REPORT OF
DETECTION OF NEW PSYCHOACTIVE SUBSTANCES AMONG SUBSTANCE ABUSE TREATMENT SEEKERS IN INDIA

A MULTICENTRIC STUDY

CONDUCTED BY

National Drug Dependence Treatment Centre

All India Institute of Medical Sciences, New Delhi

SUPPORTED BY

Department of Revenue
Ministry of Finance

Government of India
New Delhi

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विवेक अग्रवाल, आई.ए.एस.
अतिरिक्त सचिव एवं मु. स. अ.
VIVEK AGGARWAL, IAS
Additional Secretary & C.V.O.

वित्त मंत्रालय
राजस्व विभाग
भारत सरकार
Ministry of Finance
Department of Revenue
Government of India
Room No. 146-C, North Block,
New Delhi-110001
Tel. : +91-11-23092264, Fax : 23092750
E-mail : revas-mof@gov.in

Foreword

Dated 26th December, 2022

New psychoactive substances (NPS) are a major source of concern for law enforcement agencies involved in the control of illicit drugs. NPS are 'substances of abuse', either in pure form or a preparation, that are not controlled by the Single Convention on Narcotic Drugs of 1961 or the 1971 Convention on Psychotropic Substances, but may pose a significant public health threat. The illicit drug market is constantly coming out with newer NPS, whose detection remains an ongoing challenge for law enforcement agencies dealing with narcotic control. In the period 2009-2021, UNODC has received information about 1127 such substances from 134 countries across the world. NPS are a fluid category and are constantly being put under international control.

Detection of NPS is a big challenge for the laboratories involved in drug detection. In this background, the present multicentric study was conducted by the National Drug Dependence Treatment Centre (NDDTC), All India Institute of Medical Sciences (AIIMS), New Delhi with support from the Department of Revenue, Ministry of Finance, Government of India. It is really creditable for the investigator's team to assess 1476 drug users in five different parts of the country, from the far east Imphal (Manipur) to Amritsar (Punjab) at the Indo-Pak border, Bhubaneshwar (Odisha), Rishikesh (Uttarakhand) and Delhi. The study faced many interruptions during the Covid pandemic, which affected the recruitment of subjects due to the closure of health facilities. This also led to difficulties in the transportation of samples. The urine samples were stored locally and transported later to the NDDTC, where these were analysed by the state-of-the-art laboratory.

I would like to especially congratulate the team of investigators led by Professor Rakesh Chadda and Professor Raka Jain and their team members and the investigators from the other centres for completing this voluminous work.

This report is a catalytic attempt towards improving analytical strategies in enhancing the detection of these substances as a valuable tool for medicinal chemists, toxicologists, clinicians, forensic scientists, law enforcement agencies and policymakers in the country.

Vivek Aggarwal, IAS

PREFACE

Substance use has been a major challenge faced by health professionals and policymakers all across the world. The pattern of drug use varies from time to time depending on the availability of new illicit drugs. Over the last decade, the world is witnessing an alarming new drug problem. Most of these new drugs are legal and marketed as "legal highs", "plant food", "research chemicals", "smart drugs" and "bath salts". These are collectively called New Psychoactive Substances (NPS). By definition, NPS are substances of abuse, either in a pure form or in the form of preparations, that are not controlled under the Single Convention on Narcotic Drugs of 1961 or the Convention on Psychotropic Substances of 1971, but that may pose a threat to public health.

NPS can be analogues of the existing controlled drugs or newly synthesized compounds, which have been designed to mimic the psychoactive effects of the controlled drugs. As a category, NPS are rapidly evolving group, with newer compounds being added and some of the older ones being removed. Over the last 15 years, a total of 1127 such substances have been identified by the national authorities and forensic laboratories from 134 countries.

In the above background, the present study was planned by the National Drug Dependence Treatment Centre (NDDTC) at the All India Institute of Medical Sciences (AIIMS), New Delhi, with objectives to assess the extent and pattern of NPS use among treatment-seeking substance users and to develop and establish urine testing procedure for "New Psychoactive Substances" among treatment-seeking substance users. The study was supported by the Department of Revenue, Ministry of Finance, Government of India. Considering that India is a vast country, it was proposed to have centres from all over the country including Punjab, Manipur, Goa, Mumbai, Himachal Pradesh and Kerala. Later due to logistic reasons, some of the centres needed to be changed. Finally, we had five centres, Government Medical College, Amritsar, Regional Institute of Medical Sciences, Imphal, All India Institute of Medical Sciences, Rishikesh and All Indi Institute of Medical Sciences, Bhubaneswar, besides the NDDTC. The process involved taking ethics approval from all the study centres. All the peripheral centres were provided initial sensitisation and training by the investigator team from the NDDTC.

Initially, the study was proposed for the period 2016-2019, but it could begin in 2017. Since the study needed the acquisition of some new state-of-the-art equipment like LC system (ultra-high pressure liquid chromatography) and also reference standards for analysing NPS, it got delayed. Later, the Covid pandemic further slowed down the study. Thus, we have been able to complete the study in October 2022.

This report is the first attempt in establishing objective evidence of the occurrence of NPS among treatment seekers in India. It is important to highlight that the current study began in 2017, and some of the compounds detected in patients' urine samples were later placed under international control. A novel finding of this study is that some of the identified substances are currently not under international control.

This report provides insight into several issues at the drug control policy levels, such as product purity, manufacturing process, alternative manufacturing routes, use of precursors and the efficacy of precursor control measures, impurity profiles, the role of cutting agents, market dynamics, and an understanding of the flow of controlled substances from producers to consumer markets.

The report addresses a mixed group of readers such as medicinal chemists, forensic scientists, toxicologists, clinicians, healthcare professionals, law enforcement agencies, and policymakers. It is hoped that the findings and recommendations from this report to be informative and meaningful in addressing the challenges posed by newer psychoactive drugs in the country. We recommend that future research and policy interventions should be geared toward addressing this emerging problem of the NPS.

The Team of Investigators
New Delhi, October 2022

ACKNOWLEDGEMENTS

We would like to express our deepest appreciation to all those who provided us with the possibility to complete this study and draft this report. It is our radiant sentiment to place on record our best regards and deepest sense of gratitude to all the co-investigators for their careful and precious guidance, which was extremely valuable for this study, both theoretically and practically. However, it would not have been possible without the kind support and help of many individuals and organizations. We would like to extend our sincere thanks to all of them.

We are highly indebted to the Department of Revenue, Ministry of Finance, Government of India for the funding and constant support throughout the course of the study. Furthermore, we would also like to acknowledge with much appreciation the crucial role of the staff of Government Medical College Amritsar, Punjab; Regional Institute of Medical Sciences, Imphal Manipur; All India Institute of Medical Sciences, Bhubaneswar, Orissa; All India Institute of Medical Sciences, Rishikesh, Uttarakhand; and the National Drug Dependence Treatment Centre, All India Institute of Medical Sciences New Delhi, along with all the patients that participated, without whom this study could not have been completed.

Special thanks are due to the laboratory team of the NDDTC, AIIMS, New Delhi for their hard work throughout the study without which the completion of this work would not have seen the light of the day.

Finally, we would like to extend our sincere acknowledgments to the staff at the NDDTC, AIIMS for coordinating the administrative aspects of the project.

Team of Investigators
NDDTC, AIIMS, New Delhi
October 2022

ABBREVIATIONS

4-MEC	4-Methylethcathinone
AMT	α-Methyltryptamine
APINACA	N-(Adamantan-1-yl)-1-Pentyl-1H-Indazole-3-Carboxamide
ATS	Alternate Trading System
ATS	Amphetamine Type Stimulants
BAT	Biochip Array Technology
BZP	Benzylpiperazine
CB1	Cannabinoid Receptor type 1
CNS	Central Nervous System
DBS	Dried Blood Spots
DIA	Data Independent Acquisition
DMAA	1,3-Dimethylamylamine
DMT	N-N-Dimethyl Tryptamine
EMCDDA	The European Monitoring Centre for Drugs and Drug Addiction
EWA	Early Warning Advisory
GABA	Gamma Amino Butyric Acid
GC-MS	Gas Chromatography Coupled to Mass Spectrometry
LC-MS	Liquid Chromatography Coupled to Mass Spectrometry
LC QTOF MS	Liquid Chromatography with Quadrupole Time of Flight Mass Spectrometry
LLE	Liquid-Liquid Extraction
LSD	Lysergic Acid Diethylamine
mCPP	1-(3-chlorophenyl) Piperazine
MDAI	5,6-Methylenedioxy-2 Aminoindane
MXE	Methoxetamine
NMDA	N-methyl-D-aspartate
NPP	N-Phenethyl-4- Piperidone
NPS	New Psychoactive Substance
PCP	Phencyclidine
SCRA	Synthetic Cannabinoid Receptor Agonists
SPE	Solid Phase Extraction
THC	Tetrahydrocannabinol
UNODC	United Nations Office on Drugs and Crime
α –PVP	α- Pyrrolidinopentiophenone

CONTENTS

TEAM OF INVESTIGATORS

Coordinating Centre

National Drug Dependence Treatment Centre, All India Institute of Medical Sciences, New Delhi

- Prof. R.K. Chadda (Principal Investigator)
- Prof. Raka Jain (Co-Principal Investigator)
- Prof. Rakesh Lal (Co-Principal Investigator)
- Dr. Ravindra Rao (Co-Investigator)
- Dr. Rizwana Quraishi (Co-Investigator)

Participating Centres

Government Medical College, Amritsar, Punjab

- Prof. P.D. Garg (Co-Investigator)
- Dr Neeru Bala (Co-investigator)

Regional Institute of Medical Sciences, Imphal, Manipur

- Prof. R.K. Lenin Singh (Co-Investigator)

All India Institute of Medical Sciences, Bhubaneswar, Orissa

- Dr. Sushant Padhy (Co-Investigator)

All India Institute of Medical Sciences, Rishikesh, Uttarakhand

- Dr. Aniruddha Basu (Co-Investigator)
- Dr Vijay Krishnan (Co-investigator)

Research Staff

- Dr. Rahul Raghav
- Dr. Satish Kumar
- Sachin Rana
- Arun Kumar

EXECUTIVE SUMMARY

Introduction

New psychoactive substances (NPS) are "substances of abuse", either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat. In the last decade, an alarming 'new drug' problem has been encountered globally. Most of these 'new drugs' are a complex and diverse group of substances commonly known as 'designer' or 'synthetic drugs' or 'legal highs'. A rapid increase in NPS creates major challenges among researchers, forensic toxicologists, healthcare systems, and policymakers as a 'growing worldwide epidemic'.

Broadly, the NPS include stimulants, synthetic opioids, synthetic cannabinoids, dissociatives, hallucinogens and sedatives/hypnotics. NPS have similar effects as substances under international control like cannabis, cocaine, heroin, LSD or methamphetamine. Most NPS reported until December 2021, showed effects like stimulants, followed by synthetic cannabinoid receptor agonists and hallucinogens while an increase in synthetic opioids and benzodiazepines has been observed in recent years.

The emergence of NPS has been reported in around 134 countries and territories worldwide. According to the EMCDDA, the most common among the categories of NPS reported to the European Union are cannabinoids (25%), followed by cathinones (22%) and other synthetic compounds (EMCDDA 2020). The UNODC Early Warning Advisory on NPS received reports of 1,124 substances from various governments, forensic laboratories and partner organisations across the world from 2009 to 2021. The NPS market is in a constant state of influx and outflow with every passing year certain new NPS get added to the pool and some previously known substances disappear from the market. In addition, pharmacokinetic and metabolic data are not yet available formost NPS (mainly due to the frequent appearance of new compound son the market), leaving many unknowns.

There is very limited scientific information available on the prevalence of NPS in India. However, there are regular reports from the media regarding their seizures. An upsurge in synthetic cathinone like mephedrone is reported among teenagers. Recently, in August 2022, the Mumbai police reported one of the biggest haul of more than 700 kg of mephedrone seized from a manufacturing unregulated laboratory in Mumbai.

India, however, has been identified as a booming pharmaceutical and precursor exporter by the UNODC. Along with its scientific expertise and manufacturing capabilities, it may pose significant challenges for authorities in exercising control over the diversion, clandestine manufacture and trafficking of precursor chemicals. There have been reports of trafficking of ATS (amphetamine-type stimulants) precursors from India, and also seizures of other synthetic drug precursors including N-acetylanthranilic acid, N-phenethyl-4- piperidone (NPP) and 2-bromo-4-chloropropiophenone.

Testing for NPS in clinical and forensic settings can be a complex task. Typical methods used in general unknown analyses include immunoassay, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry. These approaches, however, may not be sufficient in detecting current and emerging NPS that continue to appear on the illicit market. Among the many methods available, liquid chromatography (LC) coupled with quadrupole time of flight mass spectroscopy (MS) (LC QTOF-MS) has gained popularity due to its ability to measure accurate masses and operate in data-independent acquisition (DIA) modes. This acquisition technique offers a comprehensive full scan of MS and MS/ MS that can be retrospectively interrogated for new

analytes of interest. Moreover, the sample amount needed for the analysis is very small, and sample preparation is minimal.

In addition to the paucity of analytical research data on NPS, the inaccessibility of scientific literature, as well as the lack of scientific reference standards from a scientific standpoint, pose challenges to the toxicological laboratory in NPS identification.

In the above background, the current study was conceptualised to investigate the use of NPS in the India drug using population with the following objectives:

- To assess the extent and pattern of new psychoactive substances (NPS) use among treatment-seeking substance users.
- To develop and establish a urine testing procedure for NPS among treatment-seeking substance users.

Methodology

The study was planned as a prospective cross-sectional observational study on patients seeking treatment for psychoactive substance use. It included five sites:

- National Drug Dependence Treatment Centre (NDDTC), All India Institute of Medical Sciences (Nodal Site)
- Regional Institute of Medical Sciences (RIMS), Imphal, Manipur
- Government Medical College (GMC), Amritsar, Punjab
- All India Institute of Medical Sciences (AIIMS), Rishikesh, Uttarakhand
- All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha

A team of investigators from the NDDTC visited the other participating centres to sensitise them about the study methodology, recruitment of subjects, sample collection and storage and its subsequent transport to the NDDTC.

Subjects attending the deaddiction services of the institutions were screened for the study. To be included, the subjects needed to be of male gender, aged 18-60, and fulfil ICD-10 criteria for harmful use or dependence syndrome, and be willing to provide consent to take part in the study and to provide a biological sample for laboratory analysis. Those seeking treatment primarily for alcohol use disorder were not included. It was proposed to include 1000 subjects from the NDDTC and 200 each from the other four centres, but due to the study being interrupted due to the Covid-19 pandemic, though we were able to recruit 1000 patients at the NDDTC, only 476 subjects could be recruited from the other four centres. Ethics approval was taken from the ethics committee of all the participating centres.

Basic sociodemographic and clinical information including substance use history was taken on a study proforma. The risk status of the subjects was also assessed on the WHO-ASSIST questionnaire. Five ml of urine was collected from each subject in a leak-proof plastic container by the project laboratory staff, under close supervision to prevent the risk of tampering. After proper labelling and sealing, the urine sample was sent to the NDDTC Drug Abuse Testing laboratory for analysis. All samples were stored at -20^{o}C till analysis to prevent degradation. Samples from each site were transported in liquid nitrogen to the Drug Abuse Screening Laboratory, NDDTC, AIIMS. The samples from each site were screened for all the drugs of abuse and NPS.

Following the receipt of the samples in the Drug Abuse Screening Lab at the NDDTC, AIIMS, routine urine testing was performed to confirm the presence of the substance abuse using the Cassette test (ABON Multi-

drug one-step screen test panel, Abon Biopharm Co. Ltd, China). Further, all the urine samples were screened for NPS by Bioarray Chip technology (BAT) based kits using an Evidence Investigator Analyser (Randox Laboratories Ltd. UK). Based on the findings of screening results, all the positive samples of different groups of aforesaid NPS were further confirmed by liquid chromatography-QTOF-mass spectrometry (LC–QTOF-MS). Instrumentation included an EXION AD LC system (ultra-high pressure liquid chromatography) equipped with an autosampler and two micropumps, and coupled to the AB Sciex Triple TOF 5600+ System. Samples were considered true positive (TP), if the BAT assay and LC- QTOF -MS were positive. Stock solutions of reference standards and internal standards were prepared in methanol (MeOH) to a concentration of 1mg/mL and were stored at -20^{o}C till analysis. A sensitive and specific method for the analysis of NPS in urine was developed using LC-QTOF-Mass spectrometry.

Results

The study sample consisted of 1476 subjects with a mean age of 28.2 years (SD: 8.4). Most (92%) were current users of opioids. Nearly 95% of the subjects were using tobacco. Alcohol and cannabis use was present in more than 70% of the subjects. Most (91.3%) of the opioid users came in the high-risk category as assessed on the WHO-ASSIST. Twenty-nine percent (n=424) of the participants reported injecting psychoactive substance at least once in their lifetime. Heroin was the most common opioid injected followed by buprenorphine injections. Eight percent (n=122) of the injection drug users (IDUs) reported sharing needles/syringes ever in their lifetime. Forty-three (2.9%) subjects reported using a substance that was not a commonly used drug in the local population. Of these, 21 reported using MDMA, 17 reported using 'meow meow', 5 reported using LSD, while one reported using ketamine.

On the initial urine screen, the drugs detected included morphine (63.8%), benzodiazepine (41.99%), cannabinoids-THC (40.9%), tramadol (34.06%), buprenorphine (21.5%), barbiturates (1.36%), cocaine (0.95%) and amphetamine (0.47%). There were some minor variations in the individual positivity rates across the different centres. There was concomitant use of one or more drugs in these patients. The laboratory results also indicate a high detection rate of prescription drugs, like buprenorphine, tramadol and benzodiazepines at the collaborating sites. It is likely that some of the individuals were receiving treatment and had been prescribed these medications.

Analysis indicated that the percentage of NPS-positive tests varied from 57.11% to 0.06% among different categories of drug types. These includes:

Synthetic cannabinoids: AB-CHIMNACA (0.27%), AB-PINACA (0.61%), JWH-018 (4.51%),

Stimulants: 1-Phenylpiperazine (5.67%), 2-Phenylpiperazine (22.09%), Benzylpiperazine (2.25%)

Synthetic Cathinones: Mephedrone (3.35%), Alpha-PVP (11.35%)

Fentanyl analogs: Actetylfentanyl (1.16%), Ocfentanil (0.27%), Carfentanil (6.97%)

Benzodiazepine analog: Etizolam (4.17%)

Other Synthetic Opioids: AH-7921 (0.06%), MT-45 (0.27%), W-19 (2.46%)

Plant-based products: Mescaline, 57.11% (Hallucinogen), Mitragynine, 2.59% (Stimulant),

Salvinorin A, 0.06% (Dissociative)

Also, analytical findings of urine specimens were indicative of multiple drug use and combination pattern at all sites.

Discussion

The present study is the first study from India to report the NPS objectively in an addiction treatment setting. Morphine was detected in the majority of samples followed by benzodiazepines, cannabinoids-THC, tramadol, buprenorphine, barbiturates, cocaine and amphetamine.

Three synthetic cannabinoids (AB-CHIMNACA, AB-PINACA, JWH-018 were detected in the patient's urine sample. All three are new-generation synthetic receptor agonists (SCRAs). These have cannabimimetic effects that are more potent than THC, which is listed as a schedule II substance in accordance with the Convention on Psychotropic Substances of 1971. All these synthetic cannabinoids have no therapeutic usefulness. The study also showed the presence of three piperazine derivatives (1-phenylpiperazine, 2-phenylpiperazine and benzylpiperazine) in the patient's urine. Our test results showed a high percentage of positives for two synthetic cathinones (mephedrone and alpha-PVP). It is also interesting to note that in some parts of India, seizure data and other indirect measures of use have raised concerns about the increasing use of synthetic cathinones, such as mephedrone. The current study samples also showed the presence of three fentanyl analogs (actetylfentanyl, ocfentanil and carfentanil) and two synthetic opioid analgesics (AH-7921and MT-45). Etizolam, a designer benzodiazepine, was detected in some urine samples. Mescaline was detected in more than half of the urine samples. The reasons behind this have yet to be fully investigated.

At the national level, other categories of drugs detected were mitragynine-type stimulant salvinorin A like Dissociative, and W-19 like synthetic opioid drug and piperazine derivatives such as 1-Phenylpiperazine and 2-Phenylpiperazine (22.09%). A novel finding of this study is that these substances are currently not under international control.

