FORENSIC PROFILING OF PROPRIETARY PSEUDOEPHEDRINE PRECURSORS AND OXIDATIVE PRODUCTS IN TANDEM WITH CHEMOMETRIC ANALYSIS

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by

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LIST OF SYMBOLS

c	Velocity of light in vacuum
λ	wavelength
C	Carbon
CO	Carbon monoxide
CO_2	Carbon dioxide
eV	electronvolt
g	Gram
h	Planck's constant
H	Hydrogen
H_2	Hydrogen gas
He	Helium
Js^{-1}	Joules per second
m	Meter
mg	Milligram
min	Minute
ms^{-1}	Meter per second
m/z	Mass-to-charge ratio
N	Nitrogen
N_2	Nitrogen gas
NO_x	Nitrous oxide
O	Oxygen
S	Sulphur

LIST OF ABBREVIATIONS

APIs	Active Pharmaceutical Ingredients
ATR	Attenuated Total Reflectance
ATS	Amphetamine-type Stimulant
$CuSO_4$	Copper (II) sulphate
3-CMC	3-Chloromethcathinone
DCM	Dichloromethane
DEA	Drug Enforcement Administration
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EPH	Pseudoephedrine
EtOH	Ethanol
EU	European Union
EWA	Early Warning Advisory
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
d. H_2O	Distilled water
HCA	Hierarchical Cluster Analysis
HCl	Hydrochloric acid
HMQC	Heteronucleur Multiple-Quantum Correlation
H_2SO_4	Sulphuric acid
IAEA	International Atomic Energy Agency
ICP-MS	Inductively Couple Plasma-Mass Spectrometry
IRMS	Isotope Ratio Mass Spectrometry
IS	Internal standard
$K_2Cr_2O_7$	Potassium dichromate
KH_2PO_4	Potassium phosphate monobasic
$KMnO_4$	Potassium permanganate
KIEs	Kinetic isotopic effects
LC-MS	Liquid Chromatography-Mass Spectrometry
LDA	Linear Discriminant Analysis
LOD	Limit of detection
LOQ	Limit of quantification

MA	Methamphetamine
MeOH	Methanol
MET	Methcathinone
3-MMC	3-Methylmethcathinone
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium hydrogen carbonate
$Na_2HPO_4.2H_2O$	Sodium phosphate dibasic
NaOH	Sodium hydroxide
NIR	Near-Infrared Spectroscopy
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
NPS	New Psychoactive Substance
PAC	Phenylacetylcarbinol
OTC	Over-The-Counter
PC	Principle Component
PCA	Principal Component Analysis
PLSR	Partial Least-Square Regression
PSE	Pseudoephedrine
R*f*	Retention factor
RSD	Relative standard deviation
RT	Retention time
SIM	Selected ion monitoring
S/N	Signal to noise ratio
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
TIC	Total ion chromatogram
TIEs	Thermodynamic isotopic effects
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
UNODC	United Nations Office on Drugs and Crime
US	United States

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Appendix A ^{1}H and ^{13}C NMR of sample LGMn

Appendix B ^{1}H and ^{13}C NMR of sample AACr

Appendix C ^{1}H and ^{13}C NMR of sample CACr

Appendix D EI-MS of 1-Phenyl-1-propanone

PEMPROFILAN FORENSIK PREKURSOR PSEUDOEFEDRINA PROPRIETARI DAN HASILAN OKSIDATIF SEIRING DENGAN ANALISIS KEMOMETRIK

ABSTRAK

Prekursor pseudoefedrina (PSE) telah disalahgunakan secara berleluasa untuk penghasilan dadah sintetik. Ahli kimia tidak bertauliah boleh mengakses bahan ini samada daripada sumber haram atau diekstrak secara haram daripada formulasi proprietari meskipun pengeluar berusaha menghadkan pengekstrakan semula PSE tersebut. Jika tindak balas penurunan PSE menghasilkan metamfetamina (MA), tindak balas pengoksidaan pula mampu menghasilkan metkatinon (MET), sejenis bahan psikoaktif baharu (NPS). MET kini mendapat perhatian sebagai pengganti dadah MA; walau bagaimanapun, terdapat laporan yang sangat terhad dalam kesusasteraan saintifik mengenai analisis bahan ini terutamanya berkenaan profil organik dan isotopnya yang mungkin mengehadkan keupayaan komuniti forensik untuk mengesan dan mengawal bahan ini. Kajian awal melibatkan pengoptimunan pengekstrakan PSE daripada pelbagai sumber proprietari, diikuti pencirian dan pemprofilan forensik hasil PSE tersebut. Pengekstrakan asid-bes terbukti merupakan kaedah yang berupaya menghasilkan PSE yang agak tulen. Seterusnya, lima jenis sumber prekursor PSE simulasi yang berbeza telah dioksidakan menggunakan dua laluan sintetik haram iaitu laluan kromat dan manganat. Produk oksidatif yang disintesis kemudiannya dicirikan secara forensik diikuti dengan pemeriksaan profil organik dan isotopik mereka. Spektroskopi inframerah transformasi fourier-jumlah pemantulan terlemah (ATR-FTIR) dan gas kromatografi-spektrometri jisim (GC-MS) digunakan untuk pemprofilan bendasing organik manakala spektrometri jisim nisbah isotop (IRMS)

memprofil isotop stabil ^{13}C:^{12}C dan ^{15}N:^{14}N. Laluan kromat dan manganat, masing-masing menghasilkan sebatian khusus U1 (RT 5.701, m / z 51, 77, 105, 91 dan 207) dan U3 (RT 9.013, m/z 70, 85, 117). Selain itu, PSE yang tidak habis bertindak balas turut ditemui dalam semua sampel dari laluan manganat. Analisis nisbah isotop ^{13}C:^{12}C dan ^{15}N:^{14}N dapat membezakan sampel mengikut laluan sintetik dan sumber prekursor dengan isotop nitrogen memberikan hasil yang terbaik. Data daripada spektrum ATR-FTIR dan kromatogram GC-MS digunakan bersama-sama dengan analisis kemometrik iaitu analisis kluster hierarki (HCA), analisis komponen utama (PCA), dan analisis diskriminasi linear (LDA), untuk menyiasat sumber prekursor dan laluan sintetik yang digunakan. Hasil perkumpulan terbaik diperolehi daripada LDA, di mana semua sampel PSE yang diekstrak daripada sumber yang berbeza dikesan kembali kepada sumbernya, dan MET berjaya dibezakan berdasarkan laluan sintetik dan sumber prekursor PSE yang digunakan. Berdasarkan set data FTIR, LDA mencatatkan kadar pengkelasan yang betul untuk sampel PSE dan MET, masing-masing ialah 90.0% dan 78.6% manakala bagi set data GC-MS, LDA mencatatkan kadar pengkelasan yang betul iaitu 100% untuk sampel PSE dan 95.2% untuk sampel MET. Pemprofilan kimia dadah seiringan dengan analisis kemometrik adalah berguna dalam menentukan jenis prekursor dan kaedah sintetik yang digunakan dalam penghasilan dadah dalam makmal haram.

