 Qualitative Analysis

Qualitative analysis has been done with colour tests and TLC technique.

3.3.1 Materials

3.3.1(1) Equipments and Chemicals

Spot plates, United Nations Office on Drugs and Crime (UNODC) Colour test kit, Water, Glass rod or Spatula, Reagent A1, Reagent A2, Reagent B, Reagent B2, Reagent B3, Reagent C1, Reagent C2, Reagent D1, Reagent D2, Reagent E1, Reagent

E2, Test tubes, Ammonia, Ethyl acetate, methanol, Fast blue salt or tetrazotized or O-dianisidine, Iodoplatinate spray reagent, TLC plates, Chromatography tank, capillary tube, pencil, Chloroform, UV lamp.

3.3.2 Colour tests

Using UNODC drug kit different colour tests were conducted for qualitative analysis of drug samples (UNODC drug kit).

Drug	Test	Reagents	Time
Opium	Test A	A1, A2	Few seconds
Morphine	Test A	A1, A2	Few seconds
Amphetamine	Test A	A1, A2	Few seconds
Heroin	Test A	A1, A2	Few seconds
Hashish	Test B	B1, B2, B3	2 minutes
Methamphetamine	Test C	C1, C2	Few seconds
Barbiturates	Test D	D1, D2	Few seconds
Cocaine	Test E	E1, E2	10 seconds

3.3.3 Thin layer chromatography (TLC)

For the detection of drug present in the sample, thin layer chromatography was employed. It is quick, responsive, open method to various substances and visualization practices and is economical.

3.3.3(a) Preparation of samples and standard solutions

3.3.3(b) Standard solutions

Standard solutions of heroin, morphine was prepared at a concentration of 1mg/ml in methanol. All standards were combined into a single standard solution. 5µl spot of the standard solution(s) was spotted to the TLC plate (UNIDCP, 1998).

3.3.3(c) Sample preparations

5 mg of sample was dissolved for each 1 ml of methanol, and placed 5µl spot onto the plate (UNIDCP, 1998).

3.3.3(d) TLC plates

Commercially available TLC plates, coated with activated silica gel G having thickness of 0.25 mm was used. Plate size was 20 x 20 cm (UNIDCP, 1998).

3.3.3(e) Spotting

The ‘’spotting line’’ was made 1 cm from the bottom of the plate. The samples was spotted about 0.8 cm apart from each other. The spot size was ≤ 2 mm. The spots were dried by cold or hot air between applications (UNIDCP, 1998).

3.3.3(f) TLC tank

Clear glass TLC tank and lid was used. The tank was contained with developing solvent. The developing solvent in the TLC tank was 0.3 and 0.5 cm in depth. Plates were placed in the tank (UNIDCP, 1998).

3.3.3(g) Developing solvent

For the developing systems, the solvent was renewed after a maximum of 3 runs, or, ideally, after each development. Three solvent systems were used. System A contain toluene (45 ml), acetone (45 ml), ethanol (7 ml), conc. ammonia (3 ml), solvent system B contain ethyl acetate (85 ml), methanol (10 ml), conc. ammonia (5 ml), solvent system C contain methanol (100 ml), conc. ammonia (1.5 ml) (UNIDCP, 1998).

3.3.3(h) Development line

Development line was 14 cm long. An analysis was terminated when the solvent migrated to the development line. The plates were removed from the developing tank as soon as the solvent reaches the development line (UNIDCP, 1998).

3.3.3(i)Visualization

Plates were dried prior to visualization. Drying was accomplished at room temperature.

1. Ultraviolet (UV) light, usually at 254nm was used.
2. Acidified potassium iodoplatinate spray reagent was used.

The data obtained was recorded at each stage, including the colours and retardation factor (*R*f) values of the compounds observed at each stage of the visualization process (UNIDCP, 1998).

3.4 Quantitative analysis

3.4(1) High performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) were employed for the investigation of heroin and its metabolites by using in street samples with various analytical methods e.g. HPLC coupled with ultraviolet (UV), fluorescence, or electrochemical detection (Mahdy *et al.*, 2012).

3.4(2) Experimental

3.4(2a) Chemicals and reagents

Heroin samples were supplied by anti-narcotics force of Pakistan. Reference standards includes: pure caffeine, paracetamol, diazepam, dextromethorphan, morphine, cocain, acetic anhydride and ephedrine were purchased from PDH laboratories (Pvt) limited, Lahore, Pakistan. HPLC-grade methanol was used, distilled water, produced by NIH laboratory. Dioctyl Sulfosuccinate sodium salt and sodium acetate were obtained from PDH laboratories (Pvt) limited, Lahore, Pakistan. All other types of materials and reagents were of analytical grade and provided from commercial sources.

3.4(2b) Apparatus

The method development was performed with the Shimadzu HPLC system (Japan) consisting of LC-9A, LC-20AT pump coupled with UV-Vis detector SPD-6AV at 285 nm, with system controller SCL-6B. The data and peak areas were processed with chromatopac C-R4A. The analytical column was octadecyl sulphonate (ODS) CTO-6A (stationary phase) with particle size of 5µm maintained at room temperature 35-37˚C. The samples and standards were injected with an HPLC syringe having capacity of 20µl sample loop. All types of the calculations related to quantitative

analysis were carried out with external standardization by the computing the peak areas.

3.4(3) Procedure

3.4(3a) Preparation of samples and standard solutions

i. Heroin

Heroin samples were taken accurately by weighing 20 mg in volumetric flask and dissolved in 5 ml mobile phase. After sonication for 2 minutes, 1 ml from solution was taken and again dissolved in 10 ml mobile phase. Dilution of 400 µl was prepared (Zelkowicz *et al.,* 2005).

ii. Morphine

Morphine samples were taken accurately by weighting 10 mg in volumetric flask and dissolved in 10 ml of mobile phase. After sonication for 2 minutes, 1 ml from solution was taken and again dissolved in 10ml mobile phase. Dilution of 400 µl was prepared (Zelkowicz *et al.,* 2005).

iii. Operating conditions

The operating conditions includes detector having UV at 285nm, temperature 35-37˚C, run time 40min, flow rate 1ml/min, injection volume 20µl, mobile phase contain 0.01M Dioctyl Sulfosuccinate sodium salt in methanol (60%) i.e. 1.33g in 300ml of methanol and 0.005M Sodium acetate in distilled water (40%) i.e. 0.136g in 200ml of distilled water. Pump system was isocratic pump system (B.P, 2012).

iv. Cocaine

Samples were taken accurately by weighting 10 mg in volumetric flask and dissolved in 10 ml of mobile phase. After sonication for 2 minutes, 1 ml from solution was taken and again dissolved in 10ml mobile phase. Dilution of 400 µl was prepared (Zelkowicz *et al.,* 2005).

v. Acetic anhydride

2g of acetic anhydride was accurately weighed in 50 ml of N/1 sodium hydroxide (NaOH) in a stopper flask and allowed to stand for 1 h. Excess of alkali was titrated

with N/1 hydrochloric acid (HCl) using phenolphthalein solution as indicator (BP, 1968).

vi. Operating conditions

Column used was octadecylsilica reversed-phase column, mobile phase contain phosphate buffer (0.01 M; pH 2.1) containing tetrabutyl-ammonium hydroxide (0.2 mM) and acetonitrile (6%, v/v), flow rate 1.5 ml/min, detection with UV at 233 nm (UNIDCP, 1995).

3.4(4) Method

3.4.4(a) Standard operating instructions (SOI)

Main instrument which includes system controller, pumps, detector, column oven and integrator was switched on. Mobile phase was put according to the samples to be analysed. Wavelength, flow rate, pressure, and temperature and run time was adjusted. Switched on the integrator, set date, language, title and was connected with main instrument and was controlled with co-inter using monitor. After running some time required pressure and base line was achieved. Standard /sample solutions were prepared. Standard or sample was injected while injector was at load position using HPLC syringe. It was brought to the inject position.

3.4.4(b) Standard operating procedure (SOP)

Main button, system controller, chromatopac, column and pumps were switched on. Suction tubes were put in already prepared mobile phase. Parameters i.e. rate of flow, pressure, temperature; wavelength etc was set in system controller and pressed start. Instrument was tuned at auto zero and weighted for few minutes till the base line was clear on monitor. Base line was acquired and then injected the standard through HPLC syringe at inject position and then took it to load position with jerk. Again instrument was tuned at auto zero and checked the peaks on monitor and took out print. Same was done with sample. Peaks were observed and were calculated. After assay, column was washed with mobile phase for few minutes. Suction tubes were kept in HPLC grade methanol.

4.1. Microbial analysis

4.1.1 Bio burden

The first part of our research was the microbial analysis of heroin samples. This was further divided into two parts i.e. estimation of total aerobic count on Trypton soya agar and estimation of microbial growth on different media and the second portion was conduction of biochemical tests. In the first case the samples having total aerobic count was done. Those samples having range of 1- 15 colonies of microbes were 55 in numbers, those having more than 15 colonies were 7 in numbers. In this analysis some samples have numerous growth i.e. uncountable growth were 20 in numbers and those having no growth i.e. negatives were 22 in numbers as shown in figure 4.1. All this data is given in Table 4.1(1). For estimation of microbial growth on their respective media gram-negatives bacteria show their growth on MacConkey agar, fungus species grow on Sabouraud dextrose agar while *Streptococcus* and *Staphylococcus* species showed their growth on Vogel-Johnson agar. These results indicated that heroin samples contain several microbes that arrive during the drug preparations in laboratories. Unclean and unhygienic handling of drug also assists in their growth. The samples of gram-negative bacteria having single colony were 17 in numbers, those having double colonies were 14 in numbers, numerous growth were 7 in numbers while negative growths were 60 in numbers. This is shown in figure 4.3. For fungus species the samples having single colony were 12, those with double colonies were 10, numerous growth were 18 and negative growth were 57 in numbers. This is clear in figure 4.4. For *Streptococcus* and *Staphylococcus* species samples with single colony were 12, with double colonies were 5, with numerous growth were 35 and with negative growth were 48 in numbers as indicated in figure 4.5. All this is shown in Table 4.1(2).

4.1.2 Biochemical test:

For *S. aureus* catalase test showed active bubbling while coagulase test indicated the clumping within 10 seconds. Similarly for *Salmonella* species in TSI test acid, gas and hydrogen sulphide was produced which was marked by yellow colour butt, active bubbling in butt and black colour of the tube. While Indole and Urease test gave negative results that confirmed the presence of *Salmonella* species. This indicated in table 4.1(3) and 4.1(4).

4.2. Chemical analysis

1. Colour Test

The second part of the research was the chemical analysis of drug samples. The first portion of this part was the colour test. Different colour tests were performed for different drugs which were confiscated by the anti narcotic force of Pakistan. The colour tests were performed by using UNODC drug kit, containing specific reagents for each drug samples. Using UNODC drug kit, each drug gives its respective results. Five types of colour test were performed such as Test A, B, C, D and E, recommended for particular drug. The results for each drug are shown in Table 4.2.

2. Thin layer chromatography (TLC)

The second portion of chemical analysis was the thin-layer chromatography. TLC for heroin and morphine samples was done. In three different solvent systems each gives its respective Rf values. The data is shown in Table 4.3.

3. High-performance liquid chromatography (HPLC)

The last portion of chemical analysis was the quantitative analysis of heroin, morphine, cocaine and acetic anhydride samples. HPLC gives quantitative values of drugs along with their adulterants. Samples were quantified by using formula:

Sample/ Standard x 100

a. Percentage of Heroine samples

According to our results heroin samples which were adulterated with paracetamol was 20, with diazepam were 10, with dextromethorphan were 35 and those which cannot be quantified were 34 samples as shown in figure 4.12. The data is given in the Table 4.4.

b. Percentage of Morphine samples

Morphine was also subjected to HPLC for quantitative analysis. Among morphine samples those having concentration between 1-5% were 49, from 6-10% were 99 and more than 10% were 39 in numbers as shown in figure 4.14. The data of morphine samples is given in Table 4.5.

c. Percentage of Cocaine samples

10 cocaine samples were subjected to HPLC for quantitative analysis. Those having concentration up to 10 % were 3 samples and those having concentration beyond the 10 % were 7 in numbers as shown in figure 4.16. The data is given in Table 4.6.

d. Identification and Quantification of Acetic Anhydride

20 acetic anhydride samples were subjected to HPLC for quantification. According to figure 4.18 those samples having concentration up to 10 % were 4, up to 20 % were 5, up to 30 % were 7, up to 40 % were 2 and up to 50 % were also 2 in numbers. The data is shown in the Table 4.7.

e. Samples of acetic anhydride adulterated with HCl

30 samples of acetic anhydride adulterated with HCl were quantified by HPLC. According to figure 4.20 there were no sample of acetic anhydride having concentration up to 10 %, up to 20 % were 11 and up to 30 % were 19 in numbers. Acetic anhydride samples adulterated with HCl ranges from 30-35%. From 30-31, there were 10, from 32-33 were 16 and from 34-35 were 4 samples. The overall data is given in Table 4.8.

