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ADVANCED REVIEW

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Identification of synthetic cathinones in seized materials: A review of analytical strategies applied in forensic chemistry

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Abstract

Synthetic cathinones are a class of novel psychoactive substances, commonly tested in seized materials by forensic chemistry laboratories. These drugs have been found in different types of seized materials including powders, tablets, liquids, herbs, and blotters. A comprehensive review of the literature shows that the analyses of synthetic cathinones have been performed by a diverse repertoire of techniques, from more traditional to emerging methods. Techniques commonly used in the analysis of "traditional" illicit drugs, such as color and microcrystalline tests, chromatography, capillary electrophoresis, mass spectrometry (MS), and infrared (IR), and Raman spectroscopies, have been used for screening and/or confirmatory purposes, depending on the technique and laboratories preference. However, other emerging techniques, such as nuclear magnetic resonance, high-resolution MS, direct analysis in real-time MS, gas chromatography with IR detection, and so on, have been drawing the attention of forensic scientists due to the advantages of these techniques in providing important structural data. It is important to consider that each technique presents analytical benefits and limitations, which should be considered when analyzing synthetic cathinones in seized materials. Furthermore, the combination of more than one analytical technique is always recommended, especially when an unknown synthetic cathinone is suspected and/or encountered in the sample.

This article is categorized under:

Forensic Chemistry and Trace Evidence > Controlled and Emerging Drug Compounds

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KEYWORDS

analytical techniques, forensic chemistry, novel psychoactive substances, seized drugs, synthetic cathinones

1 | INTRODUCTION

Novel psychoactive substances (NPS) are drugs emerging on the recreational drug market that are generally not controlled or scheduled. The United Nations Office on Drugs and Crime (UNODC) defines NPS as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" (UNODC, 2021). UNODC also highlights that these drugs are not necessarily new drugs and NPS can be drugs that were discovered a long time ago but only recently emerged on the drug market (UNODC, 2021; Zapata et al., 2021). Similarly, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) defines NPS as "a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions" (EMCDDA, 2021b). Some NPS are developed through modifications to the chemical structure of previously emerged NPS or other "traditional" drugs of abuse (Krotulski et al., 2020). NPS have been used and marketed aiming to mimic other "traditional" drugs of abuse (e.g., cocaine and methamphetamine) (EMCDDA, 2020; Rinaldi et al., 2020) and proposed to be "legal alternatives" to illicit drugs as they can circumvent national and international drug legislations (EMCDDA, 2020; Krotulski et al., 2020; Rinaldi et al., 2020). Thus, the production and distribution of NPS can be facilitated if a new drug is not yet scheduled or controlled, either nationally or internationally.

The NPS phenomenon has been causing challenges for forensic chemists and toxicologists, physicians, and public health and safety professionals (Krotulski et al., 2020). Many recently emerged NPS have short- and long-term effects on health only poorly described or completely unknown (Corkery et al., 2020). Therefore, it is often difficult to diagnose an acute intoxication or to estimate the long-term consequences of NPS on the health of users. This is alarming considering that highly potent NPS with potential to cause acute intoxications and death have been reported (EMCDDA, 2020). In addition, another potential source of health risk is the presence of toxic adulterants or cutting agents mixed into drug materials with NPS. Another issue is that the NPS market is highly dynamic, diverse, and subject to geographical differences with new drugs frequently emerging, some being prevalent on the market only for short periods of time, and others being prevalent on the market for long or sustained periods of time (Tettey & Crean, 2015). Most NPS are designed and developed based on slight modifications of chemical structures of known drugs or prototypes with suspected in vivo receptor activity. This can lead to the formation of various analogues and also sometimes isomers. This can add another level of complexity in the analysis of NPS, requiring constant method developments and updates, or general assessments by the forensic laboratories, in order to monitor a high number of ever-changing NPS accurately, which can be a demanding process. Methods for the analysis of "traditional" drugs of abuse, such as cocaine, methamphetamine or heroin, are more widely available in forensic laboratories whereas methods for NPS testing might still be more limited (Tettey & Crean, 2015). In addition, certified reference materials of these NPS might not be readily obtainable due to either commercial unavailability or to high costs (Laks et al., 2004; Tettey & Crean, 2015), preventing the identification based on comparison between analytical data (e.g., the chromatographic retention time, mass spectrum) of reference materials versus suspected analytes (Laks et al., 2004).

Although there are several challenges in the detection and identification of a NPS in a forensic specimen, it is noteworthy to mention that many scientific advances have been achieved over the years in this regard. In the literature, there are several methods, monographs and reports available for a wide range of NPS, which is highly useful to forensic laboratories. Certified reference materials' manufacturers have been constantly improving their research and development capabilities, offering an ever-increasing number of NPS standards in the market. In addition, instrumental libraries are available for some platforms (mainly gas chromatography–mass spectrometry [GC-MS]), allowing the identification, even in the absence of the reference material. More recently, in silico strategies have been developed to identify "new" NPS without reference materials and based on chemometrics tools obtained from previously acquired experimental data for other NPS from the same class (e.g., synthetic cannabinoids) (Polettini, Kutzler, Sauer, Bleicher, & Schultis, 2021; Polettini, Kutzler, Sauer, Guber, & Schultis, 2021). Portable instrumentations have been developed as well, enabling the miniaturization of instrumentations and possibility of detection in the field.

NPS encompasses a large number of substances with high chemical and pharmacological diversity. Therefore, these substances can be classified according to either their chemical structures or their pharmacological effects (Rinaldi et al., 2020). Considering their chemical structure, NPS comprise synthetic cannabinoids, synthetic cathinones, synthetic opioids, designer benzodiazepines, phenethylamines, phencyclidine-like substances, novel piperazines, novel tryptamines, and other substances (Rinaldi et al., 2020; UNODC, 2021). On the other hand, with regard to their

pharmacology, NPS can be classified as stimulants, depressants, hallucinogens, anesthetics, and dissociatives, among others (Rinaldi et al., 2020).

2 | SYNTHETIC CATHINONES

Synthetic cathinones are a class of NPS with high interest for forensic toxicologists and chemists, clinicians and public safety and health, and other professionals (Schifano et al., 2020), which have become more popular as recreation drugs since the beginning of the 2000s (Krotulski et al., 2018). At a high level, these substances are derived from the alkaloid cathinone which is naturally present in the plant *Catha edulis* (Baumann et al., 2018; de Campos, 2020; La Maida et al., 2021; Logan et al., 2017). Synthetic cathinones are phenylalkylamines (or beta-keto phenethylamines) possessing a phenyl ring bonded to an aminoalkyl side chain containing a beta-carbonyl group (Prosser & Nelson, 2012) and can be seen as beta-keto derivatives of amphetamine (Baumann et al., 2018; Schifano et al., 2020) (Figure 1). The presence of chiral centers in the structure of synthetic cathinones lead to the existence of two enantiomers, which might differ in pharmacological properties (Merola et al., 2014).

In general, synthetic cathinones exhibit stimulant properties, similar to those induced by 3,4-methylenedioxymethamphetamine (MDMA) and other amphetamine-like drugs (de Campos, 2020; La Maida et al., 2021; Prosser & Nelson, 2012). However, structural modifications of these substances, with the introduction of different