FORENSIC PROFILING OF PROPRIETARY PSEUDOEPHEDRINE PRECURSORS AND OXIDATIVE PRODUCTS IN TANDEM WITH CHEMOMETRIC ANALYSIS

ABSTRACT

Pseudoephedrine (PSE) precursors are rampantly abused for the illicit production of synthetic drugs. Clandestine chemists can access this substance either from illegal sources or clandestinely extracted from proprietary formulations despite efforts from manufacturers to limit the PSE's re-extraction. While the reduction reaction of PSE will produce methamphetamine (MA), the oxidation reaction will afford methcathinone (MET), a new psychoactive stimulant (NPS). MET is currently gaining attention as a possible replacement for MA drugs; however, there is a minimal report in the scientific literature regarding the analysis of this substance, particularly regarding its organic and isotopic profiles, which may limit the forensic community's ability to detect and regulate this substance. Initial studies involved optimisation of PSE extraction from various proprietary sources, followed by characterisation and forensic profiling of the extracted PSE products. Acid-base extraction proved to be the most viable method to obtain considerably pure PSE. Subsequently, five different sources of simulated PSE precursors were oxidised following two clandestine synthetic routes, namely chromate and manganate routes. The synthesised oxidative products were then forensically characterised, followed by an examination of their organic and isotopic profiles. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) were used for organic impurity profiling, while isotope ratio mass spectrometry (IRMS) profiles ^{13}C:^{12}C and ^{15}N:^{14}N stable isotopes. The chromate and manganate

routes produce compounds specific to U1 (RT 5.701, m / z 51, 77, 105, 91, and 207) and U3 (RT 9.013, m/z 70, 85, 117). Additionally, unreacted PSE was found in all the samples from the manganate route. Isotope analysis of ^{13}C:^{12}C and ^{15}N:^{14}N ratios differentiated the samples by the synthetic route and precursor sources with a nitrogen isotope, providing the best results. Data from ATR-FTIR spectra and GC-MS chromatograms were used in conjunction with chemometric analysis, namely hierarchical cluster analysis (HCA), principal component analysis (PCA), and linear discriminant analysis (LDA), to investigate the sources of precursors and the synthetic routes used. The best grouping results are obtained from LDA, where all PSE samples extracted from different sources are traced back to the source, and MET are successfully differentiated based on their synthetic routes and sources of PSE precursor used. Based on the FTIR data set, LDA recorded the correct classification rate for PSE and MET samples of 90.0% and 78.6%, respectively, while for GC-MS datasets, LDA recorded a correct classification rate of 100% for PSE and 95.2% for the MET samples. Forensic chemical profiling in tandem with chemometric analysis is beneficial in advocating the type of precursor and synthetic route used for drug manufacturing in illicit laboratories.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The widespread abuse of illegal drugs has become a serious problem plaguing most nations, including Malaysia. Particularly over the last 10 to 15 years, abuse of plant base illegal drugs such as opiates and cocaine has gradually shifted to synthetic or designer drugs. Synthetic drugs are human-made designer drugs; as such, they are produced using specific chemical compounds known as precursors and reagents that undergo chemical reactions to produce the drugs. A synthetic drug is defined as a drug with properties and effects that are comparable to those of well-known stimulants, depressants, hallucinogens, or narcotic drugs but has a slightly altered chemical structure, especially one that was created to avoid current drug control measures (Christophersen, 2000). Due to its synthetic nature, it is relatively easy to produce or manufacture, a vital factor that explains the constant supply and is possibly less expensive, thus contributing to increased availability and uptake among drug users (Singh *et al.*, 2013; Shaffi *et al.*, 2020; Linh, 2022).

The rapid emergence of designer drugs of new or Novel Psychoactive Substances (NPS) has gained the attention of drug users as a viable alternative to other stimulant illicit drugs that are heavily regulated by the drug acts. Methcathinone (MET) is one of the NPS from the cathinone family with stimulant effects similar to methamphetamine (MA) and is twice as potent as cathinone (De Ruiter *et al.*, 1994; Zhingel *et al.*, 1991). Recently, its popularity and abuse have increased globally, and a growing number of cases involving disability or even death have been reported in several countries, raising public concern. One example of a case study of MET intoxication that has been documented involves a 29-year-old woman who was

brought into the emergency room due to a coma caused by an overdose of MET dissolved in alcohol taken with bromazepam (Belhadj-Tahar & Sadeg, 2005). According to a case study in China, MET accounted for 95.8% of the synthetic drug cases in a local area in 2017 (Zhao, 2020). This substance was also identified as among most of the 31 NPS reported within the country between 2019 and 2020 (UNODC, 2021).

Synthetic drugs rely on starting materials or precursors to initiate chemical reactions to obtain the product. Methods for the clandestine manufacture of MET involve the extraction and subsequent reaction of precursors such as ephedrine (EPH) or pseudoephedrine (PSE) salts with other essential chemicals. Theoretically, chemical reactions modify the structure of the precursor, thus producing a new compound that retains some parts of the molecular structure of the precursor. Along with the target product, the unreacted precursor, impurities, and by-products are also formed in the illicit drug end product, which can be considered a fingerprint specific to that type of drug. In turn, this benefits forensic drug investigation.

