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Review Article

A review of emerging substance use and their analytical detection techniques

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Abstract

Around the globe, the abuse of emerging drugs under various substance classes has emerged as a serious health challenge. These include novel psychoactive substances (NPS) and designer drugs that comprise constantly advancing chemical structures, and because of that, it is even more difficult to identify and detect them. Thus, they usually easily escape from legal consequences. Public health is at stake because of the inconsistency in the timely and precise identification of these substances. This review aims to assess the emerging substances of abuse according to their substance classes, particularly emphasizing their pharmacokinetics, metabolism, and chemical properties. The article reviews synthetic cannabinoids, synthetic cathinone, NPS, designer drugs, and anabolic steroids, as well as the principle and working mechanism of the advanced analytical tool for detecting them. Google Scholar and PubMed were utilized to perform a broad, extensive literature review, considering studies published from 2000 to 2024. Synthetic cannabinoids mainly target cannabinoid receptors of the endocannabinoid system. Synthetic cathinones inhibit neurotransmitter reuptake. NPS hinders the monoamine transporters' reuptake, designer drugs antagonize N-methyl-D-aspartate (NMDA) receptors, and anabolic steroids bind to androgen receptors in cells. Even low concentrations of drugs in the sample can be detected by GC-MS, LC-MS/MS, LC-QTOF MS, and SERS. At the same time, nanoparticle-based NMR chemo sensing, IR, IRMS, and DART-MS carry out structural analysis, molecular fingerprinting, and rapid field identification. Advancements in analytical detection techniques, along with public education and holistic rehabilitation efforts, are necessary to fight and overcome the grave challenge posed by emerging substance abuse.

Keywords: Substance use, Substance abuse detection, Synthetic cathinones, Gas Chromatography-mass spectrometry, LC-MS, Forensic toxicology.

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1. Introduction

An uncontrollable urge to consume drugs, irrespective of hazardous consequences, affecting individuals and people around them, is called Substance use. According to the diagnostic and statistical manual of mental disorders, substance use and substance dependence are different. Psychological dependence, which includes tolerance and withdrawal symptoms, denotes substance dependence, whereas such psychological aspects need not be essentially associated with substance use. A vicious cycle of abuse gets tailored because of repeated exposures to the substance, triggering a loss of cognitive control and resulting in compulsive drug-seeking behavior.[1]

In 2016, approximately 5.6% of the global population aged 15-64 years had consumed drugs at least once, a disturbing statistic showcasing the prevalence of substance use across the global population profile. It is highlighted by the Global Burden of Disease study that about 14% of the total health burden in young men is due to drug abuse.[2] A multistage random sampling total of 40,697 males from randomly selected households aged between 12 to 60 years across 25 states of India surveyed that the frequency of consuming any drug ever was 63.7%.[3] An alarming concern has been raised due to increasing addiction rates in India and Globally, leading to severe health issues. This emergency has even escalated due to regional variations and an insufficient database.[4,5]

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Stress, burnout, and depression are often found as the cause of substance use among healthcare professionals. Some individuals self-medicate without medical supervision due to emotional distress caused by perfectionism and high job demands. In the place of consulting professionals for help, individuals may cope with stress, anxiety, depression, or other mental health disorders by abusing substances.[6] Prominent social issues are related to substance use, including violence, homelessness, and relationship breakdowns. In Western countries, a large fraction of the prison population is found to have a dual diagnosis, consisting of substance use and legal involvement.[7] In 2010, a study estimated the economic output lost nearly US dollars 8.5 trillion because of mental, neurological, and substance use disorders globally, anticipating that without any impactful approach, it could approximately double by 2030. Substance use disorders not only affect the workforce productivity of individuals but also gravely impact the family, which seeks to provide healthcare.[8]

To confront the crisis of substance use and drug addiction, the Indian government has put various initiatives into action, such as Nasha Mukt Bharat Abhiyan (NMBA) and the Narcotic Drugs and Psychotropic Substances (NDPS) Act. The NDPS Act outlines penalties for violations outlined in the Act for regulating activities related to narcotic drugs and psychoactive substances. Based on a national survey and insights from the Narcotics Control Bureau, 272 districts referred to as most vulnerable to substance use are targeted by the NMBA.[9]

This article aims to illustrate emerging drugs of abuse according to their substance class and dedicates efforts to explain the available analytical detection techniques. Synthetic cannabinoids are powerful man-made drugs that resemble the effects of natural cannabis. Cathinone is found in the Catha edulis (khat plant) as a natural stimulant, and its chemical derivative is known as synthetic cathinone. To escape existing laws against illicit drugs, Novel Psychoactive Substances have emerged recently, replicating the effect of traditional illicit drugs. Designer drugs comprise a wide range of various stimulants that are generally sold in markets under different names to dodge laws and regulations. For muscle-building and strength-boosting, Anabolic steroids are abused, primarily by athletes.[10-13] The available analytical methods of detection of these emerging substance uses are also explained in this article. Substance use is a danger that requires practically effective and urgent efforts. The use of advanced techniques and trained personnel to operate them should be promoted to challenge this critical adversity.[14]

