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Identification and Profiling of street samples of Mephedrone and its commonly encountered adulterants through Chromatographic techniques

Akanksha Singh[1*] & Bharti Arya[2]

[1]*Forensic Professional, Chemistry Division, Central Forensic Science Laboratory, Bhopal, India.*
[2]*Deputy Director, Chemistry Division, Central Forensic Science Laboratory, Bhopal, India.*
Corresponding Author (Akanksha Singh) Email: singh.akanksha1695@gmail.com[*]

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ABSTRACT

Drug abuse has become a global concern for the society. The illicit market of drugs is increasing at a faster rate thus, making it difficult for the law enforcement agencies to regulate the manufacturing and trading of these controlled substances. The drug traders/ smugglers adulterates core drug compound with other chemical moieties or adulterants to deceive regulatory and law enforcement agencies in order to enhance potency of the drug, to escalate it's tempt for consumption by making it addictive thus to gain commercial benefits. The purpose of this research was to identify Mephedrone and its adulterants and to check the presence of adulterants in illicit samples seized. The street samples of Mephedrone were isolated, screened and identified using GC-MS technique. Besides other consistent additives, 12 unusual adulterants were detected in seized Mephedrone samples in Madhya Pradesh region of India. The study will update forensic chemical experts in detection of new adulterants in seized drugs, besides disseminating awareness and knowledge among those concerned with drug regulation, monitoring and policy making.

Keywords: Mephedrone; Illicit; Adulterants; Addictive; Enforcement agencies.

1. Introduction

Drug abuse is increasing at an alarming rate and has become a global concern despite stringent measures on its manufacturing, supply and trade. The illicit market of drugs consistently witnesses experiments to deceive law regulatory agencies during manufacturing and trading of these regulated drugs [1]. New psychoactive substances (NPS) are generally known in the market or sold via internet under slang such as 'legal highs', 'designer drugs', 'bath salts', 'herbal highs' and many more which comprises a large and expanding class of psychoactive drugs posing high risk to public health and challenges to the scientific community because of emerging illegal alternatives to traditional controlled drugs, while having similar effects and sometimes more toxic than the controlled drugs [2]. Psychoactive compounds that mainly contain a phenylethylamine structure especially β-keto-amphetamines analogues known as synthetic cathinones (such as amphetamine-type stimulants and synthetic cathinones) are one of the major classes of stimulants on the recreational drug market. These types of drugs tend to stimulate the central nervous system and offer hallucinogenic and psychedelic effects, which make their use attractive. It is generally observed that Amphetamine-type stimulants (ATSs) and synthetic cathinones are taken together because of their similar mechanism of action [3].

The abuse of psychoactive drugs have created a demand for sensitive, robust and reliable analytical methods for their identification and quantification in different matrices [4]. Cathinone are extracted from the leaves of an African and Middle Eastern plant Catha edulis or 'Khat'. The main alkaloid content of the fresh leaves is responsible for its psychoactive stimulating effects [5]. 4-methylmethcathinone commonly known as Mephedrone sometimes called as 'meow', 'mcat' and 'bubbles. The IUPAC name is: (RS)-2-methylamino-1-(4-methylphenyl) propan-1- one. It is a synthetic ring-substituted cathinone closely related to the phenethylamine family, which is

different only by a keto functional group at the beta carbon. The molecular formula for mephedrone is C11H15NO and its molecular weight is 177.242 g/mol. Mostly mephedrone has been found to contain adulterants such as benzocaine, lidocaine, caffeine, paracetamol, other synthetic cathinones like butylone, methylone, ethylcathinone, 10 fluoromethcathin-one, methylenedioxypyrovalerone/MDPV) and/or other recreational drugs for example MDMA, mCPP (meta-Chlorophenylpiperazine), ketamine [6]. Methylenedioxymethamphetamine (MDMA), often sold as 'Ecstasy' or 'Molly', is commonly used at rave parties or music festivals are itself used as an adulterant in various synthetic cathinones and often adulterated with various other drugs which increases morbidity and mortality [7]. Mephedrone is usually administrated nasally or orally, although sometimes reports of intravenous administration have also been encountered. Depending on the dosage and route of administration, the onset of its effects is within 10–30 minutes [8]. Mephedrone mimic similar stimulant effect such as such as euphoric, entactogenic and hallucinogenic properties [9].

Detection of Mephedrone from different biological samples such as blood and urine through liquid-liquid extraction and derivatization of the extract with MSTFA prior to analysis in GCMS have been developed [10]. Mephedrone can also be detected from the hair sample to detect chronic abuse which is incorporated into hair (concentrations in the ng/mg range) like other stimulant drugs such as amphetamines or cocaine [11]. Adulterants are often intentionally added to bulk, dilute, complement or to enhance the effects of drugs, but it is sometimes simply present as a result of poor manufacturing practice [12].

There are many instances in which various medicines in the market have been tested for the illegal adulteration of drugs such as caffeine, indomethacin, ethoxybenzamide, chlorzoxazone and diazepam which were detected using reversed phase HPLC quantitative analysis [13]. A lot of single-pill combinations (SPC) are manufactured and used as a promising choice in treatment of signs and symptoms of diseases which has become a serious challenge to drug analysts because of the difficulty in the analysis of two or more drugs in presence of each other therefore, it is necessary to find suitable analytical methods for simultaneous determination of the co-administered drugs in same dosage form and also in the presence of their related substances and impurities [14]. The infrastructure that is used to detect illicit drugs and co-existing adulterating species is mainly based on chromatography, spectrometry, and spectroscopy (frequently combined) techniques, the evaluation of the frequency of occurrence of the chemical impurities detectable with GC-MS [15].

Profiling and Identification of adulterants in street samples is not only of clinical value but also important from forensic point of view to relate geographical origin of the drug. Some contaminants are derived from the refining procedure of manufacturing. The adulterated product can produce unexpected reactions in intoxicated patients and may be fatal [16]. Forensic chemist in drug profiling extracts the chemical signatures that can be used to establish the degree of commonality of seizures with their origins or a specific group of other samples. Impurity profiling of drugs is based on precursors, synthetic routes, and chemical modifications, changes in the synthetic pattern result in new impurity profiles. The chemical analysis of illegal drugs provides valuable information about the conspiracy links and trafficking routes, categorizing the seizures based on the signatures, thereby identifying their origins [17], [18]. Thus, this study at Central Forensic Science Laboratory, Bhopal (CFSL) aims to uncover the newly detected adulterants in Mephedrone mainly encountered from the Madhya Pradesh region of India.

2. Experimental Analysis

2.1. Materials and Methods

2.1.1. Chemicals and Reagents

Chemicals- Methanol & Acetone GC Grade purchased from Finar, Sanand, Ahmedabad (Min Assay 99.9 %), Conc. Sulphuric acid (Finar, Sanand, Ahmedabad), sodium nitrite, bismuth subnitrate, potassium iodide, glacial acetic acid Pallav Chemicals & Solvent Pvt. Ltd. Tarapur, Boisar, n- butanol (Loba Chemie Pvt. Ltd.).