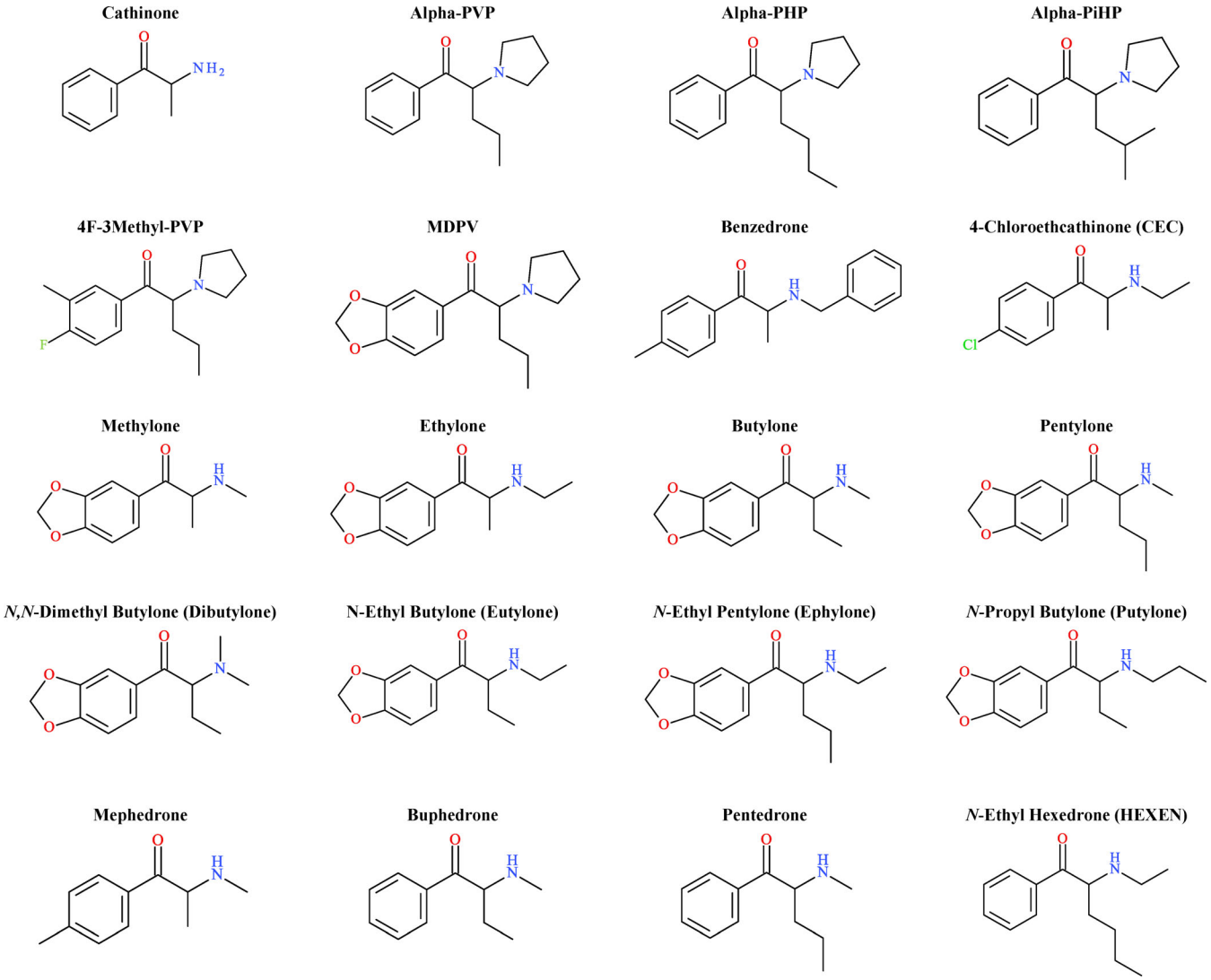

FIGURE 1 Chemical structures of several synthetic cathinones commonly reported by forensic laboratories

substituents (such as alkyl groups and halogens), induce variations in the pharmacological potency of these drugs (La Maida et al., 2021), in part due to size and lipophilicity. The pharmacology of cathinones is mainly associated with the interaction with monoamine (dopamine, serotonin, and norepinephrine) transporters in the brain (Baumann et al., 2018; Logan et al., 2017). The main effects associated with the use of synthetic cathinones are cardiac and neurological signs and symptoms, including euphoria, tachycardia, motor excitation, and increased blood pressure (de Campos, 2020; Logan et al., 2017; Prosser & Nelson, 2012).

Many agencies and countries frequently report this class of NPS in forensic casework, which continue to be found in seized materials. Although these drugs are "novel" psychoactive substances, some have been known for a long time, and only many years later have become manufactured and used as recreational drugs. An example is mephedrone (4-methylmethcathinone [4-MMC]), which was first synthesized in 1929 and started to be reported as recreational drug in 2007 (Schifano et al., 2020). The emergence of synthetic cathinones in the drug market dates back at least to 2003 (La Maida et al., 2021). According to the UNODC, between 2009 and 2019, synthetic cathinones, along with phenethylamines, were the most frequently reported NPS class (UNODC, 2020c). In the European Union, in 2019, synthetic cathinones and synthetic cannabinoids were detected in 60% of the drug seizures and the European Union Early Warning System currently monitors 156 synthetic cathinones (EMCDDA, 2021a). In the United States, the Drug Enforcement Administration (DEA) reports synthetic cathinones in seizures and in 2020, an increase of around 9% was observed in relation to 2019 (DEA, 2020).

Synthetic cathinones are usually found in seized drug materials such as powders, crystalline solids, tablets, capsules, and liquids, some of which resemble "Ecstasy," "Molly," or MDMA (de Campos, 2020; Schram et al., 2021; UNODC, 2020b). However, these drugs may also appear in alternative forms. For example, alpha-pyrrolidinovalerophenone (alpha-PVP) has been found in herbal materials in Poland (Byrska et al., 2017), and ethylone, 3′,4′-tetramethylene- alpha-PVP (TH-PVP), 4-methylethcathinone (4-MEC), dibutylone, and *N*-ethylpentylone were found in blotters seized in Brazil (de Souza Boff et al., 2020). In addition, synthetic cathinones have also been detected in contents recovered from syringes (EMCDDA, 2021a).

3 | OVERVIEW OF THE ANALYSIS OF SYNTHETIC CATHINONES IN SEIZED MATERIALS

Many laboratories around the world have been monitoring and detecting synthetic cathinones in biological fluids (collected from intoxication cases) or seized materials (apprehended by law enforcement). Drugs in general can be seized in many settings, such as the apprehensions in ports of entry or in the possession of a person and the detection of NPS in such materials can be very valuable for the investigations. For example, if a person exhibits signs of acute intoxication or is found dead, having in his/her possession a suspected illicit substance, identifying any drug, including a NPS, in this material can provide some clues for elucidating the cause of the intoxication. On the other hand, if NPS are detected in materials being shipped to a country, this information can help in monitoring the international illicit market for these substances.

In general, the analytical workflow for any type of seized drug material is based on sampling, sample preparation, analysis, documentation, and reporting (Scientific Working Group for the Analysis of Seized Drugs [SWGDRUG], 2019). The general analytical workflow for the determination of synthetic cathinones in a seized material is usually similar to that employed for the analysis of other routinely encountered drugs. The main differences can depend on the type and amount of material submitted for analysis. Bulk solids, tablets, plants, blotters, and liquids may be processed differently prior to analysis. If a seemingly homogeneous and non-segmented sample is submitted for testing (e.g., a few grams of an unknown powder), the entire sample may be subjected to single or direct analysis. On the other hand, when a large number of samples are seized (e.g., tons of unknown powder or pills), it may not be feasible to test all iterations and laboratories may employ a sampling method to select a reduced amount of representative samples to be analyzed in order to enable a time and cost-effective testing regime. Using a practical example, in a report of multiple similar samples of capsules, wrap packages, and cigarettes submitted for analysis (which later revealed the presence of synthetic cathinones), all the materials were weighted and one representative sample was selected for testing (Johnson et al., 2020).

Prior to the analysis of synthetic cathinones in seized drug materials, a sample preparation step may be required, such as dissolution, filtration, and/or extraction. The preparation method depends on the material type and the ultimate analytical technique used. For example, gas and liquid chromatography (LC) analysis requires samples in liquid phase for injection (Lesiak & Shepard, 2014). In general, for chromatographic analysis, samples of powders and crystalline solids are subjected to dissolution in organic solvents such as ethanol, methanol or acetonitrile, or buffer (for LC),

which may be complemented by centrifugation, sonication, and/or dilution (Błażewicz et al., 2017; Johnson et al., 2020; Majchrzak et al., 2016; Pasin et al., 2017; Reitzel et al., 2012; UNODC, 2020b). Extraction with solvents combined to centrifugation was also employed in the analysis of synthetic cathinones in herbal materials (Byrska et al., 2017) and blotters (de Souza Boff et al., 2020). Moreover, in addition to dilution, extractions with solvents under controlled pH have also been employed (NPS Discovery, 2021; UNODC, 2020b). For chromatographic analyses, dissolution of the samples prior to injection is usually adopted due to its simplicity and fast execution but only if sample is homogeneous (e.g., powders) and the concentrations of the drug are expected to be elevated in the material (which might require a further dilution to avoid detector contamination/saturation). Filtration or centrifugation will be required depending on the composition of the sample as well, for example, if there are particulate materials that are not soluble in the solvent. Extraction methods are more recommended when less sensitive techniques are used and/or when complex samples are being tested (e.g., herbal materials), making more difficult to perform only a simple dilution prior to analysis. Extraction with solvents and dilution have also been used in the analysis of seized drug materials by ion mobility spectrometry (IMS) and direct analysis in real-time mass spectrometry (DART-MS) (Gwak & Almirall, 2015). Dissolution in water was also reported for microcrystalline tests (UNODC, 2020b). Spectroscopic techniques, such as infrared (IR) spectroscopy, may require either the preparation of a pellet containing the sample and a salt (e.g., KBr) (Majchrzak et al., 2016) or may be performed without any sample preparation using attenuated total reflection IR spectroscopy (ATR-IR) (Johnson et al., 2020; Machado et al., 2019). With regard to nuclear magnetic resonance (NMR) analyses, samples are usually diluted in deuterated solvents such as chloroform, methanol, and dimethylsulfoxide (DMSO) (Błażewicz et al., 2017; Gaspar et al., 2015; NPS Discovery, 2021; Power et al., 2011; Yovanovich et al., 2018), to avoid interferences from hydrogen or carbon atoms from the solvent depending on the type of NMR being used. Other techniques such as color tests require no sample pretreatment and the analysis is performed directly on the sample (Cuypers et al., 2016), which can be adapted if unconventional materials are submitted for testing.