Apart from drug seizures and enforcement to combat the proliferation of illegal drugs and NPS on the black market, law enforcement agencies and forensic experts also perform intelligence-gathering initiatives such as chemical profiling via Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) to obtain information that may useful in illicit drug-related cases. More recently, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Isotope Ratio Mass Spectrometry (IRMS) is also receiving attention as other potential additional techniques for drug profiling purpose (Nicdaeid *et al.,* 2012, 2013;).

Chemical drug profiling focuses on the analysis of impurities and by-products to obtain valuable information for the operational work of law enforcement agencies. It has been applied extensively, mainly in countries with serious drug threats like Thailand, Japan, Australia, the United States (US), and the Philippines (Abdullah *et al.*, 2014; Valente *et al.*, 2014). United Nations Office on Drugs and Crime (UNODC) has defined drug profiling as the method of investigation of the chemical and/or physical properties of a drug seizure for comparing seizures for intelligence and evidential purposes (UNODC, 2001). Various valuable information can be obtained from drug profiling studies, including chemical links between samples, sample origin, output from illicit laboratories, the common method of synthesis, precursor trends, as well as drug trafficking patterns and distribution networks (Meola *et al.*, 2021).

Chemical data obtained from the characterisation and profiling of precursors and illegal drugs are helpful for classification (grouping) or discrimination (batch comparison) purposes. Chemometrics is the technique employed in forensic investigation and its use has increased dynamically in the last two decades (Popovic *et al.,* 2019). Despite the potential, the application of chemometric methods for the classification and discrimination of synthetic NPS, i.e., MET, has never been reported, although a similar technique is widely applied for other samples of forensic interest (Ismail *et al.,* 2014; Jais *et al.,* 2020; Sandran *et al.,* 2020; Shadan *et al.,* 2018). One related work used Fourier Transform Infrared Spectroscopy (FTIR) in combination with the partial least-square regression (PLSR) technique to perform an analysis of real seized cocaine samples (Groberio *et al.,* 2015), while another work determined heroin by using diffuse reflectance Near-Infrared Spectroscopy (NIR) with PLSR (Moros *et al.,* 2008).

1.2 Problem Statements

Misuse of synthetic drug precursors are possible especially in small scale clandestine operations. EPH, and its isomer, PSE is one of the most commonly abused precursor chemicals in the illicit production of MA and more recently, MET (Huang *et al.,* 2022; Simpson *et al.,* 2022). These precursors were conveniently isolated from the pharmaceutical preparations, i.e., cold medication products, through chemical process by the clandestine laboratories. However, the extraction process of these precursors from pharmaceutical products is poorly understood by forensic scientists. Understanding the precursor extraction process is critical for forensic scientists to effectively investigate and combat various types of illegal drug activities related to precursors extraction.

MET is a prevalent NPS used illicitly in some countries (Simpson *et al.* 2022). Although MET has not yet become a serious problem in Malaysia, a detailed investigation into the method for producing MET covertly from EPH/PSE precursor is required because there is the possibility of future epidemic abuse considering that this substance has been detected in greater frequency lately (UNODC, 2021). Additionally, since MET has stimulant properties that are nearly identical to those of MA, which is currently subject to strict regulation by law enforcement agencies, this drug may end up replacing MA in the near future by clandestine drug manufacturers and users.

As earlier countermeasures, forensic scientists need to have knowledge of the type of chemicals and precursors commonly used to synthesise clandestine as well as the synthetic reaction mechanisms of MET. Forensic scientists also need to develop, optimise, and validate tests and analytical methods for reliable and robust

characterisation and identification of these substances since there is a lack of scientific literature regarding the analysis of these substances using robust laboratory methods.

Regulatory and enforcement agencies are also faced with challenges in getting the necessary information to understand and possibly disrupt illegal drug network operations, such as the sources of supply, chemical links between each seized drug, and the variations between drug production batches. Chemical profiling using traditional GC-MS techniques of the impurities and by-products stemming from the synthetic drug processes, as well as more recent isotopic profiling by IRMS, is valuable for identifying trafficking patterns and distribution network, chemical links between samples, origin of samples, output from clandestine laboratories, and trends in a covert operation. Various illicit drugs ranging from natural and amphetamine type-stimulant (ATS) groups such as amphetamine, MA, ecstasy, and many others were successfully characterised and profiled using the techniques mentioned above. However, there are significant gaps in the present literature reporting investigations into the potential for source identification, linking between seizures and discrimination of drug batches via organic and isotopic profiling of clandestine synthesised NPS for forensic purposes. The insufficient scientific knowledge of specific NPS (particularly cathinone analogues) by chemical profiling analysis hinders the forensic community's ability to detect and regulate these substances. In addition, the lack of present scientific scrutiny of NPS may indirectly contribute to their potential escalation into a perilous epidemic comparable to ATS use.

The capability to link back the PSE precursors and their synthesised products back to their source will enable the investigators to monitor and regulate certain brands of pharmaceutical products that have been misused, hence enabling the control of the

production or applied more stringent policy towards possession the products. To the best of our knowledge, there is no report yet in the scientific literature emphasising the organic and isotopic profiling of NPS, for example, MET, compared to other ATS drugs.

1.3 Research Questions

This study was conducted to answer the following questions:

1) Can the precursors from the proprietary tablets be used to synthesise substances using clandestine methods?
2) What are the chemical characteristics of the products synthesised through oxidation reaction of the precursors?
3) What are the organic impurities and the isotopic profiles of the oxidative products?
4) Can the precursors and their oxidative products be linked back to its original sources use these chemical data?

1.4 Research Objectives

The overall aim of this study was to establish links between batches of PSE precursors and their oxidative products derived from different sources and further discriminate and classify these samples back to their original sources. In order to achieve this goal, a few other specific objectives are also addressed, as outlined below:

I. To extract and physicochemically characterise the extracted PSE precursors from various sources of proprietary cold medication tablets.

The identification of the extracted PSE samples was firstly done by preliminary screening tests, including colour test, melting point test, and TLC, followed by conformational analysis by ATR-FTIR and GC-MS.

II. To synthesise and physicochemically characterise the PSE oxidative products from various sources of PSE precursors.

The identification of the PSE oxidative products was done by preliminary colour and melting point tests before confirmational analysis using ATR-FTIR, NMR, and GC-MS to confirm the identity of MET in the synthesised products.