Reagents-Libermann's reagent, Zimmermann's reagent, Dragondroff reagent and ninhydrin reagent were prepared according to the UNODC manual.

2.1.2. Sample Size

A number of suspected Mephedrone samples were examined for identification and analysis of chemical constituents of Mephedrone sent by various investigating agencies in M.P region.

2.2. Methodology

2.2.1. Sample and standard preparation

The samples were dissolved in methanol and were subjected for further analysis.

2.2.2. Chemical tests

Name of Test	Method	Reference
Zimmermann test	Suspected sample + 2 drops of 1% w/v 1,3 nitrobenzene in methanol +2 drops of 15% w/v Potassium hydroxide in water.	UNODC
Liebermann test	Suspected sample + 1g of Potassium/Sodium Nitrite + 10 ml conc. Sulphuric acid.	
Microcrystal test	Suspected sample + aqueous solution of mercury chloride (10g/L).	

2.2.3. Thin Layer Chromatography

2.2.3.1. Developing Solvent systems

System: Ethyl acetate: Methanol: Ammonia (85:10:5).

2.2.3.2. Sample and standard solution preparation and spotting

5 mg each of suspected Mephedrone were dissolved in 1 ml methanol and vortexed. The sample was then filtered and 5 microliter of each sample was spotted on the TLC plate (pre coated silica gel 60 F254 Aluminium sheet TLC plates, layer thickness 200µm make by Merck Darmstadt, Germany). Ultra-violet lamp operating at wavelength 254 nm was used for the location of spots after development in solvent system. The separated spots were then visualized using 2% Ninhydrin solution or Dragendroff reagent.

2.2.4. GC-MS operating condition

Sample Preparation	Solution of samples were prepared at a concentration of 1mg/100 ml
System used	GC- MS Nexis 2030 of Shimadzu
Column	Capillary column of SH-Rxi- 5 Sil MS (Crossbond®, similar to 5% diphenyl/95% dimethylploysiloxane) 30 meter, 0.25 mm ID, 0.25 um df
Oven	Temperature programming 90^0C , 1 min hold, rate 8^0C/min to 300^0C for 10 min
Injector	Splitless Injection temp.- 225^0C Sampling time 1min
Carrier gas	Helium, 1.39 ml/min, constant flow
MS parameters	Ionization mode : EI mode, 70 ev Ion source temp: 230^0C Interface temp: 300^0C Scan parameters: TLC, scan range: 35 to 500 amu
Sample injected	1 microliter

3. Observation and Results

3.1. Physical appearance

The colour of the seized/ suspected sample varied from off white, pure white, transparent crystals, brown, light brown, creamish (Figure 1). The morphological texture of confiscated Mephedrone samples varied from powdery material to coarse/ crystalline lumps to sticky/moist substances.

Figure 1

3.2. Presumptive Chemical tests for Thin layer Chromatography

Visualization of separated spots of Mephedrone TLC plates were carried out by spraying 2% Ninhydrin solution. It was observed that there were few more separated spots on TLC plates apart from Mephedrone. For further identification and confirmation of different separated spots, the samples were subjected to GC-MS analysis.

3.3. Gas Chromatography- Mass Spectrometry

Gas Chromatography Mass Spectroscopy (GC-MS) is a hyphenated technique for the analysis of drugs in Forensic examination. In this technique the gas chromatography is used to separates different components mixture in a substance and mass spectroscopy measures molecular mass of the ion separated from the column as determined by their mass to charge ratio (m/z).

In this study GCMS is used to identify different adulterants detected along with Mephedrone in the seized/suspected forensic samples. The common adulterant compounds detected along with Mephedrone were Amphetamine, Methamphetamine, MDMA (methylenedioxymethamphetamine), Lefatamine, Chlorzoxazone, Fludiazepam, Aminorex, Nalbuphine, Olanzapine, Dimethyl Sulfone, Caffeine and Acetaminophen.

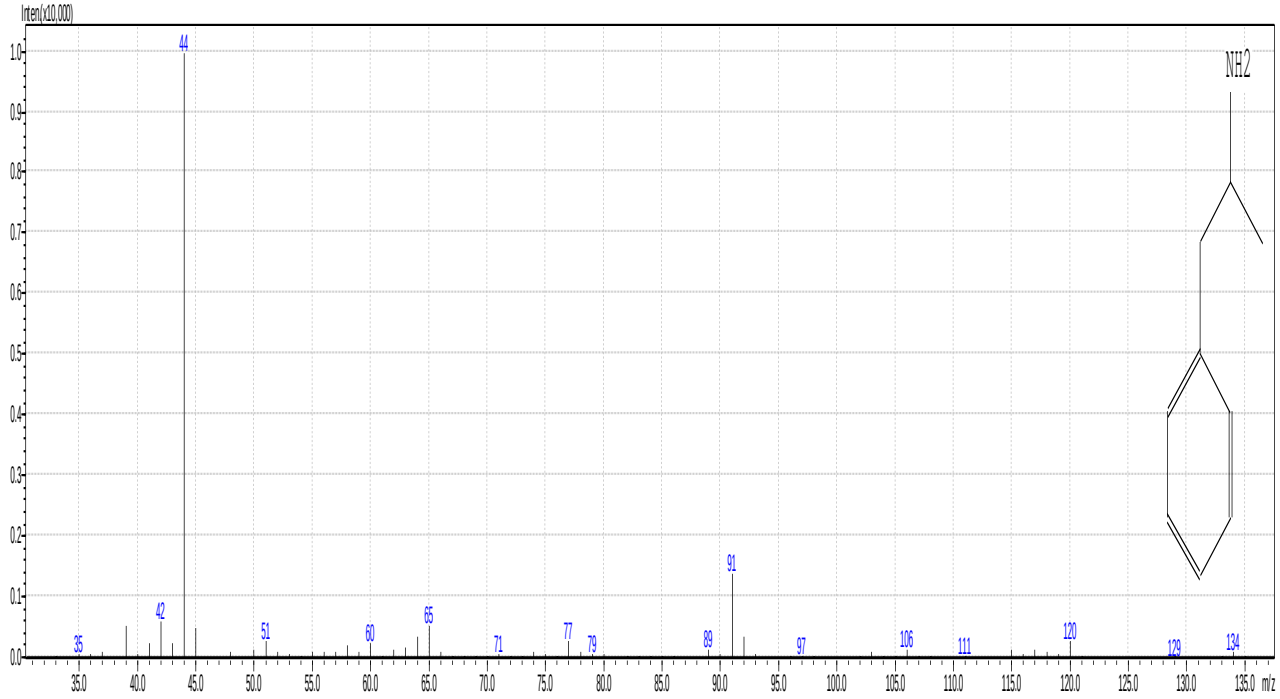
Table 1. m/z and details of adulterants detected in Mephedrone samples