The detection of synthetic cathinones in seized materials can be performed by using the same techniques traditionally used in routine testing of "traditional" illicit drugs (e.g., cocaine, methamphetamine). However, additional techniques may be required for more in-depth structural elucidation, especially when an unknown cathinone is suspected. Several techniques have been reported for the characterization of synthetic cathinones, such as GC-MS, LC-MS, Fourier transform IR spectroscopy (FTIR), and NMR, described in a report by the UNODC (UNODC, 2013). GC, LC, and capillary electrophoresis (CE) are some of the common techniques used in the separation of isomers or enantiomers (Tai & Morrison, 2017), and GC and LC have been widely adopted in the separation of synthetic cathinones (including their isomers) present in seized drugs. Each analytical technique has advantages and benefits but also limitations that scientists must remain aware of (Strano Rossi et al., 2014; UNODC, 2013).

4 | COLOR TESTS

Color tests or spot tests have been historically adopted as presumptive tests for the analysis of drugs (O'Neal et al., 2000), performed as the first type of analysis carried out on a seized material (Bono, 2007). According to SWGDRUG guidelines, color tests can provide analytical selectivity through obtaining general or chemical class-related information (SWGDRUG, 2019). These tests present some analytical benefits including easy and quick execution, no need for instrumentation, low costs for execution and the possibility of execution in diverse settings, including on-site, outside the laboratory environments (Clancy et al., 2020; Philp & Fu, 2018). In general, color tests exhibit good sensitivity and can reach the microgram range, which depends on the type of drug and reagent (Harper et al., 2017). However, the low selectivity is a limitation of these tests and cross-reactions might generate false-positive results, which may be attributable to the presence of a different drug or adulterants, diluents, and contaminants (Bono, 2007). In addition, the interpretation of a color change is subjective and can be biased by the analyst perception of color or the environmental conditions where test was performed (e.g., poor luminosity on-site), which could result in false-positive results with legal impacts (Elkins et al., 2017). This reiterates that these tests must be used only as presumptive tests and require confirmation by instrumental analysis. Moreover, this test demands analyzing positive controls with a reference standard, simultaneously to the samples.

The detection of synthetic cathinones in complex mixtures (i.e., in the presence of other similar drugs, contaminants, and cutting agents) by color tests can be complicating and challenging (Namera et al., 2015). As previously approached, many NPS are structurally related to other "traditional" drugs of abuse. Therefore, there is a potential issue with regard to the selectivity of color tests when an NPS may be involved (Clancy et al., 2020), with the traditional, common color tests being poorly specific to these new drugs (Philp & Fu, 2018), especially considering that many synthetic cathinones are

similar to other traditional, well-known drugs, such as the amphetamines. Moreover, a color test might not be available yet for a class of NPS or not be appropriate (Sisco et al., 2021), which applies to the synthetic cathinones as well. A single-color test might not be able to differentiate between structurally-related synthetic cathinones.

Several conventional reagents used in color tests to detect "traditional" illicit drugs have been shown to react with synthetic cathinones **(**Table 1**)**. A study comparing the results obtained via color tests and FTIR demonstrated a good correlation for mephedrone (eight samples), methylone (one sample) and 3,4-methylenedioxypyrovalerone (MDPV) (one sample) (Toole et al., 2007). According to the authors of the study, the tests of Marquis and Liebermann were the most appropriate for detecting methcathinone and its analogues in preliminary analyses (Toole et al., 2007). By application of the test of Marquis or the test of Chen, methylenedioxy analogues of cathinones give positive results (Namera et al., 2015). The UNODC also recommends the Zimmermann test for the presumptive identification of synthetic cathinones (in the form of hydrochloride or hydrobromide salts) (UNODC, 2020b). This test enables the identification of many cathinones and also can provide the differentiation between halogen-substituted methcathinones and ketamine (which is not possible via Marquis, Simon, and modified Scott tests) (UNODC, 2020b). The combination of tests of Marquis, Ehrlich, Simon, Lieberman, and Mandelin can be useful for the determination of a synthetic cathinones (Namera et al., 2015). In another study, the authors also proposed conventional color test-based workflows, combining multiple commercial kits from a company to test several NPS, including 4-ethylmethcathinone, butylone, methylone, and MDPV (Cuypers et al., 2016). More recently, a test with copper(II)-2,9-dimethyl-1,10-phenanthroline (Cu(II)-neocuproine) was proposed for mephedrone and other synthetic cathinones (Philp et al., 2016). To a small amount of sample (between 0.1 and 0.2 mg), five drops of Cu(II) nitrate solution (at 5.0×10^{-3} mol/L) are added, followed by the addition of two drops of neocuproine solution (at 5.12×10^{-3} mol/L), and two drops of sodium acetate buffer (2 mol/L). The mixture is heated, and potential color changes should be observed within 10 min. This test was useful in the detection of mephedrone hydrochloride and other synthetic cathinones, with good selectivity for several synthetic cathinones and using a safer chemical reagent (Philp et al., 2016).

5 | MICROCRYSTALLINE TESTS

Microcrystalline tests can provide several analytical gains for presumptive tests: fast analyses, easy execution, and high sensitivity (L. Elie et al., 2012; Quinn et al., 2020; UNODC, 2020b). These methods are also cost-effective, requiring low

TABLE 1 Conventional color tests and applicability to synthetic cathinones reported in the literature

Test	Reagents	Reactive synthetic cathinones (change of color)	References
Marquis	Concentrated sulfuric acid and formaldehyde	Methylone, MDPV, and butylone	Cuypers et al. (2016), Toole et al. (2007)
Liebermann	10% Sodium nitrite in sulfuric acid (w/v)	Cathinone, methcathinone, *N*,*N*-dimethylcathinone, mephedrone, methedrone, methylone, MDPV, and butylone	Toole et al. (2007)
Zimmermann	1,3-Nitrobenzene in methanol and aqueous solution of potassium hydroxide	Benzedrone, 4-BMC, butylone, 4-CMC, ethylone, eutylone, flephedrone, MDPV, mephedrone, methcathinone, methedrone, methylone, 2-MMC, 3-MMC, 4-MEC, alpha-PVP, and 4-(trifluoromethyl) methcathinone	UNODC (2020b)
Simon	Mixture of sodium nitroprusside, sodium carbonate, and acetaldehyde	4-Ethylmethcathinone, butylone, flephedrone, MDPV, methylone, and bk-IVP	Cuypers et al. (2016), Toole et al. (2007), Yovanovich et al. (2018)
Mecke	Mixture of selenous acid and concentrated sulfuric acid	Butylone, MDPV, and methylone	Cuypers et al. (2016)
Mandelin	Mixture of ammonium vanadate and concentrated sulfuric acid	Butylone, MDPV, and methylone	Cuypers et al. (2016)

Abbreviations: bk-IVP, 1-(2,3-dihydro-1H-inden-5-yl)-2-(ethylamino)pentan-1-one; 4-BMC, 4-bromomethcathinone; 4-CMC, 4-chloromethcathinone; MDPV, 3,4-methylenedioxypyrovalerone; 4-MEC, 4-methylethcathinone; 2-MMC, 2-methylmethcathinone; 3-MMC, 3-methylmethcathinone; alpha-PVP, alpha-pyrrolidinovalerophenone.

amounts of reagents and simple instrumentation (L. E. Elie et al., 2011; M. P. Elie & Elie, 2009; Quinn et al., 2020) and are less affected by subjectivity (Bono, 2007). However, this method relies on the direct comparison with reference standards performed simultaneously to the samples or with photographs (L. E. Elie et al., 2011; UNODC, 2020b). The results can be affected by highly diluted analytes or "expired" reagents, leading to no formation of crystals (Bono, 2007). For further presentation of the results, the photographs are the only possibility of registering the data of a particular sample (Bono, 2007). Moreover, microcrystalline tests are not able to provide quantitative analyses (M. P. Elie & Elie, 2009). According to SWGDRUG, microcrystalline tests are a Category B technique and need to be combined with at least one Category A technique (e.g., GC-MS) to provide identification in seized drugs (Quinn et al., 2020; SWGDRUG, 2019).

The applications of microcrystalline tests to synthetic cathinones analyses are limited in the current literature. A method using aqueous solution of $HgCl_2$ is reported in the literature for the identification of mephedrone, exhibiting a limit of detection of 3 g L^{-1} and a proposed cut-off of 5 g L^{-1} (L. Elie et al., 2012), and is recommended by UNODC (, 2020b). This technique method has also been recently proposed in combination to Raman spectroscopy for the analysis of 2-MMC, 3-MMC, and 4-MMC (mephedrone) (L. Elie et al., 2016).