It is important to note that the current study commenced in the year 2017 and some of the substances detected in patients' urine samples were placed under international control thereafter. Some of the NPS were present in very low percentages. One probable explanation is that these compounds could be adulterants, and people who use recreational substances may have been unintentionally exposed to these newer psychoactive chemicals, either alone or in combination with other substances, raising the risk of potential harm.

The report provides objective evidence of the occurrence of newer NPS among treatment seekers in India. The urinalysis findings have important policy implications. Future research and policy initiatives should be geared toward addressing this emerging drug problem.

Recommendations

- NPS research necessitates multidisciplinary approaches that include epidemiology, pharmacology, and prevention.
- Drug detection laboratories need to employ analytical methodologies that are both flexible and robust while meeting workload demands.
- Newly developed analytical methods for detecting NPS must be made widely available to assist in the identification of novel substances as they appear in the recreational drug marketplace.
- The challenges posed by the NPS necessitate the use of epidemiological monitoring systems to rapidly identify emerging substances and alert policymakers and health professionals in a timely manner.

INTRODUCTION

In the last decade, an alarming 'new drug' problem has been encountered globally. Most of these 'new drugs' are a complex and diverse group of substances commonly known as 'designer' or 'synthetic drugs' or 'legal highs' (Luethi & Liechti, 2020). These are collectively called 'New Psychoactive Substances (NPS)'. The term 'designer drugs' defines synthetic and other psychoactive substances that mimic the effects of illicit drugs. 'Legal highs', 'herbal highs', 'research chemicals' and 'bath salts' are also common names used for NPS as a legal alternative to controlled drugs (Peacock et al, 2019). NPS can be either an analogue of existing controlled drugs and pharmaceutical products or newly synthesized chemicals (O'Hagan A & McCormack 2019). These are manufactured specifically to mimic the actions and psychoactive effects of controlled substances or medicines (Batisse et al, 2020).

UNODC defines "new psychoactive substances (NPS)" as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" (UNODC EWA, 2021).

A rapid increase in NPS creates major challenges among researchers, forensic toxicologists, healthcare systems, and policymakers as a 'growing worldwide epidemic' (Zawilska & Andrzejczak, 2015). Over the last decade, NPS have been introduced in alternate trading system (ATS) markets through various modes of distribution. This includes the internet with online sales, 'head' or 'smart shops', street-level drug traffickers, and rave parties or music festivals as legal alternatives to illicit drugs (Palamar et al, 2016).

New Psychoactive Substances: Pharmacology and Effects

The psychoactive substances which are controlled under the international drug conventions can produce their pharmacological effects by different mechanisms, like by their interactions with opioid receptors and inhibitory neurotransmitters, or by the activation of the cannabinoid receptor type 1 (CB1), or by action at the GABAA, or NMDA receptor, or by altering the levels and action of monoamine neurotransmitters. Using this approach majority of the NPS are assigned into six main 'effect' groups (UNODC EWA, 2021).

1. **Stimulants:** These act as CNS stimulants by mediating the actions of monoamine neurotransmitters, producing entactogenic and hallucinogenic effects. Some of the NPS under this group are phenylethylamines, cathinone, aminoindanes and piperazines. Currently, synthetic stimulants are among the largest group of NPS monitored by the UNODC. These substances mimic the effect of traditional drugs, like cocaine, amphetamine, methamphetamine and ecstasy. These can be made into a variety of chemical formulations. The various ways to use them can be insufflation, swallowing, inhalation, smoking, injection or rectally; the most common route is orally in form of pills/tablets (Karila et al, 2015).

   Synthetic stimulants have been reported to be used as cognitive enhancers or 'nootropics'. These help students with their exams. These are also used to cope with stress in stressful work environments, to maintain and enhance attention, and as part of weight loss regimens. Acute harms associated with the use of synthetic stimulants are agitation, nausea, vomiting, headache, palpitations, tachycardia, hypertension and hyperthermia. Severe adverse effects are less commonly observed and include significant peripheral organ damage and rhabdomyolysis. In some cases, deaths have been linked to hypertensive crises, hyperthermia, cardiac arrest and/or serotonin syndrome.
2. **Synthetic Opioids:** They act as CNS depressants. They bear structural features that allow binding to opioid receptors resulting in morphine-like effects including analgesia. NPS belonging to this group

include carfentanil and other synthetic opioids. The adverse effect with their use includes nausea, vomiting, constipation, dizziness. More severe effects like respiratory and central nervous system depression are also reported (Helender et al, 2017). Noncardiogenic pulmonary oedema, acute lung injury and diffuse alveolar haemorrhage are associated with intoxication (Nash et al, 2019). Cases of toxicity with the use of MT-45 (a synthetic opioid) reported hearing loss and/or deafness in addition to typical opioid-like toxicity (Helender et al, 2020).

3. **Synthetic cannabinoid receptor agonists (SCRA, synthetic cannabinoid).** Drugs belonging to this group bind to cannabinoid receptors and produce an effect similar to those of delta-9-THC. These are often laced with herbal products and commonly sold as spice, K2, kronic, etc. These are used by either mixing with tobacco or smoked directly, inhalation being the main route of use. As per UNODC, more than 280 synthetic cannabinoids had been identified by the end of 2019.

   The adverse effects associated with the use of synthetic cannabinoids include cardiovascular and respiratory complications, renal injury and cerebrovascular accidents. Commonly observed adverse effects in emergency rooms are nausea, vomiting, agitation, drowsiness, dizziness, confusion, hypertension, tachycardia and chest pain (Zimmer et al, 2019). Severe morbidity and mortality have also been reported from prisons, homeless and other secure settings (Joseph et al, 2019).

4. **Dissociatives:** This group of hallucinogens modulates the effect of NMDA receptors and produces a feeling of dissociation. Substances in this group include controlled substances like PCP and ketamine. These can be used by inhalation, insufflation, orally or by intravenous injection. Adverse effects include nausea, hypertension, tachycardia, renal impairment, agitation, disorientation, confusion, slurred speech, hallucinations, ataxia and muscle rigidity. Reports from the emergency room include cerebellar toxicity, severe kidney and bladder damage and fatal intoxication (Hutton, 2020).

5. **Classic Hallucinogens:** This is a chemically diverse group of substances that mediates specific serotonin receptors and have hallucinogenic effects. Substances in this group mimic the effect of traditional drugs like LSD and DMT. Some classic hallucinogens are tryptamines, lysergamides and phenethylamines. Common routes of use include inhalation, nasal insufflation, oral ingestion, sublingual/buccal administration and intravenous injection. Adverse effects with non-clinical use include agitation, aggression, tachycardia, hypertension, hyperthermia, hallucinations, drowsiness and confusion. Multi-organ failure, seizures and serotonin syndrome are some of the serious adverse effects associated with the use of phenethylamine derivatives. The use of tryptamine derivatives may lead to delusions, renal failure and reported fatalities (Iwersen-Bergmann et al, 2019).

6. **Sedatives/Hypnotics:** These are CNS depressants, benzodiazepine analogues, acting on the GABA receptor complex in the brain. These drugs may mimic the effect of benzodiazepines. Reasons for use include hypnotic and anxiolytic effects or to self-treat withdrawal symptoms. Reports suggest experience of muscle relaxant, anticonvulsant and amnesic properties by the users (El Balkhi et al, 2020).

   The use of synthetic sedatives and hypnotics, especially the benzodiazepine analogues gives adverse effects like confusion, dizziness, drowsiness, fatigue, auditory and visual hallucinations, delirium, seizures, deep sleep and coma. Withdrawal symptoms such as anxiety, panic attacks, restlessness and convulsions are also observed by abrupt cessation of these drugs (Carpenter et al, 2019). Slow onset of action and longer half-life of this group of drugs may lead to overdose and related fatalities (Koch et al, 2018). Chronic hepatitis has been associated with bentazepam use (Ren et al, 2019).

GLOBAL PREVALENCE OF NPS

The emergence of NPS has been reported in around 134 countries and territories all over the world. According to the EMCDDA, the most common among the categories of NPS reported to the EU are cannabinoids (25%), followed by cathinones (22%) and other synthetic compounds (EMCDDA, 2019). The UNODC Early Warning Advisory on NPS received reports of 1,124 substances from various governments, forensic laboratories and partner organisations across the world from 2009 to 2021 (UNODC EWA, 2021). The global emergence of NPS has shown a steady increase in the number of NPS reported each year until 2015. While stabilization was observed, though at a high level, after 2015 (UNODC EWA, 2021). The NPS market is constantly changing with new NPS getting added to the pool and some previously known substances disappearing from the market.

NPS have similar effects as substances under international control like cannabis, cocaine, heroin, LSD or methamphetamine. Most NPS reported until December 2021, showed effects like stimulants, followed by synthetic cannabinoid receptor agonists and hallucinogens while an increase in synthetic opioids and benzodiazepines has been observed in recent years.

During the last few years, the main substance groups of NPS available in the market are aminoindanes, e.g. 5,6-methylenedioxy-2 aminoindane (MDAI), phencyclidine-type substances, e.g. methoxetamine (MXE), phenethylamines e.g. 2C-E and 25H-NBOMe, synthetic cannabinoids, e.g. APINACA, JWH-018, synthetic cathinones, e.g. 4-methylethcathinone (4-MEC) and á- pyrrolidinopentiophenone (á –PVP), piperazines, e.g. benzylpiperazine (BZP) and 1-(3-chlorophenyl) piperazine (mCPP), plant-based substances e.g. kratom, salvia divinorum and khat, tryptamines e.g. á-methyltryptamine (AMT), and other substances e.g. 1,3-dimethylamylamine (DMAA) (UNODC EWA, 2021).

According to the UNODC EWA report as on18.5.2022, the total number of NPS reported from the US and Sweden was about 500, while the European countries (UK, Poland) and Canada reported about 400 NPS. From Japan 300-399 NPS were documented. Countries like China, Russia and Denmark observed 200-299 NPS during this time period (UNODC EWA, 2021).

Over the years, the number of new NPS detections has decreased since 2015. In addition, the nature of the market has changed. A relative decrease in the number of new stimulants and synthetic cannabinoids has been observed, while an increase in the number of new opioids and benzodiazepines was reported (EMCDDA, 2019). The rapidly changing NPS market raises concerns about their chemical, metabolic and toxicity profile. This may include the physical, social and mental health harms associated with their use (Al-Banna et al, 2020; Rinaldi et al, 2020; Dinis Oliveira & Magalhaes, 2020).

NPS - INDIAN PREVALENCE

In India, there is very limited scientific information available on the prevalence of NPS in the Indian market. However, there are regular reports from the media regarding their seizures. An upsurge in synthetic cathinone like mephedrone is reported among teenagers. Recently, in August 2022, the Mumbai police reported one of the biggest haul of more than 700 kg of mephedrone seized from a manufacturing unregulated laboratory in Mumbai. Indian states reporting mephedrone seizures are Maharashtra, followed by Delhi, and Gujarat. Clandestine drug laboratories in India and China manufacture mephedrone which is mainly popular at rave parties among teenagers. Law enforcement agencies in India report both structured or unstructured channels for ketamine. Seizure of more than 1 kg of ketamine was observed between 2009-2012 in India (UNODC Global Smart programme, 2013). As per UNODC 2013 reports, the primary region of origin of NPS was identified to be Asia, followed by Europe, the Americas, Africa and Oceania. In Asia, China and India are

frequently named as sources of NPS (UNODC, 2013). The total number of reported NPS from India falls between 1-9, which is similar to South African countries (UNODC EWA, 2021).

A recent update by UNODC (2022), reported

- India is a booming pharmaceutical and precursor exporter. Along with its scientific expertise and manufacturing capabilities, the emergence of the NPS may pose significant challenges for authorities in exercising control over the diversion, clandestine manufacture and trafficking of precursor chemicals.
- Trafficking of ATS (amphetamine-type stimulants) precursors from India remains significant.
- Seizures of other synthetic drug precursors including N-acetylanthranilic acid, N-phenethyl-4- piperidone (NPP) and 2-bromo-4-chloropropiophenone, trafficked from or within India, were also reported.

LABORATORY DETECTION OF NPS

The laboratory detection of the NPS is quite challenging due to their rapidly changing chemical structure. In general, there is a lack of knowledge regarding the chemical composition of the rapidly emerging NPS among the professionals performing analytical analysis. The analytical methodologies are still not sufficient to detect the presence of all of the NPS in the analysed samples and many laboratories lack appropriate analytical equipment for their recognition (Zamengo et al, 2011). Additionally, the unprecedented speed of appe arance and distribution of the NPS worldwide brings technical difficulties in the development of analytical procedures and risk assessment in real-time. Thus, the emergence of novel psychoactive substances is an ongoing challenge for analytical toxicologists too (Jain & Verma, 2022).

Biological Samples

The various biological matrices such as blood, urine, hair, nails and oral fluids can be used to analyse NPS (Wagmann & Maurer, 2018). Among others, urine and blood are the most commonly preferred samples in clinical settings. Recently some non-conventional matrices like dried blood spots (DBS) have also gained attention in clinical and forensic toxicology. DBS provides the advantages of a longer detection time window, less invasive sample collection and easy storage and shipping (Quraishi et al, 2017). Urine is the most preferred matrix for drug testing in clinical settings for being non-invasive, readily available, and has a longer detection window compared to blood. However, in emergency departments, blood might be preferred over urine for reasons like blood being a common matrix for analysis of most of the analysis, and the patients may not be willing / not able to provide urine. The concentration of the drug may get affected by the amount of liquid consumed. Thus, it is important to include the metabolites along with the parent compound while performing the analysis.

The preparation of biological samples is an important step before the sample analysis. It protects the instrument and gives better results. Selection of the sample preparation technique depends on the matrix, the physical and chemical properties of the analytes, and the level of sensitivity and specificity required for the assay. For blood samples, the extraction methods include protein precipitation, liquid-liquid extraction (LLE) and solid phase extraction (SPE), while for urine samples, the extraction can be performed either by SPE or LLE. There are reports of using a simple dilution of the urine samples for some assays.

Analytical Techniques

The detection and identification of psychoactive drugs consist of two analytical steps, a preliminary screening

and a confirmation test (Jain & Verma, 2022). Preliminary screening is used to filter presumptive positive samples. It helps to decide the selection of subsequent confirmation techniques to be used for further identification (Graziano et al, 2019). The preliminary screening saves time, energy and resources facilitating testing of all the samples with the more limited available confirmation methods.

The conventional screening methods include immunological-based psychoactive drug screening tests. Immunoassays are biochemical tests based on selective antigen-antibody binding. These determine qualitatively the presence or absence of a compound. Immunoassays provide fast and low cost analysis. For NPS analysis, immunoassays have certain limitations like the fewer availability of antibodies specific to a wide array of new drugs. Also, these have low sensitivity and cross-reactivity which may lead to false positive or false negative results (Jain & Verma, 2022).

Biochip Array Technology (BAT), an advanced technique, is the next step in immunoassay drug screening. It has revolutionised the practice of forensic and clinical toxicology worldwide. A wide variety of sample matrices, like blood, urine, oral fluids, hair, tissue and meconium can be used for testing. It works on the chemiluminescence principle. BAT is multi-analyte testing platform is used allowing simultaneous quantitative or qualitative detection of a wide range of analytes. It is cost-effective and a less labour-intensive technique. BAT technique has excellent sensitivity and specificity ensuring accurate results. There are commercially available arrays in BAT for the detection of NPS. Many laboratories in the world have tested this technique for many NPS drugs (Bulska et al, 2020).

In recent years, a number of chemical analysis techniques are being used to identify NPS. As per the UNODC questionnaire on NPS analysis, most of the respondents stated using gas or liquid chromatography coupled to mass spectrometry (GC-MS or LC-MS). These techniques allow the separation of mixtures of molecules into individual components, followed by their identification and quantification. The data collected from electron ionization in mass spectrometry is confirmed from the fragmentation libraries. These methods have their limitations for NPS detection. For example, when using GC-MS, it may not be possible to differentiate between synthetic cannabinoids from JWH-18. Identification of the active compound of NPS is further complicated by the presence of isomers and possible similarities between various compounds of the same class.

Liquid chromatography with quadrupole time of flight MS (LC-QTOF-MS) provides some dominance to GCMS in detecting NPS (Leuthi & Liecht, 2020). LC-QTOF-MS bears the exclusive feature of predicting the chemical formula of the compound from accurate ion mass measurements and unique isotopic patterns. Additionally, low sample volume and minimal sample preparation make this technique very effective. Modern LC-QTOF mass spectrometers have high chromatographic and mass resolutions with high mass accuracy measurements of both parent and fragment ions. A database of spectral information from known NPS chemical structures is currently being built and validated. This allows for the identification of known and potentially unknown NPS (Bulska et al, 2020).

Mass Spectrometry (LC-QTOF-MS) is a promising solution for the analytical testing of NPS. The benefits of high-resolution mass spectroscopy (LC-QTOF-MS) for NPS investigations include:

- High sensitivity can detect very low levels of unknown compounds.
- SWATH® analysis may detect NPS as they emerge into forensic toxicology. It is the only data-independent acquisition technique (DIA). This allows the quantitation and detection of virtually all the detectable compounds in a sample.
- It utilizes the MS/MS fragmentation information for accurate chemical characterization.
- It screens simultaneously for known and unknown compounds in the same analysis.

Reference Standards

The availability of reference standards is an important tool in the identification of NPS. Reference standards are certified samples of high quality and purity, and serve as a reference base for the measurement. NPS identification is based mainly on the match done through mass spectra libraries. Reference standards may be obtained through available commercial sources. In-house reference materials can be obtained from internal sources like seized materials.

The commercial availability of NPS reference standards is restricted mainly due to the following reasons. Firstly, with every passing day, a new NPS emerges in the market and thus the laboratories need to maintain a huge stock to keep up to date with the latest NPS. Secondly, there is a high cost involved for laboratories, particularly in developing countries. Lastly, the preparation of in-house reference standards from seizures may present challenges like validation and legal issues. The UNODC survey among the participating laboratories has reported issues like the non-availability and difficulty in obtaining NPS reference standards with regard to NPS detection.

RATIONALE OF THE STUDY

The rapid rise in the NPS in the illicit drug market poses a serious challenge for the users, and the health care and drug controlling agencies. The heterogeneity of NPS, their drug compositions, concentrations, and chemical components make the detection even more challenging (Zamengo et al, 2011). Neither the drug users nor the traffickers are aware of the chemical nature of the drug. Additionally, NPS are not always detected by traditional toxicology screenings. Thus, to keep a check, drug control agencies and forensic laboratories need to remain up-to-date with new developments and trends. Developed countries report a higher number of NPS detected, as confirmed from the updated detection system (including drug control agencies, health care professionals and toxicology laboratories) about the NPS hitting the market. However, for developing countries, this data is very low, with India reporting less than 10 drugs during the 2022 EWA report.

Research on most NPS is very scant. There is a scarcity of available comprehensive scientific data on NPS toxicity. Most literature is either based on studies in animals or the fatal poisonings observed in patients in emergency rooms. NPS toxicity, abuse liability and risks associated with long-term use are largely unknown. Most reports point towards the use of NPS among high-risk drug users, people who inject drugs and the homeless and prison populations. The presentation of NPS in the emergency rooms presents a need for healthcare professionals to remain up-to-date with the clinical features of NPS use. Evidence-based approaches to harm reduction and treatment of dependence syndrome need to be developed.

There is relatively little evidence about NPS use in lower- or middle-income countries. In India, there is no scientific data with regard to the extent and prevalence of NPS use and the associated harms. This calls for a systemic multicentric research study to understand the extent and pattern of NPS use among the treatment-seeking substance abusing population in India. Such an exercise would help in establishing detection methods for NPS. The study would be able to provide an evidence base for the regulatory authorities and policymakers to frame policies and programmes to address the issue of NPS in India.

Objectives

1. To assess the extent and pattern of new psychoactive substances (NPS) use among treatment-seeking substance users.
2. To develop and establish a urine testing procedure for NPS among treatment-seeking substance users.

Methodology

I. **Collaborating sites of study:** The study was conducted at five sites:

i. National Drug Dependence Treatment Centre (NDDTC), All India Institute of Medical Sciences (Nodal Site)

ii. Regional Institute of Medical Sciences (RIMS), Imphal, Manipur

iii. Government Medical College (GMC), Amritsar, Punjab

iv. All India Institute of Medical Sciences (AIIMS), Rishikesh, Uttarakhand

v. All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Orissa

II. Study Design: A prospective cross-sectional observational study involving patients seeking treatment for psychoactive substance use

Inclusion criteria for patient recruitment:

- Male gender
- Age 18 – 60 years
- Patients fulfilling ICD-10 criteria for harmful use or dependence syndrome
- Willing to provide consent to take part in the study and to provide a biological sample for laboratory analysis

Exclusion criteria for patient recruitment:

- Seeking treatment primarily for alcohol use disorder

Study Samples: The cases comprised of the patients from NDDTC, Ghaziabad, N=1000; RIMS Imphal N=200; GMC, Amritsar N=200; AIIMS, Rishikesh N=200, and AIIMS Bhubaneswar N-200) coming to the outpatient clinics of the deaddiction/psychiatry OPD of the respective institutions.