	Adulterant	**Molecular weight (g/mol)**	**Molecular formula**	**Base Peak**	**Fragment Ions**	**Ref.**
Mephedrone is the main narcotic detected in suspected sample	Amphetamine	135.21	C9H13N	44	91,42,65,77,120,51,129,89	NIST Library
	Methamphetamine	149.2337	C10H15N	58	91,59,30,42,103,117	

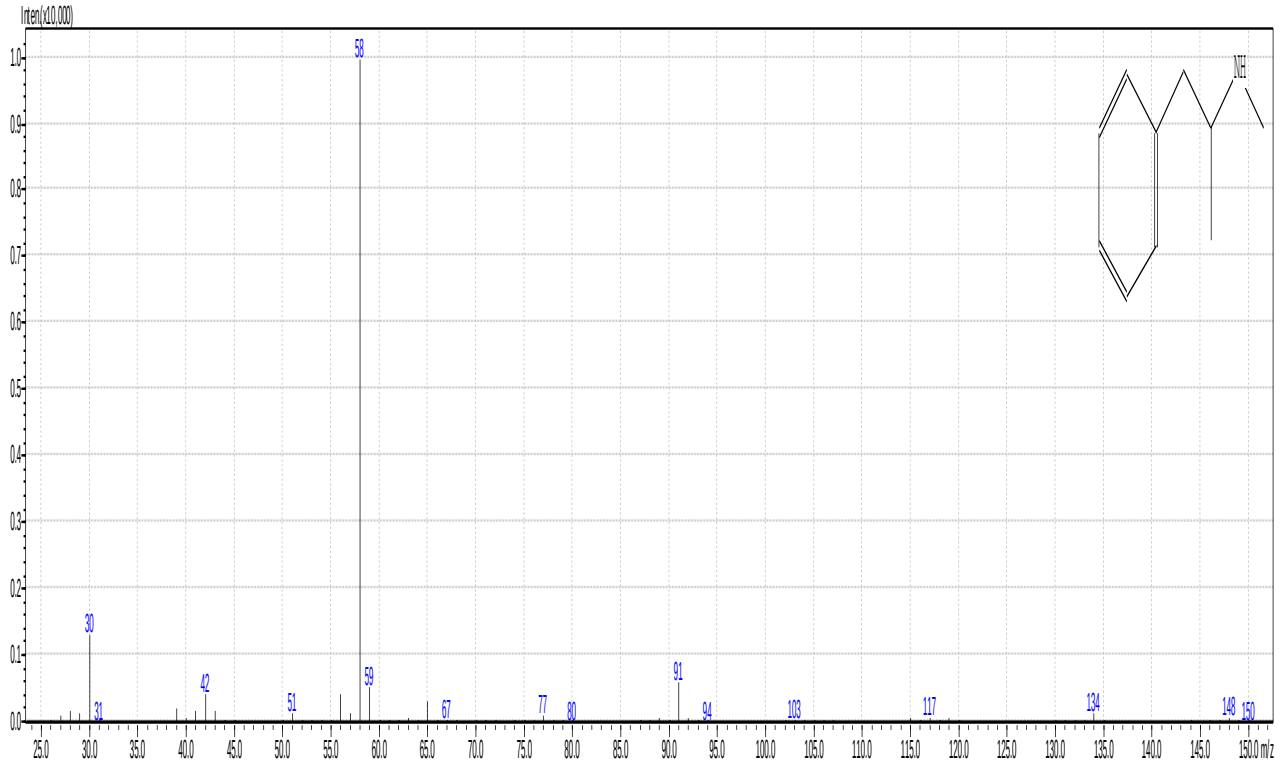
	MDMA (methylenedioxymethamphetamine)	193.25	C11H15NO2	58	135,178,193,30,39,42,51,77,88,105	
	Lefatamine	225.329	C16H19N	134	91,135,77,65,118,178,165,152,103,42	
	Chlorzoxazone	169.565	C7H4ClNO	169	78,113,51,63,76,36,39	
	Fludiazepam	302.73	C16H12ClFN2O	274	275,301,239,75,109,125,183, 137,150,211	
	Aminorex	162.19	C9H10N2O	56	118,91,162,44,51,65,107,108,128,132,145	
	Nalbuphine	357.443	C21H27NO4	302	357,41,55,77,91,115,128,161,173,185,214,228,254,272	
	Olanzapine	312.44	C17H20N4S	242	229, 213, 254, 312,198,99,43,57,71,84,99	
	Dimethyl Sulfone	94.13	C2H6O2S	79	94,15,18,28,45,48,63,26,32	
	Caffeine	194.19	C8H10N4O2	67	55,109,42,67,82,194,137,165	
	Acetaminophen	151.163	C8H9NO2	109	151,43,53,80,27	