III. To determine the organic impurities and isotopic profiles based on ^{13}C:^{12}C and ^{15}N:^{14}N isotope ratios of the PSE oxidative product.

The organic impurities profiles of the oxidative products were determined by GC-MS, while IRMS was used to determine the isotopic profiles of the samples.

IV. To determine the feasibility of discriminating and classifying the PSE precursors and their oxidative products using the HCA, PCA, and LDA analysis techniques in combination with FTIR and GC-MS datasets.

Unsupervised and supervised chemometric techniques namely HCA, PCA, and LDA were applied to the spectroscopic and chromatographic data obtained from FTIR and GC-MS analysis of the samples to assess which technique and which analytical data will provide most meaningful discrimination and classification of the PSE precursors and its oxidative products according to their sources of origin and synthetic routes.

1.5 Significance of the Study

Chemical forensic drug characterisation and profiling studies can be one of the many initiatives for Malaysian law enforcement authorities. In general, drug profiling can provide scientific information vital for the identification of a specific type of drug. Understanding the chemical profiles of synthetic drugs may shed light on various issues, including dealer-user relationships, drug sources, distribution networks, trafficking routes, manufacturing processes, and precursors used (UNODC, 2001).

This work involved the characterisation of samples suspected to contain MET (synthesised from the various sources of PSE precursor). Understanding the clandestine process and further characterisation of synthesised MET will provide valuable chemical information concerning the products of PSE precursor and its chemical reactions, impurities, and by-products involved in illicitly manufacturing drugs from a similar precursor. Additionally, this chemical information will hopefully demonstrate an association of drugs from various sources of precursors. Knowledge about the type of chemicals and precursors used for MET synthesis may also help the investigators determine the drug the clandestine operators will produce.

This research emphasises the science behind chemical profiling from organic and isotopic perspectives for evaluating synthetic procedure elucidation, establishing source identification, and linking and/or discriminating drug batches. Concurrently, it aims to address gaps in knowledge about using selected stable isotopes (e.g., ^{15}N:^{14}N and ^{13}C:^{12}C) for forensic drug investigations and ultimately provide an additional means to enhance control of NPS under the current legislation.

The research findings are useful to the operational work of forensic laboratories and law enforcement agencies in providing relevant, consolidated up-to-date, and

supportive information about illicit synthetic MET drugs from EPH or PSE precursors. Additionally, it is hoped that the knowledge gleaned from this study will be useful in addressing the serious threat posed by illegal manufacture, trafficking, and distribution, thus facilitating policing and helping policymakers assess the local NPS situation and decide on appropriate intervention and prevention measures.

1.6 Scope of the Study

The core activities of this work employed forensic drug analysis to qualitatively identify precursors and their reaction products using combinations of analytical methods. ATR-FTIR, GC-MS, and IRMS were employed to acquire information about the precursor, their oxidative products, and synthetic route details. Ultimately, using chemometric analysis (e.g., PCA, HCA, and LDA) in combination with the chemical data obtained, linkages among the precursors, products, and synthetic routes of the synthesised products can be discovered using robust chemical methods. Specifically, this work presents the benefits of combined techniques for forensic source determination of clandestine substances.

1.7 Thesis Outline

This study is outlined in six main chapters. The first chapter, i.e., the introduction to the study, presents an overall view of the synthetic drug scenario and drug profiling approach to combat the aforementioned problems. The problem statements, research questions, objectives, and significance of the study are elaborated in this chapter.

Chapter 2 is the literature review which discusses the general overview of synthetic cathinone drugs including the PSE precursors. Several analytical techniques

commonly applied for the detection and profiling of illicit synthetic drugs such as FTIR, NMR, GC-MS and IRMS are also addressed in this chapter. Chemometric techniques utilised in the study including HCA, PCA, and LDA are also addressed briefly.

Chapter 3 outlines the extraction of PSE precursors from various sources of proprietary cold medication tablets using simple and acid-base extraction methods. The chapter includes optimisation of simple and acid-base extraction followed by characterisation of the extracted product. Presumptive screening tests, including colour test, melting point test, and TLC followed by confirmatory test procedures used for the characterisation and identification of the extracted products by ATR-FTIR and GC-MS are elaborated. The validation of FTIR and GC-MS instruments used for the characterisation of PSE was also described.

In Chapter 4, the synthesis of MET from five different PSE precursor sources, following the clandestine methods adapted from the internet, i.e., chromate and manganate routes is described. Identification and confirmation of the identity of the synthesised products by preliminary screening tests, including colour test and melting point test followed by confirmatory test procedures by ATR-FTIR, NMR and GC-MS are elaborated. Later in the chapter, the results are provided and further discussions are deliberated according to the methods involved.

Chapter 5 outlines the organic impurities and isotopic profiling by GC-MS and IRMS, respectively. In this chapter, the applicability of GC-MS and IRMS in providing information relating to the precursor sources, synthetic routes and production batches is investigated.

In Chapter 6, the spectroscopic and chromatographic data obtained from FTIR and GC-MS analysis were subjected to HCA, PCA and LDA to assess which data analysis techniques provide meaningful discrimination of the PSE precursors and the MET products. The discrimination of the MET samples on the basis of IRMS data is compared to that afforded by the GC-MS impurity profiling method.

The overall conclusion from this work is summarised in Chapter 7, and suggestions for future works that can be extended to advance the field of MET profiling are also included.

CHAPTER 2

LITERATURE REVIEW

2.1 New Psychoactive Substances

NPS refers to a complex and diversified category of substances that have witnessed an explosive surge in non-medical abuse and prominence in the recreational drug market at the beginning of the 21st century. UNODC defined NPS as a "substances of abuse, either in pure form or preparation, which are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but still may pose a significant danger to the health and safety of the public" (Pieprzyca *et al.,* 2020). However, definitions of NPS can differ from country to country due to differences in national legislation rather than in pharmacological or structural classification. The term "new" does not necessarily indicate new inventions; in fact, numerous NPS were initially synthesised more than four decades ago; rather, it refers to substances that have only very recently been commercially available (UNODC, 2021). These substances are structurally chemically altered as they are derivatives of currently controlled drugs and pharmaceutical products or synthesised as new chemicals created to replicate the actions and psychoactive effects similar to that of licensed medications and other regulated substances (Kuropka *et al.,* 2022). Thus, they have a similar effect on the human central nervous system (CNS) as well-known drugs like amphetamine, cannabis, heroin, or lysergic acid diethylamide (LSD) (Pieprzyca *et al.,* 2020).