Drug samples from different cities of Pakistan were confiscated by anti-narcotics force of Pakistan. The ratio of these drugs obtained from different cities is given in the Table 4.9.

Table No. 4.1(1) Total aerobic count of microbes on Trypton Soya Agar.

S.No	T.count (TSA) cfu/gm	S.No	T.count (TSA) cfu/gm	S.No	T.count (TSA) cfu/gm	S.No	T.count (TSA) cfu/gm
1	Num*	26	05	51	-ve	76	Num
2	10	27	-ve	52	11	77	Num
3	25	28	01	53	-ve	78	Num
4	20	29	02	54	-ve	79	Num
5	10	30	-ve	55	03	80	Num
6	70	31	-ve	56	01	81	10
7	100	32	-ve	57	-ve	82	07
8	-ve	33	06	58	01	83	05
9	-ve	34	01	59	-ve	84	Num
10	85	35	05	60	-ve	85	10
11	-ve	36	-ve	61	03	86	08
12	-ve	37	17	62	05	87	Num
13	-ve	38	-ve	63	02	88	Num
14	15	39	01	64	03	89	Num
15	10	40	01	65	02	90	Num
16	02	41	01	66	03	91	15
17	02	42	03	67	02	92	05
18	Num	43	03	68	03	93	03
19	-ve	44	02	69	11	94	Num
20	04	45	17	70	10	95	05
21	-ve	46	-ve	71	07	96	09
22	-ve	47	05	72	05	97	Num
23	13	48	03	73	Num	98	Num
24	06	49	03	74	10	99	Num
25	10	50	-ve	75	08	100	Num

-ve = No growth, *Num = Numerous (uncountable).

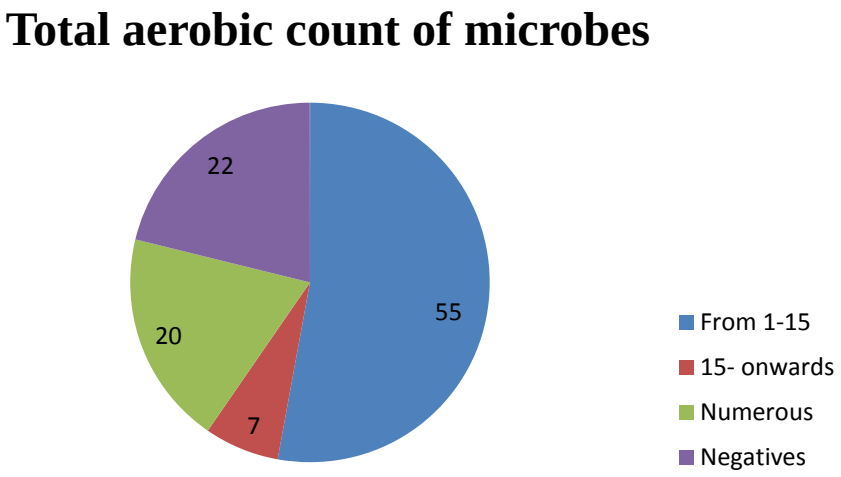

Figure 4.1: Figure showing total aerobic count of micobes

Tryptone Soya Agar is used for total aerobic count of microorganisms.

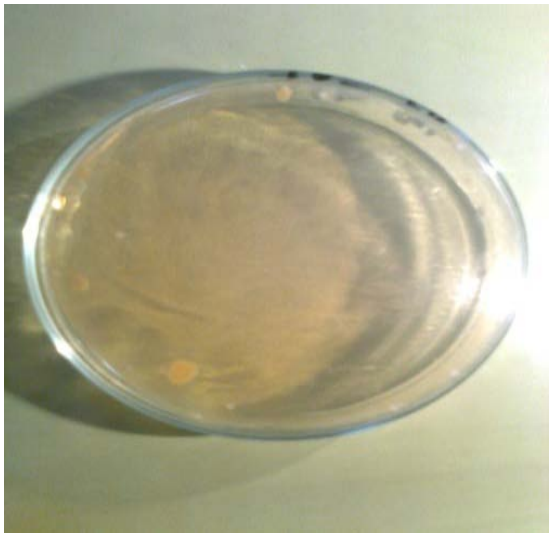

Figure 4.2: Tryptone Soya Agar plate

Table No. 4.1(2) Growth of microbes present in heroin samples, on different culture media.

S. No	Gram -ve (MacConkey Agar)	Fungus sp (SDA)	*Strep* and *staph* sp (VJA)	S. No	Gram -ve (MacConkey agar)	Fungus sp (SDA)	*Strep* and *staph* sp (VJA)
1	-ve	-ve	-ve	35	-ve	+ve	-ve
2	++	-ve	-ve	36	-ve	-ve	-ve
3	++	-ve	++ve	37	-ve	+ve	+ve
4	++	-ve	+ve	38	-ve	-ve	-ve
5	-ve	-ve	+ve	39	-ve	-ve	-ve
6	-ve	+ve	+ve	40	-ve	-ve	-ve
7	-ve	-ve	++ve	41	-ve	-ve	-ve
8	-ve	-ve	-ve	42	-ve	-ve	-ve
9	-ve	-ve	-ve	43	-ve	-ve	-ve
10	-ve	-ve	+ve	44	-ve	+ve	-ve
11	-ve	-ve	-ve	45	-ve	-ve	-ve
12	-ve	-ve	-ve	46	+ve	-ve	-ve
13	-ve	-ve	-ve	47	-ve	-ve	+ve
14	-ve	-ve	+ve	48	-ve	-ve	-ve
15	-ve	-ve	+ve	49	+ve	-ve	-ve
16	-ve	-ve	-ve	50	+ve	+ve	-ve
17	-ve	-ve	-ve	51	+ve	-ve	-ve
18	-ve	-ve	-ve	52	-ve	-ve	-ve
19	-ve	-ve	-ve	53	+ve	-ve	-ve
20	-ve	-ve	+ve	54	-ve	-ve	-ve
21	-ve	-ve	-ve	55	-ve	-ve	-ve
22	-ve	-ve	-ve	56	-ve	-ve	-ve
23	-ve	-ve	-ve	57	-ve	-ve	+ve
24	-ve	+ve	-ve	58	-ve	-ve	-ve
25	-ve	-ve	-ve	59	-ve	-ve	-ve
26	-ve	-ve	-ve	60	+ve	+ve	-ve
27	-ve	-ve	-ve	61	+ve	Num	Num
28	-ve	-ve	-ve	62	+++ve	Num	Num
29	-ve	-ve	-ve	63	++ve	+ve	Num
30	-ve	-ve	-ve	64	++ve	++ve	Num
31	-ve	-ve	-ve	65	+ve	++ve	++ve
32	-ve	-ve	-ve	66	-ve	++ve	++ve
33	-ve	-ve	-ve	67	+ve	++ve	+ve

34	-ve	-ve	-ve	68	-ve	-ve	+ve
69	+ve	++ve	Num	85	+++ve	+++ve	Num
70	+ve	++ve	Num	86	++ve	-ve	Num
71	+ve	+ve	Num	87	+ve	+ve	Num
72	-ve	+ve	Num	88	-ve	-ve	Num
73	++ve	Num	Num	89	+ve	-ve	Num
74	-ve	Num	Num	90	++ve	+ve	Num
75	-ve	Num	Num	91	+++ve	+++ve	Num
76	+ve	Num	Num	92	++ve	++ve	Num
77	+ve	Num	Num	93	-ve	+++ve	Num
78	Num	Num	Num	94	-ve	Num	Num
79	Num	Num	Num	95	+ve	Num	Num
80	Num	Num	Num	96	++ve	++ve	Num
81	++ve	++ve	++ve	97	-ve	Num	Num
82	++ve	Num	Num	98	++ve	Num	Num
83	++ve	Num	Num	99	Num	Num	Num
84	+++ve	++ve	Num	100	Num	Num	Num

+ve = Single colony, -ve = No growth, ++ve = Double colonies, +++ve = More than two colonies, Num = Numerous (uncountable).

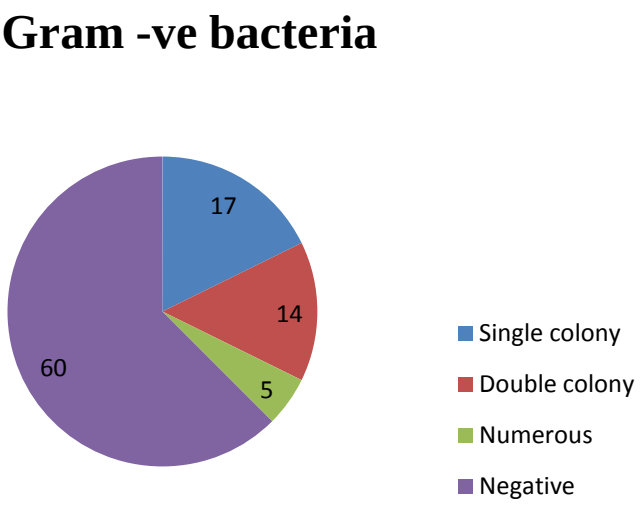

Figure 4.3: Figure showing gram –ve bacteria colonies

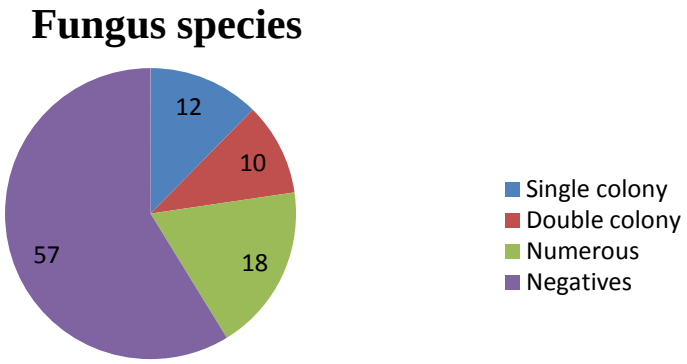

Figure 4.4: Figure showing fungus species colonies

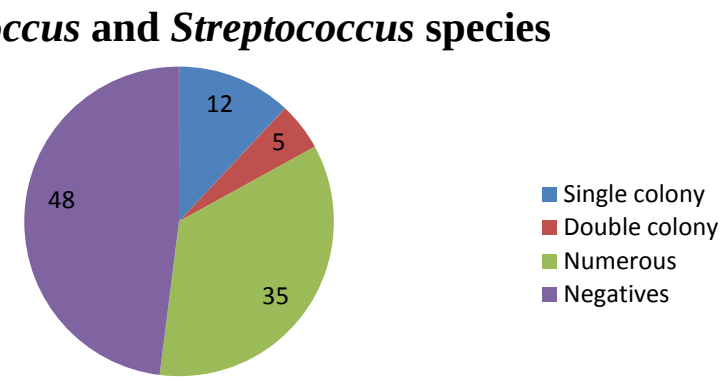

Figure 4.5: Figure showing ratio of *Staphylococcus* and *Streptococcus* species

MacConkey agar is selective for gram –ve bacteria and is a differential medium that differentiate between lactose fermenters and non-lactose fermenters for gram –ve bacteria. In lactose fermenters it gives yellow colonies (*E.coli*) while in case of lactose non-fermenters it gives pink colonies (*Salmonella typhi*).

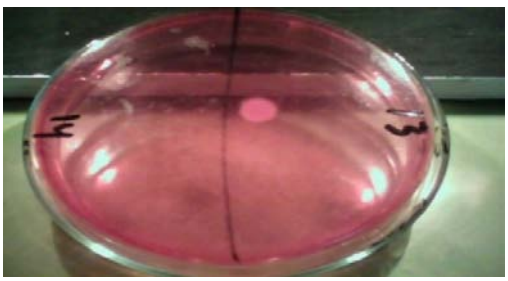

Figure 4.6: MacConkey Agar plate

Vogel-Johnson agar is selective media for *streptococcus* and *staphylococcus* species.