6 | THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a simpler chromatographic technique that can be easily performed for the separation and identification of drugs (Y. Liu et al., 2020). Simple, fast, and reduced cost analyses can be achieved by TLC, with the possibility of screening a high number of samples and thus reducing the number of confirmatory analyses (e.g., by GC-MS) (Jain, 2000; Y. Liu et al., 2020; UNODC, 2020a; Yu et al., 2016). It also exhibits good sensitivity and the specificity depends on the complexity of the mixture (Harper et al., 2017). TLC can be considered more environmentally friendly than LC because it requires reduced use of solvents (Y. Liu et al., 2020). Similarly to color tests, TLC mainly provides qualitative results (Harper et al., 2017) but quantitation via TLC might be possible. Coupling TLC to ambient pressure mass spectrometry (MS) techniques might be possible and useful in detecting a high range of compounds (UNODC, 2020). In the literature, there are data available on the retention factor for several drugs in different solvents but this may be limited due to noninterchangeability between different laboratories, which is not uncommon (Bono, 2007), and due to the inexistence of NPS standards. This technique requires standard materials for comparison of retention times to be run simultaneously with the samples (UNODC, 2020). Furthermore, even with the simultaneous analysis of standards, co-eluting compounds or compounds producing similar color spots during revelation can be a limitation for TLC analysis (Howlett & Steiner, 2011). TLC may also be subjected to poor resolution, which could be circumvented by the use of high-performance TLC (HPTLC) (Y. Liu et al., 2020). Overall, TLC-based methods cannot provide the definitive identity of a synthetic cathinone, especially in the case of unknown drugs (Fowble et al., 2018).

TLC has been reported for the preliminary analyses of methylone, mephedrone, 4-MEC, and MDPV in a drug testing service in Spain; results were confirmed by GC-MS methods (Caudevilla-Gálligo et al., 2013). TLC was performed using silica gel plates and three solvent systems (methanol/25% ammonia solution [100:2.5 v/v], methanol, and acetone), with colors produced via Marquis and p-dimethylaminobenzaldehyde (p-DMAB) tests (Caudevilla-Gálligo et al., 2013). Another report on the analysis of methcathinone and other analogues by TLC employed a commercial assay (DeRuiter et al., 1994). The UNODC has proposed a TLC-based method for the identification of synthetic cathinones using a silica gel plate and ethyl acetate, methanol and 25% ammonia solution (89.5:10:0.5 v/v/v), and visualization with ethanolic solution of ninhydrin (2%) under ultraviolet (UV) light (at 254 nm) (UNODC, 2020b). This method was shown applicable to a range of synthetic cathinones including eutylone, butylone, and methylone (UNODC, 2020b).

7 | GAS CHROMATOGRAPHY–MASS SPECTROMETRY

GC-MS is a hyphenated technique that combines the advantages of superior chromatographic separation provided by GC with the highly specific identification by MS, producing very useful data for analytical characterization (Špánik & Machyňáková, 2018; UNODC, 2020). Based on SWGDRUG guidelines, MS is considered a category A technique (which enables "selectivity through structural information") whereas GC is classified as a category B technique (which enables "selectivity through chemical and physical characteristics") (SWGDRUG, 2019). This is one of the techniques most

frequently utilized in seized drug testing by many laboratories (Davidson et al., 2018), including NPS (Kelly & Bell, 2018), and especially using electron impact (EI) ionization (Stuhmer et al., 2020). The UNODC includes the recommendation of GC-MS methods in confirmatory analysis of seized cathinones (UNODC, 2020b). Moreover, it is possible to use GC coupled to tandem mass spectrometry (GC-MS/MS) and/or high-resolution mass spectrometers (GC-HRMS) (Dei Cas et al., 2019; Levitas et al., 2018). GC coupled to time-of-flight mass spectrometry (GC-TOF-MS), to quadrupole-time-of-flight mass spectrometry (GC-QTOF-MS) or to quadrupole-orbitrap MS, with EI and chemical ionization, are examples of GC-HRMS currentLY available (Špánik & Machyňáková, 2018). The identification by GC-MS can be performed by comparing the results obtained for a given analyte with the retention time and mass spectrum of a reference material, under the same instrumental conditions (Stuhmer et al., 2020; SWGDRUG, 2019; UNODC, 2020). The analysis can also be performed by comparison of the analytical findings within a sample to comprehensive instrumental libraries, such as SWGDRUG and NIST (Kelly & Bell, 2018). If a certified reference material is unavailable in-house, there are some alternative chromatographic strategies that can be used such as retention time locking, relative retention time or retention index for comparison with data from a partnering laboratory (UNODC, 2020; Zacca et al., 2021). Recently, a GC-MS method based on linear retention indexes was developed and validated for 22 drugs, including the synthetic cathinones *N*-ethylpentylone, resulting in a selective method with potential to be implemented in routine testing (Zacca et al., 2021).

GC-MS is a platform that is widely available and widely adopted for drug identification in many forensic laboratories (Krotulski et al., 2020; UNODC, 2020). Analysis by GC-MS can provide important information on the chemical structure of synthetic cathinones, with high sensitivity and selectivity. As many laboratories have been using GC-MS methods for detecting NPS, including synthetic cathinones, there are many GC-MS databases available, and frequently updated, based on the emergence of new compounds, such as SWGDRUG Mass Spectral Library (SWGDRUG, 2021), NPS Discovery (, 2021), Cayman Spectral Library (Cayman Chemical, 2021) and others. An advantage is that the sample is not required to be of high-purity and multiple compounds can be detected and separated within the same analysis (Bono, 2007), especially considering the database of instrumental libraries. Another advantage of GC-MS is the reduced cost for acquiring an instrument, in comparison to other techniques (Kyle, 2017). The possibility of using Fast-GC offers faster analysis times due to narrower and shorter capillary columns, providing more efficient separations (Dei Cas et al., 2019). Another benefit is that GC-MS maintenance is not complex and usually is not very expensive in comparison to other techniques (such as LC-MS) (Choi et al., 2013). The possibility of chemical ionization mode can enable soft-ionization conditions for labile drugs, reducing extensive fragmentation of molecular ions (Gwak et al., 2015; Mokhtar et al., 2016). The use of GC-MS/MS can also provide some analytical benefits. GC-MS/MS employing triple quadrupole MS renders highly selective and sensitive analyses, with multiple reaction monitoring mode allowing the selection and detection of multiple ions for qualitative or quantitative analyses (Mokhtar et al., 2016). Furthermore, GC-HRMS provides excellent chromatographic separation combined to with high-sensitivity and accurate analyses, with elevated data acquisition rates (Špánik & Machyňáková, 2018).

GC-MS methods are limited by the fact that drugs must be relatively volatile and thermally stable (generally, substances with low to moderate polarity) (Cruces-Blanco & García-Campaña, 2012; Kyle, 2017; Mokhtar et al., 2016). Therefore, in many cases, samples might need to undergo some level of processing prior to the analysis, which may involve extraction and/or derivatization (Kyle, 2017). In GC, some analytes may not be amenable to the high temperatures in the injection port, requiring derivatization procedures at times or other modifications to the method (Bono, 2007). Another concern is the possibility of the formation of artifacts by GC-MS (Brandt & Kavanagh, 2017; Köppel & Tenczer, 1995). More specifically, synthetic cathinones undergo extensive fragmentation during GC-MS analysis, especially using EI, and the molecular ion may not be detected (Fowble et al., 2018; Lesiak & Shepard, 2014; Sisco et al., 2021). However, the use of cold electron ionization or chemical ionization (as previously mentioned) might be an alternative to reach higher intensities of molecular ions in GC-MS (Gwak et al., 2015; Levitas et al., 2018). Oxidative degradation can also occur (due to high temperatures in the system), generating enamine or imine species that can co-elute with the parent cathinone (Carnes et al., 2017; UNODC, 2020b). These artifacts will result in a shift of 2 Da in the mass spectra profile, attributable to the loss of two hydrogen atoms by the parent (Kerrigan et al., 2016; UNODC, 2020b). However, this degradation may be reduced by decreasing the temperature and residence time at the injection port and avoiding active sites in the flow path (Kerrigan et al., 2016; Tsujikawa et al., 2013). For example, for alpha-PVP, the selection of split injection and the use of a new, deactivated liner were useful in preventing the thermal degradation of this cathinone (Tsujikawa et al., 2013). Another limitation is that GC-MS may not provide the full distinction between closely related isomers (Sisco et al., 2021; UNODC, 2020), due to their similar retention times and mass spectra and the potential unavailability of certified reference materials (Kranenburg, Verduin, et al., 2020; Sisco

et al., 2021). This can hamper the unequivocal identification of the correct isomer based on visual inspection only (Stuhmer et al., 2020). Moreover, this technique is not able to provide mass resolution below one unit and will not distinguish between two compounds with mass differences of 0.01 Da by mass alone, for example (Wu et al., 2012). Another limitation of GC-MS is with regard to the potential variations of retention times between different instrumentations and the need for reference standards. As previously discussed, certified reference materials might not be easily available for some NPS and updates in instrumental libraries may not follow the pace of the emergence of NPS (Kelly & Bell, 2018). The coupling of GC and HRMS instrumentations also shows limitations. GC-TOF-MS can exhibit a narrow dynamic range, limiting the analysis of complex mixtures with drugs at various levels (especially high concentrations) (Špánik & Machyňáková, 2018).