III. Study procedure

The study was conducted after obtaining approval from the institutional ethical committee of the AIIMS, New Delhi, and other collaborating sites. All the subjects for the study were selected as per the inclusion and exclusion criteria mentioned above. A valid written informed consent was taken from all the patients before inclusion in the study. Conditions of confidentiality and anonymity were ensured throughout the study. The project staff at each collaborating site were trained by the NDDTC AIIMS project investigators to interview patients to collect clinical details and collect urine samples.

For each patient, socio-demographic profile (Age, Sex, Marital status, Employment, Education), including medical history, drug use history, and current drug use was recorded using a semi-structured proforma prepared for the purpose of the study (Data Collection tool attached-Annexure-I). Patients were also asked whether they have used any NPS in their lifetime. If the patient reported the use of any NPS, details of the NPS in terms of the frequency, mode of use, effect of NPS, mode of procurement, etc. were also collected. Additionally, brief clinical history and diagnosis, the type of specimen to be tested, the substances used in the past 72 hours, and information regarding the use of any medication were also recorded on the urine screening request form

(as per Annexure-I). Thereafter, five ml of urine was collected from each subject in a leak-proof plastic container by the project laboratory staff, under close supervision to prevent the risk of tampering. After proper labelling and sealing, the urine sample was sent to the NDDTC Drug Abuse Testing laboratory for analysis. All samples were stored at -20^{0}C till analysis to prevent degradation.samples from each site were transported in liquid nitrogen to the Drug Abuse Screening Laboratory, NDDTC, AIIMS. The samples from each site were screened for all the drugs of abuse and NPS.

Laboratory Investigations at NDDTC

I. Screening of urine for drugs of abuse

Following the receipt of the samples in the Drug Abuse Screening Lab at the NDDTC, AIIMS, routine urine testing was performed to confirm the presence of substance of abuse using the Cassette test (ABON Multi-drug one-step screen test panel, Abon Biopharm Co. Ltd, China). The Cassette test is an immunochromatography-based one-step in vitro test. Briefly, the test is based on the principle of specific-immunochemical reaction between antibodies and antigens to analyse specific substances in human urine specimens. The assay depends on the competition between drug conjugate and free drug, which may be present in the urine specimen being examined, for binding antibody. This testing looked for the presence of various substances such as opioids (morphine, buprenorphine, dextro-propoxyphene, tramadol), benzodiazepines, barbiturates, amphetamines, cocaine, and cannabinoids (THC). The cut-off levels for tramadol, morphine, buprenorphine, benzodiazepine, cannabinoids (THC), amphetamine, barbiturates, cocaine, and propoxyphene are 200, 300, 10, 300, 500, 50, 1000, 300, 300, 300 (ng/mL) respectively.

II. Screening of Newer Psychoactive Substances in urine

All the urine samples were screened for NPS by Bioarray Chip technology (BAT) based kits using an Evidence Investigator Analyser (Randox Laboratories Ltd. UK).

The biochip array technology enables multiple competitive immunoassays simultaneously. The drug in the sample and the drug labelled with horseradish peroxidase (HRP) compete for binding sites on the immobilized polyclonal antibody. A signal reagent is added to the biochip to generate a chemiluminescent signal that is compared to the intensity of the calibrator. The chemiluminescent signal is detected with a digital imaging technique, i.e. charged coupled device (CCD) camera, with readings compared to calibrator signals. The signal intensity is inversely proportional to the urinary drug analyte concentration. The higher the drug concentration in urine, the lesser unbound antibody is available for the HRP-labelled drug to elicit a chemiluminescent response. The signal intensity of the samples was compared with the signal intensity of the calibrator and concentrations were determined based on the calibration curve.

In the current study, Randox Newer Psychoactive for NPS I and II test assays were used. Table-I lists the urinary NPS drugs screened and the manufacturer's cut-off values.

II. Confirmatory test for Newer Psychoactive Substances in urine

Based on the findings of screening results, all the positive samples of different groups of aforesaid NPS were further confirmed by liquid chromatography-QTOF-mass spectrometry (LC–QTOF-MS). Samples were considered true positive (TP), if the BAT assay and LC- QTOF -MS were positive.

Sample preparation procedure for standards and test specimens

Stock solutions of reference standards and internal standards were prepared in methanol (MeOH) to a

Table-1. Newer Psychoactive Substances and their Cut-Offs in urine

Analyte	Cut-Offs
JWH-018	10ng/mL
AB-PINACA	5ng/mL
AB-CHMINACA	2ng/mL
Mephedrone	5ng/mL
Alpha-PVP	7.5ng/mL
Salvinorin	1ng/mL
Benzylpiperazines	10ng/mL
Mescaline	0.5ng/mL
Phenylpiperazines 1	7.5ng/mL
Phenylpiperazines II	7.5ng/mL
Acetylfentanyl	1ng/mL
Carfentanil	0.25ng/mL
Ocfentanyl	2ng/mL
AH-7921	1ng/mL
MT-45	2ng/mL
U-47700	10ng/mL
W-19	2ng/mL
Etizolam	2ng/mL
Mitragynine	1ng/mL

concentration of 1mg/mL and were stored at -20^{0}C till analysis. Standard working solutions were serially diluted from stock solutions with MeOH/MeOH:H_20 (v/v). One ml of urine was centrifuged at 14,000 rpm for 5 minutes at 4^{0}C. Following centrifugation, 200µl of urine specimen was diluted with 800µl of deionized water and methanol (50:50) /methanol. The 250 µl of diluted sample was transferred to an autosampler vial, and 10 µl was injected onto the LC column. All the urine samples were processed in a similar manner. All the reagents used were of LC-MS grade.

Instrumentation and experimental conditions

A sensitive and specific method for the analysis of NPS in urine was developed using LC-QTOF-Mass spectrometry. Instrumentation included an EXION AD LC system *(*ultra-high pressure liquid chromatography) equipped with an autosampler and two micropumps, and coupled to the AB Sciex Triple TOF 5600+ System. Chromatographic separation was achieved with Kinetex® C-8 column (2.6 µm 100 x 3mm i.d Phenomenex) with a guard column (Security Guard™ ULTRA C-8, 2.6 mm Phenomenex). The mixture of 0.1% formic acid

in water (A) and 0.1% formic acid in 95 % methanol (B) was used as a mobile phase for all the NPS drugs except for carfentanil where a mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was used as a mobile phase. The gradient program was 0- 2.5 min, 5% B; 2.5-8.8 min, 90% B; 8.8-10.4 min, 90% B; 10.4-11.0 min 90% B; 11.0-13.0 min, 90% B; 13.0-14.0 min, 90% B; 14.0- 14.4 min, 5% B; 14.4- 15.0 min, 5% (B). The gradient flow rate was 0.3ml/min for 0-2.5 min and then 0.5m/ml for 2.5-10.5min. The column temperature was maintained at during the analysis and the injection volume was 10µl. The total run time was 15 min. Analytes were detected by high-resolution mass spectrometry in positive electrospray ionization (ESI) mode. For the positive ionization method, the source parameters were: curtain gas, 40 psi; nebulizing gas (GS1), 50 psi; heater gas (GS2), 50 psi; interface temperature, 50°C; collision energy (CE), 35V; collision energy spread (CES), 15V; declustering potential (DP), 80 V; ion spray voltage (ISVF), 5500 V. The TOF mass range was 50-500 (Da). All data were acquired and processed using the AB Sciex Analyst TF 1.7.1 software.

Statistical analysis

Descriptive Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 22 for Windows (Chicago, Illinois, USA).

Results

Subjects:

Data were collected from a total of five centres spread across the country including a total of 1476 patients. The centres and the number of patients recruited from each centre included are shown in table 2.

Table 2. Distribution of the Sample (N=1476)

City	Name of the centre	Number of participants	Percentages
Ghaziabad (Uttar Pradesh)	NDDTC, AIIMS	1000	67.8%
Bhubaneshwar (Odisha)	AIIMS	194	13.1%
Imphal (Manipur)	Regional Institute of Medical Sciences	111	7.5%
Rishikesh (Uttarakhand)	AIIMS	66	4.4%
Amritsar (Punjab)	Government Medical College	105	7.1%

Sociodemographic profile

The mean age of the participants was 28.2 years (SD: 8.4). All participants were males. Fifty-three percent (783) of the subjects were unmarried and 44.5% (N=657) were married and living with their wives. A small number (36, 2.4%)) were either divorced, separated, or widowers. Eighty percent of the subjects had received for varying years till the 12th standard. About 5% (49) of the subjects were illiterate and 15% (221) were graduates. About 30% (n=436) were currently unemployed, while the rest were either fully or part-time employed. Sixty- nine percent (n=1018) of the subjects were residing in urban areas with about 5% (n=72) in urban slums. Sixty-eight percent (n=1002) of the subjects were Hindus and 20.9% (n=309) belonged to the Muslim religion. The mean monthly family income was INR 35,280/- (SD: 14148), while mean monthly income of the subjects was INR 17600/- (SD: 14139/-). For most respondents, the most common source of their own income was income from job (38.1%, n=563) followed by business (21.8%, n=322).

SUBSTANCE USE HISTORY

The most common psychoactive substance used by the participants in their lifetime was tobacco (95.7%, n=1412), followed by opioids (93.8%, n=1385).

Table-3 provides the lifetime and past three-month use of various psychoactive substances.

Table-3. Lifetime and past three-month use of various psychoactive substances. (N= 1476)

Sl. No.	Substance	Ever Use N (%)	Past three months N (%)
1	Tobacco products	1412 (95.7%)	1394 (94.4%)
2	Alcoholic beverages	1114 (75.5%)	870 (58.9%)
3	Cannabis	1059 (71.7%)	939 (63.6%)
4	Cocaine	110 (7.5%)	49 (3.3%)
5	Amphetamine type stimulants	40 (2.7%)	20 (1.3%)
6	Inhalants	166 (11.2%)	66 (4.4%)
7	Sedatives or sleeping pills	443 (30%)	338 (22.8%)
8	Hallucinogens	73 (4.9%)	16 (1.1%)
9	Opioids	1385 (93.8%)	1356 (91.8%)

Twenty-sixpercent (n=385) of the subjects had a family member with history of substance use. The most common substance usedby family members was alcohol (10%, n=147).

RISK AS ASSESSED ON WHO-ALCOHOL, SMOKING AND SUBSTANCE INVOLVEMENT SCREENING TEST (ASSIST)

WHO-ASSIST was also applied to the participants to assess the risk category of various substances. Low risk denotes the need for no active intervention, while moderate risk denotes that the participant is

Table 4. Risk status of the subjects as assessed on the WHO ASSIST

Substance	Low-Risk (n, %)	Moderate-Risk (n, %)	High-Risk (n, %)
Alcohol	894 (60.6%)	186 (12.6%)	152 (10.3%)
Cannabis	317 (21.5%)	630 (42.7%	264 (17.9%)
Cocaine	1025 (69.4%)	46 (3.1%)	9 (0.6%)
Amphetamine type stimulants	1065 (72.2%)	19 (1.3%)	1
Inhalants	1009 (68.4%)	62 (4.2%)	24 (1.6%)
Sedatives or sleeping pills	801 (54.3%)	180 (12.2%)	155 (10.5%)
Hallucinogens	1055 (71.5%)	21 (1.4%)	2
Opioids	19 (1.3%)	49 (3.3%)	1280 (91.3%)

in harmful use category, while high risk denotes that the participants is dependent on the substance in question, with both needing intervention. The risk category of various substances is shown in table 4.

Most of the subjects were at 'high-risk' of opioid use indicating dependence on opioids. Similarly, most subjects were also at moderate or high risk of cannabis use. About 31% (n=465) of the subjects reported having ever been abstinent from psychoactive substances for at least one month in their lifetime. Similarly, 17% (n=253) of the subjects reported taking treatment for substance use disorder in the past.

Injecting drug use

Twenty nine percent (n=424) of the participants reported injecting psychoactive substance at least once in their lifetime. Heroin was the most common opioid injected followed by buprenorphine injections. Eight percent (n=122) of the injection drug users (IDUs) reported sharing needles/syringes ever in their lifetime. Thirteen percent (n=195) of the IDUs reported reusing needles/syringes, and 7% (n=105) reported having abscess or ulcers, while 14% (n=210) had vein related complications due to their injecting drug use. Fifteen percent (n=225) of the IDUs reported having overdosed on opioids. Fifty eight percent (n=245) of the IDUs reported getting tested for HIV, with 17 having being detected as HIV positive. Fourteen subjects were on regular anti-retroviral treatment. Five percent (n=74) of the total sample reported being Hepatitis-C positive, while 2.7% (n=40) reported being Hepatitis-B. Only twenty one of them reported being treated for Hepatitis in their lifetime.

New psychoactive substances

Forty-three (2.9%) subjects reported using a substance that were not the most commonly used drugs. Of these, 21 reported using MDMA, 17 reported using 'meow meow', 5 reported using LSD, while one reported using ketamine. Mean age of these subjects was 23.1 years. While ketamine, LSD, and meow-meow were used only a couple of times by majority of participants, MDMA was used more frequently – multiple times in their lifetime. Most of the patients had used these substances within the past one year.

MDMA was used by 21 participants. The average age of use of MDMA was 22.5 years. Many of them had used the substance up to 10 to 12 times in their lifetime in the previous 2-3 months. All the participants reported that MDMA is available in solid form. Almost all participants had snorted the substance, except one who had injected it. All had obtained the drug either from a user or from a drug dealer. The average cost for one dose was 3755 INR. Almost all participants felt active after using the drug, while five participants experienced hallucinations. The effect of the drug lasted for almost 24 hours. Two users reported negative effect due to the drug who experienced vomiting after consuming the drug. Three subjects developed compulsions to consume the drug. Six subjects indulged in risky behaviour after consuming MDMA. The risky behaviour included indulging in aggressive behaviour, unprotected sexual intercourse, and risky driving. Three subjects reported being caught by police for using the substance. None of them felt that using MDMA was harmful.

Seventeen subjects reported using meow-meow, available in either powder form or as a solid, and almost all consumed it through snorting route. Two subjects reported injecting the substance. Eleven users reported obtaining it through a drug dealer, while 6 obtained it from another user friend. The mean cost of one dose was around 3500 INR. Almost all felt very active after using it. The effect of one dose lasted for at least 24 hours. Most of the users did not have any negative effect of the substance after consumption or after the effect of the substance wore down. Of the 4 users, who experienced negative effects, weakness, extreme sleepiness, vomiting, and illusions were reported by one participant each. Only 3 out of 17 participants reported that they felt compulsion to take the substance. Six subjects reported indulging in risky behaviour after consuming the substance, the most common being increased sexual intercourse due to increased sexual desire. Two subjects reported getting caught by police for consuming the substance. Roughly half of the subjects felt that the substance use is harmless while the rest felt it is harmful.

Five participants reported use of LSD.

In the current study, overall, 1462 urine samples were analysed from five sites. Figure 1 shows the urinalysis results of drugs in patient samples (N=1462) of all sites that were found to be positive. Overall, the drugs detected were morphine (63.8%), benzodiazepine (41.99%), cannabinoids-THC (40.9%), tramadol (34.06%), buprenorphine (21.5%), barbiturates (1.36%), cocaine (0.95%) and amphetamine (0.47%).

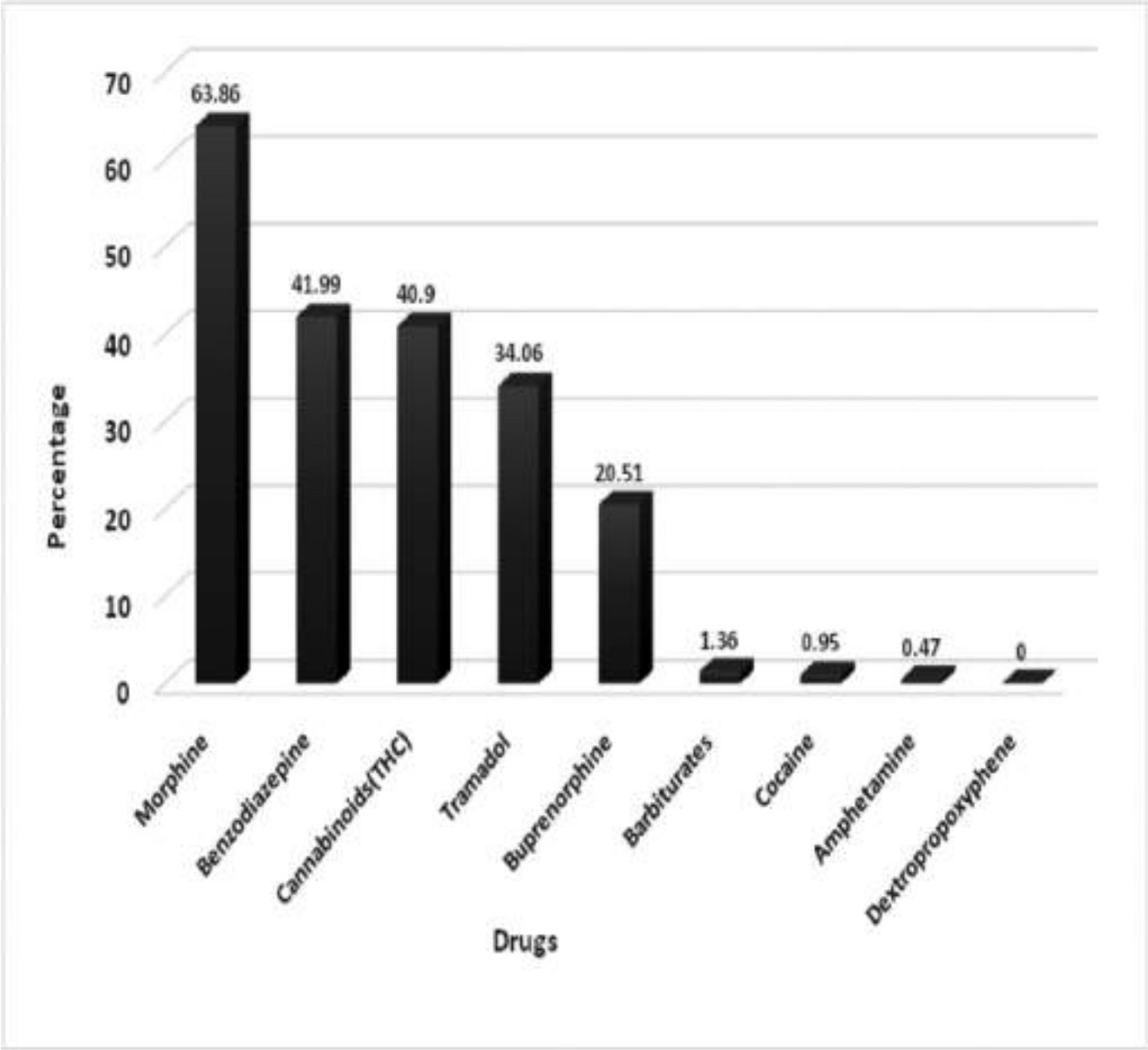

Figure - 1. Urine Analysis Results of Drugs (N=1462)

Figure 2 depicts urinalysis results of drugs screened by cassette test in urine samples (N=1000) collected at NDDTC, Ghaziabad (Nodal site). Analysis indicated that percentage of positive tests varied from 69.0% to 0.2% among different drug types: morphine (68.7%), cannabinoids-THC (52.2%), benzodiazepines (42.0%), tramadol (24.6%), buprenorphine (15.2%), barbiturates (1.7%), cocaine (0.9%), amphetamine (0.4%) and dextro-propoxyphene (0.2%).