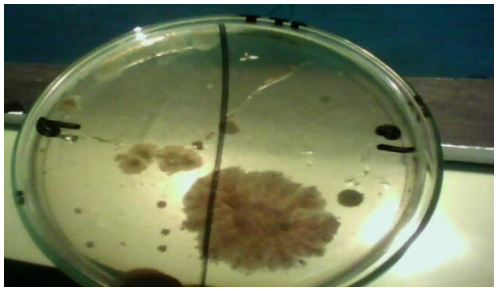

Figure 4.7: Vogel-Johnson agar plate

Sabouraud Dextrose agar is used for detection of molds, fungi (*C. albicans* and *Aspergillus* sp) and yeasts.

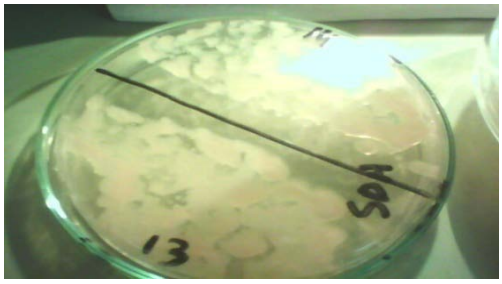

Figure 4.8: Sabouraud Dextrose Agar plate

Table no. 4.1(3) Biochemical test for *S. aureus.*

Biochemical test	Result
Catalase test	Active bubbling
Coagulase test	Clumping within 10 seconds

Table no. 4.1(4) Biochemical test for *Salmonella* species.

Biochemical test	Results
TSI test (acid production)	Yellow butt
TSI test (gas production)	Active bubbling in butt
TSI test (H_2S production)	Black colour
Urease test	Yellow
Indole test	Yellow ring

Table No. 4.2: Colour tests of different drug samples.

Drugs	Test A Result	Test B Result	Test C Result	Test D Result	Test E Result
Opium	Purple colour	NR	NR	NR	NR
Heroin	Pink to dark purple	NR	NR	NR	NR
Morphine	Light purple to dark grey	NR	NR	NR	NR
Amphetamine	Yellow to brown colour	NR	NR	NR	NR
Methamphetamine	NR	NR	L. blue to d. blue colour	NR	NR
Hashish	NR	Maroon to red colour	NR	NR	NR
Cocaine	NR	NR	NR	NR	Prominent blue colour
Barbiturates	NR	NR	NR	Purple colour	NR

NR= Not recommended

Figure 4.9: UNODC Drug kit

Table No. 4.3: Thin-layer chromatography of heroin and morphine samples.

Sample	Solvent system A		Solvent system B		Solvent system C	
	Standard	Sample	Standard	Sample	Standard	Sample
Heroin	57	57	49	49	47	47
Morphine	19	19	20	20	37	37

Solvent system A:

Toluene (45 ml), acetone (45 ml), ethanol (7 ml), conc. ammonia (3 ml)

Solvent system B:

Ethyl acetate (85 ml), methanol (10 ml), conc. ammonia (5 ml)

Solvent system C:

Methanol (100 ml), conc. ammonia (1.5 ml) (UNIDCP, 1998).

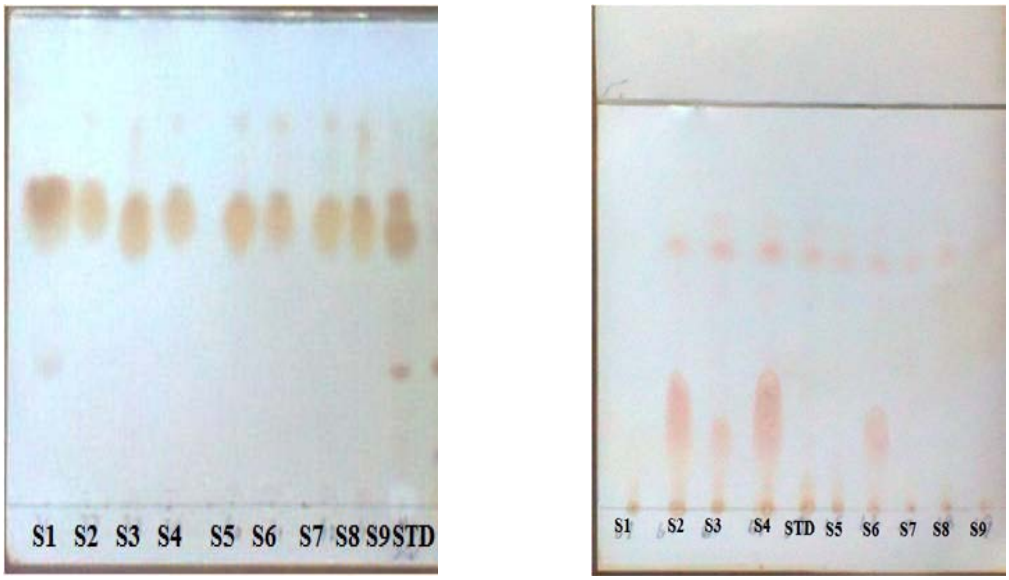

Figure 4.10: TLC of Heroin and Morphine samples

Table No. 4.4: Percentage of heroin with its adulterants.

S.No	Appearance	Heroin %	Paracetamol %	Dextromethorphan %	Diazepam %
1	Brown powder	38	17.98	NI	NI
2	Brown powder	23.09	15.55	NI	NI
3	Brown powder	38.45	13.53	NI	NI
4	Brown powder	58.08	14.66	NI	NI
5	Brown powder	32.00	18.09	NI	NI
6	Brown powder	24.44	29.00	NI	NI
7	Brown powder	29.00	24.54	NI	NI
8	Brown powder	34.66	12.43	NI	NI
9	L. Brown powder	46.77	12.65	NI	NI
10	L. Brown powder	59.22	14.77	NI	NI
11	Brown powder	49.11	22.76	NI	NI
12	Off-white powder	47.77	16.98	NI	NI
13	Off-white powder	46.77	15.78	NI	NI
14	Off-white powder	62.22	11.76	NI	NI
15	Off-white powder	36.02	16.65	NI	NI
16	Off-white powder	40.11	23.44	NI	NI
17	Off-white powder	55.88	21.56	NI	NI
18	Brown powder	59.00	18.95	NI	NI
19	Brown powder	53.55	20.09	NI	NI
20	Brown powder	14.55	25.75	NI	NI
21	Br solid pieces	12.77	NI	5.09	NI
22	Br solid pieces	13.33	NI	7.66	NI
23	Off-white powder	12.66	NI	10.88	NI
24	Off-white powder	12.77	NI	12.96	NI
25	Off-white powder	41.07	NI	5.08	NI
26	Brown powder	28.07	NI	5.66	NI
27	Brown powder	51.01	NI	4.36	NI
28	Brown powder	59.33	NI	11.01	NI
29	Brown powder	13.88	NI	8.98	NI
30	Brown powder	20.08	NI	10.00	NI

31	Brown powder	20.00	NI	3.89	NI
32	Brown powder	12.07	NI	5.56	NI
33	Brown powder	27.02	NI	5.67	NI
34	Brown powder	60.55	NI	12.87	NI
35	Brown powder	58.55	NI	6.78	NI
36	Brown powder	51.55	NI	3.44	NI
37	Off-white powder	22.22	NI	9.98	NI
38	Off-white powder	12.55	NI	9.01	NI
39	Off-white powder	53.22	NI	4.55	NI
40	Off-white powder	14.66	NI	2.88	NI
41	Off-white powder	13.55	NI	0.98	NI
42	Brown powder	30.66	NI	1.10	NI
43	Brown powder	53.55	NI	10.58	NI
44	Brown powder	18.77	NI	9.76	NI
45	Brown powder	22.77	NI	7.92	NI
46	Brown powder	23.88	NI	11.11	NI
47	Brown powder	12.11	NI	15.98	NI
48	Brown powder	16.44	NI	5.86	NI
49	Brown powder	57.88	NI	7.82	NI
50	Brown powder	45.11	NI	5.19	NI
51	Off-white powder	47.88	NI	7.00	NI
52	Off-white powder	27.08	NI	4.53	NI
53	Brown powder	15.05	NI	8.98	NI
54	Off-white powder	22.00	NI	10.01	NI
55	Off-white powder	30.00	NI	10.55	NI
56	Brown powder	44.33	NI	NI	10.22
57	Brown powder	30.33	NI	NI	5.6
58	Brown powder	25.44	NI	NI	9.89
59	Brown powder	18.22	NI	NI	13.45
60	Brown powder	37.66	NI	NI	15.16
61	Brown powder	41.55	NI	NI	12.78
62	Brown powder	14.07	NI	NI	14.00
63	Brown powder	29.22	NI	NI	9.98
64	Brown powder	17.71	NI	NI	11.36
65	Brown powder	13.66	NI	NI	11.98

66	Br solid pieces	22.22	NI	NI	NI
67	Br solid pieces	20.66	NI	NI	NI
68	Br solid pieces	9.66	NI	NI	NI
69	L. Brown solid pieces	54.44	NI	NI	NI
70	Brown powder	29.22	NI	NI	NI
71	L. Brown powder	58.00	NI	NI	NI
72	Brown powder	48.00	NI	NI	NI
73	Brown powder	28.88	NI	NI	NI
74	Brown powder	35.06	NI	NI	NI
75	Brown powder	20.55	NI	NI	NI
76	Brown powder	35.44	NI	NI	NI
77	Off-white powder	47.05	NI	NI	NI
78	Off-white powder	28.77	NI	NI	NI
79	Off-white powder	13.02	NI	NI	NI
80	Brown powder	35.44	NI	NI	NI
81	Brown powder	33.85	NI	NI	NI
82	Brown powder	37.66	NI	NI	NI
83	Brown powder	36.11	NI	NI	NI
84	Brown powder	30.55	NI	NI	NI
85	Brown powder	16.55	NI	NI	NI
86	Brown powder	14.11	NI	NI	NI
87	Brown powder	59.44	NI	NI	NI
88	Brown powder	52.22	NI	NI	NI
89	Brown powder	17.11	NI	NI	NI
90	Brown powder	13.47	NI	NI	NI
91	Brown powder	59.66	NI	NI	NI
92	Brown powder	18.44	NI	NI	NI
93	Brown powder	25.33	NI	NI	NI
94	Brown powder	30.33	NI	NI	NI
95	Brown powder	44.00	NI	NI	NI
96	Brown powder	44.00	NI	NI	NI
97	Brown powder	57.00	NI	NI	NI
98	Brown powder	10.22	NI	NI	NI
99	Brown powder	24.55	NI	NI	NI
100	Brown powder	52.66	NI	NI	NI

NI = Not identified.

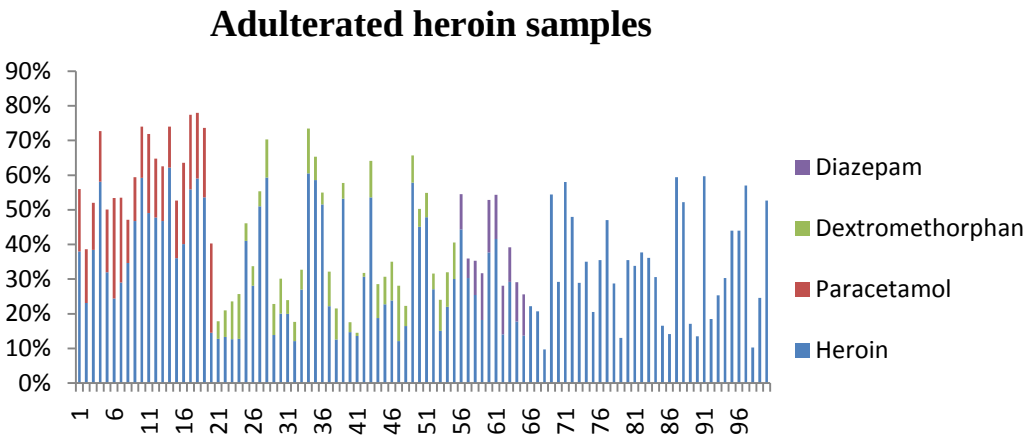

Figure 4.11: Figure shows adulterated heroin samples

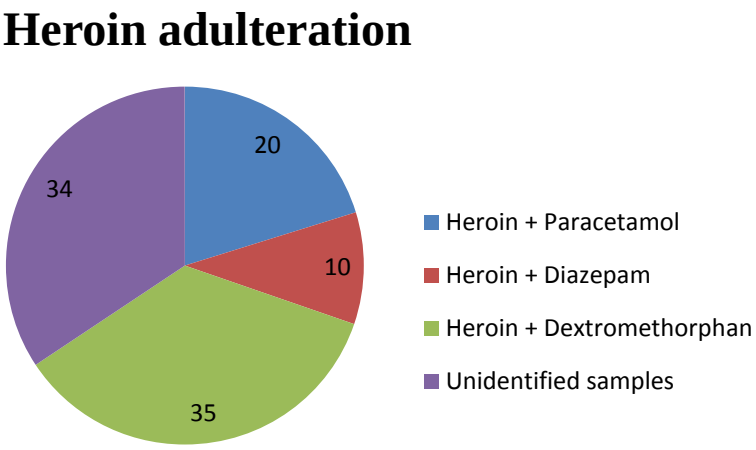

Figure 4.12: Figure showing ratio of adulterants in heroin and unidentified samples

Table No. 4.5: Percentage of Morphine samples.