2. Methodology

This review was performed by strategically searching the literature on emerging substances of abuse and their analytical detection methods. Google Scholar and PubMed are the databases used to delve into authentic sources. For the substance class, the following terms were frequently used in combination and permutations while searching literature: “Synthetic Cannabinoids introduction,” “Pharmacological absorption of synthetic cannabinoids,” “Mechanism of action of synthetic cathinones,” “Metabolism and excretion of designer drugs,” “Newer drugs of abuse under anabolic steroids”, and so forth. These searches were repeated with each of the five core substance classes discussed: Synthetic Cannabinoids, Synthetic Cathinones, Novel Psychoactive Substances (NPS), Designer Drugs, and Anabolic Steroids. Alongside, Principle and working mechanism of analytical methods were searched with keywords like “Principle and working of GC-MS,” “Detection of NPS using LC-MS/MS,” “Application of DART-MS in toxicology,” “Nanoparticle-based NMR spectroscopy working mechanism,” “Surface-enhanced Raman spectroscopy in principle and working mechanism,” and more. Other keywords were used so as to understand the findings for the Indian forensic scene:“Availability of GC-MS in CFSLs and SFSLs,” “Status of forensic science instrumentation in India,” “Analytical toxicology instrumentation in India,” "Forensic labs using IRMS India and others related to infrastructure and regional accessibility. Added to that, a cross-comparative search strategy was carried out, in which separate searches were conducted for each of the ten analytical methods to find out whether they were capable of detecting each of the five substance classes. Keywords like “Can GC-MS detect synthetic cannabinoids,” “LC-QTOF-MS application in detecting anabolic steroids,” “NMR detection of designer drugs,” and similar formulations were extensively used.

The inclusion criteria were Peer-reviewed journal articles, government and institutional reports, original studies, case reports, and systematic reviews published between 2000 and 2024 that specifically outlined pharmacological features or analytical detection methods. Only articles that were relevant to toxicological or forensic science were included, and sources that showed practical application in substance detection were given consideration.

Among the exclusion criteria were redundant information, research with no analytical dimension, solely clinical articles without any forensic applicability, and publications written in languages other than English.

This way, basic foundational studies and recent advancements were reviewed.

3. Discussion

3.1. Newer drugs of abuse according to substance class

3.1.1. Synthetic cannabinoids

Synthetic cannabinoids (SCBs) are a class of psychoactive substances that are man-made chemicals. They are formulated to reproduce the effects of the active components of the cannabis plant, such as delta-9-tetrahydrocannabinol (THC). These are mainly used for recreational activities. They are non-polar and soluble in lipids and easily evaporate

when smoked. SCBs generally look like fine powders in their pure form, which can be white or yellow and have a texture like powdered sugar or flour.[15] They interact with the receptors in the body's complex endocannabinoid system, which regulates mood, appetite, and pain. Cannabinoid receptor type 1 (CB1R) and cannabinoid receptor type 2 (CB2R) are two important receptors that synthetic cannabinoids primarily target. CB1R is present in the brain and associated with psychoactive effects such as feeling high, whereas CB2R is found in the body's immune system, regulating pain and inflammation. Synthetic cannabinoids activate the receptors, causing stronger effects, and are often full agonists. On the other hand, Δ9-THC (the main psychoactive component in cannabis) is a partial agonist, activating the receptors partially, not as strongly as SCBs. Thus, SCBs can cause more severe adverse effects as compared to cannabis. Smoking, vaping, or ingesting are some of the common ways of consumption of SCBs. Based on the method of administration and their chemical structure, the pharmacological absorption of SCBs differs.[16] Upon binding SCBs with cannabinoid receptors, the release of neurotransmitters from presynaptic neurons is hindered. This alters the transmission of messages with the nervous system, affecting mood, memory, and reward systems.[17] After consumption, the body breaks SCB rapidly, so while testing urine, the byproducts or metabolites of the breakdown process are usually found instead of the original drug consumed. The type of chemical linker influences the metabolic pattern of SCBs in them. SCBs with amide linker get metabolized mainly through hydrolysis to form carboxylic acids, with additional hydroxylation occurring on the amino-oxo butane moiety. The key path of excretion for SCB is urine. Multiple metabolites from different SCBs in urine can complicate the testing results because a single SCB may be present in blood, but various metabolites are found in urine.[18] The examples of SCBs that have emerged as new drugs of abuse comprise Spice, K2, Eclipse, Mojo, UR-144, KUR-144, JWH-250, HU210, and XLR-11.[19]

3.1.2. Synthetic cathinone

The key psychoactive component of Catha edulis (khat plant) is cathinone; thus, synthetic cathinones are a chemically similar group of psychoactive substances. The chemical structure of Synthetic cathinone is related to amphetamines, which is why both produce similar sympathomimetic effects.[20] Snorting and oral ingestion are methods of consuming synthetic cathinone. Through snorting, the effects can peak within 10-20 minutes, whereas it takes 20-40 minutes of slower onset for peak effect in case of oral ingestion.[21] The absorption of synthetic cathinone is influenced by its stability and solubility, as well as the pH level of the stomach/intestine. Synthetic cathinone's ionization, which depends upon the host's pH, affects the ability to cross the cell membrane and be absorbed into the bloodstream.[22] Synthetic cathinone inhibits the reuptake of norepinephrine, which leads to an increased level of this neurotransmitter in the synaptic cleft, which causes enhanced alertness and high energy levels.[23] Synthetic cathinones go through Phase II metabolism, mainly methylation and conjugation. The catechol-O-methyltransferase enzyme facilitates methylation. Conjugation involves adding glucuronic acid (Glucuronidation) and Sulfation. Most metabolites formed during Phase II are excreted in urine, a common pathway for eliminating drugs from the body. Some substances may also be excreted unchanged, but this is less common for synthetic cathinones.[24] Emerging drugs of abuse under the class of synthetic cathinone are bath salts, MCAT, Bubbles, Isoethcathinone, and Isopentedrone.[25]