GC-MS is widely applied to the analysis of synthetic cathinones in seized materials, commonly using EI (usually at 70 eV) and with MS operating in full scan mode (Błażewicz et al., 2017; Byrska et al., 2017; Frison et al., 2021; Gaspar et al., 2015; Göl & Çok, 2019; Johnson et al., 2020; C. Liu et al., 2017; Machado et al., 2017, 2019; Maheux et al., 2016; Reitzel et al., 2012; Rojkiewicz et al., 2017; Strano Rossi et al., 2014; Yovanovich et al., 2018; Zuba et al., 2013). This same approach was also reported for synthetic precursors of cathinones, such as 3′,4′-methylenedioxy-2,2-dibromobutyrophenone (Armenta et al., 2020). With regard to isomers separation, there are some applications of GC-MS methods, especially using multivariate analysis. A study with reference standards has shown that multivariate statistical approaches, in particular principal component analysis (PCA), can provide the discrimination between *ortho*-, *meta*-, and *para*-isomers of ethylmethcathinone with up to 16 ions at 99.9% confidence level (Stuhmer et al., 2020). However, the authors highlight that further research is need for studying the potential effect of concentration and tune parameters in this discrimination (Stuhmer et al., 2020). Another study observed that even with the derivatization, the isomers 2-, 3-, and 4-MMC (the latter also known as mephedrone) were not directly, visually differentiated but the application of chemometric techniques, in particular PCA and linear discriminant analysis (LDA) made possible the unequivocal identification (Kranenburg, Verduin, et al., 2020). The application of GC-MS/MS and GC-HRMS analysis of synthetic cathinones has also been reported. For example, fast-GC-TOF-MS was able to detect NPS, including mexedrone, buphedrone, 3-MMC, 4-MEC, MDPV, alpha-PVP, and alpha-PHP, in seized samples, in a 10-min run, by comparison with reference standards or data available in SWGDRUG Spectral Library (Dei Cas et al., 2019). Another example is the application of GC-MS/MS, using cold EI, for the analysis of 35 cathinones (Levitas et al., 2018). This platform showed increased discriminatory power in cases of cathinones exhibiting low-intensity molecular ions. However, due to the low energy in the collision cells, less fragments were observed in comparison to conventional single MS approach (Levitas et al., 2018). GC-MS/MS employing triple quadrupole MS with EI and chemical ionization modes was reported for the detection of synthetic cathinones, including 3-fluoromethcathinone, mephedrone, 4-MEC, 3,4-dimethylmethcathinone (3,4-DMMC), methedrone, methylone, and MDPV (Gwak et al., 2015). The limits of detection for mephedrone and MDPV were 123.5 and 162.9 ng/ml using chemical ionization and 37.5 and 63.5 ng/ml using EI, showing that the latter mode provided more sensitive analysis (Gwak et al., 2015). However, as previously mentioned, the use of chemical ionization can avoid the problem of extensive fragmentation (and loss of molecular peak) (Gwak et al., 2015). GC-MS/MS was reported for the differentiation between the positional isomers 2-, 3-, and 4-fluoromethcathinone (Murakami et al., 2019). Employing energy-resolved mass-spectrometry (ERMS), the study reported that the fragments corresponding to fluorophenyl (95 Da) and fluorobenzoyl (123 Da) ions showed differences in the relative abundance and thus were used in unequivocal identification of each isomer (Murakami et al., 2019). The method was also successfully applied in the analysis of three brown-colored seized powders, which were all found to contain 4-fluoromethcathinone (*para*-fluoromethcathinone) (Murakami et al., 2019).

8 | LC WITH UV–VISIBLE DETECTION

LC with UV-visible detector (LC-UV) or LC with photodiode array detection (LC-DAD) combines the advantages of LC to UV–Vis spectroscopy. These techniques can provide insightful information regarding the presence of some functional groups (absorbing the light in a given wavelength) (Błażewicz et al., 2017; Li & Lurie, 2014). LC-DAD also offers the advantage of full scanning of the entire UV spectrum of the sample (Bono, 2007), instead of monitoring only specific wavelengths. In addition, LC-DAD and LC-UV can enable the differentiation between positional isomers (Li & Lurie, 2014; UNODC, 2020b). This technique can be used either in qualitative or quantitative analyses of synthetic cathinones (UNODC, 2020b). However, the analysis by this technique is not able to provide definitive, unequivocal determination of chemical identity (Li & Lurie, 2014). Instrumental libraries containing NPS data are also usually

unavailable for this type of instrumentation, requiring reference materials. Moreover, for the identification using UV–Vis detection, the analyte must possess the ability to absorb the UV–Vis radiation (by the presence of specific functional groups in the structure) (Bono, 2007) and sample preparation is usually required (e.g., dilution in a proper solvent).

In the literature, there are some reports describing the usefulness of LC-UV or LC-DAD methods for the analysis of synthetic cathinones. Ultrahigh-performance liquid chromatography with photodiode array detection (UPLC-DAD) was employed in the screening (in the range of 200 to 400 nm) and quantitation (at 254 nm) of alpha-PVP in herbal materials (Byrska et al., 2017). Screening by UPLC-DAD (between 190 and 320 nm) has been also used as a complementary technique for the identification of hexedrone, 4-bromoethcathinone, 4-chloro-alpha-pyrrolidinopropiophenone, and 4-bromo-alpha-pyrrolidinopentiophenone (Błażewicz et al., 2017). The method provided important additional information such as the maximum absorption peak at 265 nm for an unknown cathinone, coherent with 4-bromo substitution in the aromatic ring, which was further confirmed by other techniques to be 4-bromoethcathinone (Błażewicz et al., 2017). UPLC-DAD coupled to single quadrupole MS was also described in the screening of 4-MEC, alpha-PVP, pentedrone, and pentylone in seized materials, obtaining retention time, mass fragmentation and UV absorption profiles in a single run (Li & Lurie, 2014). This technique in combination with GC-MS is also suggested as a solid analytical approach in the determination of different classes of drugs, isomers, and analogues (Li & Lurie, 2014). However, there are some caveats in this coupled approach. For example, the presence of formic acid in the mobile phase, required in electrospray ionization (ESI) for MS, may interfere in the detection of compounds with weak absorbance in the UV (Li & Lurie, 2014). It is also interesting to highlight that while coupling LC to UV–Vis detectors is a common approach, the use of UV–Vis spectroscopy (without being coupled to a separation technique) has been used, in combination with several techniques. High-performance liquid chromatography with photodiode array detection (HPLC-DAD) and UV–Vis spectroscopy have both been employed in the analysis of seized materials containing 4-fluoro-PV9 and alpha-PHP (Majchrzak et al., 2016). 4-Fluoro-PV9 exhibited maximum absorption peak at 254 nm (by HPLC-DAD) and 253 nm (by UV–Vis spectroscopy), whereas alpha-PHP showed maximum absorption peak at 252 nm (by LC-DAD) and 251 nm (by UV–Vis spectroscopy) (Majchrzak et al., 2016). Another example is the identification of 1-(2,3-dihydro-1H-inden-5-yl)-2-(ethylamino)pentan-1-one (bk-IVP) by UV–Vis spectroscopy (200 to 400 nm), in combination with other analytical methods (Yovanovich et al., 2018). However, it is important to emphasize that UV–Vis spectroscopy alone will not provide definitive structural confirmation and its use is limited to providing additional information, which can complement other analyses.

Reports on the detection of synthetic cathinones isomers by LC-DAD or LC-UV are also available. An example is the reported separation of 49 cathinones using a LC with diode-array detection, employing a chiral stationary phase with cellulose tris-(3,5-dimethylphenyl-carbamate) (Hägele et al., 2020). This method was applicable to authentic specimens such as in the identification of *N*-ethylpentylone in a sample suspected to be 3-MMC (Hägele et al., 2020). Another method used LC-UV employing a cellulose tris(3-chloro-4-methylphenylcarbamate) chiral stationary phase was shown able to separate 17 cathinones and a partial resolution of only 2 cathinones was achieved (Taschwer et al., 2017). The method was also applicable to the separation of 2-MMC, 3-MMC, 4-MMC (or mephedrone), 6-(2-aminopropyl)benzofuran (6-APB), 4-chloromethcathinone (4-CMC), and 4-MEC in seized materials (Taschwer et al., 2017). More recently, a low-flow, portable LC-UV instrumentation with dual capillary columns (C8 and biphenyl) and detection at 255 and 275 nm, was used in the detection of 16 cathinones (May et al., 2020). The limits of detection using this approach varied between 0.39 and 2.76 ppm (at 255 nm) and between 0.64 and 5.22 ppm (at 275 nm). Based on the advantages of this system, this portable instrumentation is proposed as a promising alternative for the presumptive analysis of seized materials (May et al., 2020).

9 | LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

LC-MS/MS is a sensitive and specific technique that has been commonly employed by forensic chemistry and, more so, toxicology laboratories, combining both the separation provided by LC and mass spectral data provided by MS/MS (Chiang et al., 2019). LC can be performed either via UPLC or HPLC. Comparatively, UPLC can provide faster analyses and higher peak capacities than HPLC (Marginean et al., 2015). The combination of MS/MS to UPLC or HPLC is an advantageous analytical approach, which leads to an increase in the potential of MS/MS to determine the mass measurements of a drug (Błażewicz et al., 2017). There are several MS/MS platforms available, using different ionization

modes (e.g., ESI, matrix-assisted laser desorption ionization, and atmospheric pressure chemical ionization) and mass analyzers (e.g., quadrupole, TOF, orbitrap, and hybrid analyzers such as quadrupole-time-of flight and quadrupole-orbitrap) (Kang, 2012; Meyer et al., 2014; Pitt, 2009).