S.No	Appearance	Morphine	S.No	Appearance	Morphine
1	Off White powder	12.80%	36	White powder	5.44%
2	White powder	14.08%	37	White powder	6.14%
3	White powder	18.35%	38	White powder	6.78%
4	White powder	10.01%	39	White powder	5.96%
5	White powder	16.15%	40	White powder	11.37%
6	Off White powder	9.75%	41	White powder	10.03%
7	White powder	13.55%	42	White powder	5.05%
8	White powder	8.87%	43	White powder	9.17%
9	White powder	11.37%	44	White powder	14.78%
10	White powder	5.11%	45	White powder	12.36%
11	White powder	10.00%	46	White powder	7.46%
12	White powder	10.07%	47	White powder	5.78%
13	White powder	16.00%	48	White powder	13.00%
14	White powder	11.02%	49	White powder	8.14%
15	White powder	9.04%	50	White powder	12.46%
16	White powder	8.05%	51	White powder	12.78%
17	White powder	10.01%	52	White powder	13.00%
18	White powder	10.09%	53	White powder	9.46%
19	White powder	7.01%	54	White powder	8.78%
20	White powder	5.08%	55	White powder	10.46%
21	White powder	6.07%	56	White powder	14.36%
22	White powder	9.08%	57	White powder	11.99%
23	White powder	8.01%	58	White powder	9.75%
24	White powder	8.07%	59	White powder	7.54%
25	White powder	7.15%	60	White powder	12.49%
26	White powder	6.14%	61	White powder	13.34%
27	White powder	5.11%	62	White powder	8.26%
28	White powder	10.10%	63	White powder	7.22%
29	White powder	12.04%	64	White powder	8.46%
30	White powder	11.11%	65	White powder	10.43%
31	White powder	9.17%	66	White powder	10.78%
32	White powder	7.20%	67	White powder	11.97%
33	White powder	7.87%	68	White powder	9.18%
34	White powder	10.53%	69	White powder	8.71%
35	White powder	11.01%	70	White powder	7.41%

71	White powder	9.66%	108	White powder	10.75%
72	White powder	10.42%	109	White powder	13.87%
73	White powder	8.82%	110	White powder	9.46%
74	White powder	8.35%	111	White powder	7.23%
75	White powder	7.88%	112	White powder	10.77%
76	White powder	10.37%	113	White powder	8.42%
77	White powder	6.72%	114	White powder	10.78%
78	White powder	11.01%	115	White powder	12.08%
79	White powder	12.20%	116	White powder	6.44%
80	White powder	14.78%	117	White powder	7.11%
81	White powder	9.81%	118	White powder	5.92%
82	White powder	7.73%	119	White powder	5.36%
83	White powder	13.05%	120	White powder	7.55%
84	White powder	8.42%	121	White powder	5.00%
85	White powder	7.26%	122	White powder	4.29%
86	White powder	5.12%	123	White powder	5.16%
87	White powder	10.72%	124	White powder	4.77%
88	White powder	10.33%	125	White powder	3.06%
89	White powder	9.79%	126	White powder	6.12%
90	White powder	7.46%	127	White powder	4.69%
91	White powder	8.44%	128	White powder	6.75%
92	White powder	10.79%	129	White powder	7.19%
93	White powder	10.71%	130	White powder	5.12%
94	White powder	11.44%	131	White powder	5.56%
95	White powder	8.78%	132	White powder	4.05%
96	White powder	8.72%	133	White powder	3.73%
97	White powder	9.33%	134	White powder	4.17%
98	White powder	12.55%	135	White powder	7.55%
99	White powder	9.88%	136	White powder	7.15%
100	White powder	11.72%	137	White powder	5.76%
101	White powder	12.10%	138	White powder	5.36%
102	White powder	12.43%	139	White powder	7.07%
103	White powder	4.36%	140	White powder	6.95%
104	White powder	7.42%	141	White powder	5.04%
105	White powder	7.49%	142	White powder	4.09%
106	White powder	11.36%	143	White powder	5.08%
107	White powder	8.43%	144	White powder	6.04%

145	White powder	6.02%	167	White powder	6.95%
146	White powder	6.09%	168	White powder	5.76%
147	White powder	6.02%	169	White powder	7.19%
148	White powder	7.19%	170	White powder	5.06%
149	White powder	6.07%	171	White powder	6.04%
150	White powder	5.06%	172	White powder	4.17%
151	White powder	5.04%	173	White powder	3.73%
152	White powder	6.04%	174	White powder	7.19%
153	White powder	5.02%	175	White powder	5.00%
154	White powder	5.09%	176	White powder	6.02%
155	White powder	5.04%	177	White powder	4.17%
156	White powder	7.01%	178	White powder	5.02%
157	White powder	5.72%	179	White powder	5.04%
158	White powder	5.02%	180	White powder	0.70%
159	White powder	6.06%	181	White powder	0.80%
160	White powder	3.09%	182	White powder	0.90%
161	White powder	6.04%	183	White powder	14.71%
162	White powder	5.00%	184	White powder	9.78%
163	White powder	7.55%	185	White powder	6.07%
164	White powder	5.99%	186	White powder	14.05%
165	White powder	7.05%	187	White powder	2.92%
166	White powder	4.09%			

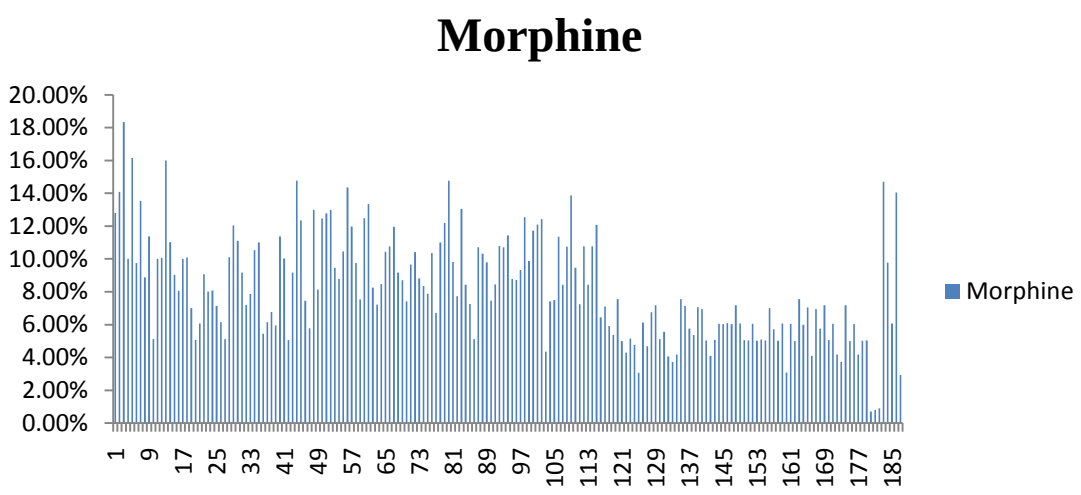

Figure 4.13: Figure showing concentration of morphine samples

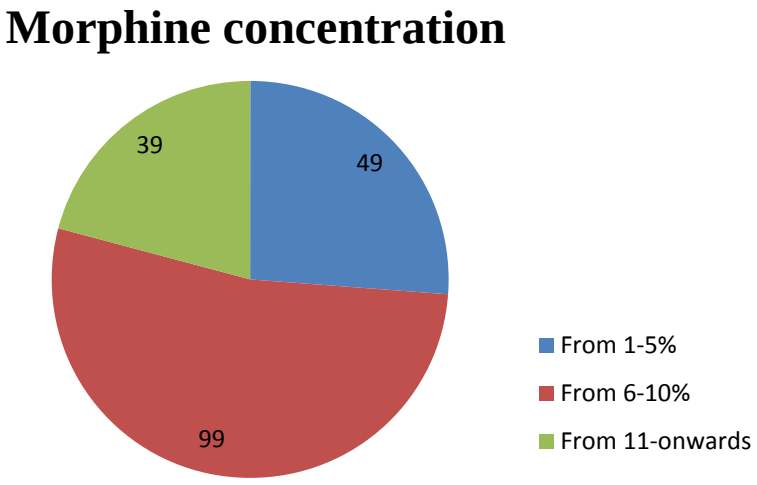

Figure 4.14: Figure exhibiting morphine concentration

Table 4.6: Percentage of cocaine samples.

S. No	Appearance	Cocaine
1	White shiny crystal	11.45%
2	White shiny crystal	12.09%
3	White shiny crystal	17.44%
4	White shiny crystal	15.34%
5	White shiny crystal	22.01%
6	White shiny crystal	13.56%
7	White shiny crystal	10.11%
8	White shiny crystal	12.33%
9	White shiny crystal	7.67%
10	White shiny crystal	9.55%

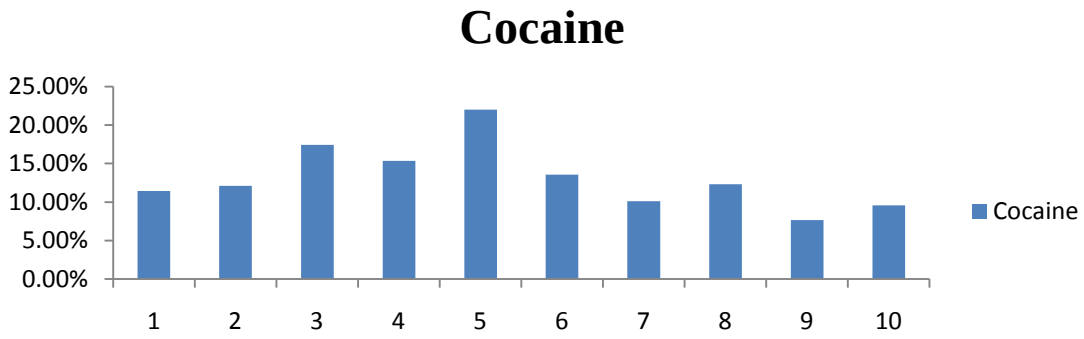

Figure 4.15: Figure showing cocaine concentration

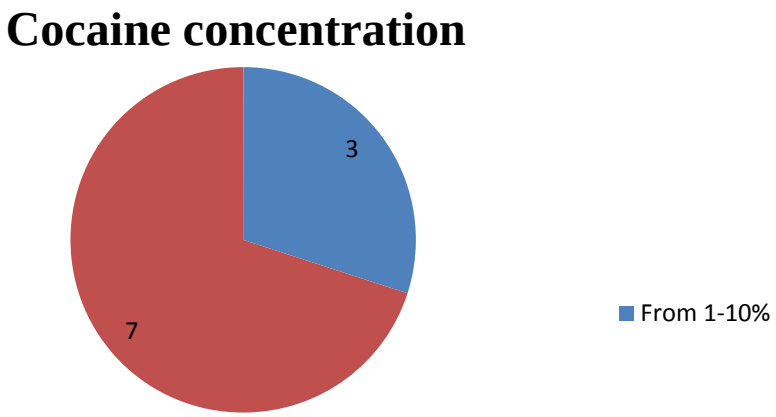

Figure 4.16: Figure exhibiting concentration of cocaine

Table 4.7: Percentage of acetic anhydride samples.