3.1.3. Novel psychoactive substances

Novel psychoactive substances (NPS) are synthetic compounds that are manufactured to imitate the effects of traditional recreational drugs. They are popularly cited as "Legal Highs' as they were earlier designed to find a clever way of avoiding difficulty with existing drug laws. Creators of NPS often change the chemical structure of known drugs to produce new substances that remain legal.[26] The mechanism of action of Novel Psychoactive substances is mainly linked to their interaction with neurotransmitters in the brain. Many NPS deploy their effect by inhibiting Monoamine transporter. These proteins in the neurons' membrane regulate neurotransmitter levels in the brain. Dopamine Transporter, Norepinephrine Transporter, and Serotonin Transporter are primary transporters responsible for the reuptake of dopamine, norepinephrine, and serotonin, respectively. NPS blocks the reabsorption of neurotransmitters back into presynaptic neurons by inhibiting these transporters. Due to this, neurotransmitters accumulate in the synaptic cleft, enhancing their actions on post-synaptic receptors.[27] The absorption of NPS differs based on their mode of intake and chemical structure.[26,28] Orally ingested NPS passes through the gastrointestinal tract, and from there, it gets absorbed into the bloodstream. In the case of snorting, substances rapidly get absorbed into the bloodstream via the lungs' alveoli. After getting absorbed, they are distributed throughout the body via the bloodstream. The distribution is affected by several factors, such as the drug's solubility, molecular size, and the presence of specific transport proteins.[28] The liver metabolizes these NPS using special proteins called cytochrome P450 enzymes. 2-Methiopropamine is metabolized by enzymes such as CYP1A2 and CYP2D6. These enzymes catalyze the removal of a methyl group (N-demethylation) and the addition of a hydroxyl group (hydroxylation) at the side chain and thiophene ring. The main metabolite product of this process is hydroxy-aryl metabolite, and they are found in greater quantities than the original drugs in urine. Methyl phenyl-amphetamines (2-MA, 3-MA, 4-MA) are three isomers mainly metabolized by hydroxylation with the help of the enzyme CYP2D6. After metabolism, the body excretes the metabolites and the form of a drug that has not undergone any chemical transformation through urination.[29] Quetiapine,

Loperamide, Pregabalin, Gabapentin, Clenbuterol, and Olanzapine are some examples of emerging drugs of abuse under NPS.[30]

3.1.4. Designer drugs

As per the Designer Drug Enforcement Act of 1986, a designer drug is defined as "a substance other than a controlled substance that has a chemical structure substantially similar to that of a controlled substance in schedule I or II or that was specifically designed to produce an effect substantially similar to that of a controlled substance in schedule I or II." In India, the Narcotic Drugs and Psychotropic Substances (NDPS) Act, 1985 mainly concentrates on scaling down the supply and availability of these illicit designer drugs and regulates the cultivation, manufacture, sale, possession, and use of narcotic drugs and psychotropic substances.[9] To create these drugs, chemists modify the existing structures of drugs to increase their potency or change their effects, which leads to the production of new designer drugs.[31] The effect of a drug depends upon how it is absorbed. The quicker the absorption, the greater the effects that a person involved in such recreational activities desires the most. Designer drugs, such as Methylenedioxy-substituted amphetamines (MDMA) and Paramethoxyamphetamine (PMA), have specific chemical modifications that change their properties. Such changes considerably influence how the drug interacts with the body. Cytochrome P450 enzymes catalyze the metabolism of many designer drugs. These enzymes break down drugs in the liver. Metabolites of designer drugs come out of the body via urine. It is essential for discarding the drugs from the body, and it can differ largely among individuals because of genetic differences in enzyme expression.[32] N-methyl-D-aspartate (NMDA) receptor antagonism is a significant mechanism of action for Designer drugs. NMDA is a receptor protein in the brain that transmits signals between nerve cells, significantly impacting learning, memory, and overall cognitive function. Designer drugs like methoxetamine act as NMDA receptor antagonists. It blocks normal signals that are transferred through these receptors. Thereby, they cause confusion, hallucination, and other psychologically disturbing effects that are caused similarly by other dissociative anesthetics like phencyclidine.[33] Kratom, Salvia divinorum, 4-Fluoroamphetamine (4-FA), 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT) are some of the notable examples of emerging designer drugs.[34]