Table 2. Mass data of the following adulterants

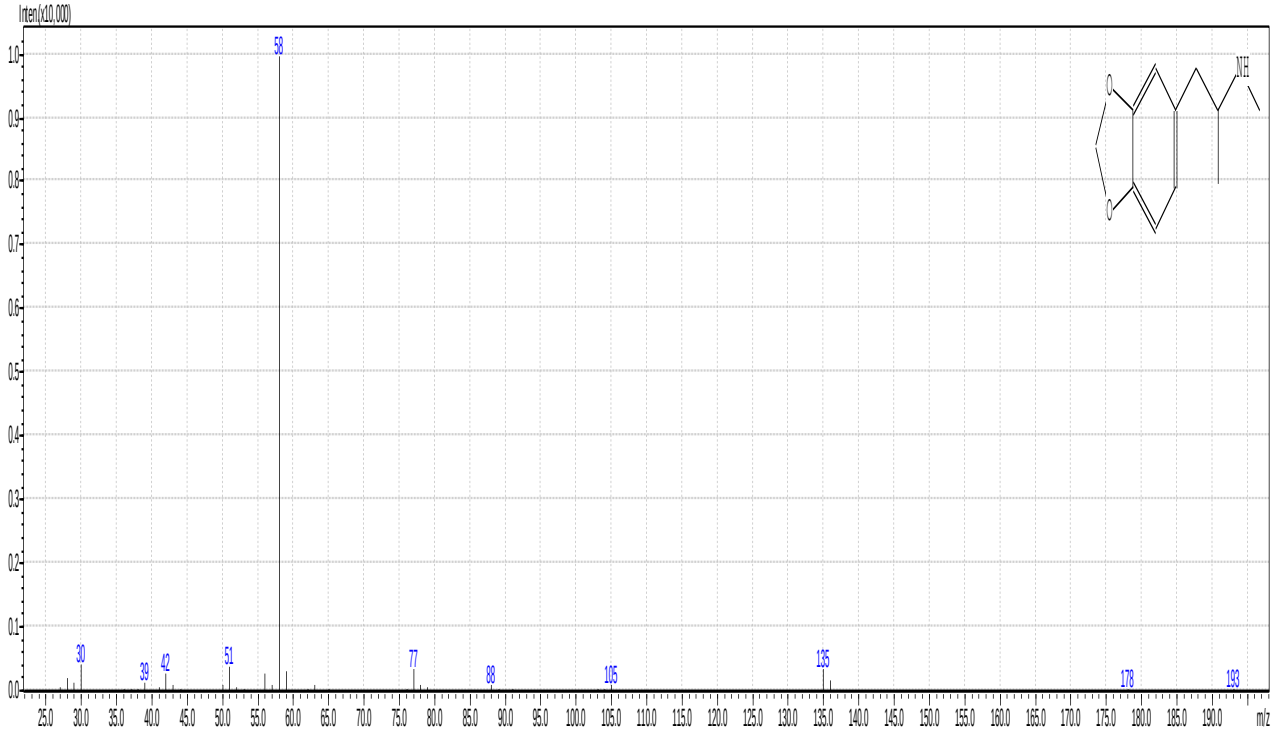
Amphetamine



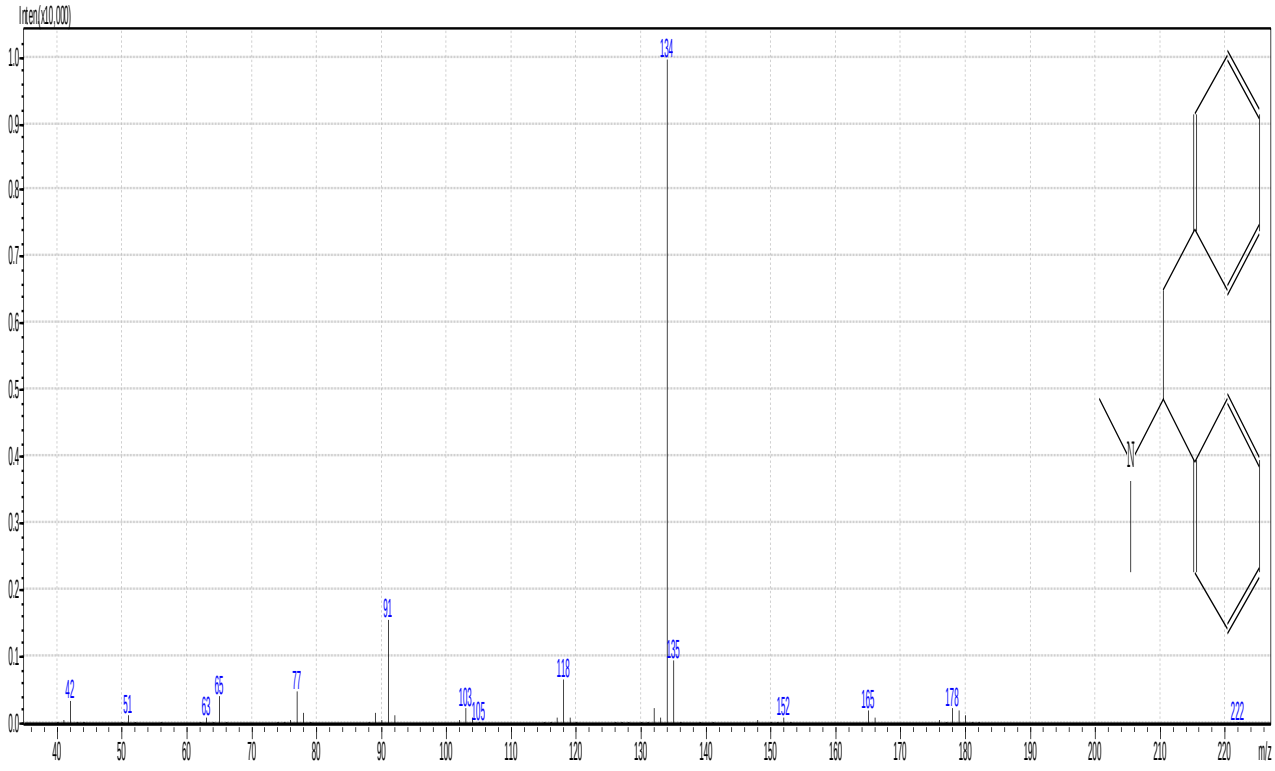
Methamphetamine



N-Methyl-3,4-methylenedioxyamphetamine (MDMA)