S.No	Appearance	A. anhydride	S.No	Appearance	A. anhydride
1	Colourless liquid	21.09%	11	Colourless liquid	46.04%
2	Colourless liquid	43.00%	12	Colourless liquid	20.04%
3	Colourless liquid	25.76%	13	Colourless liquid	51.08%
4	Colourless liquid	62.02%	14	Colourless liquid	24.27%
5	Colourless liquid	17.71%	15	Colourless liquid	22.44%
6	Colourless liquid	11.02%	16	Colourless liquid	12.75%
7	Colourless liquid	27.00%	17	Colourless liquid	15.35%
8	Colourless liquid	51.00%	18	Colourless liquid	25.75%
9	Colourless liquid	56.00%	19	Colourless liquid	35.23%
10	Colourless liquid	36.05%	20	Colourless liquid	22.01%

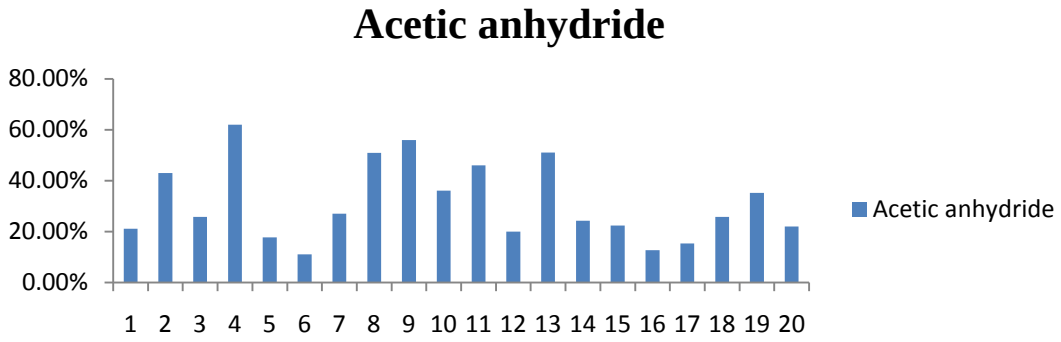

Figure 4.17: Figure showing acetic anhydride concentration

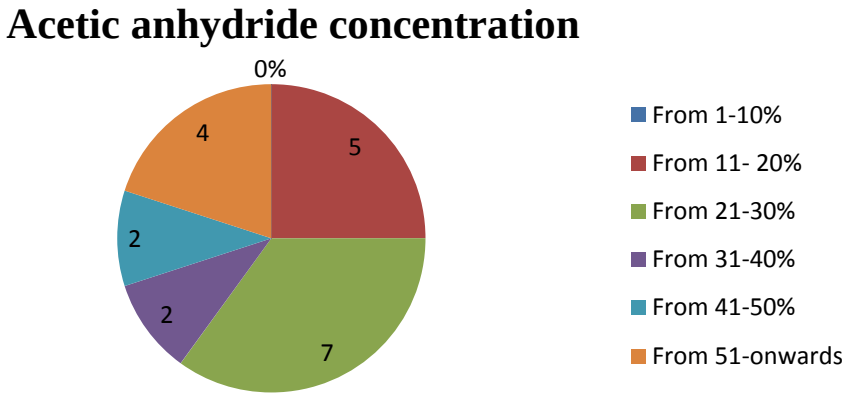

Figure 4.18: Figure exhibiting concentration of acetic anhydride

Table 4.8: Percentage of acetic anhydride adulterated with HCl.

S.No	Appearance	A.anhydride	HCl	S.No	Appearance	A.anhydride	HCl
1	Colourless liquid	18.24	33.57	16	Colourless liquid	25.05	33.67
2	Colourless liquid	20.56	34.05	17	Colourless liquid	21.65	31.23
3	Colourless liquid	27.18	31.27	18	Colourless liquid	18.06	33.45
4	Colourless liquid	28.14	32.18	19	Colourless liquid	18.83	31.55
5	Colourless liquid	25.87	31.06	20	Colourless liquid	28.11	33.32
6	Colourless liquid	19.55	33.08	21	Colourless liquid	22.31	31.67
7	Colourless liquid	21.32	32.88	22	Colourless liquid	19.87	32.54
8	Colourless liquid	22.98	32.04	23	Colourless liquid	20.00	33.12
9	Colourless liquid	18.04	33.56	24	Colourless liquid	26.14	32.61
10	Colourless liquid	23.76	31.34	25	Colourless liquid	22.45	34.38
11	Colourless liquid	23.14	31.06	26	Colourless liquid	25.01	31.09
12	Colourless liquid	24.88	34.44	27	Colourless liquid	19.87	32.74
13	Colourless liquid	26.72	30.55	28	Colourless liquid	26.56	33.84
14	Colourless liquid	18.24	32.44	29	Colourless liquid	26.52	31.56
15	Colourless liquid	26.52	34.32	30	Colourless liquid	19.24	32.00

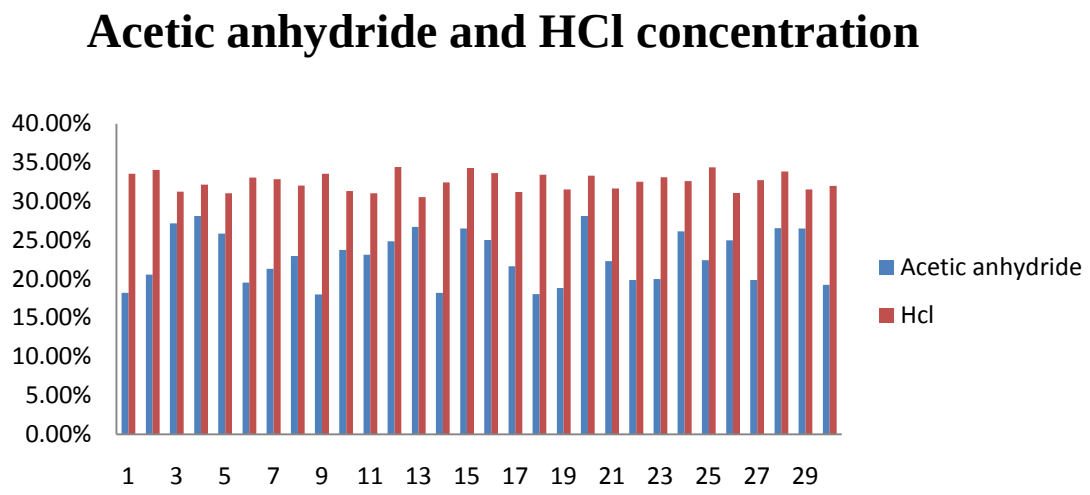

Figure 4.19: Figure showing acetic anhydride and HCl concentration

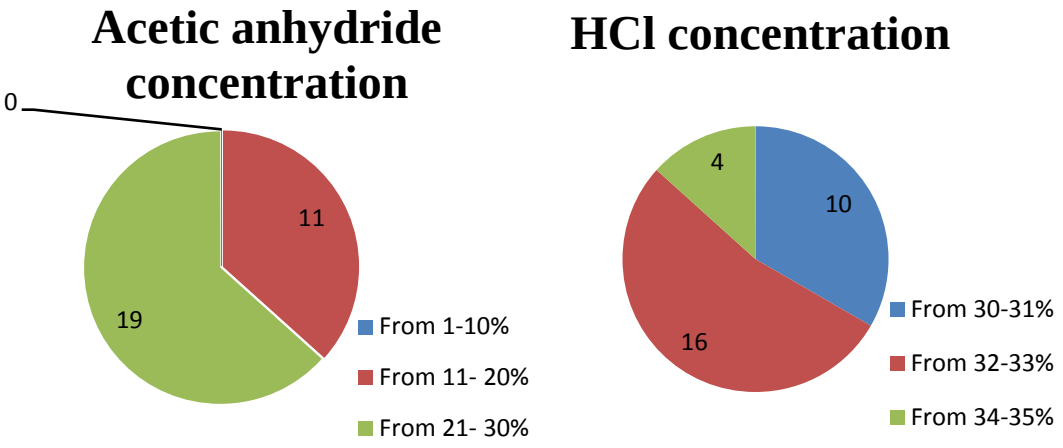

Figure 4.20: Figures exhibiting concentration of acetic anhydride and HCl

Table 4.9: City wise distribution of drug samples.

Number of samples of drugs obtained				
Cities	Heroin	Morphine	Cocaine	Acetic anhydride
Quetta	40	87	5	19
Karachi	30	50	3	01
Islamabad	10	20	0	30
Lahore	20	30	2	0

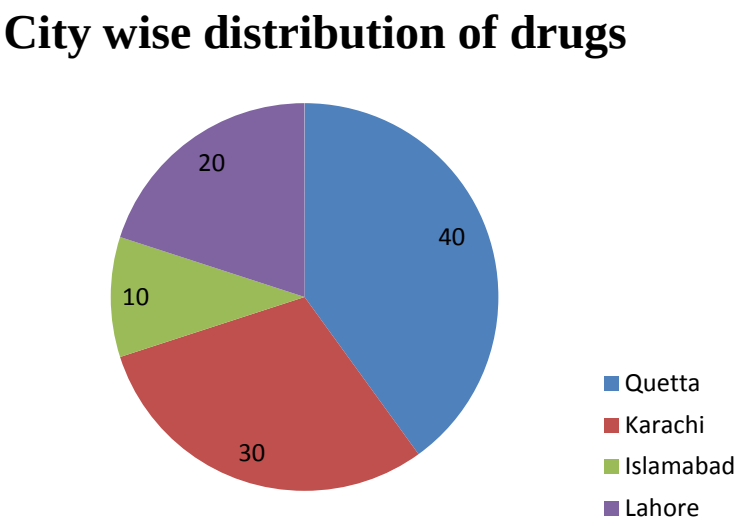

Figure 4.21: Figure showing city wise distribution of drug samples

The current study aimed to examine the different drug samples that were used by the youngsters in the whole world and also in Pakistan. The strength of this study was the microbial analysis and chemical analysis of drug samples. These samples were obtained from different districts of the country. They were from Quetta, Karachi, Lahore and capital of the country. These drugs were confiscated by the anti-narcotics force of Pakistan. Different number of drug samples obtained from these cities indicated drug prevalence in particular city. According to our samples Quetta was leading in heroin and morphine samples which were then followed by Karachi.

The main findings of our research were divided into two parts, one was the estimation of microbial analysis or bio burden of heroin samples along with biochemical tests and the other part was related to the chemical analysis. The heroin samples used for microbial analysis were randomly chosen. In our samples various colonies of microorganisms were produced on their respective medium which indicated the biotic contamination in the drug samples.

Our results show that the microbial flora has also been present in the samples. The 100 different samples were selected randomly for microbial analysis. Mostly they contain gram –ve, *Staphylococcus*, *Streptococcus* and fungal species. These were the result of unclean and unhealthy process of manufacturing in the laboratories. Using TSA the total aerobic count of microbes having range of 1-15 colonies of microbes were 55 in numbers, those having more than 15 colonies were 7 in numbers, numerous growth i.e. uncountable growth were 20 in numbers and those having no growth i.e. negatives were 22 in numbers as shown in figure 4.1. Samples numbers used for the estimation of microbial growth on different agar media were 100 that were selected randomly. Those samples having single colony of the microbes were 17, with double colony were 14, and with numerous growth were 5 and those having no growth were 60 in numbers. Black colonies with yellow zone and shiny black colonies were produced in certain samples of the drug which highlighted the presence of *S. aureus* and *Salmonella* species. These colonies were then confirmed by various biochemical test specific for *S. aureus* and *Salmonella* species. Catalase and coagulase test were conducted for *S. aureus* which confirmed its presence by producing active bubbling and clumping within 10 seconds. Similarly for *Salmonella* species triple sugar iron (TSI) test, indole test and urease test was performed. In TSI

test acid, gas and hydrogen sulphide was produced which confirmed its presence by giving yellow colour of butt, active bubbling in butt region and black colour of the tube. For indole and urease test it produced yellow colour and yellow ring instead of giving red colour which confirmed *Salmonella* species presence. These results can also be compared with the study conducted by McLauchin in 2002 on micro flora of heroin. According to him different microbes i.e. *Bacillus* sp, *Clostridium* sp, *Streptococcus* sp, *Staphylococcus* sp etc were present in heroin samples (McLauchin *et al.,* 2002).

The second part was the chemical analysis of drug samples. For this part we have done different colour tests, TLC (Thin layer chromatography) and HPLC (high-performance liquid chromatography) for quantification. The colour tests were performed by using the UNODC drug kit. This kit contain reagent specific for each drug. It gives particular colour change, specific for each drug, indicated the particular drug. For example purple colour indicate heroin while light purple to grey colour was the indication of the morphine. TLC technique was performed for heroin and morphine samples. We used three solvent systems, each drug give its own Rf value in each solvent system. The last task was the quantification of drug samples using HPLC. HPLC quantified different drugs along with their adulterants and gives their respective values. Mostly these drug samples were adulterated with diazepam, dextromethorphan and paracetamol while in rest of the samples other adulterants were also present but could not be identified. These heroin samples were also quantified along with its adulterants. Similarly samples of morphine and cocaine were also subjected for quantitative analysis. Acetic anhydride samples were adulterated with HCl and were also analysed quantitatively.