3.1.5. Anabolic steroids

Anabolic steroids, also known as anabolic-androgenic steroids, are man-made derivatives of testosterone, which is a critical hormone in the human body.[35] Athletes and fitness fanatics desire these steroids because of their anabolic effect since they promote protein synthesis, facilitating the building up of muscle tissues. Testosterone is released naturally into the body and has anabolic and androgenic effects. The androgenic effect is associated with the secondary sexual characteristics in males. Manufactured anabolic steroids primarily focus on muscle-building effects rather than progressing androgenic traits.[36] Anabolic steroids deploy their effects mainly by binding to androgen receptors in cells. Upon binding to steroids, these androgen receptors get activated, and the shape of the receptor changes, which influences how it interacts with other proteins inside the cell. Certain helper proteins connect with activated receptors and send the signals inside the cell, impacting how much protein it will produce. Thus, these steroids impart their anabolic effects by influencing gene expression that activates the genes responsible for protein production. Apart from the genomic pathway, which takes 30-60 minutes to produce the impact since it comprises how the genes function, the non-genomic pathway could begin showing its effects within seconds to minutes. One of the non-genomic pathways is a rapid surge in the level of calcium ions. Due to this increment in calcium ions within cells, the muscle fibers' contraction property strengthens, improving athletes' performance during workouts. These steroids can also interact with specific receptors in the brain, affecting brain activity and behavior almost immediately.[37] The chemical structure of steroids influences their metabolism and excretion. Anabolic steroids undergo oxidation, reduction, and hydroxylation (Phase I metabolism) and convert into more polar compounds, easing the body to get rid of them. Various isomers are formed after the metabolism of the A-ring of steroids. For a particular steroid called mestanolone, after taking it, the body first gets rid of small amounts of different forms of the steroid. Over time, the amount of one specific form increases in the urine, showing that the body is processing it differently as time goes on. These steroids generally attach to glucuronic acid or sulfate, making it easier to dissolve and excrete via urine.[38] Nandrolone, Stanozolol, Oxymetholone, 19-Norandrostenedione, and Androstenedione are some of the notable examples of anabolic steroids.[39]

3.2. Analytical detection methods for drugs of abuse

3.2.1. Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is a refined analytical procedure combining GC and MS methods for separating, purifying, and analyzing volatile and semi-volatile substances. Gas chromatography separates substances between a stationary liquid phase and the mobile gas phase. The sample is introduced via the injector, which converts it into vapor by heating it, ensuring it enters the column in the gaseous state. Separation occurs in the column, either a capillary column (narrow and provides high resolution) or a packed column (containing solid-coated stationary material). The resolution and efficacy of separation are based on the choice of the column. In GC, the separation is based on boiling point and affinity with the stationary phase. The lower boiling point compounds eluted from the column earlier because of their poor affinity with the stationary phase. Due to this differential retention, effective

separation of complex mixture is allowed.[40] After these compounds are separated in the GC stage and transferred into a Mass spectrophotometer. MS separates compounds based on the mass-to-charge ratio. Separated compounds are ionized by Electron Impact or Chemical Ionization, depending on the nature of the analytes and the desired sensitivity. An electric field and the magnetic field then manipulate these ions. The abundance of each ion is measured, producing a Mass spectrum that shows different components of the sample. A dedicated software performs analysis of large amounts of data produced in Modern GC-MS systems based on their unique mass patterns. The software compares obtained mass spectra against reference libraries, and this automation increases the accuracy of the analysis.[41] GC-MS is utilized in industrial applications, analyzing various fuel sources, identifying impurities in active pharmaceutical ingredients, tracing organic pollutants in environmental monitoring, profiling adulteration in food products, and forensic analysis of fire debris.[42] In doping prevention, GC-MS is used to analyze anabolic androgenic steroids such as nandrolone, stanozolol, etc.[43]

3.2.2. Liquid chromatography-tandem mass spectrometry

Liquid chromatography- Tandem mass spectrometry (LC-MS/MS) is a cutting-edge analytical process. Its primary stage is liquid chromatography, where individual components of a sample mixture are distinguished. The sample is passed through a column filled with the stationary phase, and various components of the sample mixture interact with the stationary phase. Based on characteristic features like polarity and size, separation over time takes place. After separation, the eluted components are ionized using ionization techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). A high voltage is applied in ESI that produces a fine mist of charged droplets, converting analytes of sample in gas-phase ions. APCI ionizes analytes by corona discharge, working at a higher temperature than ESI. Post-ionization yielded ions are moved to the mass spectrometer. This transport takes place in a vacuum environment to minimize the hindrance from air molecules and enable the streamlined transfer of ions in the direction of the mass analyzer.[44] To increase the overall sensitivity of analysis, a focusing component directs ions toward the first mass filter (Q1). The first mass filter (Q1) filters ions selectively based on their mass-to-charge ratio by employing electric and magnetic fields. Precursor ions only pass through Q1 and enter a collision chamber (Q2). Inside Q2, precursor ions collide with a low-pressure gas (typically nitrogen or argon). Due to this collision, ions are induced to get fragmented, which is called collision-induced dissociation (CID). After going through CID, Precursor ions convert into even smaller fragments of product ions. This step is necessary for understanding the structural and chemical composition of analytes. Now, product ions are sent to the second mass filter (Q3), which filters specific ions based on mass-to-charge ratio, like Q1. Only the ions with specific m/z values that match the target criteria are allowed to move to the detector, increasing the accuracy and specificity of the analysis. An electron multiplier.is generally an ion detector, which transduces the ion signals into measurable electrical signals. The strength of the signal amplitude is directly proportional to the number of ions detected, allowing quantitative analysis of the analytes in the sample.[45] LC-MS/MS is widely used in therapeutic drug monitoring, advanced toxicology screening, hormone analysis, protein quantification, and standardized reference method development.[46] LC-MS/MS is efficient in detecting synthetic cannabinoids from oral fluid samples.[47] LC-MS/MS has a limit of detection ranging from 0.1 to 0.5 ng/mL and limits of quantification (LOQ) from 0.5 to 1.0 ng/mL for the synthetic cathinone and its metabolites, which is important for the detection of even lower concentrations of analyte in the sample.[48] Pregabalin and Olanzapine are some of the NPS that LC-MS/MS can easily detect.[49] Anabolic steroids such as nandrolone and stanozolol can be determined in human hair by application of LC-MS/MS.[50]