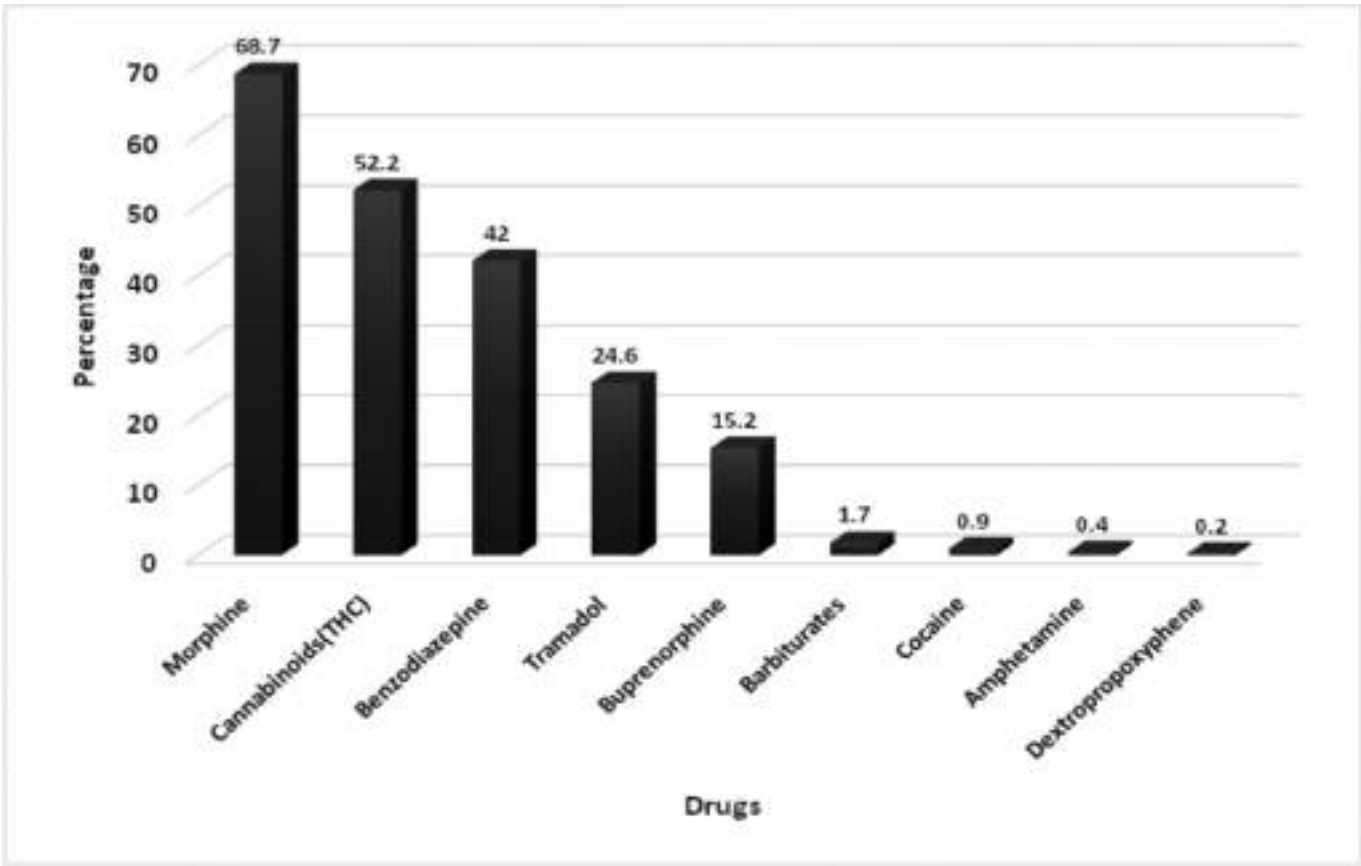

Figure - 2. Delhi site: Urinalysis results of Drugs (N=1000)

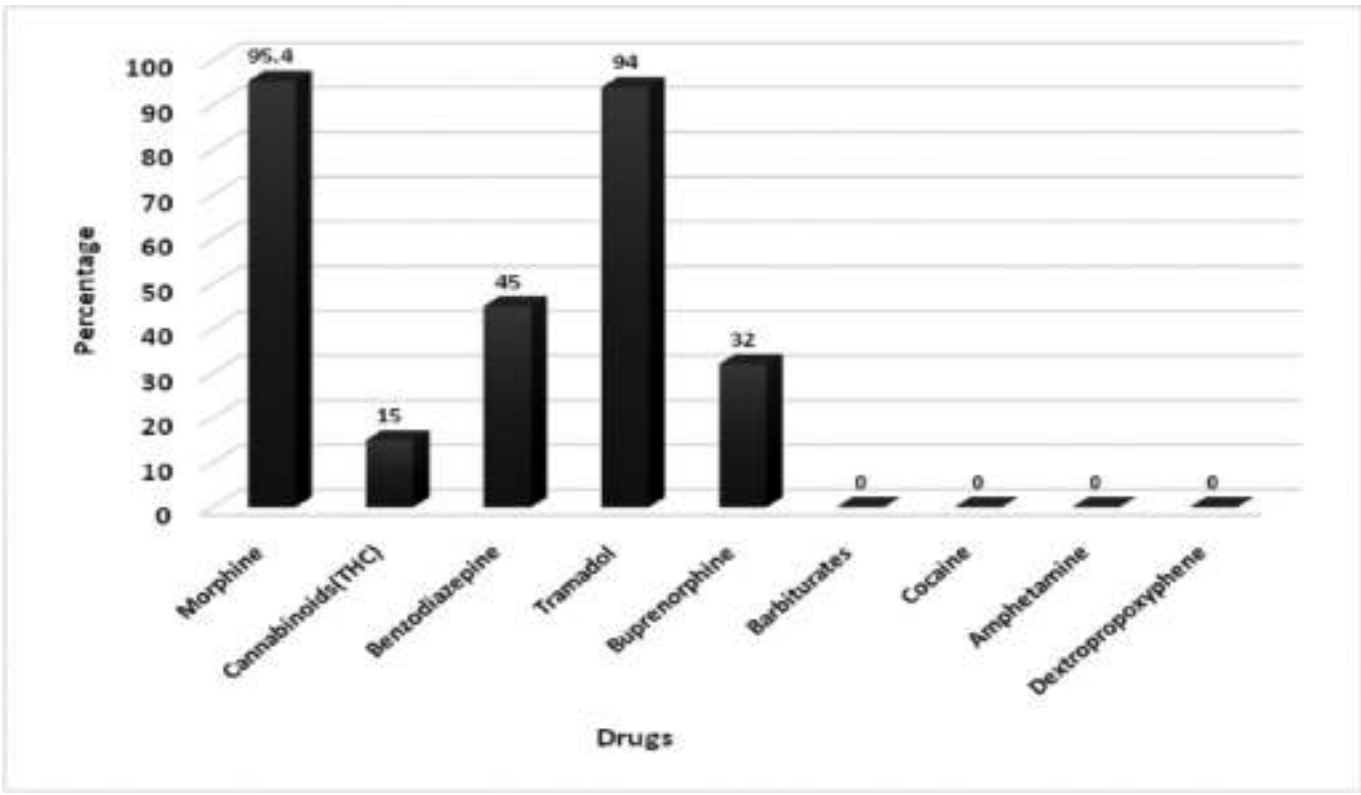

Figure - 3. Amritsar site: Urinalysis results of Drugs (N=87)

Figure 3 shows the urinalysis results of drugs screened by cassette test in urine samples (N=87) received from the Amritsar site (Punjab). Morphine was the most common drug detected constituting 95.4% of all identified drugs. The percentage of other drugs detected were tramadol (94.08%), benzodiazepine (32.0%), cannabinoids -THC (15.0%) and buprenorphine (32.0%).

Figure 4 displays the urinalysis results of drugs screened by cassette test in urine samples (N=192) received from the Bhubaneswar site (Orissa). Data from drug abuse screening positive results indicated that 35.4% were positive for tramadol. Other positive drug test results include buprenorphine (30.72%), benzodiazepine (22.91%), cannabinoids - THC (17.18%), morphine (7.71%), and barbiturates (1.04%).

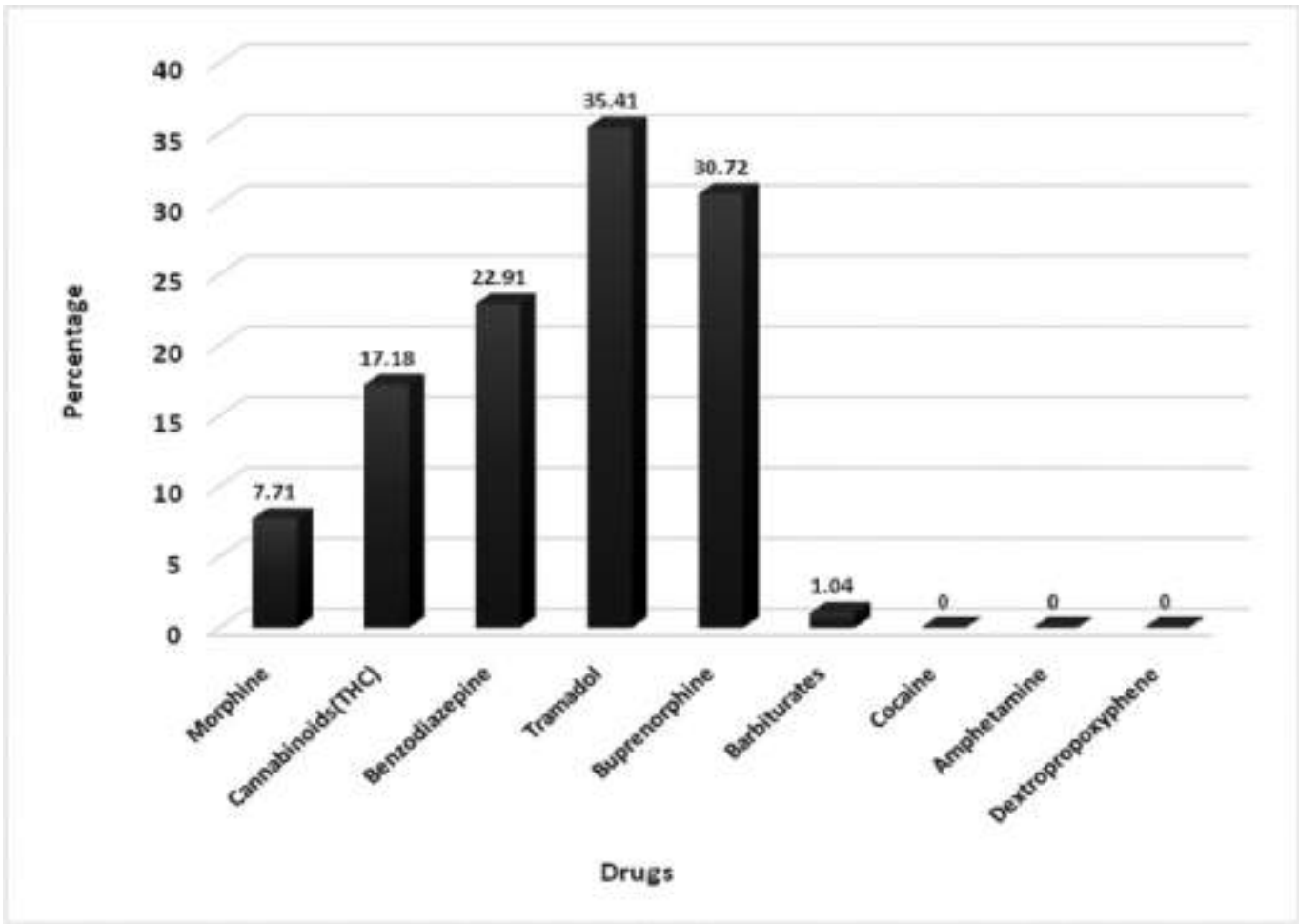

Figure - 4. Bhubaneswar site: Urinalysis results of Drugs (N=192)

Figure 5 shows the urinalysis results of drugs screened by cassette test in urine samples (N=120) received from the Imphal site (Manipur). The urine screening results showed the presence of opioids in 90.83 % of patients. Other substances that tested positive were cannabinoids (10%), benzodiazepine (53.33%), tramadol (43.33%), buprenorphine (15%) and amphetamine (1.66%).

The urinalysis results of drugs screened by cassette test in urine samples from the Rishikesh site (N=63) are shown in Figure 6. Based on urine screening results, opioids were detected in 55.55% of patients. Other drugs detected were cannabinoids (28.57%), benzodiazepine (68.25%), tramadol (79.36%), buprenorphine (68.25%) and barbiturates (1.58%).

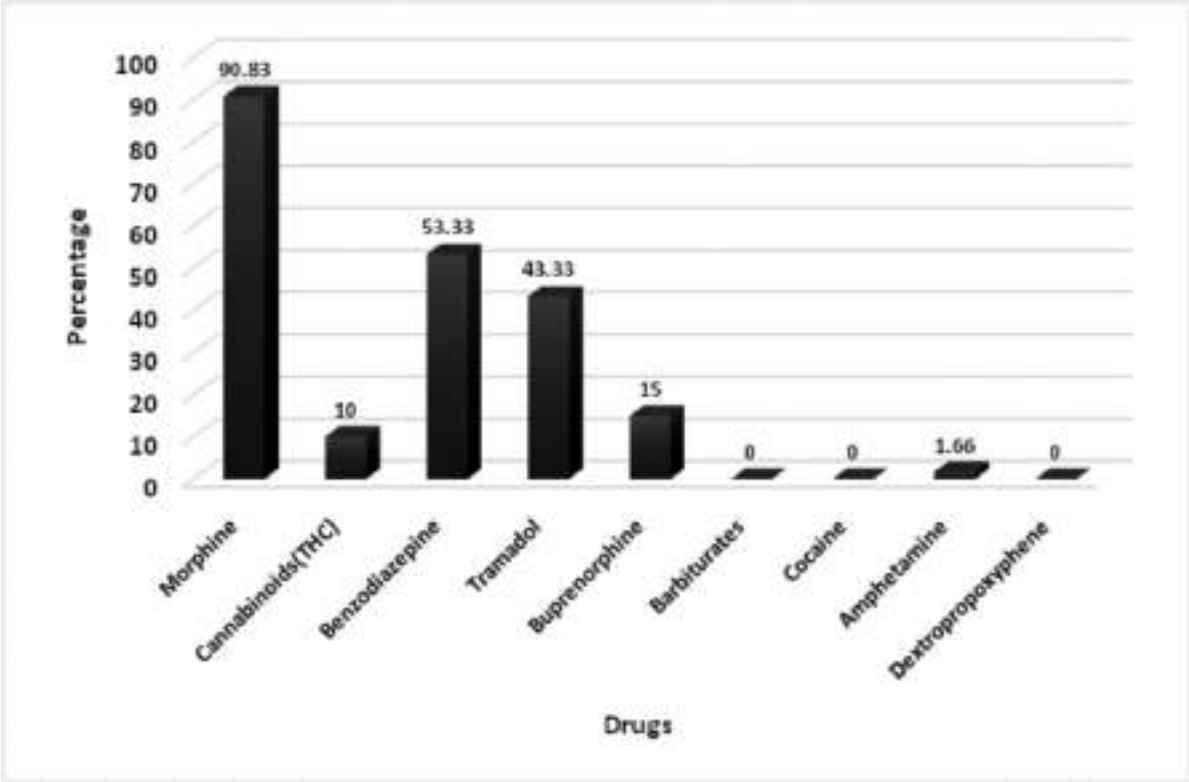

Figure - 5. Imphal site: Urinalysis results of Drugs (N=120)

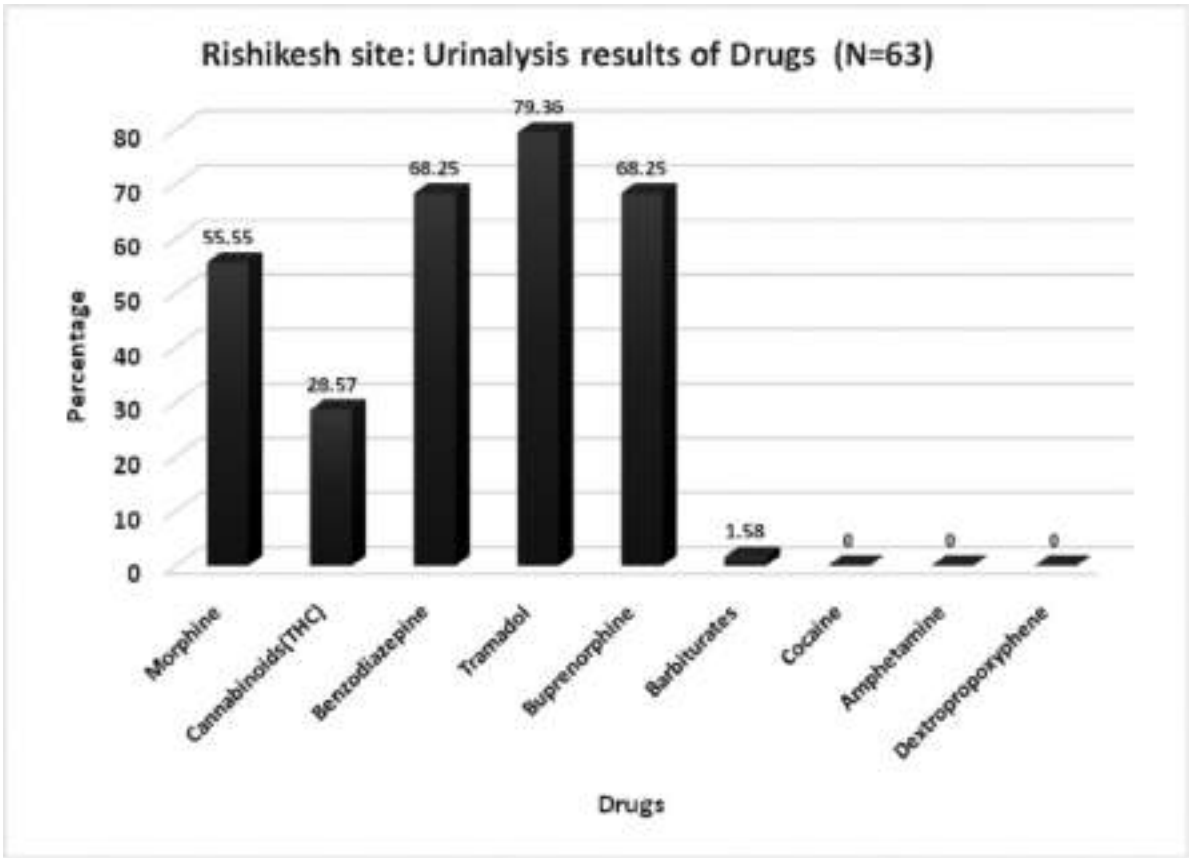

Figure - 6.

Figure 7 compares the percentage of positive drug test findings for each drug group in urine samples of patients collected from various sites. Each site had a distinct pattern of drug use as discussed below.

NDDTC Ghaziabad Site: In 68.7% of the morphine-positive samples, cannabinoids-THC (58.29%), tramadol (21.87%), buprenorphine (9.69%), and benzodiazepines (45.08%) were detected, showing polydrug use among opiate users.

Amritsar Site: The majority of samples tested positive for morphine and tramadol. The morphine-positive samples (95.4%) showed the presence of cannabinoids -THC (15.66%), tramadol (94%), buprenorphine (31.32%), and benzodiazepines (43.37%).

Bhubaneswar Site: Tramadol was the most common drug detected, constituting 35.4% of the positive drug tests. In tramadol-positive samples, other drugs like cannabinoids-THC (27.94%), morphine (7.35%), buprenorphine (52.94%) and benzodiazepines (36.76%) were also detected.