Lefetamine



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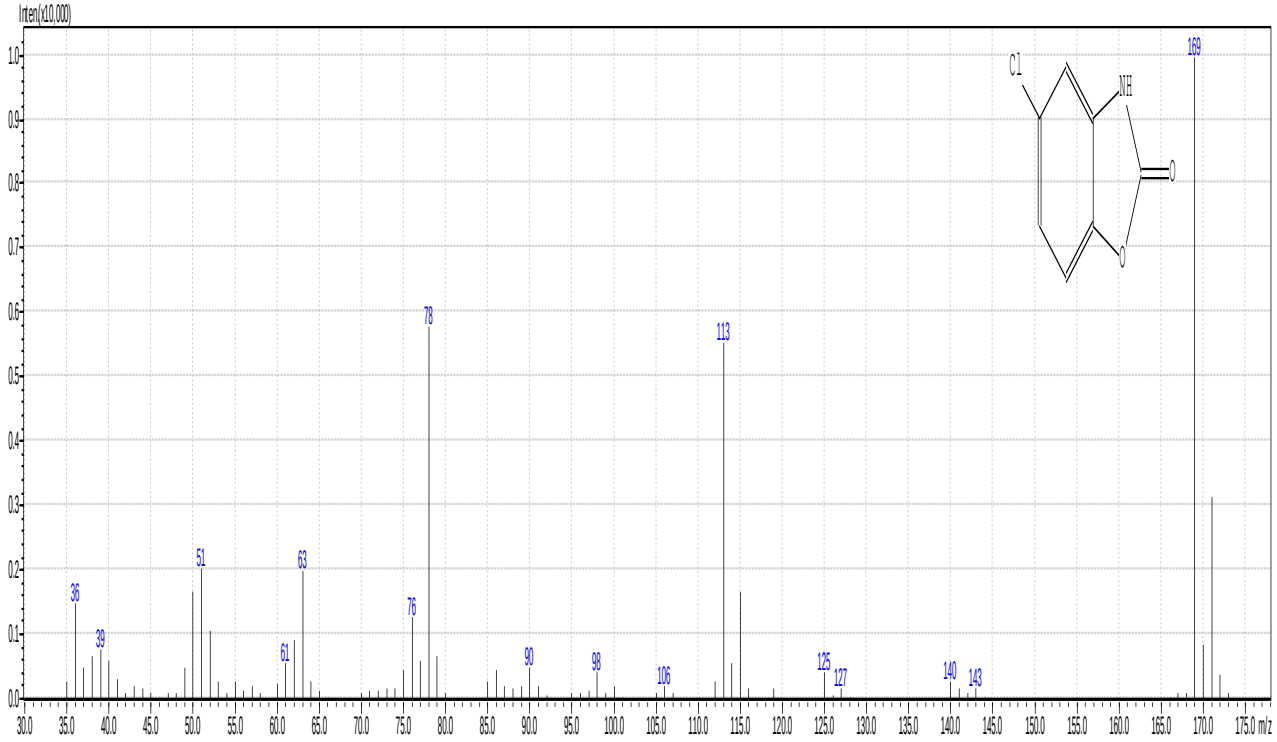
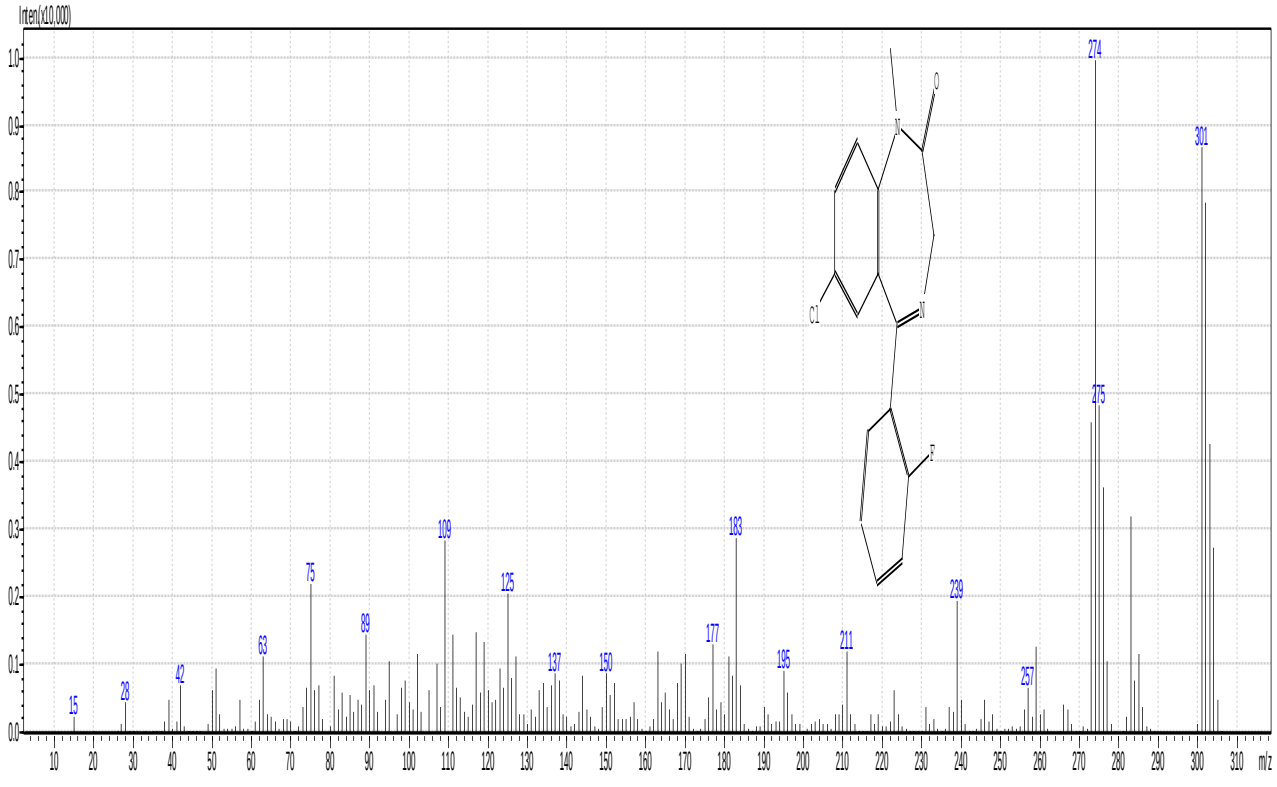
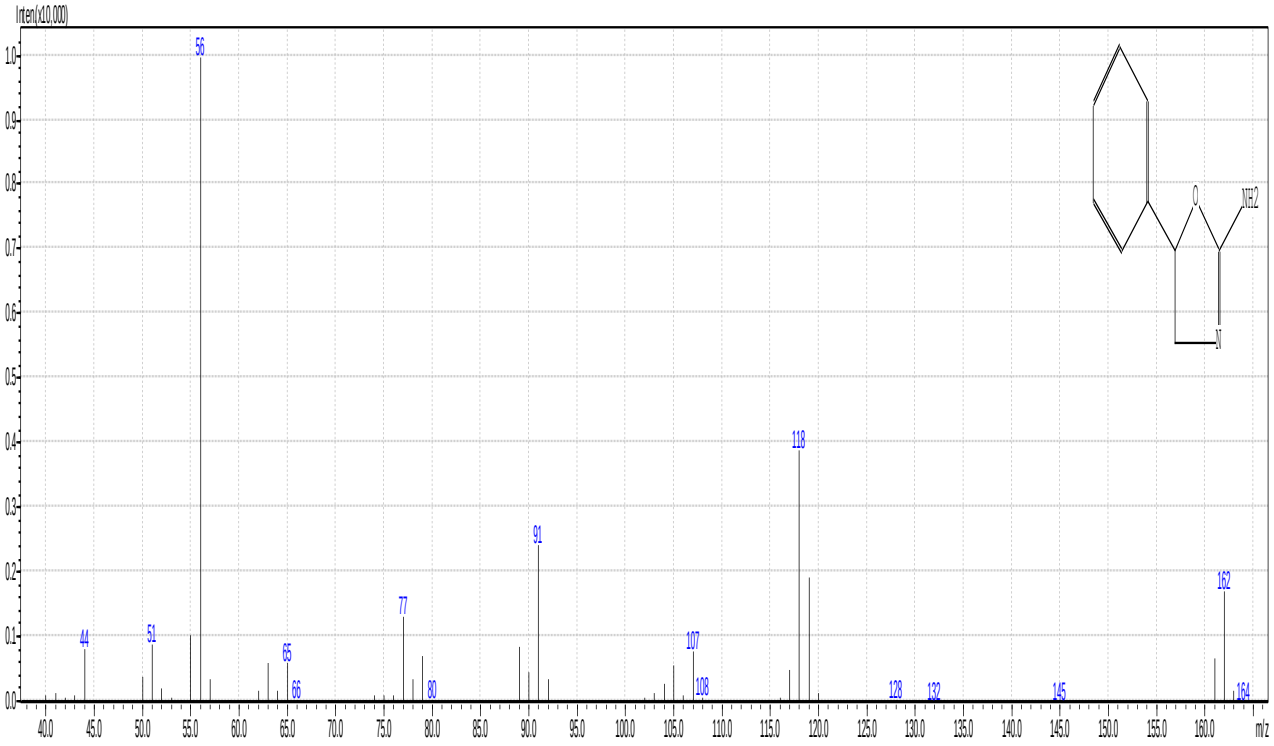
Chlorzoxazone