3.2.3. Liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

Liquid chromatography coupled with Quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) is an analytical method used for wide-ranging toxicological analysis. Initially, liquid chromatography separates the samples based on their chemical properties. This step is important as it isolates components from the mixture and allows for precise analysis. The isolated compounds are converted into ions using ESI for mass-spectrometric analysis. In ESI, the liquid sample is sprayed into a fine mist, and charged droplets form as the solvent evaporates. When these droplets break apart, ions are produced. The Agilent Jet Stream Technology increases the ionization efficiency, also aiding in detecting low-concentration analytes. The developed ions are then channeled into a quadrupole mass filter, which selectively permits ions with a specific mass-to-charge ratio to pass through, ensuring that only the analytes relevant for analysis are studied. Then, these precursor ions are fragmented into small ions via CID by colliding with a neutral gas like nitrogen. In the time-of-flight mass spectrometer, these fragmented smaller ions are accelerated, and their TOF is measured in the detector. The time taken by these ions to reach the detector helps determine mass. The feature of data-dependent acquisition allows the LC-QTOF-MS instrument to select relevant ions to analyze automatically based on their abundance. Finally, mass spectra are generated and compared against a known spectra library. The mass spectra produced from the analysis are matched with those in the library to identify compounds in the sample.[51] The LC-QTOF-MS is utilized in the metabolic fingerprinting of Human Lung tissue, identification of bioactive compounds in natural products, and uncovering new metabolic pathways related to the pathogenesis of Alkaptonuria.[52,53] UR-144 and JWH-250,

and several other SCBs are potentially detected by LC-QTOF-MS.[54]

3.2.4. Ultra performance liquid chromatography coupled with tandem mass spectrometry

Ultra Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS) is an impactful analytical method for large-scale fast-track analysis. Compared to traditional HPLC, UPLC provides higher resolution using smaller particle sizes (typically 1.8 μm) in the chromatographic column. Initially, the liquid sample is passed through a column packed with a stationary phase such as C18. Under high pressure, components of the sample are distinguished based on their interaction with the stationary and mobile phases. After the separation, compounds are directed towards a mass spectrometer. MS/MS initially selects specific ions based on mass-to-charge ratio and retention times. Then, in a collision cell, these precursor ions are allowed to collide with neutral gas. By collision energy, they are dissected into smaller ions called product ions. The fragmentation pattern is unique for each compound, aiding in precise identification. When studying analytes at low-concentration, internal standards are used to make quantification reliable.[55] Metabolite profiling and understanding metabolic processes, detecting pharmaceutical contaminants in urban wastewater, and studying the pharmacokinetics of drugs are some of the applications of UPLC-MS/MS.[56-58] With the application of UPLC-MS/MS, by preparing samples using microextraction by packed sorbent (MEPS) method, NPS such as Loperamide and Pregabalin can be detected in oral fluids.[59]

3.2.5. Surface enhanced raman spectroscopy

Surface-enhanced raman spectroscopy (SERS) is a highly influential analytical method; it strengthens Raman scattering signals of molecules that are adsorbed onto uneven metal surfaces or nanostructure. Laser light falls on the sample and interacts with the molecules in the sample. Most of the light bounces off elastically (no change in energy), whereas some fraction of light scatters inelastically (changes its energy). The resulting change in energy resembles the vibrational mode of the molecule, and this phenomenon is called Raman Scattering. However, the strength of this scattering is generally weak, so detecting analytes present at low concentrations becomes difficult. The preliminary step in SERS is creating a substrate with a nanostructure surface which is mostly composed of noble metals like silver or gold. This is achieved by nanosphere lithography and metal film over nanospheres, which develops long-lasting and reproducible surfaces for obtaining consistent experimental outcomes. Functionalization of the Substrate is the next step wherein self-assembled monolayers or atomic layer deposition techniques facilitate the coating of the nanostructured surface with specific molecules that will interact with analytes, i.e., the substances to be studied. Functionalization supports locating the analyte closer to the metal surface because SERS is highly sensitive to the distance between the analyte and substrate. Then, the analyte is introduced by drop-coating directly onto the surface, vital for amplifying Raman signals. Ideally, the analyte should be within a few nanometers of the surface to achieve maximum signal enhancement. Localized surface plasmons (LSPs) on the metal surface get excited when Laser light falls on the SERS substrate. Excitation of LSPs boosts the electromagnetic field near the surface, which increases the strength of scattered light in SERS. For detecting analytes at low concentrations, In SERS, LSP Resonance, which is a resonant oscillation of conduction electrons on the metal surface when exposed to light, strongly increases the electromagnetic field near the metal surface. A spectrometer then collects and analyzes scattered light. Detailed information on the molecule's composition and structure of the analyte is obtained from the resulting spectrum.[60] SERS finds its applications in Spectro-electrochemistry, Material characterization, and in vivo measurements and diagnostics.[61] To restrict the illegal distribution of Kratom, a designer drug, the SERS technique is used which detects even very low concentrations down to 342 nanograms per milliliter.[62]