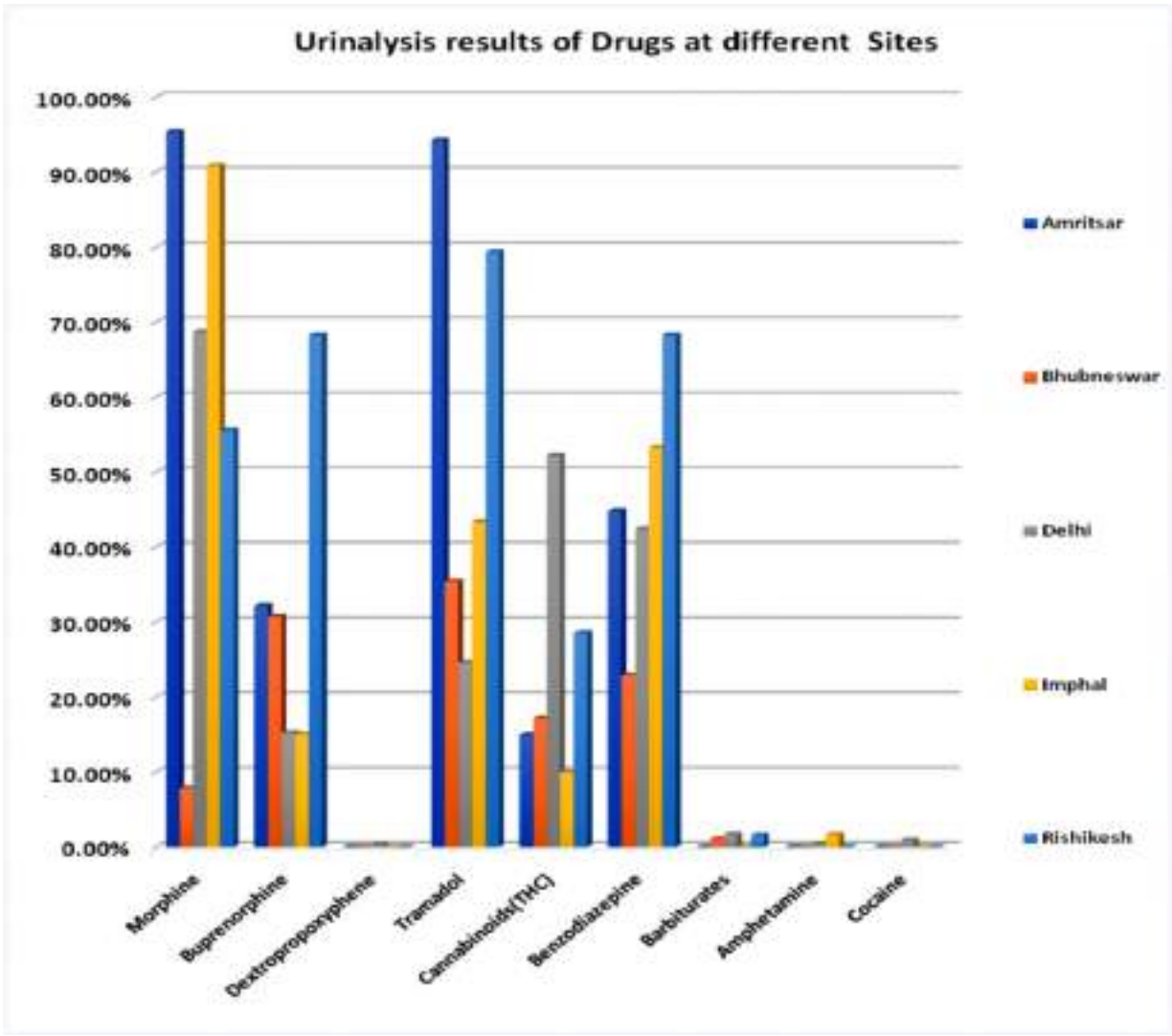

Figure 7

Figure 8 illustrates the total positive urine findings for NPS drugs in patient samples from all sites (N=1462). Urine BAT immunoassay results were confirmed by LC-QTOF-MS to evaluate the diagnostic efficiency. In LC-QTOF-MS, the cut-off for all drug categories was the same as described previously in Table 1.

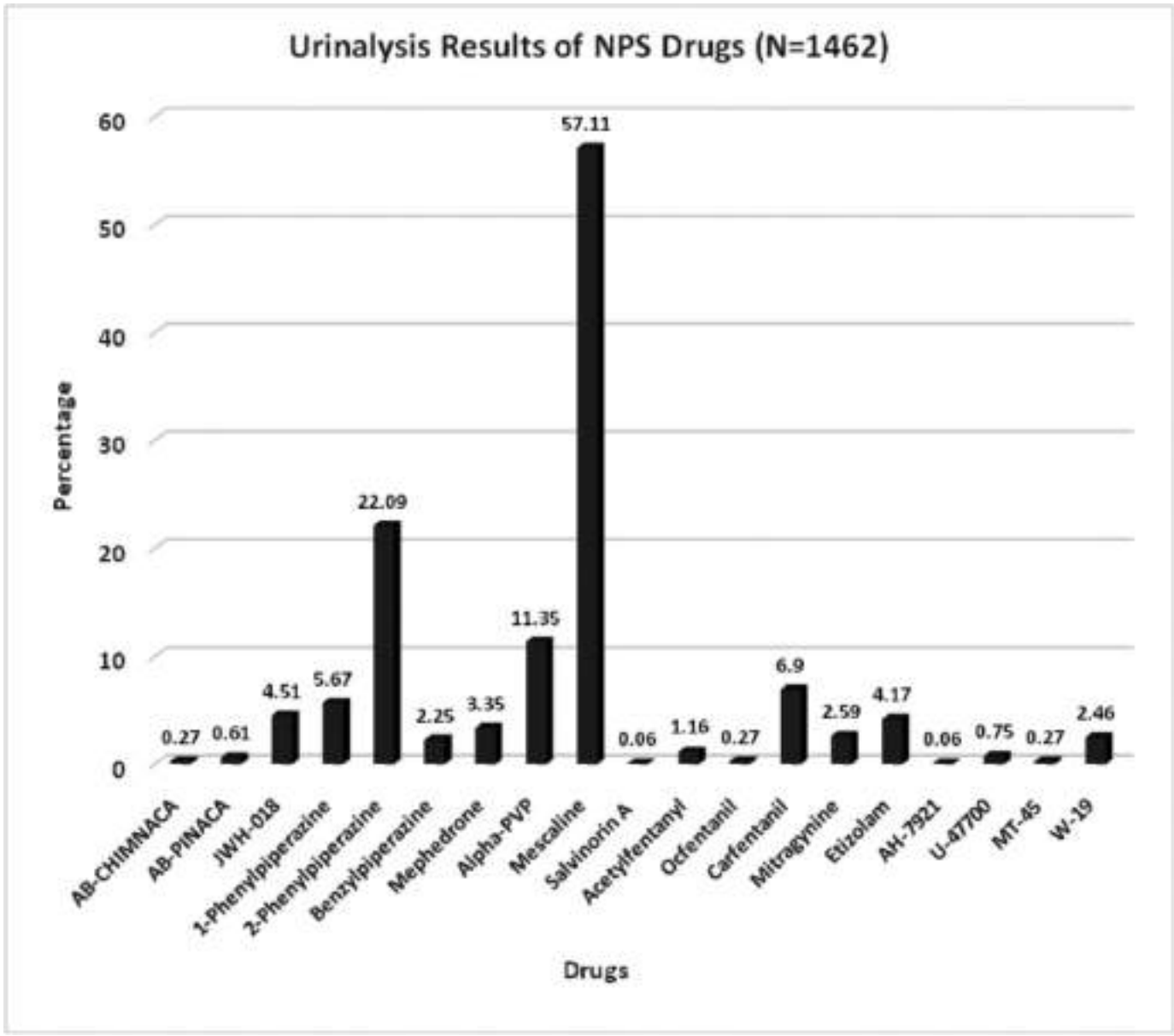

Figure - 8

Imphal Site: The majority of the samples were morphine positive. The morphine positive samples (90.83%) also showed the presence of cannabinoids-THC (8.25%), tramadol (41.28%), buprenorphine (14.67%) and benzodiazepines (51.37%).

Rishikesh Site: The most common substance found in the samples was tramadol. Other substances detected in the tramadol-positive samples (79.36%) included morphine (62.0%), buprenorphine (64%), benzodiazepines (70%), and cannabinoids-THC (28%).

These findings suggest concomitant use of one or more drugs in these patients. The laboratory results also indicate a high detection rate of prescription drugs, like buprenorphine, tramadol and benzodiazepines at the collaborating sites. It is likely that some of the individuals were receiving treatment and had been prescribed these medications. The laboratory results do not appear to reveal the precise abuse of these prescription medications.

Table 5 lists the positive urine findings and percentage of NPS drugs in patient samples from each site.

Table 5. Urine Testing Results of New Psychoactive Substances (N=1462) at each site

Sr. No.	Name of Drugs	Class of drugs	Delhi (N=1000) No. of Positives (%)	Amritsar (N=87) No. of Positives (%)	Bhubaneswar (N=192) No. of Positives (%)	Imphal (N=120) No. of Positives (%)	Rishikesh (N=63) No. of Positives (%)
1.	AB-CHIMNACA	Synthetic cannabinoids	2 (0.2)	0 (0)	0 (0)	1 (0.83)	1 (1.58)
2.	AB-PINACA	Synthetic cannabinoids	9 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)
3.	JWH-018	Synthetic cannabinoids	66 (6.6)	0 (0)	0 (0)	0 (0)	0 (0)
4.	PNP I	Phenylpiperazine I	58 (5.8)	8 (9.19)	1 (0.5)	5 (4.16)	11 (17.46)
5.	PNP II	Phenylpiperazine II	258 (25.8)	18 (20.68)	8 (4.16)	18 (15.0)	21 (33.33)
6.	BZP	Benzylpiperazine	24 (2.4)	4 (4.59)	0 (0)	3 (2.5)	2 (3.17)
7.	Mephedrone	Synthetic Cathinone (Stimulant)	44 (4.4)	1 (1.14)	0 (0)	3 (2.5)	1 (1.58)
8.	Alpha-PVP	Synthetic Cathinone (Stimulant)	144 (14.4)	7 (8.04)	0 (0)	9 (7.5)	6 (9.52)
9.	Mescaline	Phenethylamine (Hallucinogen)	793 (79.3)	7 (8.04)	8 (4.16)	4 (3.33)	23 (36.5)
10.	Salvinorin A	kappa opioid receptor agonist	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
11.	Acetyl Fentanyl	Fentanyl analog	10 (1)	0 (0)	0 (0)	3 (2.5)	4 (6.34)
12.	Ocfentanyl	Fentanyl analog	2 (0.2)	1 (1.14)	0 (0)	1 (0.83)	0 (0)
13.	Carfentanil	Fentanyl analog	73 (7.3)	7 (8.04)	2 (1.04)	13 (10.83)	6 (9.52)
14.	Mitragynine	Indole-based alkaloid	33 (3.3)	1 (1.4)	1 (0.5)	3 (2.5)	0 (0)
15.	Etizolam	Thienodiazepine derivative	47 (4.7)	4 (4.59)	1 (0.5)	6 (5.0)	3 (4.76)
16.	AH-7921	Synthetic opioid analgesic	0 (0)	0 (0)	0 (0)	1 (0.83)	0 (0)
17.	U-47700	Synthetic opioid analgesic	9 (0.9)	0 (0)	0 (0)	2 (1.66)	0 (0)
18.	MT-45	Synthetic opioid analgesic	1 (0.1)	0 (0)	0 (0)	2 (1.66)	1 (1.58)
19.	W-19	Synthetic opioid	23 (2.3)	2 (2.29)	1 (0.5)	5 (4.16)	5 (7.93)

Table 6 summarizes the MS- precursor ions, fragment ions (Q1, Q2, Q3) and retention time of the analytes in positive urine samples.

Analysis indicated that the percentage of positive tests varied from 57.11% to 0.06% among different categories of drug types as under:

Synthetic cannabinoids: AB-CHIMNACA (0.27%), AB-PINACA (0.61%), JWH-018 (4.51%)
Stimulants: 1-Phenylpiperazine (5.67%), 2-Phenylpiperazine (22.09%), Benzylpiperazine (2.25%)
Synthetic Cathinones: Mephedrone (3.35%), Alpha-PVP (11.35%)

Fentanyl analogsSynthetic cannabinoids: AB-CHIMNACA (0.27%), AB-PINACA (0.61%), JWH-018 (4.51%)

Benzodiazepine analog: Etizolam (4.17%)

Synthetic Opioid analgesic: AH-7921 (0.06%), MT-45 (0.27%)

Synthetic Opioid Drug: W-19 (2.46%).

Herbal Highs: Mescaline, 57.11% (Hallucinogen), Mitragynine, 2.59% (Stimulant), Salvinorin A 0.06% (Dissociative)

Table 6. MS-Precursor ion, Fragment ions and Retention time of analytes in positive urine samples

Sr. No.	Name of Drugs	Class of drugs	Mol. Wt.	Precursor (m/z+1)	Q1	Q2	Q3	Retention time (RT)
1.	*AB-CHIMNACA	Synthetic cannabinoids	356.4	357.2	357.1	357.2	145.1	9.92
2.	*AB-PINACA	Synthetic cannabinoids	330.43	331.22	145.1	314.1	331.2	9.22
3.	**JWH-018	Synthetic cannabinoids	341.45	342.18	155.0	144.1	119.0	10.35
4.	**PNP I	Phenylpiperazine I	162.23	163.12	117.1	120.1	77.0	6.97
5.	*PNP II	Phenylpiperazine II	162.23	163.12	90.0	102.0	128.1	6.57
6.	**BZP	Benzylpiperazine	176.2	177.14	85.1	65.0	91.1	4.49
7.	**Mephedrone	Synthetic Cathinone(Stimulant)	177.24	178.10	91.1	145.1	130.1	8.37
8.	*Alpha-PVP	Cathinone Synthetic (Stimulant)	231.33	232.20	126.1	105.0	77.0	9.19
9.	**Mescaline	Phenethylamine (Hallucinogen)	211.26	212.10	91.1	165.1	195.1	6.73
10.	**Salvinorin A	kappa opioid receptor agonist	432.46	433.19	355.2	415.2	373.2	8.72
11.	**Acetyl Fentanyl	Fentanyl analog	322.45	323.21	105.0	188.1	323.2	9.62
12.	*Ocfentanyl	Fentanyl analog	370.46	371.20	267.0	73.0	350.9	8.81
13.	*Carfentanil	Fentanyl analog	394.50	395.23	395.2	396.2	81.1	5.70
14.	**Mitragynine	Indole-based alkaloid	398.50	399.23	174.1	226.1	367.1	10.62
15.	*Etizolam	Thienodiazepine derivative	442.84	443.08	81.0	343.1	314.0	8.28
16.	*AH-7921	Synthetic opioid analgesic	329.26	329.12	127.1	67.1	119.0	9.50
17.	*U-47700	Synthetic opioid analgesic	329.26	329.12	85.0	99.0	145.1	10.74
18.	*MT-45	Synthetic opioid analgesic	348.52	349.26	141.0	181.1	105.0	7.19
19.	**W-19	Synthetic opioid	390.12	391.12	355.2	149.0	119.0	9.19

Note: *Detection of these drugs was based on Library match; ** Detection based on Reference Standards

Based on precursor ion and fragmentation pattern of the NIST library, NPS drugs AB-CHIMNACA, AB-PINACA, PNP II (2- phenylpiperazine), Alpha-PVP, Ocfentanyl, Carfentanyl, Etizolam, AH-7921, U-4770 0, MT-45 were confirmed.

Figures 9-16 displays the Chromatograms of 50ng/ml and MS/MS fragmentation of drug standards (Phenylpiperazine-1, Benzylpiperazine, Phenylpiperazine-1-D8, Benzylpiperazine-D7, W-19, Acetylfentanyl, JWH-018, Salvinorin A, Mescaline, Mitragynine, Mephedrone, Mephedrone-D3. (Figures 9–16 available at the end of the report).

Table 7-10 shows urine testing results of various categories of NPS among morphine, buprenorphine, tramadol and cannabinoids positive samples at all the sites.

Analytical findings of urine specimens are indicative of multiple drug use and combination pattern at all sites. Data derived from these urine testing results are encouraging.

Table 7. Urine testing results of various categories of new psychoactive substances among morphine, buprenorphine, tramadol and cannabinoids positive samples at Delhi site (N=1000)

Sr. No.	Name of Drugs	Morphine N=687 % Positives	Buprenor-phine N=152 % Positives	Tramadol N=246 % Positives	Cannabinoids N = 522 % Positives
1.	AB-CHIMNACA	2 (2.29)	0 (0)	0 (0)	2 (0.3)
2.	AB-PINACA	8 (1.16)	2 (1.3)	3 (1.2)	7 (1.3)
3.	JWH-018	51 (7.14)	8 (5.26)	19 (7.7)	48 (9.1)
4.	PNP I	43 (6.2)	11 (7.2)	17 (6.9)	33 (6.32)
5.	PNP II	174 (25.3)	44 (28.9)	63 (25.6)	132 (25.2)
6.	BZP	17 (2.47)	6 (3.9)	8 (3.2)	13 (2.4)
7.	Mephedrone	38 (5.5)	3 (1.9)	11 (4.4)	24 (4.5)
8.	Alpha-PVP	76 (11.0)	45 (29.6)	83 (33.7)	69 (13.2)
9.	Mescaline	615 (89.5)	108 (71.0)	180 (73.1)	431 (82.5)
10.	Salvinorin A	0 (0)	0 (0)	0 (0)	0 (0)
11.	Acetyl Fentanyl	9 (1.3)	1 (0.6)	3 (1.2)	7 (1.3)
12.	Ocfentanyl	1 (0.14)	0 (0)	0 (0)	1 (0.1)
13.	Carfentanil	55 (8.0)	13 (8.5)	24 (9.7)	54 (10.3)
14.	Mitragynine	18 (2.6)	9 (5.9)	17 (6.9)	12 (2.2)
15.	Etizolam	34 (4.9)	6 (3.9)	12 (4.8)	21 (4.0)
16.	AH-7921	0 (0)	0 (0)	0 (0)	0 (0)
17.	U-47700	7 (1.0)	1 (0.6)	5 (2.0)	6 (1.1)
18.	MT-45	1 (0.14)	0 (0)	0 (0)	0 (0)
19.	W-19	18 (2.6)	1 (0.6)	6 (2.4)	11 (2.1)

Table 8. Urine testing results of various categories of new psychoactive substances among morphine, buprenorphine, tramadol and cannabinoids positive samples at Amritsar site (N=87)

Sr. No.	Name of Drugs	Morphine N=83 % Positives	Buprenor-phine N=28 % Positives	Tramadol N=82 % Positives	Cannabinoids N = 522 % Positives
1.	AB-CHIMNACA	0 (0)	0 (0)	0 (0)	0 (0)
2.	AB-PINACA	0 (0)	0 (0)	0 (0)	0 (0)
3.	JWH-018	0 (0)	0 (0)	0 (0)	0 (0)
4.	PNP I	8 (9.6)	5 (17.8)	8 (9.7)	1 (7.6)
5.	PNP II	18 (21.6)	9 (32.1)	18 (21.9)	2 (15.3)
6.	BZP	4 (4.8)	1 (3.5)	4 (4.8)	0 (0)
7.	Mephedrone	1 (1.2)	1 (3.5)	1 (1.2)	0 (0)
8.	Alpha-PVP	6 (7.2)	4 (14.2)	6 (7.3)	1 (7.6)
9.	Mescaline	7 (8.4)	6 (21.4)	6 (7.3)	1 (7.6)
10.	Salvinorin A	0 (0)	0 (0)	0 (0)	0 (0)
11.	Acetyl Fentanyl	0 (0)	0 (0)	0 (0)	0 (0)
12.	Ocfentanyl	1 (1.2)	0 (0)	1 (1.2)	0 (0)
13.	Carfentanil	7 (8.4)	1 (3.5)	6 (7.3)	2 (15.3)
14.	Mitragynine	0 (0)	0 (0)	1 (1.2)	0 (0)
15.	Etizolam	4 (4.8)	1 (3.5)	4 (4.8)	0 (0)
16.	AH-7921	0 (0)	0 (0)	0 (0)	0 (0)
17.	U-47700	0 (0)	0 (0)	0(0)	0(0)
18.	MT-45	0 (0)	0 (0)	0(0)	0(0)
19.	W-19	2 (2.4)	1 (3.5)	2(2.4)	0(0)

Discussion

Analysis of drug use in biological fluids plays an important role in addiction treatment services. Urine is often the preferred specimen among biological specimens used to screen recent drug use due to its ease of collection, higher detectable concentrations of drugs, and metabolites.