The molecular structures of NPS are continually being tweaked in an endless variety of analogues of designer drugs and consequently contribute to the fuelling of the drug market. In an effort to slip under the radar and avoid legal impediments, they

are inherently advertised and sold under ambiguous labels that provide little information about their actual contents using more common but deceptive colloquial terms like "bath salts," "herbal high," and "legal highs" (Smith *et al.*, 2015). One of the biggest misunderstandings about NPSs, even though they are occasionally promoted as lawful, this does not indicate that they are safe for consumption. Given that NPS are relatively new and are still understudied, most users are frequently unaware of what they are taking. As a result, they do not clearly understand and are conscious of the risks associated with the use of NPSs, particularly to health effects concerning their short-term and long-term uses. As with controlled substances, the potential short-term side effects of NPSs may include paranoia, psychosis, and seizures, but their long-term health implications are still poorly understood and infrequently reported (Shafi *et al.*, 2020).

According to UNODC records, over 1100 individual NPS have been reported to the UNODC Early Warning Advisory (EWA) by 133 countries and territories. Focusing on East and Southeast Asia regions, a total of 485 different NPS have been identified by December 2020, or approximately 46 percent of the individual NPS reported at the global level thus far (Figure 2.1). The annual number of newly detected NPS in the region has continued to fall since 2015. This decline may be partially related to the inadequate forensic capabilities of some countries in the region to identify them (UNODC, 2021).

The majority of the reported NPS are synthetic cannabinoids (147), followed by synthetic cathinones (106). In terms of pharmacological effects, stimulants (182) continue to be the largest group in terms of the number of different substances, followed by synthetic cannabinoid receptor agonists (147), hallucinogens (133), and

other NPS as reported by December 2020 (UNODC, 2021) (Figure 2.2). The rate at which new NPS have surfaced on the global drugs market is unprecedented, and it was predicted that at its peak in 2015, at least one new substance would appear per week. In recent years, the frequency of new NPS detections has reduced, and the market has shifted, with a relative decline in the number of new stimulants and synthetic cannabinoids detected and an increase in the number of new opioids and benzodiazepines available (UNODC, 2021).

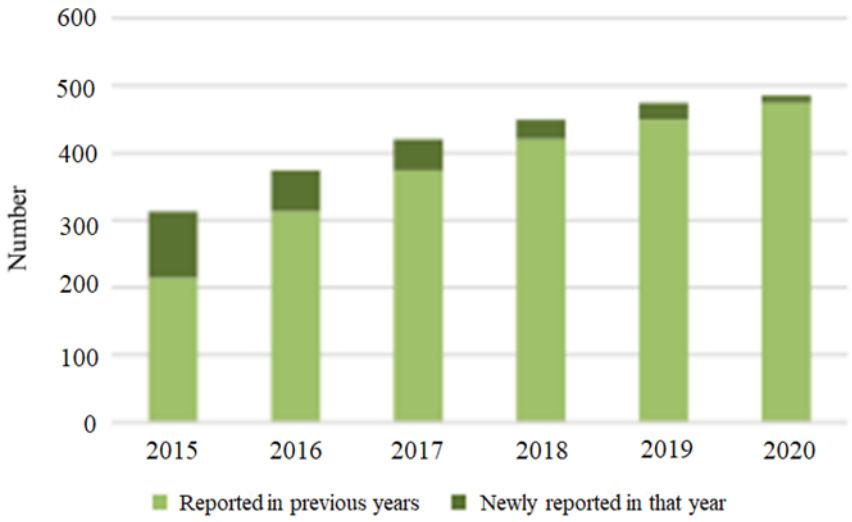


Figure 2.1 Emergence of NPS in East and Southeast Asia in 2015-2020 (UNODC, 2021)

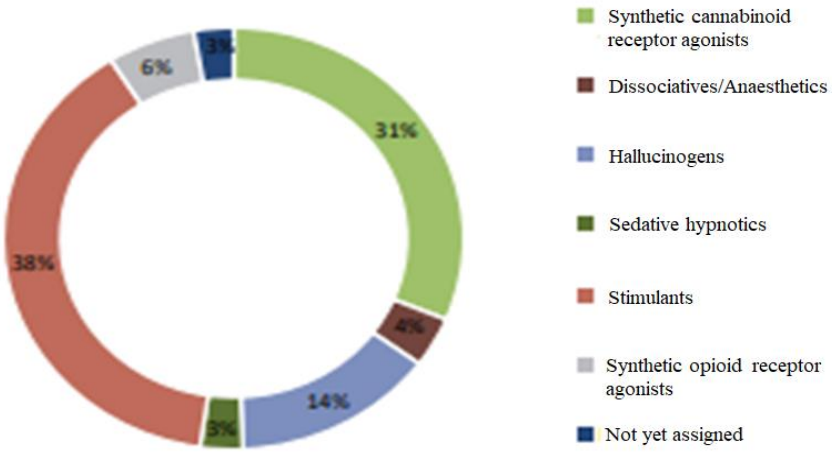


Figure 2.2 Proportion of NPS (by pharmacological effects) in East and Southeast Asia up to December 2020 (UNODC, 2021)

In terms of forensic drug characterisation, generally, NPS can be classified into six groups based on their mode of action: synthetic cannabinoids, synthetic cathinones, ketamine, phenethylamines, piperazines, plant-based substances: *Khat, Kratom, Salvia divinorum* and miscellaneous: Aminoindanes, phencyclidine, tryptamines (UNODC, 2021; Smith *et al.*, 2015). Among these NPS, synthetic cathinones and cannabinoids are considered the popular ones. European Monitoring Centre for Drugs and Drug Addiction [EMCDDA] (2022) reported that synthetic cathinones and cannabinoids accounted for almost 60% of the number of seizures reported in 2019 by European Union (EU) Member States.