In our results heroin samples adulterated with paracetamol were 20, with diazepam were 10, with dextromethorphan were 35 and those remain unidentified were 34 samples (shown in figure 4.12). Similarly morphine samples those having concentration between 1-5% were 49, from 6-10% were 99 and more than 10% were 39 in numbers (shown in figure 4.14). In the same way among 10 cocaine samples those having concentration up to 10 % were 3 samples and those having concentration beyond the 10 % were 7 in numbers (shown in figure 4.16). In acetic anhydride samples those samples having concentration up to 10 % were 4, up to 20 % were 5, up

to 30 % were 7, up to 40 % were 2 and up to 50 % were also 2 in numbers (shown in figure 4.18). Acetic anhydride samples adulterated with HCl there were 10 samples having concentration among 30-31, from 32-33 were 16 and from 34-35 were 4 samples (shown in figure 4.20).

The same type of study was also conducted by Zelkowicz and co-workers in 2005 as analysis of simulated heroin by HPLC and also by Khajeamiri *et al* in 2011 as determination of impurities in illicit methamphetamine samples seized in Iran (Zelkowicz *et al.,* 2005; Khajeamiri *et al* in 2011). According to them different adulterants were present in heroin samples like paracetamol, papaverine, acetyl-codeine, caffeine, narcotine etc (Zelkowicz *et al.,* 2005). In order to confirm the presence of morphine, codeine and 6-MAM in the samples, Dordević and Kilibarda use the multicolumn HPLC-UV method (Dordević and Kilibarda, 2007). Column-switching high-performance liquid chromatographic technique was employed by Zhang and his co-workers for the recognition of morphine and O6-monoacetylmorphine (Zhang *et al.,* 2002). Milovanović and his co-workers carry out the HPLC/MS process for usual examination and checking of heroin misuse in order to detect morphine, codeine and 6-mam (Milovanović *et al.,* 2012). In 1998 for efficient detection of 10 drugs of misuse like morphine, codeine, 6-monoacetylmorphine, heroin, levorphanol, pethidine, ethylmorphine, anadol, pentazocine and ethamivan, high performance liquid chromatography method was illustrated by the Ma *et al* (Ma *et al* in 1998). In this technique codeine was employed as interal standard (Ma *et al.,* 1998). For the accurate separation and quantification of caffeine, heroin, acetyl codeine, 6-acetylmorphine, codeine and morphine from one other, Baker and Gough employed high performance liquid chromatography method (Baker and Gough, 1981). The limitation of our study is that the samples were collected at various interval of time which may gives varied results.

According to results of our study it is suggested that as heroin availability is increasing day by day, it has been adulterated with different substances. These substances produce certain sever effects on the body along with the psychological effects produced by heroin itself. Despite significant declines in cultivation trends, the area known as the golden triangles remains a source of opiates. Opiate use has

generally stabilized in the region of late. However, since 2009, there has been an indicator to suggest that heroin use is re-emerging as a threat in the region (UNODC, 2011).

In comparison to these countries in Pakistan opiate utilisation is superior than the universal approximate while the confiscation of opium and morphine sustained to be mainly concentrated. In Pakistan information of single heroin abduction incidences propose that of the heroin delivers with a known target other than Pakistan. Their proportion planned for the Asia-Pacific region lowered from 42 per cent in 2009 to 34 per cent in 2010. In Pakistan, from 2008 confiscations of cannabis resin amplified sharply, with 212 tons being seized in 2010, almost twice the 2007 level (UNODC, 2012).

In present study, qualitative and quantitative analysis of heroin samples is done in order to establish percentage of Diacetyl Morphine (Heroin) and to assess the various types of microbes present in the drug. It will help ensure timely medical treatment of the addicts and will also provide platform for enhance public health and awareness of the risks because of its association with the potential health effects that may arise from adulteration.

Conclusions:

From our results we have concluded that the easily assessable drugs are not pure or they have been adulterated with several substances like paracetamol, diazepam, dextromethiorphan, cocaine etc. In addition to these adulterants these drugs are also loaded with microbes. Unclean and unhygienic conditions present in the laboratory during production of the drugs are the reasons for their entrance. So these drugs along with their psychoactive effects also produce severe health consequences due to presence of these microbes.

Future prospects:

We believe that effective methods should be developed for proper and accurate analysis. Efforts should be made in the future to conduct large prospective studies in drug abusers of different age groups and different geographical areas of our country in order to achieve the better understanding of their effects on the health. Effects should also be made to lessen the availability and improper use of these drugs among the people of our country.

Ahmed R. 2008. Descent into Chaos- How the War against Islamic Extremism is being Lost in Pakistan, Afghanistan and Central Asia. London. Penguin Books Ltd. **319**.

Akhgari M, Jokar F, Aleagha AE. 2011. Drug related deaths in Tehran, Iran: Toxicological, Death and Crime Scene investigations. Iranian Journal of Toxicology. **5 (1, 2).**

Altet-Gomez MN, Alcaide J, Godoy P, Romero MA, Hernandez del Rey I. 2005. Clinical and epidemiological aspects of smoking and tuberculosis: a study of 13,038 cases. Int J Tuberc Lung Dis. **9:** 430–436.

Altunkaya D, Clatworthy AJ, Smith RN, Start IJ. 1991. Urinary cannabinoid analysis: comparison of four immunoassays with gas chromatography-mass spectrometry. Forensic Sci Int. **50:** 15-22.

Alvarez M, Barturen B, Regulez P, Ponton J, Ezquerra JL, Cisterna R. 1990. Microbiological study of 30 samples of heroin. Enferm Infecc Microbiol Clin. **8**: 231–233.

Andreasen MF, Lindholst C, Kaa E. 2009. Adulterants and Diluents in Heroin, Amphetamine, and Cocaine Found on the Illicit Drug Market in Aarhus, Denmark. The Open Forensic Science Journal. **2:** 16-20.

Anon. 2000. *Clostridium novyi* and unexplained illness among injecting drug users: Scotland, Ireland and England. MMWR Morb Mortal Wkly. **49:** 543-544.

Anon. 2000. *Clostridium novyi* is likely cause of ‘serious unexplained illness’ as cases continue to be reported. Commum Dis Rep CDR Wkly. **24:** 213-216.

Anon. 2000. Serious unexplained illness among drug injectors. Commum Dis Rep CDR Wkly. **24:** 195.

Appendino G, Chianese G, Taglialatela-Scafati O. 2011. Cannabinoids: occurrence and medicinal chemistry. Curr Med Chem. **18:** 1085-1099.

Ashton CH. 2003. Pharmacolgy and effect of cannabis:A brief review. Br J Psychiatry. **45:**182-188.

Athwal BS, Gale AN, Brett MM, Youl BD. 2000. Wound botulism in UK. Lancet. **356:** 2001–2002.

Australian Health Ministers' Advisory Council. 2006. Aboriginal and Torres Strait Islander health performance framework 2006 report. Canberra.

Baker PB, Gough TA. 1981. The separation and quantitation of the narcotic components of illicit heroin using reversed-phase high performance liquid chromatography. J Chromatogr Sci. **19(10):** 483-489.

Behrman AD. 2008. Luck of the draw: Common adulterants found in illicit drugs. Journal of Emergency Nursing, **34(1):** 80-82.

Bellis MA, Beynon C, Millar T. 2001. Unexplained illness and deaths among injecting drug users in England: a case control study using Regional Drug Misuse Databases. J Epidemiol Community Health. **55:** 843–844.

Brazier JS, Duerden BI, Hall V, Salmon JE, Hood J, Brett MM, McLauchlin J, George RC. 2002. Isolation and identification of Clostridium spp. from infections associated with the injection of drugs: Experiences of a microbiological investigation team. Journal of Medical Microbiology. **51:** 985-989.

Brenneisen R, Hasler F, Wursch D. 2002. Acetylcodeine as a urinarymarker to differentiate the use of street heroin and pharmaceutical heroin. J Anal Toxicol. **26:** 561-566.

Brenneisen R, Hasler F. 2002. GC/MS Determination of Pyrolysis Products from Diacetylmorphine and Adulterants of Street Heroin Samples. J Forensic Sci. **47(4).**

Brett MM, Hood J, Brazier JS, Duerden BI, Hahne SJM. 2005. Soft tissue infections caused by spore forming bacteria in injecting drug users in the United Kingdom. Epidemiology & Infection. **133(4):** 575-582.

Brettle RP. 1992. Infection and injection drug use. J Infect. **25:** 121–131.

British pharmacopoeia (BP). 1968. By general medical council, London.

British pharmacopoeia (BP). 2012. By general medical council, London. **2.**

Brunt TM, Rigter S, Hoek J, Vogels N, van Dijk P, Niesink RJ. 2009. An analysis of cocaine powder in the Netherlands: content and health hazards due to adulterants. Addiction. **104(5):**798-805.

Brust JC. 2002. Neurologic complications of substance abuse. J. Acquir. Immune Defic. Syndr. **31(2):** S29–S34.

Büttner A, Mall G, Penning R, Weis S. 2000. The neuropathology of heroin abuse. Forens Sci Int. **113(1–3):** 435–42.

Calandra T, Francioli P, Glauser MP, Rosselet FB, Ruffieux C, Grigoriu D. 1985. "Disseminated Candidiasis with Extensive FoUiculitis in Abusers of Brown Iranian Heroin," European Journal of Clinical Microbiology. **4:** 340-342.

Carl T. 2009. "Progress in Mexico drug war is drenched in blood". Associated Press.

Cheesbrough M. 2000. District laboratory practice in tropical countries, part 2.

Childress MT. 1994. A system description of the heroin trade, Santa Monica: Rand Drug Policy Research Centre.

Christie B. 2000. Gangrene bug ‘‘killed 35 heroin users’’. BMJ. **320:** 1690.

Chung L, Liu G, Li Z, Chang Y and Lee M. 2008. J Chromatogr. **874**: 115.

Cole C, Jones L, McVeigh J, Kicman A, Syed Q, Bellis M. 2010. A guide to adulterants, bulking agents and other contaminants found in illicit drugs.

Cole C, Jones L, McVeigh J, Kicman A, Syed Q, Bellis M. 2011. Adulterants in illicit drugs: a review of empirical evidence. Drug Test Anal. **3(2):** 89-96.

Cole MD. 2003. Diamorphine and Heroin. The Analysis of Controlled Substances.

Conover C, Ridzon R, Valway S, et al. 2001. Outbreak of multidrug-resistant tuberculosis at a methadone treatment program. Int J Tuberc Lung Dis. **5:** 59–64.

Coomber R, Maher L. 2006. Street-level drug market activity in Sydney's primary heroin markets: Organization, adulteration practices, pricing, marketing and violence. Journal of Drug Issues. **36(3):** 719-754.

Coomber R. 1997c. The adulteration of drugs: What dealers do to illicit drugs, and what they think is done to them. Addiction Research & Theory. **5(4):** 297-306.

Coomber R. 1997d. Dangerous drug adulteration – An international survey of drug dealers using the internet and the World Wide Web (WWW). International Journal of Drug Policy. **8(2):** 18-28.

Cunningham J, Maxwell J, Campollo O, Cunningham K, Liu L, Lin H. 2010. Proximity to the US-Mexico border: a key to explaining geographic variation in US methamphetamine, cocaine and heroin purity. Addiction. **105:** 1785–1798.

Dams R, Benijts T, Lambert WE, Massart DL, De Leenheer AP. 2001. Heroin impurity profiling: Trends throughout a decade of experimenting. Forensic Sci Int. **123(2-3):** 81-88.

Dancer SJ, McNair D, Finn P, Kolstot AB. 2002. *Bacillus cereus* cellulitis from contaminated heroin. J Med Microbiol. **51:** 278–281.

Daughton CG. 2011. Illicit Drugs and the Environment. Occurrence, Analysis, and Fate Using Mass Spectrometry. Edited by Sara Castiglioni, Ettore Zuccato, and Roberto Fanelli.

DomÍnguez-Vilches E, Durán-González R, lnfante F, Luna-Maldonado A. 1991. Mycoeontamination of illicit Samples of Heroin and Cocaine as an Indicator of Adulteration. Journal of Forensic Sciences, JFSCA. **36(3):** 844-856.

Dordević S, Kilibarda V. 2007. Analytical confirmation of lethal heroin overdose by the use of liquid chromatography methods. Vojnosanit Pregl. **64(11):** 739-743.