This is the first study from India to report the NPS objectively in an addiction treatment setting. In the current study, all the urine samples were screened for newer psychoactive substances by Bioarray Chip technology (BAT), based on the competitive binding immunoassay principle. This technique is designed for the qualitative determination of substances in human urine specimens. The results of the analyte obtained from the screening method were confirmed by LC-QTOF-Mass spectrometry (LC-QTOF-MS). The TOF system allows the detection of all eluting analytes at very high data rates. This enables the detection of even a trace amount of psychotropic drugs. This is required since NPS are excreted from the body in small concentrations. Metabolites

Table 9. Urine testing results of various categories of new psychoactive substances among morphine, buprenorphine, tramadol and cannabinoids positive samples at Rishikesh site (N=63)

Sr. No.	Name of Drugs	Morphine/ Opioids N=35 % Positives	Buprenorphine N=43 % Positives	Tramadol N=82 % Positives	Cannabinoids N=18 % Positives
1.	AB-CHIMNACA	0 (0)	0 (0)	1 (1.2)	0 (0)
2.	AB-PINACA	0 (0)	0 (0)	0 (0)	0 (0)
3.	JWH-018	0 (0)	0 (0)	0 (0)	0 (0)
4.	PNP I	6 (17.1)	8 (18.6)	9 (10.9)	3 (16.6)
5.	PNP II	10 (28.7)	14 (32.5)	17 (20.7)	3 (16.6)
6.	BZP	1 (2.8)	2 (4.6)	1 (1.2)	0 (0)
7.	Mephedrone	1 (2.8)	0 (0)	1 (1.2)	0 (0)
8.	Alpha-PVP	3 (8.5)	3 (6.9)	6 (7.3)	1 (5.5)
9.	Mescaline	16 (45.7)	13 (30.2)	17 (20.7)	9 (50)
10.	Salvinorin A	0 (0)	0 (0)	0 (0)	0 (0)
11.	Acetyl Fentanyl	1 (2.8)	3 (6.9)	3 (3.6)	1 (5.5)
12.	Ocfentanyl	0 (0)	0 (0)	0 (0)	0 (0)
13.	Carfentanil	6 (17.1)	3 (6.9)	5 (6.0)	2 (11.1)
14.	Mitragynine	0 (0)	0 (0)	0 (0)	0 (0)
15.	Etizolam	3 (8.5)	2 (4.6)	1 (1.2)	0 (0)
16.	AH-7921	0 (0)	0 (0)	0 (0)	0 (0)
17.	U-47700	0 (0)	0 (0)	0 (0)	0 (0)
18.	MT-45	1 (2.8)	0 (0)	1 (1.2)	1 (5.5)
19.	W-19	4 (11.4)	4 (9.3)	2 (22.4)	4 (22.2)
20.	BUP	17 (48.5)	34 (79.0)	28 (34.1)	14 (77.7)

are also excreted in much smaller concentrations. Thus, the data acquired by LC-QTOF-MS provided analyte-specific results allowing significantly greater sensitivity and specificity. In the current study, both techniques yielded the same results. The characterization of NPS was further matched with the spectral library. These attributes specifically correlate mass measurements and molecular formulas to elucidate the molecular profile of an NPS. Thus, LC-QTOF-MS provides an additional level of specificity by incorporating the chemical formula into the criteria for positive identification.

The data revealed that morphine was detected in the majority of samples followed by benzodiazepines, cannabinoids-THC, tramadol, buprenorphine, barbiturates, cocaine and amphetamine. All of the samples that tested positive for morphine indicate that the patient may have used either heroin, morphine/codeine, or opium.

In the current study, the patient's medical record did not mention taking any medications prior to treatment. However, urinalysis revealed that clonazepam was present in 23.8% of all urine test samples, indicating that

Table 10. Urine testing results of various categories of new psychoactive substances among morphine, buprenorphine, tramadol and cannabinoids positive samples at Imphal site (N=120)

Sr. No.	Name of Drugs	Morphine N=109 % Positives	Buprenorphine N=18 % Positives	Tramadol N=52 Positives	Cannabinoids N=12 % Positives
1.	AB-CHIMNACA	1 (0.9)	1 (5.5)	0 (0)	0 (0)
2.	AB-PINACA	0 (0)	0 (0)	0 (0)	0 (0)
3.	JWH-018	0 (0)	0 (0)	0 (0)	0 (0)
4.	PNP I	5 (4.5)	2 (11.1)	1 (1.9)	0 (0)
5.	PNP II	17 (15.9)	4 (22.2)	6 (11.5)	1 (8.3)
6.	BZP	2 (1.8)	2 (11.1)	1 (1.9)	1 (8.3)
7.	Mephedrone	3 (2.7)	3 (16.6)	1 (1.9)	1 (8.3)
8.	Alpha-PVP	9 (8.2)	4 (22.2)	4 (7.6)	0 (0)
9.	Mescaline	4 (3.6)	3 (16.6)	2 (3.8)	1 (8.3)
10.	Salvinorin A	0 (0)	0 (0)	0 (0)	0 (0)
11.	Acetyl Fentanyl	3 (2.7)	3 (16.6)	0 (0)	0 (0)
12.	Ocfentanyl	1 (0.9)	1 (5.5)	0 (0)	0 (0)
13.	Carfentanil	12 (11.0)	6 (33.3)	0 (0)	1 (8.3)
14.	Mitragynine	3 (2.7)	2 (11.1)	1 (1.9)	0 (0)
15.	Etizolam	6 (5.5)	1 (5.5)	3 (5.7)	0 (0)
16.	AH-7921	1 (0.9)	1 (5.5)	0 (0)	0 (0)
17.	U-47700	2 (1.8)	2 (11.1)	0 (0)	0 (0)
18.	MT-45	2 (1.8)	2 (11.1)	0 (0)	0 (0)
19.	W-19	5 (4.5)	2 (11.1)	0 (0)	0 (0)

the patient may have taken it for enhancing the drug of abuse or for insomnia.

Much of the information about NPS-related health problems comes from case reports, and it is difficult to attribute adverse outcomes to a specific substance due to polydrug usage. Furthermore, there are several clinical similarities between NPS and the more established illicit substances.

In the current study, three synthetic cannabinoids (AB-CHIMNACA, AB-PINACA, JWH-018 were detected in the patient's urine sample. All three are new-generation synthetic receptor agonists (SCRAs). The most common route of administration is via smoking, but the drug can also be taken orally and by inhalation. These are sold in the form of herbal mixtures for smoking. These have cannabimimetic effects that are more potent than THC, which is listed as a schedule II substance in accordance with the Convention on Psychotropic Substances of 1971. All these synthetic cannabinoids have no therapeutic usefulness. In view of their risk to public health and safety, AB-CHIMNACA and AB-PINACA have been put under international control since 2018, whereas JWH-018 has been under International control since 2015.

The findings of the study also showed the presence of three piperazine derivatives (1-phenylpiperazine, 2-phenylpiperazine and benzylpiperazine) in the patient's urine. The psychoactive properties of the piperazine-containing compounds are caused by their ability to bind to the serotonin receptors of the human nervous system. Based on the reported psychostimulant effects, evidence of abuse, and adverse effects, benzyl-piperazine has been under international control since 2015 and is included in Schedule II of the Convention on Psychotropic Substances of 1971.

Our test results showed a high percentage of positives for two synthetic cathinones (mephedrone and alpha-PVP). It is also interesting to note that in some parts of India, seizure data and other indirect measures of use have raised concerns about the increasing use of synthetic cathinones, such as mephedrone (Jain & Verma, 2022). Considering the degree of harm to public health and society associated with the abuse, mephedrone was placed under international control in 2015 and alpha-PVP in 2016.

Analysis of the patient'surine in this study showed the presence of three fentanyl analogs (acetylfentanyl, ocfentanyl and carfentanil). These are μ receptor agonists and have effects similar to those of morphine and fentanyl which are included in Schedule I of the 1961 Single Convention on Narcotic Drugs. Due to their serious risk to public health and society, ocfentanil and carfentanil were placed under international control in 2018 whereas acetylfentanyl has been under international control since 2016.

Urinalysis data also indicated the presence of two synthetic opioid analgesics (AH-7921 and MT-45) in the patients' samples. Both these substances have "morphine-like" effects. These substances have no therapeutic applications or medical use. Recognizing the abuse of these drugs and their associated toxicity, AH-7921 was placed under international control in 2015 and MT-45 in 2016.

Etizolam, a designer benzodiazepine, was detected in some urine samples. It is a thienodiazepine derivative, with a high affinity for the benzodiazepine site in GABA receptors. In 2020, this substance has been placed under international control and is included in Schedule IV of the 1971 Convention on Psychotropic Substances.

According to urine test results, mescaline was detected in more than half of the samples. The reasons behind this are yet to be thoroughly investigated. Chemically, it is 3,4,5-trimethoxyphenethylamine, a psychedelic hallucinogen that occurs naturally in certain cacti plants. This drug is prohibited internationally by the 1971 Convention on Psychotropic Substances due to its high potential for abuse.

At the national level, other categories of drugs detected were mitragynine-type stimulant salvinorin A like dissociative, W-19 like synthetic opioid drug and piperazine derivatives such as 1-Phenylpiperazine and 2-Phenylpiperazine (22.09%). A novel finding of this study is that these substances are currently not under international control.

It is worth mentioning that the current study commenced in the year 2017 and some of the substances detected in patients' urine samples were placed under international control thereafter.

In the current study, some of the NPS were present in very low percentages. One probable explanation is that these compounds could be adulterants, and people who use reactional substances may have been unintentionally exposed to these newer psychoactive chemicals, either alone or in combination with other substances, raising the risk of potential harm. The report establishes objective evidence of the occurrence of NPS among treatment seekers in India. The urinalysis results carry significant policy implications. Future research and policy interventions should be geared toward addressing this emerging drug problem.

Limitations and Challenges

1. Installation of LC-MS-MS During COVID Period

A state of the art equipment was purchased in late 2019. The delivery of the equipment took place during the covid lockdown time (April 2020). Procurement of the equipment needed permission from various authorities due to several prohibitory measures, delaying its acquisition. The supporting accessories (AC) took further time for delivery and delayed the installation. The training of staff again got delayed due to similar reasons. Thus, the overall installation and functioning of the LC-MS-MS was delayed beyond expectation due to the covid pandemic and procedural issues.

2. Procurement of Reference and Respective Internal Standards

A list of 27 reference standards and their internal standards was prepared, based on initial screening. The procurement process was started by the NDDTC stores and permission was obtained from the concerned authorities within AIIMS to initiate the purchase. The project investigators consulted the Central Revenue Control Laboratory (CRCL), Delhi to understand the procurement process of the reference drug standards. After understanding the process thoroughly, the investigators prepared the list of standards that requires the drug import certificate for purchase. For the standards, which did not require an import certificate, the routine procurement procedure was carried out by the NDDTC store. Quotations were received from various vendors and the standards were purchased from the lowest quote.

For the standards which required an import certificate, a separate procedure needed to be followed. After seeking due permission from the concerned authorities within AIIMS, the import purchase started. The store NDDTC identified the vendor after obtaining quotations. All the documents were prepared by the NDDTC store to obtain an import certificate. The application was sent to the central controller of factories (CCF), Delhi which processes the import applications received from all over the country along with the receipt of the fee paid. Through CCF, the documents were sent to the Central Bureau of Narcotics (CBN), Gwalior. After a span of two months, we received the import certificate for 7 standards. The order was placed and the import of the first lot of drugs from an overseas supplier could be done in 3 months due to international regulatory guidelines. During the study, 14 reference and internal standards were purchased. Due to the changing regulations on the status of the NPS, there is still a pending request for the import certificate for a few of the reference and internal standards.

Since these standards were purchased for the first time in India, more so during Covid, the delivery of test standards took longer time than expected.

3. Feasibility of sample collection from the participating centers:

When the study was proposed, it was planned to collect samples from seven sites. The nodal center (NDDTC, AIIMS, Delhi) and six different centers (KEM Mumbai, Goa, Kerala, Shimla, Amritsar and Imphal) from all over India was planned to be included in the study. Thus, during the proposal, a total number of 2200 samples (1000 from the nodal center and 200 from each center) were committed to be included in the study.

At the start of the study, the training of staff was carried out in all six centers by the project investigators. As the study progressed centers from Mumbai, Kerala, and Shimla expressed their inability to participate citing reasons like non-availability of ethical clearance, or other administrative and feasibility issues.

Afterward, two other centers from AIIMS (Rishikesh and Bhuvneshwar) were included in the study. Additionally, with Covid (2020-21), the committed number of samples to be collected (200) from each participating center could not be achieved. Other issues like wear and tear and spillage add to the loss in the final sample size. Thus,

the final report incorporated 1462 samples which were from NDDTC (1000), Amritsar (87), Bhubaneswar (192), Imphal (120), and Rishikesh (63).

Listed above are some of the unexpected challenges we encountered during the implementation of this study.

Recommendations

1. NPS research necessitates multidisciplinary approaches that include epidemiology, pharmacology, and prevention.
2. NPS drug testing is complex due to the continuous emergence of these substances. The majority of NPS have limited or unpublished pharmacology data, and standards are not widely available when they are first detected in confiscated items, making identification in biological matrices difficult. To overcome these limitations, high-throughput laboratories must employ analytical methodologies that are both flexible and robust, while meeting workload demands.
3. Newly developed analytical methods for detecting NPS must be made widely available to assist in the identification of novel substances as they appear in the recreational drug marketplace.
4. Toxicologists should build and expand in-house libraries as new compounds emerge.
5. In order to control the illicit manufacture and trafficking of NPS, national monitoring and research capabilities, along with the forensic capacity to identify and report their emergence must be upgraded continuously.
6. The challenges posed by the NPS necessitate the use of epidemiological monitoring systems to rapidly identify emerging substances and alert policymakers and health professionals in a timely manner.
7. There is a need for increased awareness in light of changes in substance use trends.
8. The abuse of NPS has placed a significant burden on healthcare professionals, particularly those who provide emergency medical care. In the interest of public health and safety, better coordination among emergency medical personnel, clinical and forensic toxicologists, scientific researchers, law enforcement, and policymakers is essential in dealing with this emerging drug-problem.

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ANNEXURE: DATA COLLECTION TOOL

A. SOCIO-DEMOGRAPHIC STATUS

1. Phone number :		
2. Address:		
3. Gender	Male ☐	Female ☐
4. Age (in years):	☐ Married and staying together ☐ Never married ☐ Staying together without getting married	
5. Marital status:	☐ Divorced ☐ Separated ☐ Widower ☐ Not Known	
6. Occupation: What kind of work do you do?	☐ Highly skilled ☐ Skilled ☐ Semi-Skilled ☐ Unskilled	
7. Occupation (Specify)	_____	
1. Education: How many years of school have you attended?	☐ Illiterate ☐ Primary (up to 5 years formal education) ☐ Middle school (up to 8 years formal education) ☐ Higher school (up to 10 years formal education) ☐ Intermediate (up to 12 years formal education ☐ Graduate (up to 13 years formal education) ☐ Post graduate (up to 15 years formal education) ☐ Professional ☐ Others (Specify)	
2. Employment status: What is your current employment status?	☐ Currently employed (full-time) ☐ Currently employed (part-time) ☐ Currently unemployed ☐ Not Known	

3. Residence	☐ Urban ☐ Urban (slum) ☐ Urban (homeless) ☐ Rural
4. Religion?	☐ Hindu ☐ Muslim ☐ Sikh ☐ Christian ☐ Not Known ☐ Other (specify) _____
5. Family Income per month in Rupees	_____ Rupees
6. How many members are there in your household?	_____ excluding client)
7. How many earning members are there in your family?	_____ excluding client)
8. How many dependent members are there in your family?	_____ (excluding client)
9. What are your OWN sources of income?	☐ Income from job (including agriculture) ☐ Income from Agricultural land (not cultivated by oneself) ☐ Other farm related business activity ☐ Non-farm business (if any) ☐ Pensions (if any) ☐ Rent from any property ☐ Capital gains (interest, dividend etc) ☐ Government payout (IRDP, insurance etc) ☐ Others (Specify) _____
10. What are the sources of income for the rest of your household?	☐ Income from job (including agriculture) ☐ Agricultural land (not cultivated by oneself) ☐ Other farm related business activity ☐ Non-farm business (if any)

	☐ Pensions (if any) ☐ Rent from any property ☐ Capital gains (interest, dividend etc) ☐ Government payouts (IRDP, insurance etc) ☐ Others(Specify) _____
11. How much is your OWN average monthly income (combining all the sources)?	_____ Rs.
12. Out of your OWN income, how much do you contribute for household expenses?	_____ Rs.

B. SUBSTANCE USE HISTORY

1. In your life, which of the following substances have you ever used? (NON-MEDICAL USE ONLY)

	No	Yes
(a) Tobacco products (cigarettes,bidi, chewing tobacco, cigars, etc.)	0	3
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	3
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	3
(d) Cocaine (coke, crack, etc.)	0	3
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	3
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	3
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	3
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	3
(i) Opioids (heroin, morphine, methadone, codeine,norphine, t.d.gesic, proxyvon etc.)	0	3
(j) Other - specify:	0	3

2. In the past three months, how often have you used the substances you mentioned (FIRST DRUG, SECOND DRUG, ETC)?

	Never	Once or Twice	Monthly	Weekly	Daily or Almost daily
(a) Tobacco products (cigarettes,bidi, chewing tobacco, cigars, etc.)					
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	2	3	4	6
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	2	3	4	6
(d) Cocaine (coke, crack, etc.)	0	2	3	4	6
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	2	3	4	6
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	2	3	4	6
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	2	3	4	6
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	2	3	4	6
(i) Opioids (heroin, morphine, methadone, codeine, norphine, tidigesic, proxyvon etc.)	0	2	3	4	6
(j) Other - specify:	0	2	3	4	6

3. During the past three months, how often have you had a strong desire or urge to use (FIRST DRUG, SECOND DRUG, ETC)?

	Never	Once or Twice	Monthly	Weekly	Daily or Almost daily
(a) Tobacco products (cigarettes,bidi, chewing tobacco, cigars, etc.)	0	2	4	5	6
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	2	4	5	6
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	2	4	5	6
(d) Cocaine (coke, crack, etc.)	0	2	4	5	6
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	2	4	5	6
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	2	4	5	6
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	2	4	5	6
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	2	4	5	6
(i) Opioids (heroin, morphine, methadone, codeine, norphine, tidigesic, proxyvon etc.)	0	2	4	5	6
(j) Other - specify:	0	2	4	5	6

4. During the past three months, how often has your use of (FIRST DRUG, SECOND DRUG, ETC) led to health, social, legal or financial problems?

	Never	Once or Twice	Monthly	Weekly	Daily or Almost daily
(a) Tobacco products (cigarettes, bidi, chewing tobacco, cigars, etc.)	0	4	5	6	7
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	4	5	6	7
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	4	5	6	7
(d) Cocaine (coke, crack, etc.)	0	4	5	6	7
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	4	5	6	7
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	4	5	6	7
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	4	5	6	7
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	4	5	6	7
(i) Opioids (heroin, morphine, methadone, codeine, norphine, t.d. gesic, proxyvon etc.)	0	4	5	6	7
(j) Other - specify:	0	4	5	6	7

5. During the past three months, how often have you failed to do what was normally expected of you because of your use of (FIRST DRUG, SECOND DRUG, ETC)?