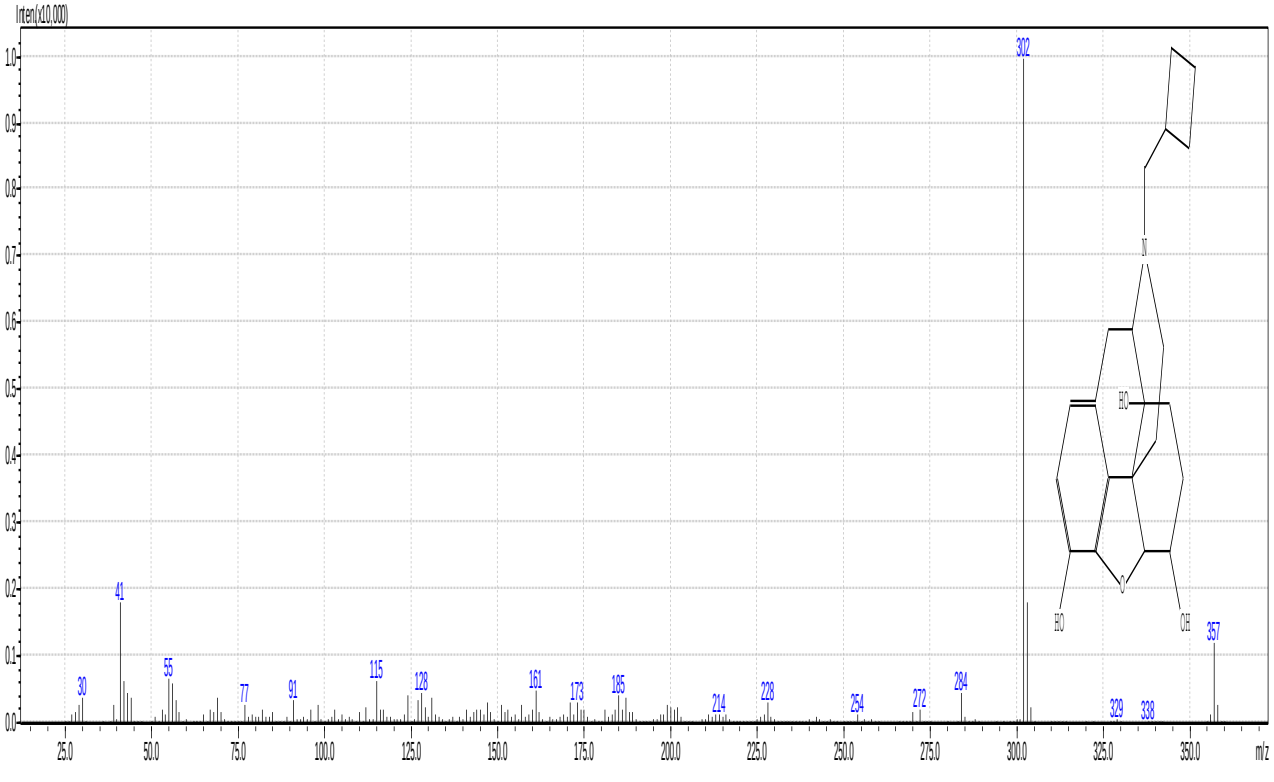
Fludiazepam



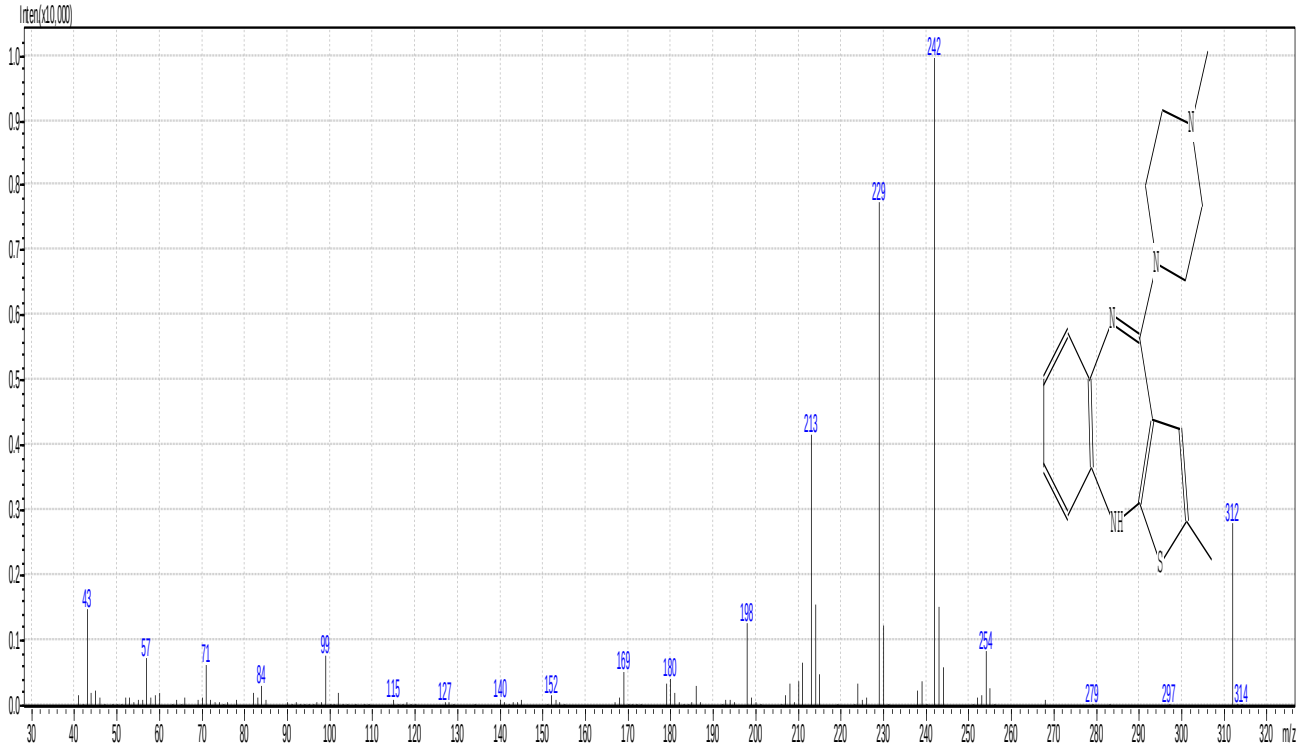
Aminorex



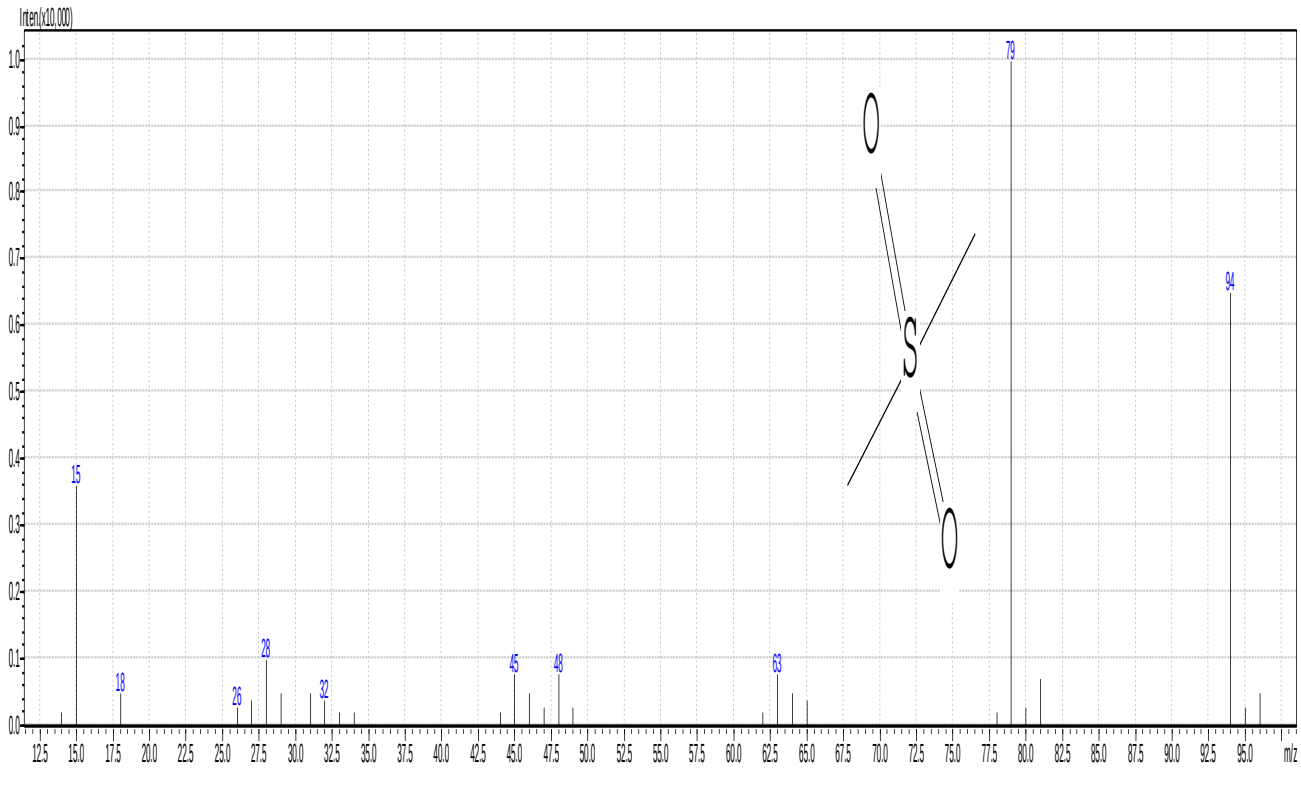
Nalbuphine



Olanzapine