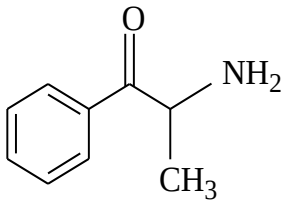
2.2 Natural Cathinones

Cathinone is a major naturally occurring psychoactive monoamine alkaloid in the leaves of the *Catha Edulis* plant, often known as Khat (Kuropka *et al.*, 2022). It is an evergreen shrub cultivated as a bush or small tree which flourishes and grows at high altitudes native to Ethiopia, East Africa, and the Southern Arabian Peninsula (Smith *et al.*, 2015; Valente *et al.*, 2014). This shrub was typically a slow-growing 2-25 m tall tree with reddish stems, sparkling green leaves, and white flowers (Figure 2.3).

The fresh leaves of the Khat tree have an aromatic scent and a mildly sweet and stringent taste which is enjoyable to chew or, less frequently, dried and consumed as a tea to achieve a state of euphoria and stimulation. It also has anorectic (appetite-reducing) side effects. Over the years, the chew of the fresh khat leaves and stalks has been a custom by the population in these regions, particularly at cultural and religious ceremonies, including funerals and weddings. In fact, this custom is now practised on a daily basis (Pieprzyca *et al.*, 2020; Soares *et al.*, 2021). It is also a common habit

among farmers and labourers to chew the Khat leaves to relieve physical fatigue or hunger, as well as among drivers and students to improve attention.

(a)



(b)

Figure 2.3 *Catha edulis* shrub (a), which contains natural cathinone compound (b)

The fresh leaves of the Khat tree have an aromatic scent and a mildly sweet and stringent taste which is enjoyable to chew or, less frequently, dried and consumed as a tea to achieve a state of euphoria and stimulation. It also has anorectic (appetite-reducing) side effects. Over the years, the chew of the fresh khat leaves and stalks has been a custom by the population in these regions, particularly at cultural and religious ceremonies, including funerals and weddings. In fact, this custom is now practised on a daily basis (Pieprzyca *et al.,* 2020; Soares *et al.,* 2021). It is also a common habit among farmers and labourers to chew the Khat leaves to relieve physical fatigue or hunger, as well as among drivers and students to improve attention.

2.3 Synthetic Cathinones

In present days, cathinone can be synthetically produced. The first synthetic cathinones were created in the 1920s as potential medicinal products. To date, synthetic cathinones and their derivatives are one of NPS's largest and most prevalent classes. The cathinone analogues were primarily designed to deliver similar

pharmacological and psychoactive effects to those produced by cathinone during khat chewing. However, because of their structural similarity to amphetamine, the potency of some of these analogues resembles amphetamines. The amphetamine-like psychostimulatory effects such as euphoria, excitement, enhanced alertness, and psychomotor hyperactivity were produced by consuming these drugs but with lower potency than that of amphetamine (Thornton *et al.,* 2012; Kraemer *et al.,* 2019). Some users report that the effects of mephedrone, a cathinone analogue, are comparable to those experienced when using 3,4-methylenedioxymethamphetamine (MDMA) (an ATS drug) (Brunt *et al.,* 2011), while others assert that it provides a higher level of satisfaction than cocaine.

Synthetic cathinones and their analogues have been street labelled as "plant foods," "bath salts," or "research chemicals," but nowadays, various names such as "conquerors of leeches," "driver's charms, "additives to sand," and "bidet refreshers" are frequently used to label those substances by distributors to circumvent legal regulations (Majchrzak *et al.,* 2018). In European countries, these substances can be legally purchased locally at convenience stores and head- or smart shops or conveniently purchased from internet suppliers in the form of odourless, white, or coloured crystalline powders and less frequently as tablets or capsules (Zawilska & Wojcieszak, 2013). In most cases, powders are shipped to distributors, who then tablet or adulterate the substance before selling it. The finished product is typically packaged in quantities ranging from 200 mg to 10 g and sold (Valente *et al.*, 2014). Most commonly, these drugs are supplied as hydrochloride salt and administered via nasal insufflation, swallow, intramuscular/intravenous injection, or rectal insertion (Vardakou *et al.,* 2011). The purity stated on some packages claims over 99% synthetic cathinone. However, analysis of these packets has shown purities of only 95%, with a

range of adulterants (such as caffeine, lidocaine, and piperazines) making up the rest of the product (German *et al.,* 2014).

Cathinones are also sold under mercurial, non-descript brand names, including Meow Meow, White Magic, Blizzard, Ivory Snow, and many others. Packets generally display warning labels such as 'not for human use', 'not tested for hazards or toxicity', ‘keep out of reach of children’, etc to avoid penalties under specific acts, for example, Analogue Enforcement Acts (Pieprzyca *et al.,* 2020) in the US. However, the composition and even the active component in a 'legal high', i.e., NPS products, can vary wildly from one another, although they are within the same brand name (Karila *et al.,* 2015). They may even contain substances other than those listed on their labels. Therefore, there are no guarantees that the customer of these NPS products may get the exact content of the psychoactive substance as advertised.

Synthetic cathinones are occasionally used in so-called "mephedrone sessions," which involve ingesting drugs repeatedly for a few hours and typically in specific social situations (such as at friend's houses, home parties, or nightclubs) (German *et al.,* 2014). Synthetic cathinone users cite a variety of factors as justifications for using these drugs, such as their legality, accessibility (mostly through the Internet), acceptable cost (less expensive than standard drugs), lack of quick screening tests to confirm use, or user preferences for particular pharmacological properties, such as the enhancement of social and sexual experiences (La Maida *et al.,* 2021). According to reports analysing the demographic information on users of synthetic cathinone, the respondents are primarily young males. In a review of data from six EU nations (Germany, Hungary, Ireland, the Netherlands, Poland, and Portugal), it was discovered that people between the ages of 18 and 25 made up the

majority of the online community interested in synthetic cathinone; however, the age profile of users of synthetic cathinone was most likely in the range of 18 to 35 years (Pieprzyca *et al.*, 2020).

Taking synthetic cathinone may cause strong withdrawal symptoms, including depression, anxiety, tremors, problems sleeping, and paranoia. Other adverse side effects include increased heart rate and blood pressure, breathing difficulties, loss of appetite, deterioration of memory, and hallucinations also occur when abused (Coppola & Mondola, 2012; Valente *et al.*, 2014). Abuse of synthetic cathinones may also lead to other negative effects such as nosebleeds, sweating, and nausea. The worst outcome of synthetic cathinone misuse may result in intoxication and further death (Lewin *et al.*, 2013).