3.2.6. Ultra-high performance liquid chromatography time-of-flight mass spectrometry

Ultra-high performance liquid chromatography time-of-flight mass spectrometry (UHPLC-TOF-MS) is a high-level analytical technique. A binary pump for solvent delivery, a vacuum degasser to eliminate air bubbles, an autosampler for sample injection, and a column compartment that controls temperature for optimal separation are the components of UHPLC. Combined efforts of these components establish a continuous smooth flow of both the mobile phase and sample through the column. The mobile phase generally comprises a mixture of water and acetonitrile. Once the sample is injected into the system, the mobile phase carries it through the chromatographic column. Based on interaction with the stationary phase, UHPLC separates compounds. Compared with HPLC, UHPLC uses smaller particle sizes (around 1.8 μm) in the stationary phase, helping achieve higher efficiency. The composition of the mobile phase slowly changes to improve the separation of compounds based on polarity and size, known as the gradient elution technique. Post separation, these compounds are moved toward the Mass spectrometer. Here, compounds are ionized using techniques like ESI, suitable for polar compounds, and Matrix-Assisted Laser Desorption/Ionization for larger biomolecules. The sensitivity and accuracy of analysis are based upon the choice of ionization technique. The determination of the mass-to-charge ratio of these ions is carried via the TOF Mass spectrometer, which measures the time these ions take to travel a fixed distance. A mass spectrometer captures data on the ions produced and then develops a mass spectrum. The produced Mass spectrum reflects the quantity of ions at different mass-to-charge ratio

values. Thus, helping quantify and identify compounds in a sample.[63] Multi-residue Screening, Untargeted Analysis, Bioanalysis, Metabolomics, Biopharmaceuticals, and Polar Compounds Analysis are some of the fields where UHPLC-TOF-MS is utilized.[64] From urine samples, UHPLC-TOF-MS detects synthetic cannabinoids, such as UR-144.[65]

3.2.7. Direct analysis in real-time mass spectrometry

Direct analysis in real-time mass spectrometry (DART-MS) is an effective and rapid method for analyzing various samples with minimal sample preparation. Preparation of the ionization source is the initial step in the DART system. A high-voltage electric field between two electrodes is created, ionizing the passing helium gas through the system. Excited helium atoms and ions are developed via this process. Then, reagent ions are created when excited helium atoms interact with water vapor or solvent vapors in the surrounding atmosphere, and these created reagent ions will interact with the sample. The sample needed to be studied can be solid, liquid, or gas. To facilitate instant interaction of the sample with reagent ions, the sample is placed directly in the path of the ion stream. A chemical ionization process is induced when reagent ions clash with the sample. Due to this collision energy, ions from the sample are produced, which is necessary for mass spectrometry analysis. Based on the chemical characteristics of the sample, the nature (positive or negative) of ions is decided. The ions produced are propelled into a mass spectrometer, where they are separated based on their mass-to-charge ratio. Data from the produced mass spectrum of these ions is then interpreted.[66] Metabolomic Fingerprinting/Profiling of food products, analysis of bulk and detonated explosives, analysis of trace evidence found at crime scenes, and analysis of inks and dyes in forgery investigations are some of the applications of DART.[67,68] Synthetic cathinones can be detected by DART-MS in whole human blood and urine samples.[69]

3.2.8. Isotope ratio mass spectrometry

Isotope ratio mass spectrometry (IRMS) is a cutting-edge analysis approach that helps evaluate the ratio of stable isotopes in various samples. Here, the sample in any form, be it Solid, liquid, or gas, is made to go through combustion chemical breakdown reactions. Since molecules can be ionized in this state, complex organic compounds can be converted into simpler gaseous products suitable for analysis, such as CO_2, H_2O, and N_2. A gas sample is now put in the mass spectrometer's ion source. The electron impaction method turns a gas sample into ions by causing electrons to come into contact with gas molecules, knocking away electrons and producing positive ions. Through the assistance of an electric field, ions are accelerated and focused into a beam, and it is attained using a series of electrodes that create potential differences. Narrowing ions into a beam is crucial to measure their mass precisely. Ions are segregated based on their mass-to-charge ratio, typically in magnetic sector mass spectrometers where ion beams are placed into a mass analyzer. After segregation, ions are sent into a detector that detects the amounts of various types of ions. Faraday cups and ion counting detectors are prevalently used detectors. Quantifying the isotopes in the sample is done because the current produced when the ions fall on the detector is directly proportional to the number of ions. Different isotope ratios are calculated by analyzing the data collected from the detector, usually by comparing ion currents of isotopes of interest.[70] IRMS helps track the movement of nutrients through ecosystems. Tracks are isotopically labeled compounds to trace metabolic processes and to determine the geographical origin of substances like drugs or explosives based on their isotopic composition.[71] IRMS can detect doping in sports through the Athlete Biological Passport, improving the detection of anabolic steroids such as Androstenedione.[72]