Dimethyl Sulfone



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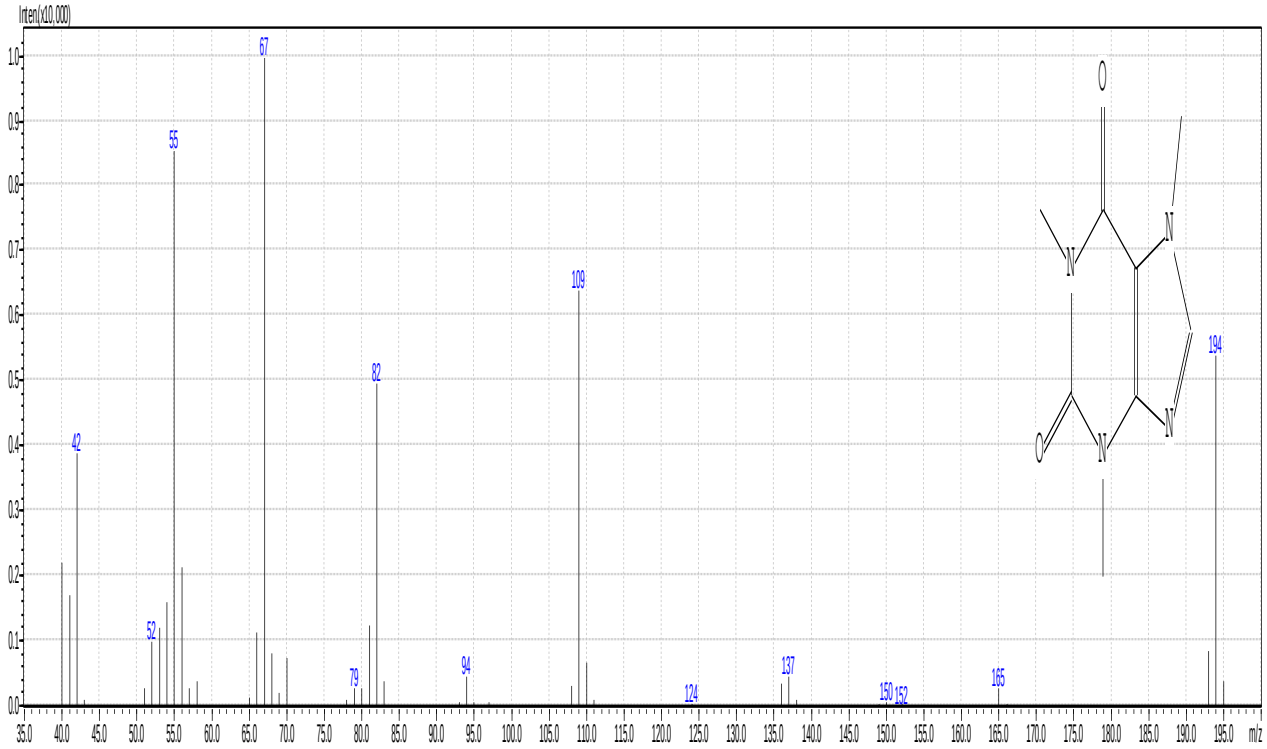
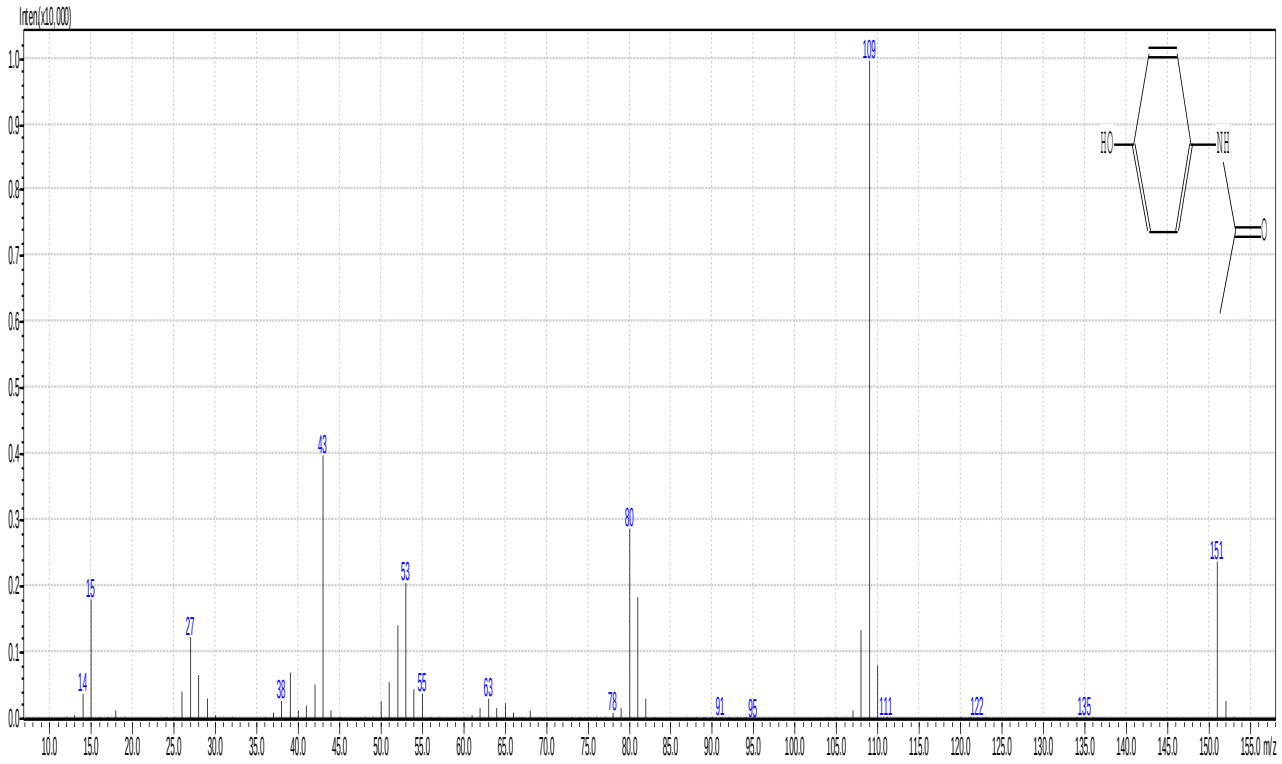
Caffeine