	Never	Once or Twice	Monthly	Weekly	Daily or Almost daily
(a) Tobacco products (cigarettes, bidi, chewing tobacco, cigars, etc.)					
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	5	6	7	8
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	5	6	7	8
(d) Cocaine (coke, crack, etc.)	0	5	6	7	8
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	5	6	7	8
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	5	6	7	8
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	5	6	7	8
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	5	6	7	8
(i) Opioids (heroin, morphine, methadone, codeine, norphine, t.d.gesic, proxyvon etc.)	0	5	6	7	8
(j) Other - specify:	0	5	6	7	8

6. Has a friend or relative or anyone else ever expressed concern about your use of (FIRST DRUG, SECOND DRUG, ETC.)?

	No, Never	**Yes, In the past 3 months**	**Yes, but not in the past 3 months**
(a) Tobacco products (cigarettes,bidi, chewing tobacco, cigars, etc.)	0	6	3
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	6	3
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	6	3
(d) Cocaine (coke, crack, etc.)	0	6	3
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	6	3
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	6	3
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	6	3
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	6	3
(i) Opioids (heroin, morphine, methadone, codeine, norphine, t.d.gesic, proxyvon etc.)	0	6	3
(j) Other - specify:	0	6	3

6. Has a friend or relative or anyone else ever expressed concern about your use of (FIRST DRUG, SECOND DRUG, ETC.)?

	No, Never	**Yes, In the past 3 months**	**Yes, but not in the past 3 months**
(a) Tobacco products (cigarettes,bidi, chewing tobacco, cigars, etc.)	0	6	3
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	6	3
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	6	3
(d) Cocaine (coke, crack, etc.)	0	6	3
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	6	3
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	6	3
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	6	3
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	6	3
(i) Opioids (heroin, morphine, methadone, codeine, norphine, t.d.gesic, proxyvon etc.)	0	6	3
(j) Other - specify:	0	6	3

7. Have you ever tried and failed to control, cut down or stop using (FIRST DRUG, SECOND DRUG, ETC.)?

	No, Never	Yes, In the past 3 months	Yes, but not in the past 3 months
(a) Tobacco products (cigarettes, bidi, chewing tobacco, cigars, etc.)	0	6	3
(b) Alcoholic beverages (beer, wine, spirits, CML etc.)	0	6	3
(c) Cannabis (bhang, charas, ganja, sulfa, marijuana, pot, grass, hash, etc.)	0	6	3
(d) Cocaine (coke, crack, etc.)	0	6	3
(e) Amphetamine type stimulants (speed, diet pills, ecstasy, etc.)	0	6	3
(f) Inhalants (nitrous, glue, petrol, varnish, paint thinner, etc.)	0	6	3
(g) Sedatives or Sleeping Pills (Valium, Serepax, nitrovet, calmopse, no.10 etc.)	0	6	3
(h) Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)	0	6	3
(i) Opioids (heroin, morphine, methadone, codeine, norphine, t.d.gesic, proxyvon etc.)	0	6	3
(j) Other - specify:	0	6	3

8. Have you ever used any drug by injection? (NON-MEDICAL USE ONLY)

	No, Never	Yes, In the past 3 months	Yes, but not in the past 3 months
	0	2	1

9. SPECIFIC SUBSTANCE INVOLVEMENT SCORE.

Scoring:

For all substances other than tobacco: Q2 + Q3 + Q4 + Q5 + Q6 + Q7

For Tobacco: Q2 + Q3 + Q4 + Q6 + Q7

	Specific Substance score
(a) Tobacco	
(b) Alcohol	
(c) Cannabis	
(d) Cocaine	
(e) Amphetamine	
(f) Inhalants	
(g) Sedatives	
(h) Hallucinogens	
(i) Opioids	
(j) Other drugs	

10. Other substance use details :

	(i) Age of 1st use	(ii) Total duration of use	(iii) Predominant route of use: Oral (1), Inhalational (2), Injecting (3), Other (pl. specify) (4)
(a) Tobacco			
(b) Alcohol			
(c) Cannabis			
(d) Cocaine			
(e) Amphetamine			
(f) Inhalants			
(g) Sedatives			
(h) Hallucinogens			
(i) Opioids			
(j) Other drugs			

11.	Any family H/O of substance use disorder	Yes ☐	No ☐
12.	If yes, relationship with you?	_____(specify)	
13.	Type of substance use disorder?	_____(specify)	

C. INJECTING DRUG USE (IDU) DETAILS:

1.	Have you ever injected drugs?	Yes ☐		No ☐
2.	If yes, at what age did you first inject drug?	_____ years		
3.	What was the predominant opioid that you inject (considering last three months)?	_____ (Specify)		
4.	Have you ever used mixed drugs with opioids?	Yes ☐		No ☐
5.	If yes, name the drugs that you mix with opioids usually (considering last three months)?	_____ (Specify)		
6.	Have you ever shared needles, syringes, etc.?	Yes ☐		No ☐
7.	Have you ever reused needles, syringes, etc.?	Yes ☐		No ☐
8.	Have you ever had abscess/ulcers?	Yes ☐		No ☐
9.	Have you ever had vein related complications? (Thrombophlebitis, vein block, varicose veins)	Yes ☐		No ☐
10.	Have you ever injected intra-arterially?	Yes ☐		No ☐
11.	Have you ever overdosed on opioids?	Yes ☐		No ☐
12.	Have you ever been tested for HIV?	Yes ☐		No ☐
13.	If yes, can you share and let me know whether you have been diagnosed as HIV +ve?	HIV positive ☐	HIV negative ☐	Don't know/ No response ☐
14.	If yes, are you on ART?	Yes ☐ Very regular ?		No ☐ (>24 d/month)
15.	If yes, what is your compliance with medications	Regular ☐ Irregular ☐		(15-24d/month) (<15d/month)
16.	Have you ever been tested for Hepatitis B or C ?	Yes ☐		No ☐

17.	If yes, can you share and let me know whether you have been diagnosed as Hepatitis +ve?	Hep B positive	☐
		Hep C Positive	☐
		Both Hepatitis Positive	☐
		Both Negative	☐
		Don't know/No response	☐
18.	If yes, have you ever been treated for Hepatitis?	Yes ☐	No ☐

D. PAST ABSTINENT ATTEMPTS

1.	Have you ever been abstinent from psychoactive substances for one month or more?	Yes ☐	No ☐
2.	If yes, how many episodes have you been abstinent?	_____times	

E. PAST TREATMENT HISTORY

1.	Have you ever been treated for substance use disorder in the past?	Yes ☐	No ☐
2.	If yes, how many times have you been treated?	_____ times	

F. NEW PSYCHOACTIVE SUBSTANCES

1.	Have you ever consumed any substance/s that was different from those listed in B1?	Yes No
2.	If Yes to above, what was the substance/s referred to as?	A. _____ B. _____ C. _____
	For each of the substance mentioned in F2,	
3.	What was the age when you consumed that substance for the first time?	
4.	How many times have you consumed the substance?	_____ times
5.	When was the last time that you consumed this substance?	
6.	How was the substance in appearance?	Capsule/tablet Powder Liquid Solid mass Any other, pl. specify_____

7.	Route of consumption of the substance	Eating Drinking
		Snorting Chasing Smoking Injecting Any other, pl. describe_____
8.	How did you obtain the substance?	From a peddler/drug dealer From a friend user From internet From a special shop Any other, pl. specify_____
9.	What was the cost of one dose?	_____INR
10.	What was the effect after consuming the substance?	Felt drowsy
		Felt active Had hallucinatory experience Felt sick Felt anxious/panicky Any other, pl. specify_____
11.	How long did the effect last?	_____hours
12.	Did you have any unpleasant physical / psychological effect after the effect of the substance was worn out?	Yes No
13.	If, yes, pl. describe the effect	
14.	Did you ever develop a compulsion to use the substance repeatedly?	Yes No
15.	Did you indulge in any risky behaviour upon using the substance?	Yes No
16.	If yes, pl. mention the risky behaviour	
17.	Were you ever caught by police for using the substance?	Yes No
18.	Did you ever seek treatment for the use of the substance?	Yes No
19.	If, yes, what was the reason for seeking treatment?	
20.	What treatment was provided during that time?	
21.	Do you think, the use of the substance is potentially harmful?	
22.	If, yes, what harm do you think can be caused?	

ANNEXURE- II

National Drug Dependence Treatment Centre, Ghaziabad
ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI-110029

Name of the Site: _____

Drug Testing Requisition Form

Patient's Name_____ Age _____ Sex _____
(In Block Letters)
NPS Study Enrolment No. _____ Name of Consultant _____

Name of Research Staff: _____
(In Block Letters)

Signature of Consultant/Research staff

Specimen collected on _____ At _____AM/PM

Nature of specimen: Urine :

Brief Clinical history and Diagnosis

Name of the drug used. Quantity of consumption, Frequency
Last 72 hrs. _____
Last 48 hrs._____
Last 24 hrs. _____
Last dose, date, time and amount _____
Medication patient is on and since when _____

Self-report : Which of the following drug have you taken in last 24, 48 and 72 hours?

	24 hours		48 hours		72 hours	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
(a) Tobacco						
(b) Alcohol						
(c) Cannabis						
(d) Cocaine						

	24 hours		48 hours		72 hours	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
(e) Amphetamine						
(f) Inhalants						
(g) Benzodiazepines						
(h) LSD						
(i) Barbiturate						
(j) Antihistaminics(Avil)						
(k) Opioids: • Heroin • Morphine • Codeine • Opium • Pentazocine • Buprenorphine • Tramadol • D-Propoxyphene (l) Other drugs (NPS): • Bath salts • Benzyl-piperazines • Mescaline • Phenypiperzines • Salvinorin • Synthetic • Cannabinoids						

For Lab Use Only

Specimen Received date_____ By_____

Lab reference No._____

Time of Receiving Specimen_____

Lab Reports:

	Positive	Negative
(a) Tobacco		
(b) Alcohol		
(c) Cannabis		
(d) Cocaine		
(e) Amphetamine		
(f) Inhalants		
(g) Benzodiazepines		
(h) LSD		
(i) Barbiturate		
(j) Anti-histaminic(Avil)		
(k) Opioids: Heroin Morphine Codeine Pentazocine Buprenorphine Tramadol Dextro Propoxyphene		
l. Other drugs (NPS):		
Bath salts Benzylpiperazines Mescaline Phenypiperzines Salvinorin Synthetic Cannabinoids		

Figure -9: Chromatogram of 50 ng/ml 1-Phenylpiperazine, 1-Phenylpiperazine-D8, Benzylpiperazine andBenzylpiperazine-D7 standard

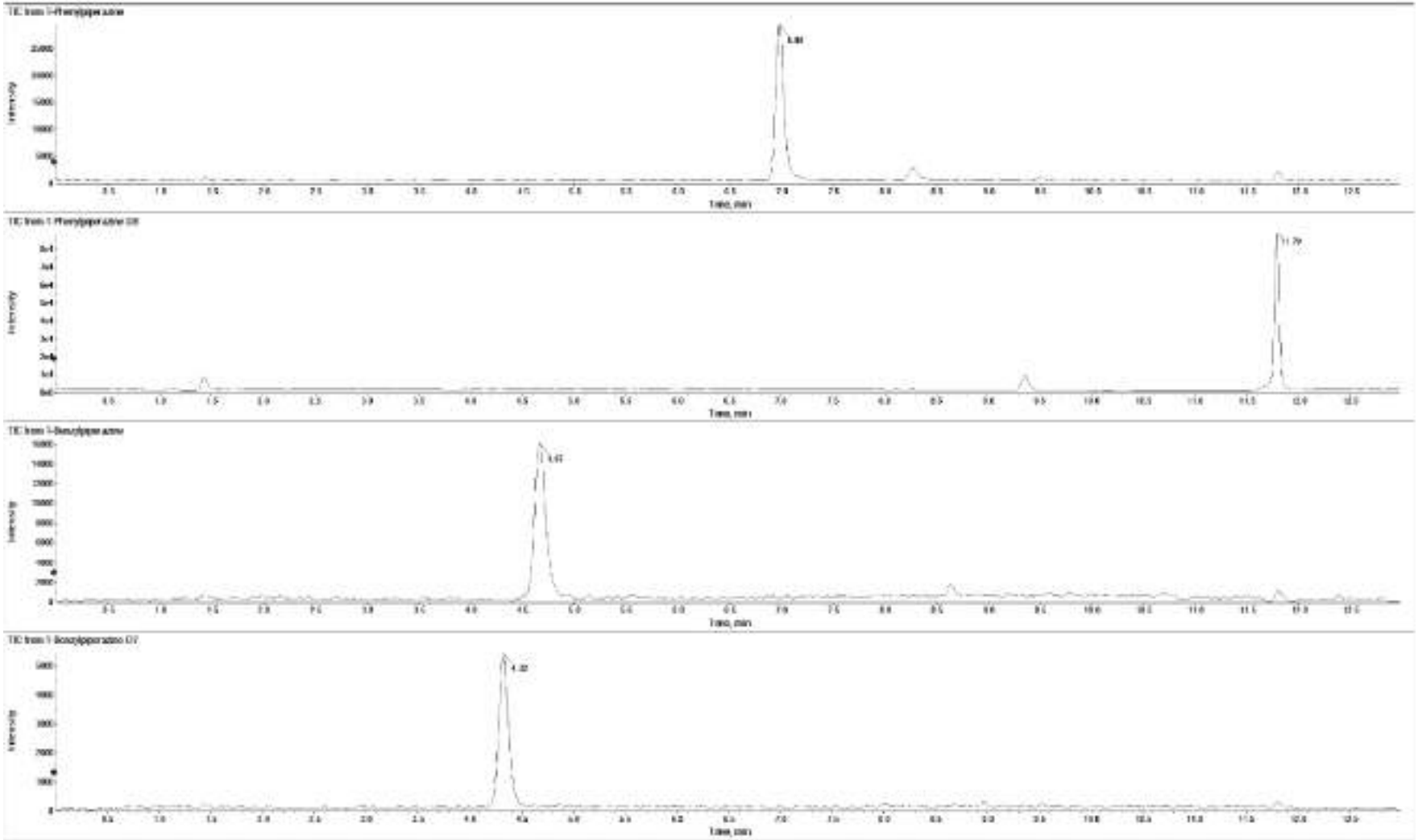


Figure- 10: MS/MS fragmentation of1-Phenylpiperazine, 1-Phenylpiperazine-D8, Benzylpiperazine andBenzylpiperazine-D7 standard