2.3.1 Chemistry of Synthetic Cathinones

Cathinone and its analogues were structurally related to the phenylalkylamine family. The difference is only with the ketone group introduced at the *β*-position of the aminoalkyl chain attached to the phenyl ring (Valente *et al.*, 2014), the reason why synthetic cathinones are frequently referred to as βk-amphetamines (Zaitsu *et al.*, 2011).

Cathinone derivatives are analogues of the natural cathinone, which are synthesised through modification of the parent cathinone structures by adding diverse substituents at different locations of the cathinone molecule (Beckett, 2015). In recent years surges of new derivatives of cathinones, some with very potent psychostimulant effects, have been mass-produced stemming from the synthetic manipulation of the cathinone.

Table 2.1 lists the most spread synthetic cathinones analogues to appear throughout the recreational drug market, according to UNODC. Depending on the substituents made on the cathinone structure, this group of 'legal high' can be divided into four prominent sub-families (Pieprzyca *et al.*, 2020):

- Group 1 represents *N*-alkylated cathinones or those with alkyl or halogen substituents at any possible aromatic ring position. Most of the first known synthetic cathinones, such as MET, mephedrone, ethcathinone, flephedrone, buphedrone, and pentedrone, were categorised into this group.

- Group 2 belongs to the cathinone analogues with methylenedioxy-substitutes compounds in which the substituents occur at any given aromatic ring position. Methylone, pentylone, and butylone are among the compounds within Group 2.

- Cathinone analogues from group 3 are distinguished by *N*-pyrrolidinyl substituents at the nitrogen (N) atom and are currently the most common substances in the recreational drug market.

- Group 4 of cathinone analogues contains both the 3,4-methylenedioxy ring substituent and *N*-pyrrolidinyl moiety (Pieprzyca *et al.*, 2020; Soares *et al.*, 2021).

Table 2.1 Structures of the most spread cathinone analogues (Valente *et al.*, 2014). Note: *Newly identified synthetic cathinones.

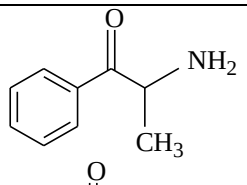
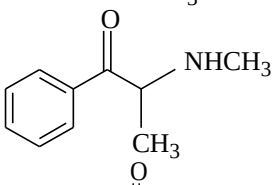
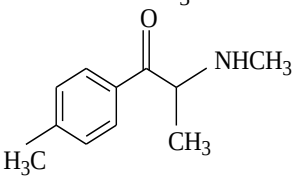
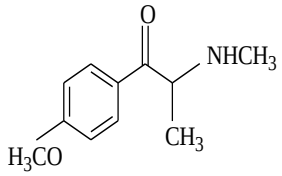
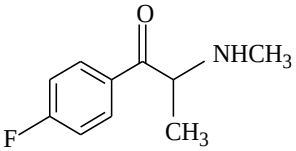
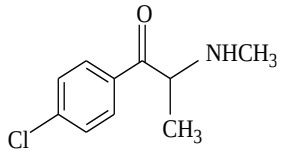
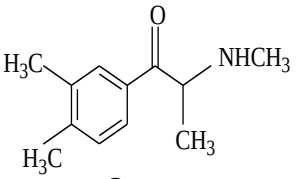
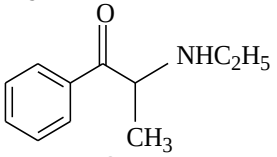
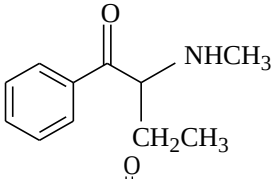
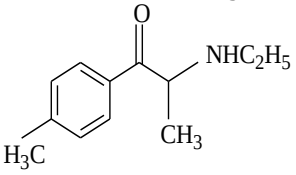
Group	Common name	IUPAC name	Molecular structure
1	Cathinone	2-Amino-1-phenyl-1-propanone	
1	MET ephedrone	2-(Methylamino)-1-(4-ethylphenyl)-1-propanone	
1	4-Methylmethcathinone Mephedrone 4-MMC	2-(Methylamino)-1-(4-methylphenyl)-1-propanone	
1	Methedrone	1-(4-Methoxyphenyl)-2-(methylamino)-1-propanone	
1	Flephedrone 4-FMC	1-(4-Fluorophenyl)-2-(methylamino)-1-propanone	
1	4-Chloromethcathinone 4-CMC	1-(4-Chlorophenyl)-2-(methylamino)-1-propanone	
1	3,4-DMMC	1-(3,4-Dimethylphenyl)-2-(methylamino)-1-propanone	
1	Ethcathinone Ethyl propion	2-(Ethylamino)-1-phenyl-1-propanone	
1	Buphedrone	2-(Methylamino)-1-phenyl-1-butanone	
1	4-MEC	2-(Ethylamino)-1-(4-methylphenyl)-1-propanone	