Drobniewski FA, Balabanova YM, Ruddy MC, et al. 2005. Tuberculosis, HIV seroprevalence and intravenous drug abuse in prisoners. Eur Respir J. **26:** 298–304.

Drug-Related Crime - Factsheet - Drug Facts. 2008. Whitehousedrugpolicy.gov.

Drummer OH. 2005. Recent trends in narcotic deaths. Ther Drug Monit. **27(6):** 738–740.

Dunn J, Laranjeira R. 1999. Cocaine-profiles, drug histories, and patterns of use of patients from Brazil. Substance Use and Misuse. **34(11):**1527–1548.

Dutta M, Ehsan MM. 2012. Illicit Drug Production in Afghanistan: a Situational Analysis. **94:** 343.57.

Esse K, Fossati-Bellai M, Traylor A, Martin-Schild S. 2011. Brain Behav Epidemic of illicit drug use, mechanisms of action/addiction and stroke as a health hazard. **1(1):** 44–54.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). 1999. Annual report on the state of the drugs problem in the European Union 1999. Lisbon, Portugal: Union Monitoring Centre for Drugs and Drug Addiction.

Fischer B, Rehm J, Kirst M, Casas M, Hall W, Krausz M, Metrebian N, Reggers L, Uchtenhagen A, Brink BWVD, Van Ree JM. 2002. Heroin-assisted treatment as a response to the public health problem of opiate dependence. Eur. j. Public Health. **12:** 228-234.

Friedman H, Newton C, Klein TW. 2003. Microbial infections, immunomodulation, and drugs of abuse. Clin Microbiol Rev. **16:** 209.

Fucci N, Giovanni N. 1998. Forensic Sci. Int. **95**: 247-252.

Fucci N. 2007. Unusual adulterants in cocaine seized on Italian clandestine market. Forensic Sci Int. **172**: e1.

Fuente De la L, Saavedra P, Barrio G, Royuela L, Vicente J. 1996. Temporal and geographic variations in the characteristics of heroin seized in Spain and their relation

with the route of administration. Spanish Group for the Study of the Purity of Seized Drugs. Drug Alcohol Depend. **40:** 185–194.

Garcia-Ferrer R. 1986. "Estudio de Contaminantes Met~licos en Muestras Ilicitas de Heroina," master's thesis, Universidad de C6rdoba, Facultad de Medicina, Cordoba, Spain.

Ghanem AEA, Bakary AAE, Hassan AA. 2008. Chemical Additives of Street Heroin in Cairo. Mansoura J. Forensic Med. Clin. Toxicol. **XVI(1).**

Gossop M, Grant M. 1990. Preventing and controlling drug abuse, Geneva, World Health Organization.

Graham CA, McNaughton GW, Crawford R. 1999. 'Popping': a cause of soft tissue sepsis in chronic drug abusers. Eur J Emerg Med. **6:** 259-261.

Grotenhermen F. 2007. The toxicology of cannabis and cannabis prohibition. Chem Bio divers. **4:** 1744-1769.

Habal R. 2009. Toxicity, Heroin.

Hall W, Darke S. 1998. Trends in opiate overdose deaths in Australia 1979–1995. Drug and Alcohol Dependence. **52(1):** 71–77.

Hall W, Zador D. 2000. Challenge of reducing drug-related deaths. Lancet. **356:** 7–8.

Hamilton RJ, Perrone J, Hoffman R, Henretig FM, Karkevandian EH, Marcus S, Shih RD, Blok B, Nordenholz K. 2000. A descriptive study of an epidemic of poisoning caused by heroin adulterated with scopolamine. J Toxicol Clin Toxicol. **38(6):**597-608.

Hannan MM, Peres H, Maltez F, et al. 2001. Investigation and control of a large outbreak of multi-drug resistant tuberculosis at a central Lisbon hospital. J Hosp Infect. **47:** 91–97.

Hay G, Gannon M, McKeganey N, Hutchinson S, Goldberg D. 2005. Estimating the National and Local Prevalence of Problem Drug Misuse in Scotland Edinburgh: Information and Statistics Division, Scotland.

Hazekamp A, Grotenhermen F. 2010. Review on Clinical Studies with Cannabis and Cannabinoids 2005-2009. Cannabinoids. **5:** 1-21.

Hinchliffe CE, Thornton GF. 1987. *Bacillus cereus* bacteremia in an intravenous drug abuser. Conn Med. **51:** 362-365.

Holzmann T, Frangoulidis D, Simon M, Noll P, Schmoldt S, Hanczaruk M, Grass G, Pregler M, Sing A, Hörmansdorfer S, Bernard H, Grunow R, Zimmermann R, Schneider-Brachert W, Gessner A, Reischl U. 2012. Fatal anthrax infection in a heroin user from southern Germany. Euro Surveill. **17(26).**

Hood J, Horne G, Sweeney G, Baird K. 2000. Third case of wound botulism in an injecting drug user. SCIEH. **34:** 200.

Horsburgh CR, Anderson JR, Boyko EJ. 1989. Increased incidence of infections in intravenous drug users. Infect Control Hosp Epidemiol. **10:** 211–215.

Hughes RA. 2000. Drug injectors and the cleaning of needles and syringes. Eur Addict Res. **6:** 20-30.

Huizer H. 1992. Samenstelling en kwaliteit van illegale heroine in Nederland, 1970–1990. T Alc Drugs. **18:** 1–12.

Hulse GK, English DR, Holman CD. 1999. The quantification of mortality resulting from the regular use of illicit opiates. Addiction. **94(2):** 221–229

Idros I.B. 2011. Development of a Method for the Analysis of Neonicotinoid Insecticides using Thin-Layer Chromatography.

Iravani FS, Akhgari M, Jokar F, Bahmanabadi L. 2010. Current trends in tramadol-related fatalities, Tehran, Iran 2005-2008. Substance Use & Misuse. **45(13):** 2162-71.

Jensenius M, Lovstad RZ, Dhaenens G, Rorvik LM. 2000. A heroin user with a wobbly head. Lancet. **356:** 1160.

Kaa E, Kempe B. 1986. Impurities, adulterants and diluents of illicit heroin in Denmark (Jutland and Funen). Forensic Sci Int. **31:** 195–210.

Kaa E. 1994. Forensic Sci. Int. **64:** 171-179.

Kalant OJ. 1972. Report of the Indian Hemp Drugs Commission, 1893-94: a critical review. Int J Addict. **7:** 77-96.

Kambiz S, Faryadi M, Akhgari M, Bahmanabadi L. 2007. Forensic Toxicology Laboratory, Legal Medicine Organization, Chemical profile of counterfeited buprenorphine vials seized in Tehran, Iran, Science direct, Forensic Science International. **172:** e4–e5.

Kapadia F, Vlahov D, Donahoe RM, Friedland G. 2005. The role of substance abuse in HIV disease progression: reconciling differences from laboratory and epidemiologic investigations. Clin Infect Dis. **41:** 1027–34.

Katz AA, Hoffman RS, Silverman RA. 1993. Ann. Emerg. Med. **22:** 1485-1487.

Kenyon SL, Ramsey JD, Lee T, Johnston A, Holt DW. 2005. Analysis for identification in amnesty bin samples from dance venues. Ther Drug Monit. **27**: 793-798.

Khajeamiri AR, Faizi M, Sohani F, Baheri T, Kobarfard F. 2011. Determination of impurities in illicit methamphetamine samples seized in Iran. Forensic Science International. **xxx:** xxx–xxx.

Khan M, Chay S, Bonner S, Brett M. 2000. Wound botulism in injecting drug users: second case in England. Eurosurveillance Weekly.

King LA. 1997. Forensic Sci. Int. **85:** 135-147.

Klemenc S. 2001. In common batch searching of illicit heroin samples – Evaluation of data by chemometrics methods. Forensic Sci Int. **115:** 43–52.

Kriegstein AR, Shungu DC, Millar WS, Armitage BA, Brust JC, Chillrud S, Goldman J. 1999. Leukoencephalopathy and raised brain lactate from heroin vapor inhalation ("chasing the dragon"). Neurology. **53(8):** 1765–73.

Kumar MB, Nath YB. 2012. Drug Abuse: A Medicolegal Aspect. Journal of Drug Delivery & Therapeutics. **2(6):** 86-89.

Lankenau SE, Schrager SM, Silva K, Kecojevic A, Bloom JJ, Wong C, and Iverson E. 2012. Misuse of prescription and illicit drugs among high-risk young adults in Los Angeles and New York. J Public health Res. **1(1):** 22–30.

Lindholst C, Andreasen MF, Kaa E. 2008. Ugeskr Lægers. **170:** 54-58.

López-Artíguez M, Cameán A, R epetto M. 1995. "Unequivocal Identification of Several Common Adulterants and Diluents in Street Samples of Cocaine by Infrared Spectroscopy, Journal of Forensic Sciences, JFSCA. **40(4):** 602-610.

Luna A, Infante F, Dominguez E. 1986. "Mycoflora of Potential Sanitary Interest Present in Illicit Heroin," Zeitschriftfiir Rechtsmedizin. **96**: 297-302.

Ma C, Duan H, Zhang H, Xu Y, Zhou T. 1998. Studies on analytical method for 10 drugs of abuse in urine using HPLC. Yao Xue Xue Bao. **33(10):** 764-767.

Mahdy T, El-Shihi TH, Emara MM, Girgi M. 2012. New HPLC method to detect individual opioids (Heroin and Tramadol) and their metabolites in the blood of rats on combination treatment, journal of chromatographic science. **50:** 658-665.

Malik A, Sarfaraz SF. 2011. Origin and Development of Drug Addiction in South Asia with Special Reference to Pakistan. Pak. J. Commer. Soc. Sci. **5(1):** 155-165.

Martin C, Schaller MD, Lepori M, Liaudet L. 1999. Cranial nerve palsies and decending paralysis in a drug abuser resulting from wound botulism. Intensive Care Med. **25:** 765–769.

Mattoo SK, Basu D, Sharma A, Balaji M, Malhotra A. 1997. Abuse of codeine-containing cough syrups: a report from India. Addiction. **92(12):** 1783–1787.

Mattos IG, Ribeiro MO, Netto IC, d'Azevedo PA. 2006. Tuberculosis: a study of 111 cases in an area of high prevalence in the extreme south of Brazil. Braz J Infect Dis. **10:** 194–198.

McGuigan CC, Penrice GM, Black M et al. 2002. Outbreak of infection with Clostridium novyi and other sporulating bacteria in Scottish injecting drug users. J Med Microbiol. **51:** 971–977.

McKetin R, Mattick RP. 1998. Attention and memory in illicit amphetamine users: comparison with non-drug-using controls. Drug and Alcohol Dependence. **50(2):** 181–184.

McKetin R, Sutherland R, Bright DA, Norberg MM. 2011. A systematic review of methamphetamine precursor regulations.

Mcketin R. 2007. Methamphetamine precursor regulation: are we controlling or diverting the drug problem? Addiction. **103:** 521–523.

McLauchlin J, Mithani V, Bolton FJ, Nichols GL, Bellis MA, Syed Q, Thomson RPM, Ashton JR. 2002. An investigation into the micro flora of heroin. J. Med. Microbiol. **51:** 1001-1008.

Milovanović V, Cirić B, Milenković J, Kilibarda V, Curcić M, Vucinić S, Antonijević B. 2012. Determination of morphine, codeine and 6-monoacetylmorphine in saliva of substance-abuse patient's using HPLC/MS methods. Vojnosanit Pregl. **69(2):**141-146.

Mokdad AH, Marks JS, Stroup DF, Gerberding J. 2004. Actual causes of death in the United States. J. Am. Med. Assoc. **291:**1238–1245.

Morozova I, Riekstina V, Sture G, Wells C, Leimane V. 2003. Impact of the growing HIV-1 epidemic on multidrug-resistant tuberculosis control in Latvia. Int J Tuberc Lung Dis. **7:** 903–6.

Moustoukas NM, Nichols RL, Smith JW, Garey RE, Egan RR. 1983. Contaminated street heroin. Relationship to clinical infections. Arch Surg. **118:** 746–749.

Mulleague L, Bonner SM, Samuel A et al. 2001. Wound botulism in drug addicts in the United Kingdom. Anaesthesia. **56:** 120–123.

National Institute on Drug Abuse. 2000. Epidemiologic trends in drug abuse. Advance report, December 1999. Community Epidemiology Work Group. Bethesda, USA: National Institutes of Health, National Institute on Drug Abuse.