3.2.9. Infrared radiation spectroscopy

Based on how molecules absorb Infrared radiation (IR), IR spectroscopy, a robust analytical method, identifies molecules. The first step is sample preparation, which is dissolved into a suitable solvent (in case of a solid sample) or formed into a thin film (in case of a liquid sample). It is essential to make sure that IR traverses through it efficiently. IR, from an Infrared light source, falls on the sample. Lasers or heated filaments are typically used as IR sources since they produce a broad range of infrared wavelengths. Molecular bonds in the sample absorb specific IR frequencies when they pass through the sample. The rest of the radiations that are not absorbed move through the sample. A detector then detects the intensity of the transmitted wavelength of radiation. The absorbed wavelengths are recorded, and a spectrum is generated, plotting absorption intensity versus frequency. Identification of functional groups in the sample is carried out by examining the generated spectrum. Vibrational modes of chemical bonds in the sample are represented by peaks in the spectrum, aiding in understanding the molecular structure and composition of the sample. Clinical diagnosis of various diseases, monitoring drugs, and metabolism of medicine are various applications of IR Spectroscopy.[73] Emerging SCBs can be detected with IR Spectroscopy.[74]

3.2.10. Nanoparticle-based nuclear magnetic resonance chemosensing

Based on integrated principles of quantum mechanics and electromagnetic theory, Nuclear magnetic resonance (NMR) Spectroscopy is a complex and innovative analytical technique that helps study molecular structure. Due to the characteristic of "SPIN," nuclei, when placed in a strong magnetic field, act as small magnets. A sample with nuclei like hydrogen or carbon is inserted in a strong Magnetic field. The spins of nuclei align them either with or against the magnetic field. This leads to the creation of different energy levels. Then, radiofrequency (RF) pulses are applied to the sample. The energy difference between aligned nuclei states

matches with the RF pulse's set frequency. RF pulse provides energy to the nuclei, which move from lower to higher energy states. The excited nuclei revert to their original state when the RF pulse is switched off. Once they return to their original state, they release the energy through radio waves, known as relaxation. When nuclei relax, they send out signals that can be detected. Analog signals are produced by the sample, which is digitized by an Analog-to-digital converter. In the computer memory, this digitized data is then stored. Post-digitization, with the help of the Fourier transform, the time-based signals are translated from the time domain to a frequency-based format. Fourier transform processes the collected raw data (intricate signals) and breaks them into individual frequencies, enabling observation of the NMR spectrum where peaks depict different chemical surroundings of nuclei. Each peak represents a specific type of nucleus in the specific chemical environment in the NMR Spectrum. The position and amplitude of these peaks help study molecular structures, such as the number and connectivity of hydrogen atoms.[75] Nanoparticle-based NMR chemo-sensing merges distinct properties of nanoparticles with NMR spectroscopy. A unique coating of the dimethylsilane group makes gold nanoparticles manufactured. This design increases the ability to interact with targeted analytes, such as phenethylamine derivatives, via Electrostatic and hydrophobic interactions. A monolayer protection over the nanoparticle allows them to bind with analytes selectively, minimizing the interference with other sample components. A sample of interest analytes is mixed with the nanoparticle, commonly in a deuterated solvent (e.g., deuterated water) buffered at a specific pH (e.g., pD 7.0). The nanoparticle concentration is controlled to affirm the optimal interaction of the analyte with the nanoparticle (e.g., 15 µM). A combination of nuclear overhauser effect and saturation transfer difference techniques is used in the NMR experiment setup. In the Nuclear overhauser effect step, magnetization is transferred from nanoparticles to analytes, whereas signals of the bound analytes are increased in the Saturation transfer difference technique. NMR spectrum is recorded, and peaks from nanoparticles and analytes are showcased. Certain techniques weaken the signals from nanoparticles to focus on the signals from the analyte, making it easier to spot the analyte signals. Afterward, signal isolation and clear peaks corresponding to bound analytes are revealed. Each peak's position is called a chemical shift, and each peak displays specific analytes, aiding in determining what analyte it is. The binding constant (K) measures the strength of the binding of nanoparticles with analytes. 10^5 to 10^6 M^-1 is the range of values of K. It is a highly sensitive method helping in the detection of analytes at lower concentrations, even as low as 30 µM.[76] Nanoparticle-based NMR chemo-sensing finds its application in medical diagnosis, monitoring toxins in environment and detection as well as identification of designer drugs.[76,77] Designer drugs such as 4-FA and 5-MeO-DMT are identified and detected by nanoparticle-based NMR chemosensing.[76]

3.2.11. Availability of analytical detection techniques in India

GC-MS is utilized in numerous State and Regional Forensic Science Laboratories (FSLs) and Central Forensic Science Laboratories (CFSLs) including CFSL Chandigarh and CFSL Bhopal.[78,79] Institutions like AIIMS Delhi, and NIMHANS have LC-MS/MS available but its appointment in most of the FSLs is limited due to complex operation.[80,81] The New Biological Sciences Building at IISc Bangalore has a Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) facility but it is not widely used in conventional forensic testing at Forensic Science Laboratories (FSLs).[82] Central Analytical Laboratory at BITS Pilani offers UPLC-MS/MS and its utilization in FSLs is occasional.[83] Researchers have developed and utilized SERS at several IITs like IIT Guwahati, IIT Jodhpur, and IIT Bombay, whereas the incorporation of SERS in forensic analysis is yet to be made effective.[84-86] NIPER Mohali's central instrumentation lab consists of UPLC-TOF-MS and a majority of CFSLs and SFSLs have yet to make use of it.[87] Advanced Center for Materials Science at IIT Kanpur has a Stable Isotope Ratio Mass Spectrometer (IRMS) facility.[88] Delhi FSL has infrared (IR) spectroscopy facilities, and it is commonly available in various CFSLs like CFSL Chandigarh.[78,89] Nanoparticle-Based Nuclear Magnetic Resonance (NMR) Chemosensing and DART-MS have not been extensively employed for forensic analysis because of infrastructural problems. Approximately 0.7-0.8 million cases were found pending in Forensic laboratories as of 2021, and about 40% of the scientific positions were left vacant in Forensic laboratories. Some of the premier research institutes and central labs in AIIMS, IITs, and Selected CFSLs only have sophisticated detection techniques, such as DART-MS or UPLC-QTOF-MS, installed.[90] This gap shows that there is an urgent need for financial support from the government to upgrade the technology facility in Forensic laboratories, which generally suffer from inadequate funding. By enhancing widespread accessibility and utilization of these detection techniques, the ability of our nation to monitor the dynamic scenario of substance use would be strengthened.