Table 2.1 Continued

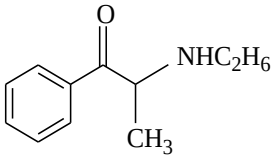
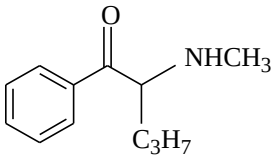
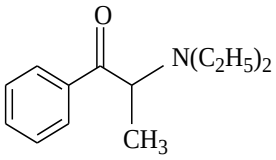
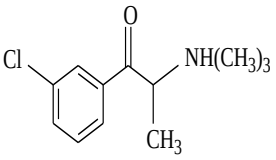
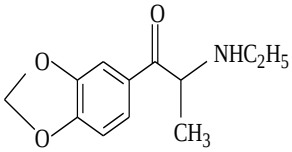
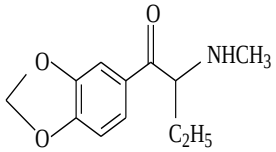
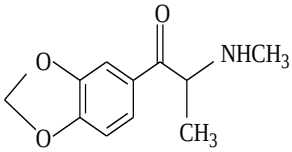
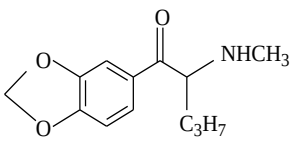
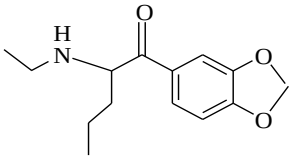
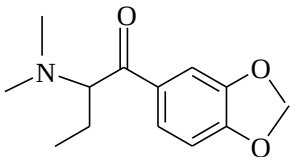
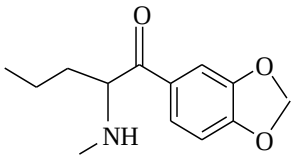
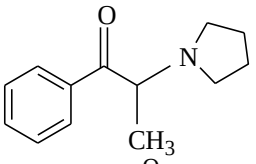
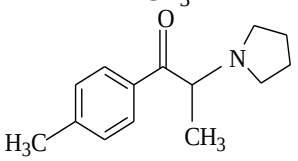
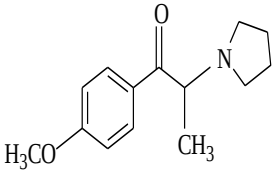
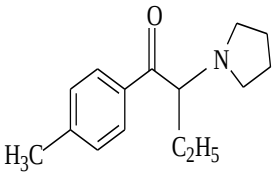
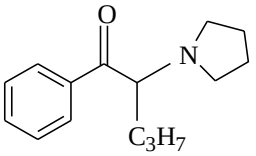
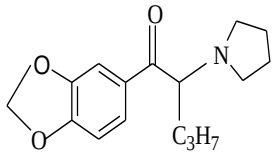
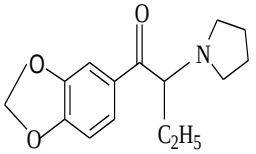
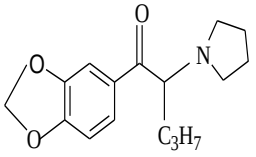
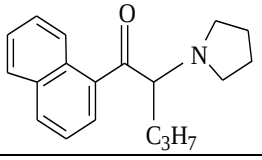
1	DMMC	2-Dimethylamino-1-phenyl-1-propanone	
1	Pentedrone	2-(Methylamino)-1-phenyl-1-pentanone	
1	Diethylpropion amfepramone	2-Diethylamino-1-phenyl-1-propanone	
1	Bupropion amfebutamone	1-(3-Chlorophenyl)-2-(*tert*-butylamino)-1-propanone	
2	Ethylone *β*k-MDEA	2-(Ethylamino)-1-(3,4-methylenedioxyphenyl)-1-propanone	
2	Butylone *β*k-MBDB	2-(Methylamino)-1-(3,4-methylenedioxyphenyl)-1-butanone	
2	Methylone *β*k-MDMA	2-Methylamino-1-[3,4-methylenedioxyphenyl]-1-propanone	
2	Pentylone *β*k-MBDP	2-(Methylamino)-1-(3,4-methylenedioxyphenyl)-1-pentanone	
2	*N*-ethylnorpentylone* Ephylone *β*k-EBDP	1-(Benzo[d][1,3]dioxol-5-yl)-2-(ethylamino)pentan-1-one	

Table 2.1 Continued

2	*β*-keto-*N*,*N*-Dimethylbenzodioxolyl butanamine* Dibutylone *β*k-DMBDB	1-(Benzo[d][1,3]dioxol-5-yl)-2-(dimethylamino)butan-1-one	
2	β-keto-*N*-Methylbenzodioxolyl pentanamine* Pentylone βk-MBDP	1-(Benzo[d][1,3]dioxol-5-yl)-2-(methylamino)butan-1-one	
3	*α*-PPP Pyrovalerone	1-Phenyl-2-(1-pyrrolidinyl)-1-propanone	
3	MPPP *α*-PVP	4'-Methyl-*α*-pyrrolidinovalerophenone	
3	MoPPP	4'-Methoxy-*α*-pyrrolidinovalerophenone	
3	MPBP	1-(4-Methylphenyl)-2-(1-pyrrolidinyl)-1-butanone	
3	PVP	1-Phenyl-2-(1-pyrrolidinyl)-1-pentanone	
4	MDPPP	1-(3,4-Methylenedioxyphenyl)-2-(1-pyrrolidinyl)-1-propanone	
4	MDPBP	1-(3,4-Methylenedioxyphenyl)-2-(1-pyrrolidinyl)-1-butanone	
4	MDPV	1-(3,4-Methylenedioxyphenol)-2-pyrrolidinyl-1-pentanone	
4	*α*-Naphyrone	1-Naphthalen-1-yl-2-pyrrolidin-1-ylpentan-1-one	

It should be noted that synthetic cathinones, like other phenethylamines, have a chiral centre, allowing them to exist in two stereoisomeric forms with varying potencies and affinity for their pharmacological targets (Soares *et al.,* 2021). Nevertheless, as with cathinone in nature, the majority of synthetic cathinones appear as racemic mixtures. Racemisation of the enantiomeric forms of these psychoactive substances may occur via keto-enol tautomerism (Coppola & Mondola, 2012). The chemical variations on the cathinone backbone structure will be responsible for each derivative's different pharmacokinetic and pharmacodynamic properties.

2.4 Prevalence and Global Seizures of Synthetic Cathinones

There is uncertainty regarding the global prevalence of synthetic cathinones as most information is based on self-reporting abuse surveys. These surveys apply to specific groups and do not accurately reflect the overall population. Furthermore, most NPS users are unaware of the drugs consumed due to inadequate product labelling (Vicknasingam *et al.,* 2020). The data from these surveys indicate the prevalence of particular synthetic cathinones in specific locations worldwide. Observations reveal that the number of cathinone derivatives, particularly MMC, has increased dramatically since 2008 and has become a popular abuse drug amongst users of "*legal high*" products (Vardakou *et al.,* 2011). The widespread and rapid prevalence of these cathinone analogues is mainly attributed to the wide availability and ease of purchase over the internet, providing uncontrolled access to users of all ages.

Synthetic cathinones are now the second-largest group of NPS tracked by EU EWA, behind the synthetic cannabinoids, according to the EMCDDA's most recent report (EMCDDA, 2022), which listed 162 synthetic cathinones under their surveillance. It was reported that between 2019 to 2022, a total of 29 new synthetic