As chromatographic separation occurs in liquid phase, one of the main advantages of LC-based methods (including LC-MS/MS) is that the analysis is not limited by the volatility, polarity or thermal stability of the analyte (Bono, 2007). Moreover, derivatization is not commonly required (Wu et al., 2012) and the mobile phase is another important component (in addition to the stationary phase) in the chromatographic separation of the analytes, in contrast to the role of mobile phase in GC (Bono, 2007). The analysis using LC-MS/MS offers more sensitivity (Wu et al., 2012) and specificity in comparison to single-stage MS techniques, such as GC-MS (Pitt, 2009). Modern LC-HRMS, such as LC coupled to TOF-MS, QTOF-MS, or orbitrap MS, can provide even higher sensitive, selective and accurate analysis, reaching high mass resolutions and making possible the determination of molecular formula and structure (Błażewicz et al., 2017; Krotulski et al., 2020; Wu et al., 2012). These systems require less complex method development and optimization, in comparison to conventional triple quadrupole mass spectrometers (Meyer et al., 2014). Moreover, it is of paramount importance the ability of LC-HRMS systems to enable retrospective analysis, which can make possible for the analyst to reinvestigate previously acquired data for data mining, using instrumental libraries (Krotulski et al., 2020; Meyer et al., 2014). Therefore, LC-MS/MS are powerful analytical techniques used in the identification and confirmation of synthetic cathinones in seized materials (UNODC, 2020b).

Although LC-MS/MS presents several advantages, there are some inherent limitations. As based in liquid-chromatographic separation, the analyte needs to be soluble in the mobile phase and a high volume of solvents is frequently required for separation. Low-resolution LC-MS/MS (such as LC–triple quadrupole MS) usually are performed using targeted qualitative/quantitative approaches, without the ability for wide-scope untargeted investigations of unknown drugs (Krotulski et al., 2020), which requires reference materials for comparison. These LC-MS/MS techniques can be used for untargeted analysis but only when performed in full scan mode, which exhibits lower sensitivity in comparison to multiple reaction monitoring (MRM) mode (Wu et al., 2012). Moreover, these particular LC-MS/MS techniques are not able to distinguish drugs with the same nominal mass (Meyer et al., 2014), which need to be chromatographically separated (Wu et al., 2012). Another drawback of LC-MS/MS is that the instrumentation is usually more expensive than GC-MS (Wu et al., 2012), especially LC-HRMS techniques (Meyer et al., 2014), and are not widely available in forensic laboratories for the analysis of seized materials (Lesiak & Shepard, 2014). The libraries of LC-HRMS instruments are not usually exchangeable between different instruments (as it occurs with GC-MS) and the laboratory needs to develop its own library or search for the availability with the vendor (Wu et al., 2012). With regard to LC-HRMS, another limitation is that the dynamic range is smaller and elevated concentrations of compounds might saturate the detector (Wu et al., 2012). Furthermore, with the ever-evolving NPS market, new methods or updates in the scope of current methods are required in order to enable the laboratory to identify new drugs, such as new synthetic cathinones emerging on the drug market (Fowble et al., 2018).

LC-MS/MS has been used in the analysis of synthetic cathinones in "bath salt" mixtures and biological samples (Fowble et al., 2018). With regard to seized materials, several LC-MS/MS techniques have been used in the detection of synthetic cathinones. Reported methods, commonly using ESI in positive mode, include UPLC-triple quadrupole-MS/MS (Machado et al., 2017, 2019), UPLC-QTOF-MS (Błażewicz et al., 2017; Fiorentin et al., 2019; C. Liu et al., 2017; NPS Discovery, 2021; Reitzel et al., 2012), UPLC-orbitrap-MS (Frison et al., 2021), LC-orbitrap-MS (Strano Rossi et al., 2014) and HPLC-ion trap-MS/MS (Majchrzak et al., 2016). LC-orbitrap-MS/MS was also reported to confirm the results when the analysis by GC-MS was not sufficient (Göl & Çok, 2019). Moreover, it is important to highlight that direct HRMS analyses (without coupling to LC systems) are also reported (Gaspar et al., 2015; Maheux et al., 2016).

10 | CAPILLARY ELECTROPHORESIS

CE is another separation technique, which has no limitations regarding polarity, volatility or thermal stability of the analytes, such as in GC (Lurie et al., 2004), and it can be used in the separation of both ions and neutral compounds (Bono, 2007). CE provides high resolution and efficiency (Baciu et al., 2015; Burrai et al., 2013; Merola et al., 2014) and fast analyses by the application of high voltage to the electrophoretic system (Bono, 2007). Analysis by CE also presents the benefits of reduced use of solvents and required sample amount (Baciu et al., 2015; Bono, 2007; Cruces-Blanco & García-Campaña, 2012; Kohler et al., 2013; Merola et al., 2014; Tagliaro et al., 1996) and high sample throughput (Cruces-Blanco & García-Campaña, 2012). In addition, capillary columns for CE are less expensive than GC or LC

columns (Bono, 2007). This technique has a potential for enantiomeric analysis using chiral selectors in the running electrolyte (Li & Lurie, 2015; Lurie et al., 2004; Merola et al., 2014). Another benefit of CE is the possibility of automation (Baciu et al., 2015; Cruces-Blanco & García-Campaña, 2012). Different CE modes are available such as capillary zone electrophoresis, micellar electrokinetic capillary chromatography (MEKC), and capillary electrochromatography (Cruces-Blanco & García-Campaña, 2012) and can be coupled to diverse detection systems (i.e., UV–Vis, fluorescence or MS) (Cruces-Blanco & García-Campaña, 2012; Tagliaro et al., 1996). However, the major reported limitation of CE is its limited sensitivity, in comparison to chromatographic techniques (Baciu et al., 2015; Bono, 2007), especially due to the reduced amount of sample injected (in nanoliters) into the system (Costa et al., 2014) or when employing UV–Vis (due to a narrow optical pathway). In addition, the collection of fractions can be hampered by the reduced volume of sample and mechanical issues (Bono, 2007). Some sample preparation techniques can be used to improve the sensitivity of CE methods but this strategy may represent more time-consuming, more complex and less reproducible analytical workflows (Baciu et al., 2015). Although these drawbacks are more commonly reported in the analysis of drugs in biological fluids, they may be limitations in seized drugs testing as well. Another limitation is regarding the unavailability of instrumental libraries for NPS in CE platforms and the need for building the database. On the other hand, CE with MS detection can improve the sensitivity and selectivity in comparison to other detection systems (Baciu et al., 2015; Cruces-Blanco & García-Campaña, 2012; Kohler et al., 2013).

CE with UV spectroscopy detection (CE-UV) and coupled to time-of-flight MS (CE-TOF-MS) using cyclodextrins were used in the analysis of 14 cathinones (buphedrone, pentedrone, methedrone, 3,4-DMMC, ethcathinone, dimethylcathinone, methylone, ethylone, mephedrone, pentylone, MDPV, and ethcathinone) (Merola et al., 2014). Limits of detection and quantification reached with CE-DAD ranged from 4.2 to 7.0 ng/ml and 13 to 21 ng/ml, respectively. Using CE-TOF-MS, the limits of detection and quantification ranged from 1.0 to 11 ng/ml and 3 to 33 ng/ml, respectively (Merola et al., 2014). The analysis of eight seized samples were performed by CE-TOF-MS, revealing the presence of methylone ($N = 8$) and 3,4-DMMC ($N = 1$), in agreement with previous GC-MS analysis (Merola et al., 2014). A study compared the separation and analysis of 24 drugs, including synthetic cathinones, by ultrahigh-performance liquid chromatography with photodiode array detection (UHPLC-DAD) and chiral CE-DAD and cyclodextrins as chiral additive (Li & Lurie, 2015). CE-DAD was able to separate regioisomers of target analytes and to resolve the majority of enantiomers. However, UHPLC-DAD provided better sensitivity and reproducibility of retention times whereas CE-DAD was more selective to alkaline drugs (Li & Lurie, 2015). Another recent study proposed the CE with laser-induced fluorescence detection in the separation and identification of 14 synthetic cathinones, based on MEKC (Emonts et al., 2021). The method exhibited high sensitivity (with limits of detection between 0.1 and 0.4 nM) and high selectivity using alkyl polyethylene glycol ether surfactants (Emonts et al., 2021).