Table 1: Summarizes various classes of substances abused and emerging drugs in each class with their adverse effects and analytical detection methods

Substance Class	Newer Drugs	Adverse Effects	Detection Methods
Synthetic Cannabinoids	*Spice, K2, Eclipse, Mojo, UR-144, KUR-144, JWH-250, HU210, XLR-11*	Anxiety, Tachycardia, Paranoia, Psychosis.	GC-MS, LC-MS/MS, UHPLC-TOF-MS (for UR144), IR Spectroscopy.
Synthetic Cathinones	*Khat, Bath salts, MCAT, Bubbles*	Agitation, Aggression, Dizziness, Seizures.	GC-MS, LC-MS, LC-MS/MS.
Novel Psychoactive Substances	*Etizzy, M-Ket, 25i, Goodfellas,*	Hypertension, Confusion, Drowsiness, Multiple Organ Dysfunction Syndrome.	LC-MS/MS, LC-QTOF-MS, UPLC–MS/MS, SERS, NMR.
Designer Drugs	*Kratom, Salvia divinorum, 4-FA, 5-MeO-DMT*	Euphoria, Insomnia, Altered Perception.	Nanoparticle-Based NMR Chemosensing, DART, UHPLC-TOF-MS, SERS
Anabolic Steroids	*Stanozolol, Methandienone, Oxandrolone, Clenbuterol*	Mood swings, Aggression, Cardiovascular Diseases.	LC-MS/MS, IRMS

4. Conclusion

The increase in the abuse of synthetic drugs has posed an alarming challenge in front of law enforcement and public health as well. These substances of abuse keep evolving, and their consumption causes potential health risks, including acute addiction and chronic health disorders, and it can be fatal, too. It is not easy to detect these substances with the help of Conventional techniques since these synthetic drugs of abuse typically consist of novel chemical compositions. Therefore, this article emphasizes the critical requirement of advanced analytical detection techniques that can keep up with the pace of these emerging abuse substances and thereby detect them. Forensic analytical methods must be refined and developed to quickly and precisely identify these synthetic drugs. More high-tech and cutting-edge technologies, such as nanoparticle-based NMR chemo-sensing and DART-MS, should be developed on a larger scale. Patients suffering from the consumption of these synthetic drugs can be treated on time by the development of these Analytical detection techniques. The laws related to the abuse of these emerging synthetic drugs could also be enforced and amended promptly. Ultimately, a holistic and diverse strategy is needed against the battle of emerging synthetic substance use. An integration of increased public awareness along with judicial actions and newer analytical detection techniques can be beneficial to combat the surge in substance use. Besides, for the sustainable recovery of individuals suffering from the abuse of these drugs, facilitation of Rehabilitation services must be advocated, providing not only medical help but psychological restorative assistance as well. Employing constant surveillance by the legal system and educating the public on the side-effects of drug consumption and the application of High-tech advanced detection tools, our society can be provided with better support and protection against the hazards of emerging substance use.

5. List of Abbreviations

NMBA: Nasha Mukt Bharat Abhiyan; NDPS Act: Narcotic Drugs and Psychotropic Substances Act; SCBs = Synthetic Cannabinoids; THC: Tetrahydrocannabinol. CB1R: Cannabinoid Receptor Type 1; CB2R: Cannabinoid Receptor Type 2; NPS: Novel Psychoactive Substances; MDMA: Methylenedioxy-substituted amphetamines; PMA: Paramethoxyamphetamine; NMDA: N-methyl-D-aspartate; 4-FA: 4-Fluoroamphetamine; 5-MeO-DMT: 5-methoxy-N,N-dimethyltryptamine; GC-MS: Gas Chromatography-Mass Spectrometry; ESI: Electrospray Ionization (ESI); APCI: Atmospheric Pressure Chemical Ionization (APCI); CID: Collision-Induced Dissociation; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; LC-QTOF-MS: Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry; UPLC-MS/MS: Ultra Performance Liquid Chromatography coupled with Tandem Mass Spectrometry; SERS: Surface-Enhanced Raman Spectroscopy; LSPs: Localized Surface Plasmons; UHPLC-TOF-MS: Ultra-High Performance Liquid Chromatography

Time-of-Flight Mass Spectrometry; DART-MS: Direct Analysis in Real-Time Mass Spectrometry; IRMS: Isotope Ratio Mass Spectrometry; IR Spectroscopy: Infrared Radiation Spectroscopy; NMR: Nuclear Magnetic Resonance; FSL: Forensic Science Laboratory; CFSL: Central Forensic Science Laboratory.

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7. Conflict of Interest

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