Ngoc Buu T, Houben RM, Thi Quy H, Thi Ngoc Lan N, Borgdorff MW, Cobelens FG. 2007. HIV and tuberculosis in Ho Chi Minh City, Vietnam, 1997–2002. Emerg Infect Dis. **13:** 1463–9.

Nishida M, Namera A, Yashiki M, Kojima T. 2002. Forensic Sci. Int. **125:** 156.

Niveau G. 2006. Prevention of infectious disease transmission in correctional settings: a review. Public Health. **120:** 33–41.

Orangio GR, Pitlick SD, Latta PD, Mandel LJ, Marino C, Guarneri JJ, Giron JA, Margolis IB. 1985. "Soft Tissue Infections in Parenteral Drug Abusers," Annals of Surgery. **199:** 97-100.

Palmero D, Ritacco V, Ambroggi M, et al. 2006. Multidrug-resistant tuberculosis in AIDS patients at the beginning of the millennium [in Spanish]. Medicina (B Aires). **66:** 399–404, 598.

Peat M, Budd J, Burns SM, Robertson R. 2000. Audit of blood borne virus infections in injecting drug users in general practice. Commun Dis Public Health. **3:** 244–246.

Peltzer K, Ramlagan S, Johnson BD, Phaswana-Mafuya N. 2010. Illicit Drug Use and Treatment in South Africa: A review. Subst Use Misuse. **45(13):** 2221–2243.

Peters A, Davies T, Richardson A. 1997. Increasing popularity of injection as the route of administration of amphetamine in Edinburgh. Drug and Alcohol Dependence. **48(3):** 227–234.

Peters AD, Reid MM. 1998. Methadone treatment in the Scottish context: outcomes of a community-based service for drug users in Lothian. Drug and Alcohol Dependence. **50(1):** 47–55.

Punnotok J, Shaffer N, Naiwatanakul T, Pumprueg U, Subhannachart P, Ittiravivongs A, Chuchotthaworn C, Ponglertnapagorn P, Chantharojwong N, Young NL, Limpakarnjanarat K, Mastro TD. 2000. Human immunodeficiency virus-related tuberculosis and primary drug resistance in Bangkok, Thailand. The International Journal of Tuberculosis and Lung Disease. **4(6):** 537-543(7).

Raharjo TJ, Verpoorte R. 2004. Methods for the analysis of cannabinoids in biological materials: a review. Phytochem Anal. **15:** 79-94.

Reed E. 2009. The prescription for eradicating meth labs: a call for states to enact stricter chemical control over precursors. Capital University Law Review. **37:** 787–818.

Reuter P, Caulkins JP. 2004. Illegal lemons: Price dispersion in cocaine and heroin markets. Bulletin on Narcotics. **54(1, 2):** 141-165.

Richter RW, Rosenberg RN. 1968. Transverse myelitis associated with heroin addiction. Journal of the American Medical Association. **206(6):** 1255-1257.

Ringertz SH, Hoiby EA, Jensenius M, Maehlen J, Caugant DA, Myklebust A, *et al*. 2000. Injectional anthrax in a heroin skin-popper. Lancet. **356(9241):** 1574-5.

Risser D, Uhl A, Oberndorfer F, Hçnigschnabl S, Stichenwirth M, Hirz R, Sebald D. 2007. Is There a Relationship Between Street Heroin Purity and Drug-Related Emergencies and/or Drug-Related Deaths? An Analysis from Vienna, Austria. J Forensic Sci. **52(5)**.

Risser D, Uhl A, Stichenwirth M. 2000. Quality of heroin and heroin-related deaths from 1987 to 1995 in Vienna, Austria. Addiction. **95:** 375–382.

Robson P, Bruce M. 1997. A comparison of 'visible' and 'invisible' users of amphetamine, cocaine and heroin: two distinct populations? Addiction. **92(12):** 1729–1736.

Rook EJ, Hillebrand MJX, Rosing H, Jan M. van Ree, Jos H. Beijnen. 2005. The quantitative analysis of heroin, methadone and their metabolites and the simultaneous detection of cocaine, acetylcodeine and their metabolites in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry. Journal of Chromatography B. **824:** 213–221.

Rook EJ, Huitemal ADR, Brink WVD, Hillebrand MJX, Ree JMV, Beijnen LH. 2006. Screening for Illicit Heroin Use in Patients in a Heroin-Assisted Treatment Program. Journal of Analytical Toxicology. **30.**

Roy É, Nonn É, Haley N. 2008. Transition to injection drug use among street youth-A qualitative analysis. Drug Alcohol Depend. **94(1-3):** 19-29.

Ryan JM, Paul J, Curtis S, Patel NK. 2001. *Clostridium novyi* infection: a fatal association with injecting drug users. Emerg Med J. **18:** 138–139.

Schneider S, Meys F. 2011. Analysis of illicit cocaine and heroin samples seized in Luxembourg from 2005–2010. Forensic Science International. **30**.

Seidi S, Yamini Y, Baheri T, Feizbakhsh R. 2011. Electrokinetic extraction on artificial liquid membranes of amphetamine-type stimulants from urine samples followed by high performance liquid chromatography analysis. Journal of Chromatography A. **1218:** 3958–3965.

Shamsuddin D, Tuazon CU, Levy C, Curtin J. 1982. *Bacillus cereus* panophthalmitis: source of organism. Rev Infect Dis. **4:** 97–103.

Sharma P, Murthy P and Bharath MMS. 2012. Chemistry, Metabolism, and Toxicology of Cannabis: Clinical Implications. Iran J Psychiatry. **7(4):** 149-156.

Slooten VEP, Helm VHJ. 1975. Forensic Sci. **6**: 83-88.

Sporer K. 1999. Acute heroin overdose. Ann Intern Med. **130:** 584–90.

Staack RF, Paul LD, Schmid D, Roider G and Rolf B. 2007. Proof of a 1-(3-chlorophenyl) piperazine (mCPP) intake: use as adulterant of cocaine resulting in drug–drug interactions? J Chromatogr B Analyt Technol Biomed Life Sci. **855**: 127–33.

State Medical Marijuana Laws. 2010. National Conference of State Legislatures.

Staub C, Marset M, Mino A, Mangin P. 2001. Detection of acetylcodeine in urine as an indicatorof illicit heroinuse: method validation and results of a pilot study. Clin. Chem. **47:** 301-307.

Stimson GV, Adelekan M, Rhodes T. 1996. The diffusion of drug injecting in developing countries. International Journal of Drug Policy. **7(4).**

Story A, Murad S, RobertsW, Verheyen M, Hayward AC. 2007. Tuberculosis in London: the importance of homelessness, problem drug use and prison. Thorax. **62:** 667–71.

Strang J, Griffiths P, Gossop M. 1997. Heroin in the UK: Different forms, different origins, and the relationship to different routes of administration. Drug Alcohol Rev. **16:** 329–337.

Strang J, Griffiths P, Gossop M. 1997. Heroin smoking by "chasing the dragon": origins and history. Addiction. **92(6):** 444–446.

Strang J, King L. 1996. Heroin is more than just diamorphine. Addiction Research. **5(1):** 3-7.

Subhan F, Khan N and Robert D. E. Sewell. 2009. Adulterant Profile of Illicit Street Heroin and Reduction of its Precipitated Physical Dependence Withdrawal Syndrome by Extracts of St John's Wort (*Hypericum perforatum*). Phytother. Res. **23:** 564–571.

Substance Abuse and Mental Health Services Administration (SAMHSA). 2010. NSDUH: Latest national survey on drug use and health, substance abuse data, SAMHSA, office of applied studies.

Talan DA, Moran GJ. 1998. Tetanus among injecting drug users: California 1997. Ann Emerg Med. **32:** 385–386.

Tang CS, Wong CS. 1996. Marijuana and heroin use in Hong Kong adolescents. Substance Use and Misuse. **31(1):**115–125.

Torrens M, San L, Peri JM, Olle JM. 1991. Cocaine abuse among heroin addicts in Spain. Drug and Alcohol Dependence. **27(1):** 29–34.

Tuazon CU, Hill R, Sheagren JN. 1974. Microbiological study of street heroin and injection paraphernalia. J Infect Dis. **129:** 327–329.

Tuazon CU, Miller H, Shamsuddin D. 1980. Antimicrobial activity of street heroin. J Infect Dis. **142:** 944.

United National International Drug Control Programme (UNIDCP). 1995. Recommended methods for the detection and assay of heroin, cannabinoids, cocaine, amphetamine, methamphetamine and ring-substituted methamphetamine derivatives in biological specimens. UN, New York.

United National International Drug Control Programme (UNIDCP). 1998. Recammended methods for testing opium, morphine and heroin manual for use by national drug testing laboratories. UN, New York.

United Nation Office on Drugs and Crime (UNODC). 2011. The Global Afghan Opium Trade.

United National International Drug Control Programme (UNIDCP). 1997. World Drug Report. New York, Oxford University Press.

United Nations Office on Drugs and Crime (UNODC). 2007. World Drug Report 2007. New York: United Nations.

United Nations Office on Drugs and Crime (UNODC). 2010. World Drug Report. Austria, Vienna: United Nations Publication.

United States Census Bureau (USCB). 2010. US census data.

United Nations Office on Drugs and Crime (UNODC). 2005. Methods for impurity profiling of Heroin and Cocaine. Manual for use by national drug testing laboratories. UN, New York.

United Nations Office on Drugs and Crime (UNODC). 2007. World Drug Report. 17, 146. Vienna.

United Nations Office on Drugs and Crime (UNODC). 2008. Illicit Drug Trends in Pakistan. The Paris pact initiative. A partnership to counter trafficking and consumption of Afghan opiates. United Nation. New York.

United Nations Office on Drugs and Crime (UNODC). 2008. Vienna International Centre, 1400 Vienna, Austria.

United Nations Office on Drugs and Crime (UNODC). 2010. World Drug Report 2010 conducted by UNODC.

United Nations Office on Drugs and Crime (UNODC). 2011. World drug report.

United Nations Office on Drugs and Crime (UNODC). 2012. World drug report.

UNODCP. 2002. Drug Abuse in Pakistan. Results from National Assessment Study of Pakistan by Anti Narcotic Force, pp. 08-10.

US Environmental Protection Agency, (USEPA). 2009b. U.S. EPA voluntary guidelines for methamphetamine laboratory cleanup. Office of Solid Waste and Emergency Response.

Van LMW, Cruts AAN, Verdurmen JEE, Van Ooyen-Houben MMJ, Meijer RF, editors. 2008. National Drug Monitor: Annual Report 2007.

Van Rie A, Zhemkov V, Granskaya J, et al. 2005. TB and HIV in St. Petersburg, Russia: a looming catastrophe? Int J Tuberc Lung Dis. **9:** 740–5.

Vries GD, Van HRA. 2006. From contact investigation to tuberculosis screening of drug addicts and homeless persons in Rotterdam. Eur J Public Health. **16:** 133–6.

Wei G, Moss J, Yuan CS. 2003. Opioid-induced immunosuppression: is it centrally mediated or peripherally mediated? Biochem Pharmacol. **65:** 1761–6.

Weller PF. Nicholson A, Braslow N. 1979. The spectrum of Bacillus bacteremias in heroin addicts. Arch Intern Med. **139:** 293-294.

Werner SB, Passaro D, McGee J, Schechter R, Vugia DJ. 2000. Wound botulism in California, 1951–1998: recent epidemic in heroin injectors. Clin Infect Dis. **31:** 1018–1024.

Wong SC, Curtis JA, Wingert WE. 2008. Concurrent detection of heroin, fentanyl, and xylazine in seven drug-related deaths reported from the Philadelphia Medical Examiner's Office. J Forensic Sci. **53(2):**495-8.

World Health Organisation (WHO). 2005. WHO Report on Neuroscience of Psychoactive Substance Use and Dependence Geneva: WHO.

Zacken, F. 1988. The encyclopedia of Psychoactive drug: Heroin the street Narcotics. Burke Publishing Company Ltd, London. 07-13.

Zelkowicz A, Magora A, Mark D. Ravreby, Rina Levy. 2005. Analysis of a Simulated Heroin Distribution Chain by HPLC. J Forensic Sci. **50(4).**

Zhang YR, Wang R, Zhang CG. 2002. Column-switching high-performance liquid chromatographic method for the determination of morphine and O6-monoacetylmorphine in urine. Fa Yi Xue Za Zhi. **18(2):** 89-91.

Zhou Y, Li X. 1999. Demographic characteristics and illegal drug use patterns among attendees of drug cessation programs in China. Substance Use and Misuse. **34(6):** 907–920.

Review of Literature

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References