11 | DIRECT ANALYSIS IN REAL-TIME MASS SPECTROMETRY

Ambient mass spectrometric techniques have been modernly explored in forensic analyses, including determination of drugs of abuse, considering they can provide structural information on the analyte (Lesiak et al., 2013). DART-MS is an example of such a technique which has been vastly applied to the analysis of drugs (Pavlovich et al., 2018). Major analytical advantages of DART-MS approaches are fast analyses at ambient conditions, without the need for sample preparation or chromatographic separation (Chernetsova & Morlock, 2011; Lesiak & Shepard, 2014; Pavlovich et al., 2018; Steiner & Larson, 2009), with an almost complete mass spectrometric profile of the sample quickly generated with high sensitivity (Lesiak & Shepard, 2014; Sisco et al., 2021; Sisco & Forbes, 2021). Accurate mass determination by DART-MS can also be reached by employing HRMS mass analyzers, such as TOF (e.g., AccuTOF) (Chernetsova & Morlock, 2011; Lesiak & Shepard, 2014). Although DART-MS analysis is limited to small polar or nonpolar molecules (Chernetsova & Morlock, 2011), it can be used in the analysis of solids, liquids, and gases by the exposure of the material to the sampling area of the instrument (Lesiak & Shepard, 2014; Steiner & Larson, 2009). Moreover, DART-MS is considered a soft-ionization technique, which is advantageous for the analysis of synthetic cathinones, in contrast to GC-MS (Lesiak & Shepard, 2014). DART-MS can also detect analytes in a large range of concentrations in a sample (Chernetsova & Morlock, 2011). Another mode of DART-MS, thermal desorption DART-MS has been proved to detected the contents of a drug package from its exterior (Sisco et al., 2019). Data analysis in DART-MS is similar to that of GC-MS or LC-HRMS, based on the comparison and inquire of mass spectra against a reference library (Sisco et al., 2021).

In contrast to the advantages mentioned above, DART-MS presents some drawbacks. The sensitivity depends on the type of analyte being investigated and the sample (Chernetsova & Morlock, 2011), and variations of ionization ability may occur among different compounds (but being more consistent among the same class) (Pavlovich et al., 2018). The analysis can be complicated by the presence of complex mixtures and/or if a low resolution MS is used as the detection system (Pavlovich et al., 2018). The conventional DART-MS approach (sample dissolution and introduction into the system) may be affected by reproducibility issues due to fluctuations in the sample introduction (Sisco et al., 2016). Quantitative analysis with DART-MS is more complex when dealing with solid materials (in contrast to liquid materials), which requires reference materials for calibration and standardization (Chernetsova & Morlock, 2011). In a similar context, reference materials are also needed for building a mass spectral library, which may be a lengthy process and involve high costs (e.g., for continuous acquisition of new standards) (Sisco et al., 2021). However, recently the NIST DART-MS Forensics Database has been developed and published (Sisco et al., 2021), which may be used by forensic laboratories and facilitate the process of DART-MS analysis of new drugs.

DART-MS has been widely used in the analysis of seized materials and more recently, it has been increasingly applied in the analysis of NPS (Sisco & Forbes, 2021). More specifically, DART-MS has been used in the detection of synthetic cathinones (Fowble et al., 2018). For example, the neutral loss spectra were successfully used for the classification of synthetic cathinones among different classes, based on chemical structures differences, including methcathinones, ethcathinones, methylenedioxy-cathinones, pirrolidine ring-cathinones, buphedrones, and unclassified, using multivariate statistics methods (Fowble et al., 2018). This method was shown to be able to classify a synthetic cathinone among a particular class in an unknown sample (Fowble et al., 2018). Another example in the literature showed the ability of in-source collision-induced dissociation (CID) DART-MS for the simultaneous analysis of 2-ethylethcathinone, diethylcathinone, isopentedrone, 3-MEC, 2-MEC, 2-fluoromethcathinone, and 2-fluoroethcathinone (Lesiak et al., 2013).

12 | IR AND RAMAN SPECTROSCOPIES

Vibrational spectroscopies, such as FTIR, near-IR spectroscopy (NIR), and Raman spectroscopy, are useful techniques for the characterization of NPS such as synthetic cathinones (Guirguis et al., 2017; Maheux et al., 2016). FTIR, NIR, and Raman can provide similar chemical data (Tsujikawa et al., 2014), used for the identification of specific structural characteristics (e.g., functional groups) (Fowble et al., 2018; Frison et al., 2021). These spectroscopic techniques can provide fast and nondestructive analyses (Moros et al., 2010; Schram et al., 2021), simultaneously analyzing multiple components in a single measurement (Marcelo et al., 2015), and requiring minimal sample preparation (Moros et al., 2008; Moros et al., 2010).

FTIR has the advantage of providing information about the chemical structure of molecules, including synthetic cathinones (Bunaciu et al., 2010; Schram et al., 2021). The detection through IR spectroscopy can generate unique spectral patterns for each compounds, the fingerprint region in the spectra, which can facilitate the differentiation between closely related drugs (Shirley Lee et al., 2019). Moreover, FTIR-based methods require minimal to no sample preparation, can be nondestructive, and provide highly sensitive analyses (Bunaciu & Aboul-Enein, 2021), reaching proper signal-to-noise ratios (Pereira et al., 2017). Samples in different states (gases, liquids, or solids) may be analyzed (Bunaciu et al., 2010; Moros et al., 2010). Particularly, the use of ATR accessories, which enables the direct analysis of solids and liquids (Pereira et al., 2017), without the need of extensive sample preparation and requiring small amount of sample, resulting in faster analyses (Bunaciu & Aboul-Enein, 2021;Eliaerts et al., 2017; Piorunska-Sedlak & Stypulkowska, 2020). ATR-FTIR can also provide good reproducibility, enabling the search of a sample's spectra in the instrumental library, without requiring certified reference standards (Eliaerts et al., 2017; Piorunska-Sedlak & Stypulkowska, 2020). There is also portable FTIR spectrometers offering fast and nondestructive analyses (Guirguis et al., 2017; Schram et al., 2021). FTIR can be used in presumptive analysis by the direct analysis of packages, without the need of handling the sample itself (Sisco et al., 2019). However, a limitation of IR spectroscopy is that the sample under testing needs to be dry and clean (Bono, 2007). Therefore, the analysis by FTIR is useful and better for high-purity seized drugs (Pereira et al., 2017; Schram et al., 2021; Sisco et al., 2019). Another limitation reported in the literature for FTIR is that the direct analysis through the package can be affected by non-transparent packaging material (Sisco et al., 2019). Interferences from adulterants and cutting agents found in seized materials may also occur, which can complicate direct analyses (Guirguis et al., 2017). For more complex matrices, the use of chemometric techniques are usually needed for data processing and analysis (Pereira et al., 2017). In addition, the identification can be

challenging, depending on the data available in the instrument's library, especially for complex mixtures (Eliaerts et al., 2017) and the identification can be more complex if a completely unknown drug is detected in the sample (Piorunska-Sedlak & Stypulkowska, 2020). The differentiation of isomers by IR spectroscopy may be challenging (Bono, 2007). Another limitation is that when using ATR, a narrow absorption range in the spectra is reached, in comparison to other FTIR techniques (Bunaciu & Aboul-Enein, 2021).

Raman is another vibrational technique that offers fast, sensitive, and nondestructive analyses (Braz et al., 2021; Quinn et al., 2020; Stewart et al., 2012; West & Went, 2011). Raman spectroscopy also provides elevated discriminatory power, minimal sample preparation and is less affected by humidity and interferents (Guirguis et al., 2017; Quinn et al., 2020). In contrast to FTIR, Raman is not sensible to water present within a sample (Hargreaves et al., 2008) Portable Raman spectrometers are also available (Braz et al., 2021; Guirguis et al., 2017) and can confront the results of a sample with spectral databases (Harper et al., 2017). Portable Raman spectrometers can make possible the direct analysis of samples through transparent or translucent packing materials but this platform should be used for screening only and confirmed by another technique (UNODC, 2020b). Raman spectroscopy can also be coupled to microscopy providing visual and spectroscopic inspection of the sample (Quinn et al., 2020). However, Raman spectroscopy is subject to some limitations. Similarly to FTIR, the testing scope may be limited when a compound present in the sample has no reference spectra in the instrument database (Jones et al., 2016). For example, this limitation has been demonstrated for FTIR and Raman, when a limited spectral library was available and used to analyze a set of seized samples; however, this was improved by the extension and update of the library combined to NMR and MS data (Jones et al., 2016). Moreover, when the direct analysis is performed through the package, the material of this package needs to be transparent or semi-transparent (Sisco et al., 2019). Contaminants or the matrix itself can generate fluorescence, leading to background signals, which allied to the weak Raman signals may lead to poor sensitivity (Braz et al., 2021; W. W. Y. Lee et al., 2016; Schram et al., 2021; Tsujikawa et al., 2014; West & Went, 2011). Similar to FTIR, Raman analysis is better for drugs exhibiting high purity in a seized material (Sisco et al., 2019). Therefore, conventional Raman spectroscopy is less sensitive and might be limited to major components of a seized sample (W. W. Y. Lee et al., 2016). However, surface-enhanced Raman spectroscopy (SERS), another Raman technique, can be an alternative due to its elevated sensitivity (W. W. Y. Lee et al., 2016; Muhamadali et al., 2019).