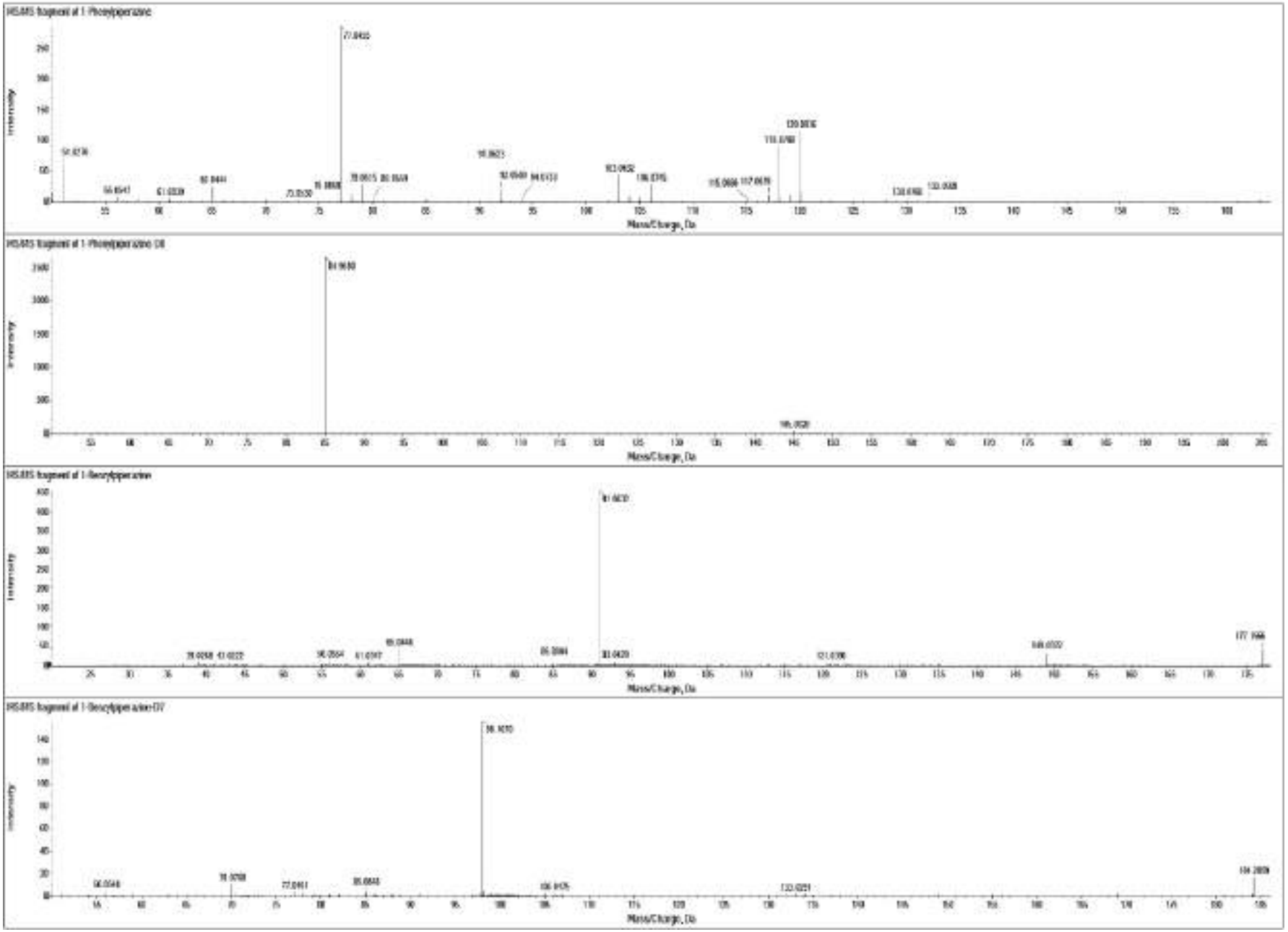


Figure -11: Chromatogram of 50 ng/ml W-19,Acetyl Fentanyl andJWH-018 standard

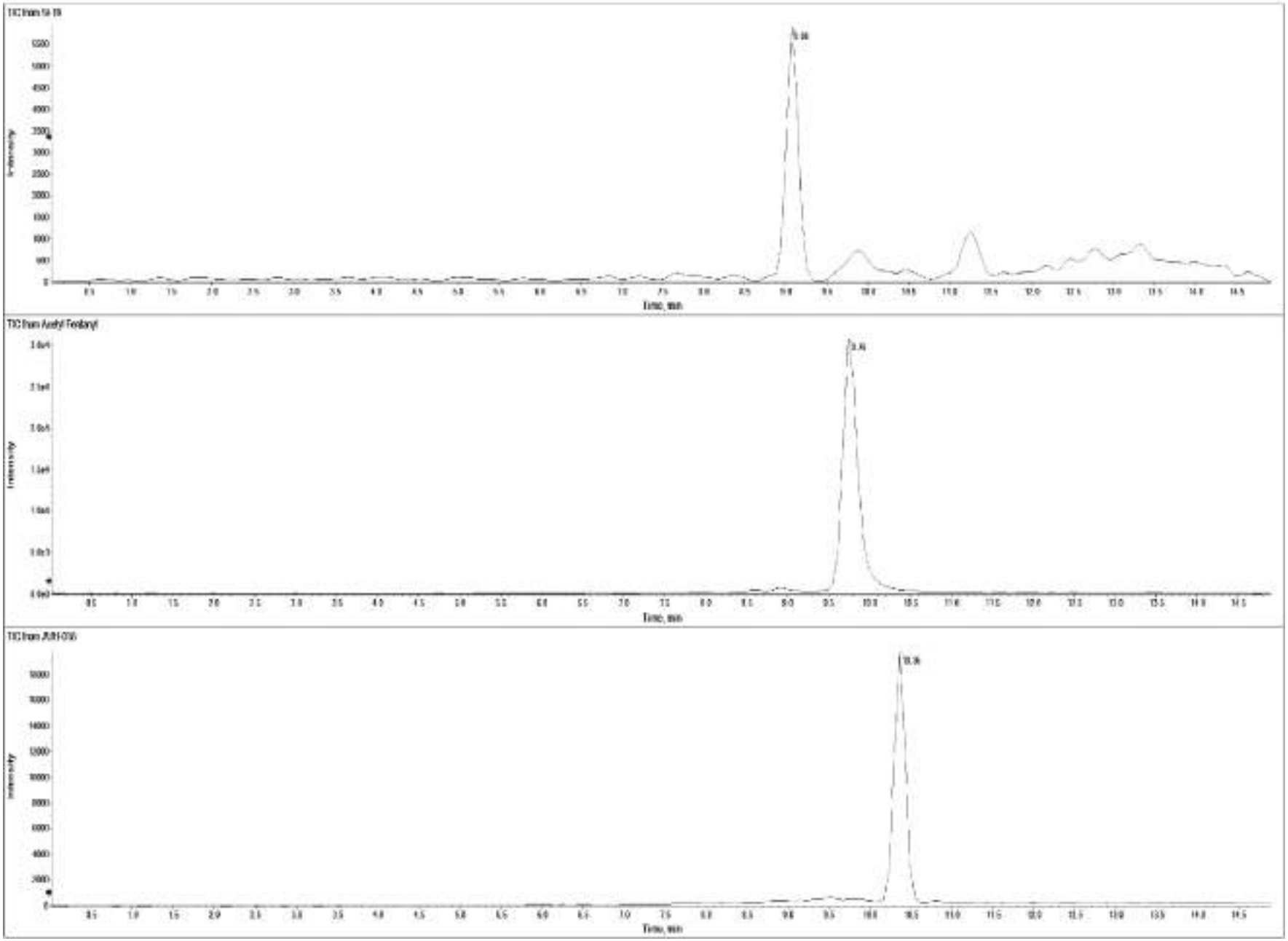


Figure -12: MS/MS fragmentation of W-19,Acetyl Fentanyl andJWH-018 standard

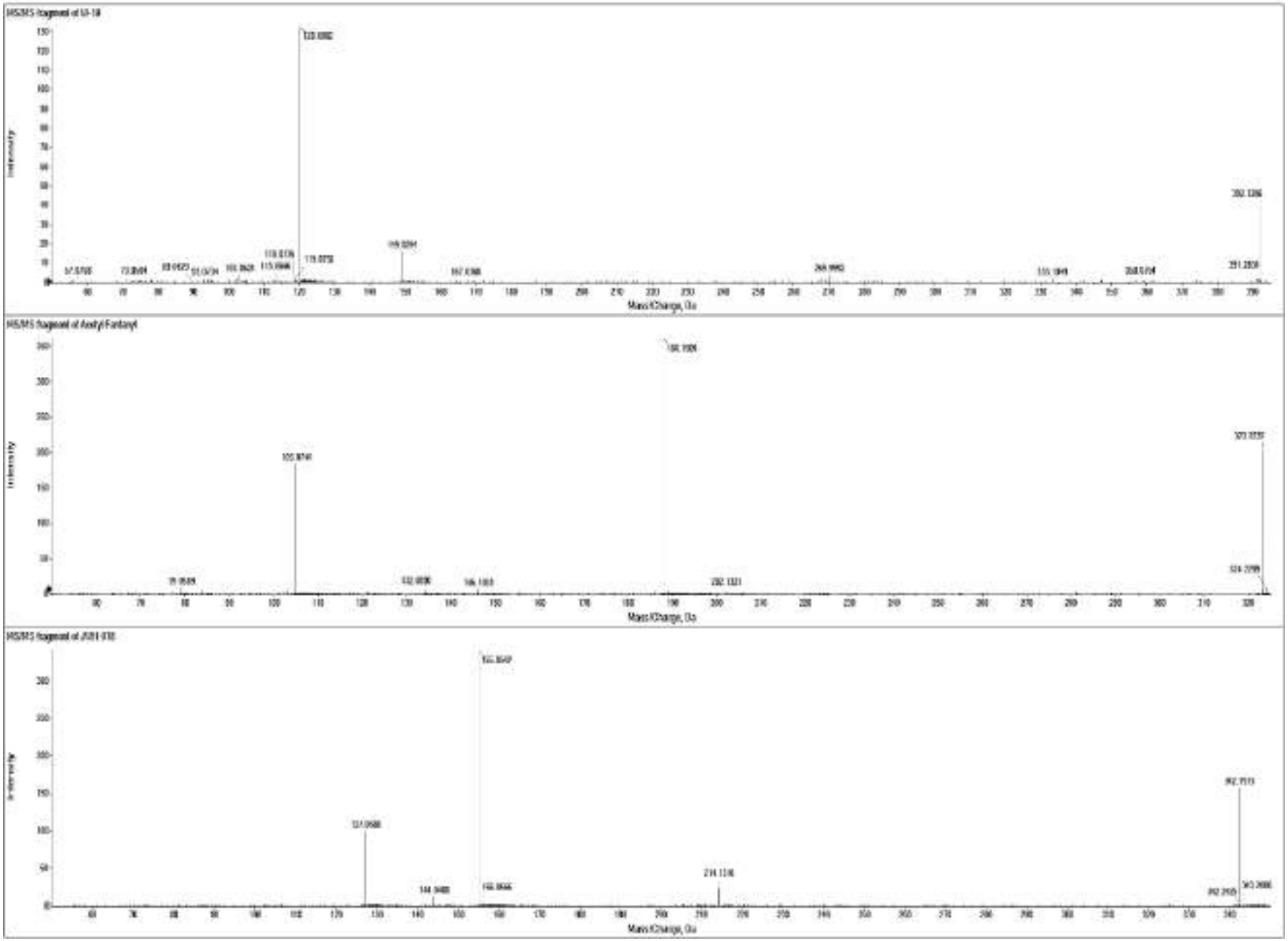


Figure-13: Chromatogram of 50 ng/mlSalvinorin A,Mescaline andMitragynine standard

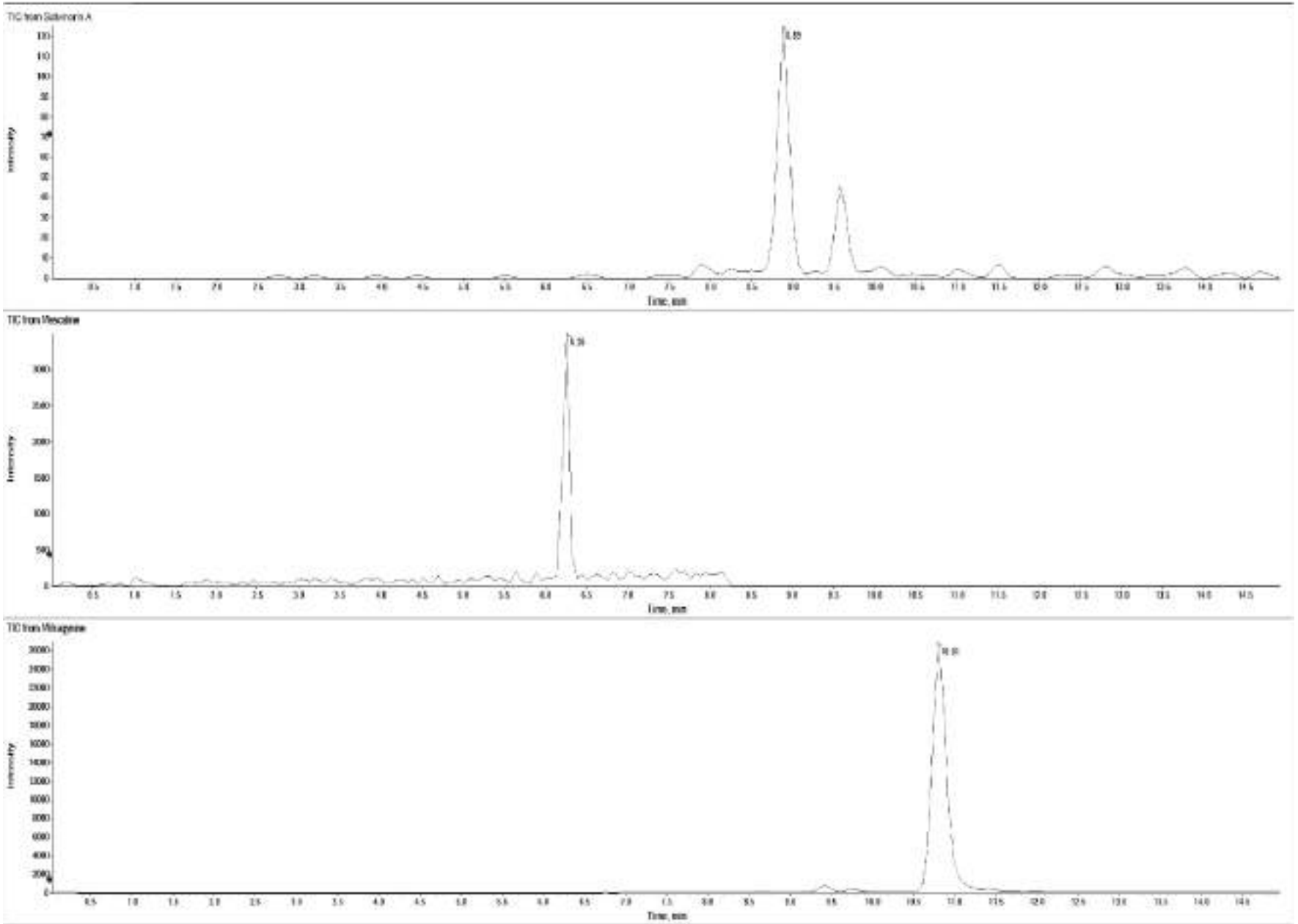


Figure-14: MS/MS fragmentation of Salvinorin A,Mescaline andMitragyninestandard

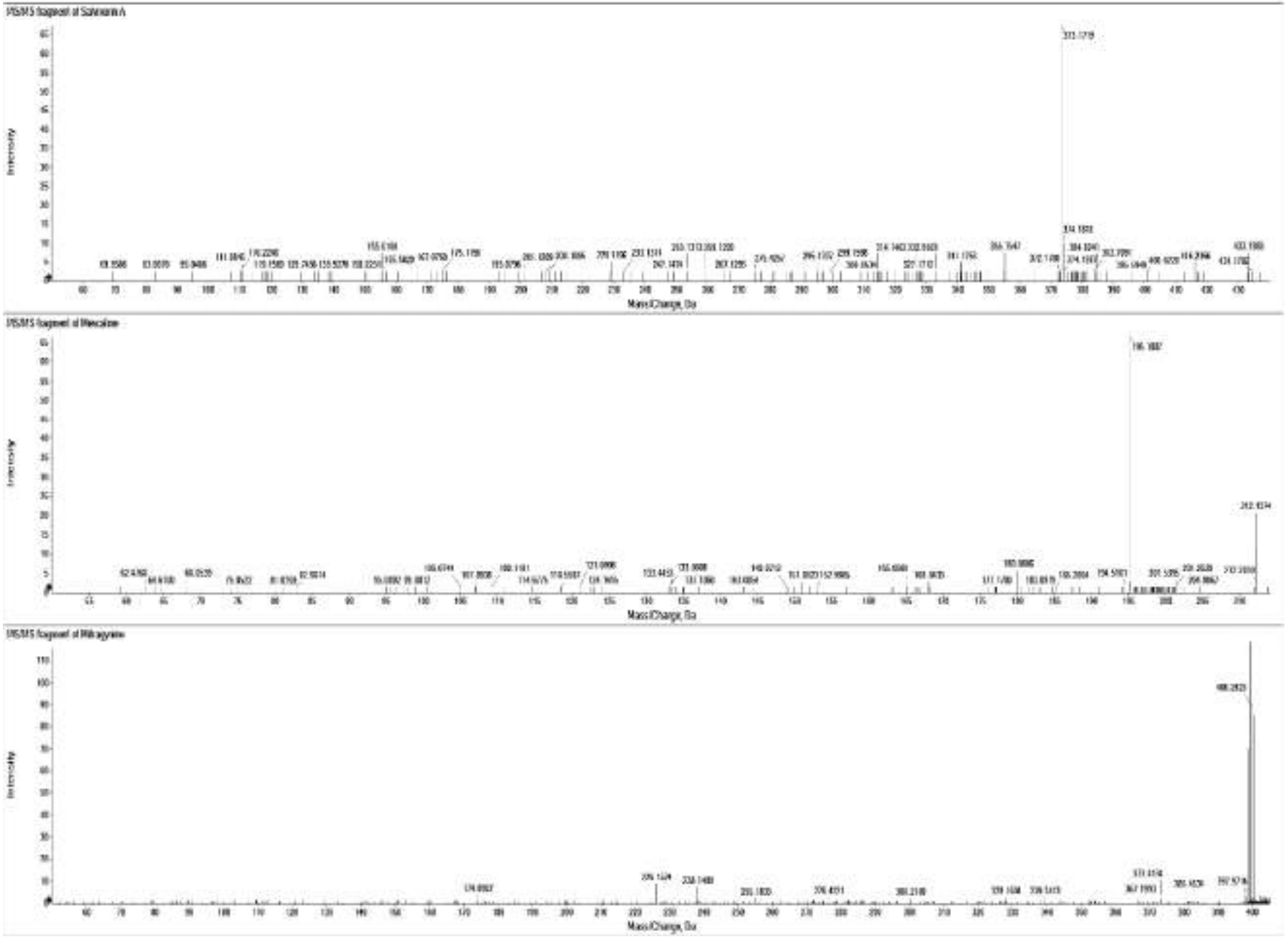


Figure -15: Chromatogram of 50 ng/ml Mephedrone and Mephedrone-D3 standard

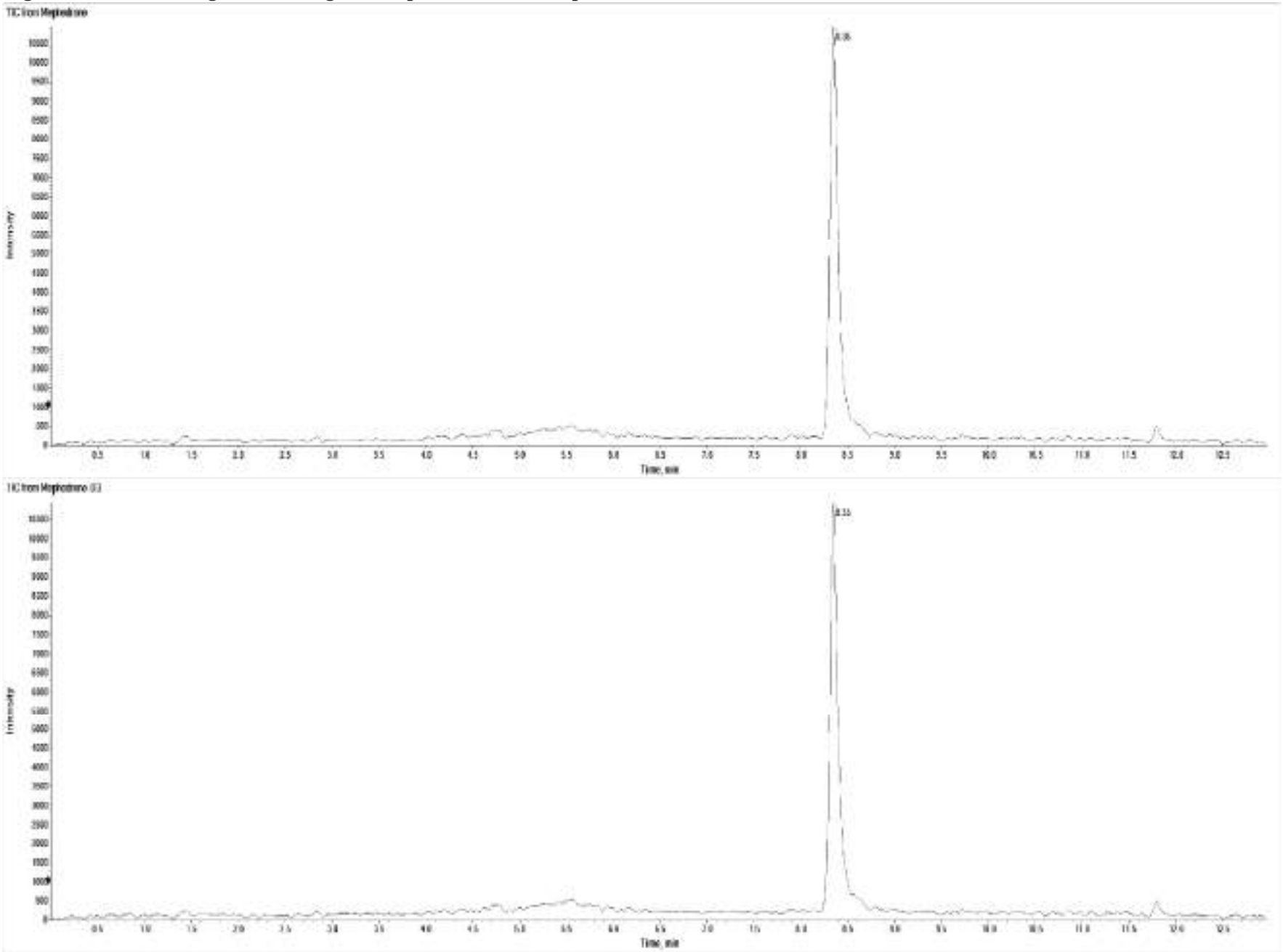
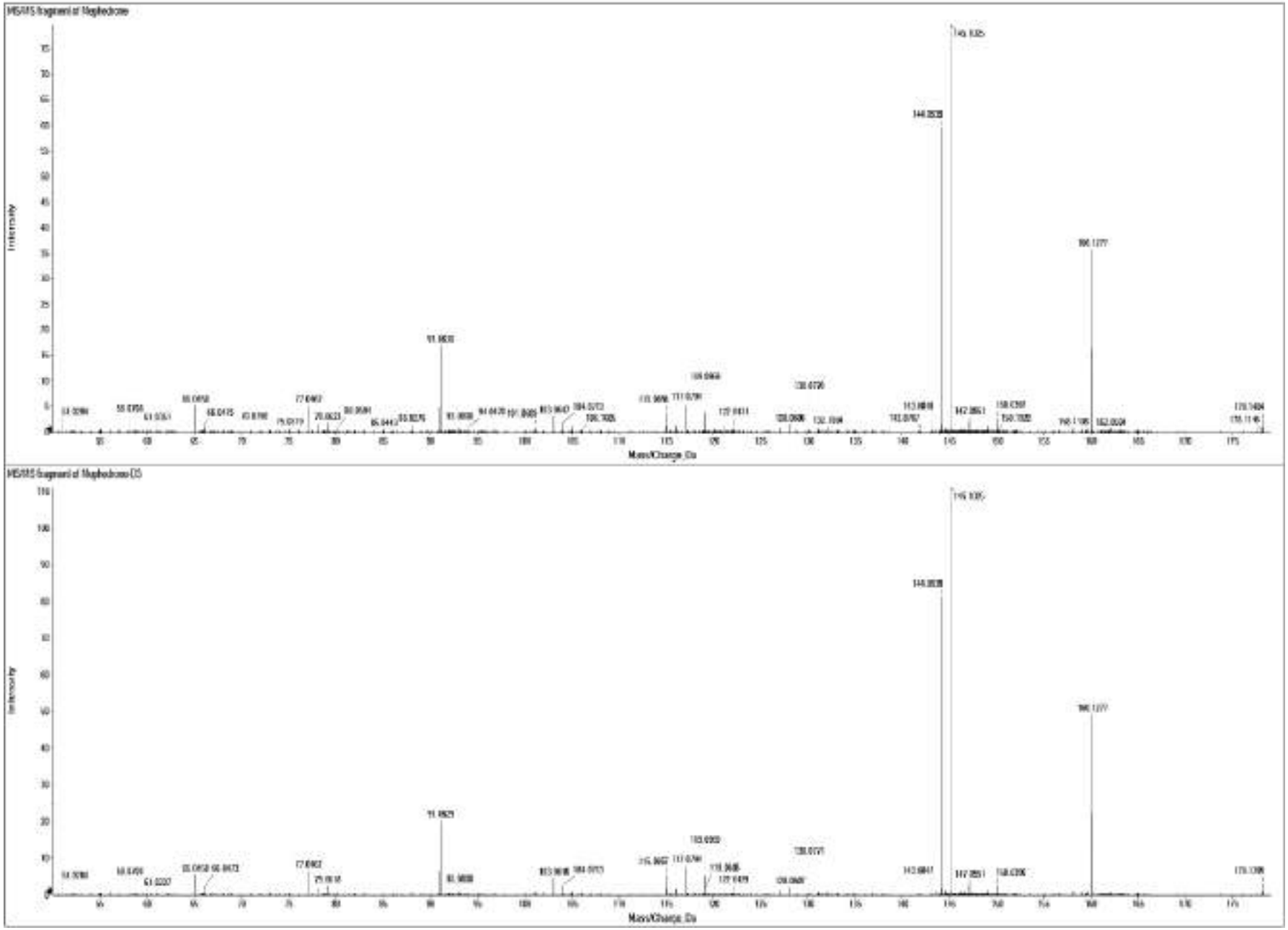


Figure-16: MS/MS fragmentation of Mephedrone and Mephedrone-D3 standard



Over the last decade, the world has witnessed an alarming new drug problem with engineered molecules, collectively called New Psychoactive Substances (NPS).

NPS constitute a rapidly evolving group, with a total of 1127 such substances currently identified by the national authorities and forensic laboratories from 134 countries. The present study was initiated by the National Drug Dependence Treatment Centre (NDDTC) at the All India Institute of Medical Sciences (AIIMS), New Delhi, to assess the extent and pattern of NPS use. Five centres, including NDDTC; the Government Medical College, Amritsar; Regional Institute of Medical Sciences, Imphal; All India Institute of Medical Sciences, Rishikesh and All India Institute of Medical Sciences, Bhubaneswar, took part.

This report establishes objective evidence of the occurrence of NPS among treatment seekers in India. The report discusses issues in drug control policy, market dynamics, and sociodemographic changes associated with the spread of NPS. It is hoped that the findings and recommendations from this report will be informative and meaningful in addressing the challenges posed by newer psychoactive drugs in the country.

CITATION:

Chadda RK, Jain R, Lal R, Rao R, Quraishi R, Garg PD, Bala N, Singh RKL, Padhy S, Basu A, Krishnan V. Detection of New Psychoactive Substances among Substance Abuse Treatment Seekers in India - Multi-Centric Study. Department of Revenue, Ministry of Finance, Government of India: New Delhi, 2023.