Acetaminophen



4. Discussions

The changing trends in the adulteration of drugs in order to mask its identity and befool legal authorities are a matter of great concern. It also makes it difficult for the forensic scientist to detect the actual compound as many adulterants interfere with the original test results. Adulterated drugs often mimic or cause synergistic or antagonistic effect and are often dangerously associated with consumption of these drugs by abusers. It is therefore felt a need to share details of identified adulterants from Mephedrone samples commonly sent to forensic laboratory in case of narcotic drug seizures. The following are the details of the adulterants mixed with the street samples of Mephedrone:

Adulterants	**Description**
Amphetamine	It is a central nervous system stimulant, used as a medication in management and treatment of ADHD and narcolepsy. It functions by increasing the amounts of dopamine, norepinephrine, and serotonin (to a lesser extent) in the synaptic cleft through a variety of mechanisms to cause its effect.
Methamphetamine	Methamphetamine is a powerful, highly addictive stimulant that affects the central nervous system. It increases the amount of the natural chemical dopamine in the brain. Dopamine is involved in body movement, motivation, and reinforcement of rewarding behaviors. The drug's ability to rapidly release high levels of dopamine in reward areas of the brain strongly reinforces drug-taking behavior, making the user want to repeat the experience [19].
3,4-methylenedioxy-methamphetamine (MDMA)	3,4-methylenedioxy-methamphetamine (MDMA) is a synthetic drug that alters mood and perception (awareness of surrounding objects and conditions). It is chemically similar to both stimulants and hallucinogens, producing feelings of increased energy, pleasure, emotional warmth, and distorted sensory and time perception [20].
Lefatamine	Lefetamine sometime also known as Santenol is a stimulant drug which act as an analgesic and has comparable effect to codeine. Lefetamine may act as an opioid partial agonist [15].

Chlorzoxazone	Chlorzoxazone is a muscle relaxant with sedative properties that is used together with rest and physical therapy to treat skeletal muscle conditions such as pain or injury.
Fludiazepam	Fludiazepam is a short-acting benzodiazepine with general properties similar to diazepam. It is used in the short-term treatment of anxiety disorders. It has anxiolytic, anticonvulsant, sedative and skeletal muscle relaxant properties.
Aminorex	It is a weight loss (anorectic) stimulant drug which causes pulmonary hypertension. It is amphetamine-like substance which is metabolized from Levamisole. It is mostly associated with ingestion of the appetite suppressant.
Nalbuphine	Nalbuphine is a synthetic opioid agonist- antagonist analgesic of the phenanthrene series It interacts with an opiate receptor site in the CNS to cause its effect.
Olanzapine	Olanzapine is a second-generation (atypical) antipsychotic medication used for schizophrenia. It exerts its action primarily on dopamine and serotonin receptors. It works by blocking dopamine from potential action at the post-synaptic receptor.
Dimethyl Sulfone	Dimethyl sulfone is a neutral substance generally used as an adulterant in methamphetamine hydrochloride due the similarity in its appearance.
Caffeine	Caffeine is a central nervous system (CNS) stimulant. It is used as mild cognitive enhancer to increase alertness and attention of performance; however its chronic consumption at high doses can cause symptoms like anxiety, nervousness, impaired thinking, sleep disturbance, heart palpitations and stomach irritation.
Acetaminophen	Acetaminophen commonly known as Paracetamol is a non-opioid analgesic and antipyretic agent used to treat fever and mild to moderate pain.

5. Conclusion

The detection and identification of Mephedrone sample viz., physical appearance , chemical tests, TLC and GC-MS analysis indicate presence of new, uncommon adulterants such as Amphetamine, Methamphetamine, MDMA (methylenedioxymethamphetamine), Lefatamine, Chlorzoxazone, Fludiazepam, Aminorex, Nalbuphine, Olanzapine, Dimethyl Sulfone, Caffeine and Acetaminophen. Awareness and knowledge about presence and detection of these adulterants in street samples of Mephedrone is very important for forensic community for accurate identification and detection of drugs. This also encourages such studies as a part of strategy to update about the recent trends emerging in illicit drug abuse. Identification of adulterants would enable the criminal judiciary system to keep vigilant eye on national and international crime.

Declarations

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Competing Interests Statement

The authors declare that there are no competing interests.

Consent for Publication

The authors declare that they consented to the publication of their original research work.

Authors' Contributions

Both the authors took part in data collection, literature review, analysis, and manuscript writing equally.

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