NIR spectroscopy is another vibrational technique, operating between the end of the visible range and the beginning of the middle IR range in the electromagnetic spectrum (Tsujikawa et al., 2014). NIR is a technique that enables fast and nondestructive analyses (Risoluti et al., 2016; Sondermann & Kovar, 1999), with minimal sample preparation and possibility to analyze samples in loco using portable instruments (Sondermann & Kovar, 1999). NIR can also be used in the direct analysis of solid samples (Moros et al., 2008). It is useful in the differentiation between positional isomers of synthetic cathinones, such as 2-, 3-, and 4-fluoromethcathinone (Tsujikawa et al., 2014). The limitations reported for NIR compass the interferences from adulterants and contaminants in seized samples and from humidity, and also usually require data treatment (Guirguis et al., 2017). In contrast to Raman, the color of the sample is not a source of interference in NIR (Tsujikawa et al., 2014).

The identifications of synthetic cathinones in seized drugs has been made possible by using both FTIR, especially ATR-FTIR (Johnson et al., 2020; Jones et al., 2016; Machado et al., 2017, 2019; Maheux et al., 2016; Pereira et al., 2018; Piorunska-Sedlak & Stypulkowska, 2020; Yovanovich et al., 2018), and Raman (Braz et al., 2021; Johnson et al., 2020; Maheux et al., 2016; Stewart et al., 2012). The combination of ATR-FTIR with chemometric methods for data processing are often reported (Pereira et al., 2018). The use of portable instrumentations has been reported as well. A study has reported that the use of a portable Raman spectrometer showed poor sensitivity and selectivity in the analysis of a large number of samples, resulting in limited application for on-site testing (Johnson et al., 2020). However, recently, benchtop and portable Raman spectrometers combined to multivariate chemometric techniques have been tested for the analysis of synthetic cathinones in seized drugs, exhibiting potential to application in screening procedures (Braz et al., 2021). The use of SERS has been reported and proposed to analyze synthetic cathinones (W. W. Y. Lee et al., 2016; Muhamadali et al., 2019). NIR has also been reported for the detection of synthetic cathinones in seized drugs.

13 | NUCLEAR MAGNETIC RESSONANCE

NMR is a powerful analytical technique that enables acquisition of data relating more specifically to the molecular structure and tridimensional disposition of atoms (Bono, 2007). High-field NMR is a powerful technique that has been

underutilized in drug analysis (Hussain et al., 2020; Serrano et al., 2020) but it seem to be drawing more interest of the forensic community in the analysis of NPS, especially when no reference material is available for comparison. This technique provides unequivocal structure determination (Castaing-Cordier et al., 2021; Serrano et al., 2020) and it can be performed using different modes such as one-dimensional proton (^{1}H) and carbon (^{13}C) NMR and two-dimensional correlation NMR (e.g., homonuclear correlation spectroscopy [COSY], nuclear overhauser effect spectroscopy [NOESY], and HMQC) (Bono, 2007; UNODC, 2020b). Fast analyses, high reproducibility, and minimal low sample preparation are some of the analytical advantages offered by NMR (Santos et al., 2018; Serrano et al., 2020), without requiring any prior separation (Ribeiro et al., 2018). Analysis by NMR is nondestructive (Castaing-Cordier et al., 2021; Ribeiro et al., 2018; Santos et al., 2018) and can enable the determination of multiple compounds within the same analysis (Castaing-Cordier et al., 2021). In addition, NMR analyses of synthetic cathinones can provide important structural data when certified reference materials are unavailable (Guirguis et al., 2017; Serrano et al., 2020). One of the great potentialities of NMR is that the identification of a single drug or a mixture as well as the differentiation of constitutional isomers can be performed (Bono, 2007; Castaing-Cordier et al., 2021; UNODC, 2020b). Coupling LC with NMR is also possible, enabling the separation of compounds and spectroscopic characterization. Quantitation by NMR is also possible for multiple compounds (Santos et al., 2018), without requiring several, pure reference standards (Pagano et al., 2013; Serrano et al., 2020), or prior separation (Santos et al., 2018). NMR can also be used in enantiomeric analysis, with several types of chiral reagents available (Stolarska et al., 2020). More recently, benchtop and portable low-field NMR instruments have been developed, circumventing some costs-related limitations (Antonides et al., 2019;Castaing-Cordier et al., 2021; Trinklein et al., 2021). These platforms operate without requiring cryogens and are easier to operate (Trinklein et al., 2021).

One of the limitations of NMR is that such instrumentations are not widely available in forensic laboratories (Fowble et al., 2018), and this instrumentation is expensive and demand for technical expertise in the operation and data interpretation (Bono, 2007). Some NMR techniques demand for high knowledge and expertise in data interpretation, such as COSY and NOESY (Bono, 2007). In addition, for NMR analysis, the analyte needs to be at elevated concentrations, in high-purity samples, which can be easily solubilized in the solvent (Fowble et al., 2018). NMR is less sensitive in comparison to MS, for example, but more specific (Santos et al., 2018). The analysis of complex mixtures by ^{1}H-NMR may be challenging, with spectral overlaps, and correlation NMR may be required (Castaing-Cordier et al., 2021; Santos et al., 2018; Trinklein et al., 2021). Moreover, the costs associated with the instrumentation are relatively higher than other instrumentations (Antonides et al., 2019; Castaing-Cordier et al., 2021; Santos et al., 2018). As previously mentioned, benchtop NMR could be an alternative to this limitation (Castaing-Cordier et al., 2021). However, benchtop low-field NMR may show lower sensitivity and spectral resolution (Castaing-Cordier et al., 2021; Trinklein et al., 2021), and an additional level of complexity may arise from second-order coupling effects between the nuclei (Castaing-Cordier et al., 2021).

NMR has been used in the analysis of synthetic cathinones, including in the elucidation of new synthetic cathinones emerging in the NPS market (Błażewicz et al., 2017; Gaspar et al., 2015; Jones et al., 2016; C. Liu et al., 2017; Machado et al., 2017; Maheux et al., 2016; Majchrzak et al., 2016; NPS Discovery, 2021; Power et al., 2011; Reitzel et al., 2012; Strano Rossi et al., 2014; Yovanovich et al., 2018). For example, NMR can provide unequivocal determination of substitution pattern on the aromatic ring of synthetic cathinones (Błażewicz et al., 2017). The correct identification of hexedrone, 4-bromoethcathinone, 4-chloro-alpha-pyrrolidinopropiophenone and 4-bromo-alpha-pyrrolidinopentiophenone in seized powders was possible by using several 1D and 2D NMR techniques (^{1}H, ^{13}C, COSY, HSQC, and HMBC), in combination to other techniques (Błażewicz et al., 2017). In a recent comparison between low-field ^{1}H-NMR and GC-MS analyses of 416 seized samples, the cathinones ethylone and mephedrone were among the detected compounds, and the correlation between the results obtained by both techniques occurred in 93% of the cases (Antonides et al., 2019). NMR is a powerful technique for the differentiation of isomers. The differentiation of enantiomers 2-MEC, 3-MMC, and 4-MMC (or mephedrone), in the form of white powders and after dissolution in deuterated chloroform, was possible by several NMR techniques and using R-(1,1′-binaphtalene)- 2,2′-diol as chiral reagent (Stolarska et al., 2020). Similarly, benchtop ^{1}H and ^{19}F NMR were used in the discrimination of isomers of synthetic cathinones: 2-, 3-, and 4-MMC (or mephedrone); 2-, 3-, and 4-fluoromethcathinone; 2-, 3-, and 4-trifluoromethylmethcathinone; and 2-, 3-, and 4-fluoroethcathinone (Hulme et al., 2021). Another interesting application reported in the literature is the use of sequential injection analysis prior to benchtop NMR analysis, integrating solid-phase extraction, NMR (for identification), and UV spectroscopy (for quantification) for the determination of synthetic cathinones (Trinklein et al., 2021). The method was successfully applied to seized samples of methcathinone, methylone, and *N*-

ethylpentylone and *N*-ethylhexedrone but the authors highlight that the low sensitivity of NMR instrumentation may pose a challenge for the detection of cathinones at low levels (Trinklein et al., 2021).

14 | EMERGING TECHNIQUES

In addition to traditional GC and LC techniques, there are other modern techniques reported in the literature for the analysis of synthetic cathinones. GC-IR is a technique with combined analytical advantages of GC separation and IR detection (H. Z. S. Lee et al., 2020;Shirley Lee et al., 2019; UNODC, 2020b). This is a powerful analytical platform for the differentiation between isomers (Shirley Lee et al., 2019; UNODC, 2020b). Therefore, GC-IR can be a powerful analytical tool, with potential to complement GC-MS and LC-MS analyses (Frison et al., 2021), with high selectivity (Praisler et al., 2000). Samples can be analyzed without derivatization, shortening the sample preparation step (H. Z. S. Lee et al., 2020). An example of application in the literature showed the ability of GC-IR in the separation and detection of the isomers 2-, 3-, and 4-MMC, dibutylone, and other cathinones (pentylone, eutylone, methylone, and butylone), using an approach of algorithm-based criteria for more objective identifications using the generated IR and MS spectra (Shirley Lee et al., 2019). GC-IR was also used in the differentiation between two analogues of MDPV (Abiedalla et al., 2017). Another type of GC technique recently reported is GC with nitrogen chemiluminescence detection coupled QTOF mass spectrometry (GC-NCD-QTOF-MS), using to atmospheric pressure chemical ionization (Mesihää et al., 2020). A method using secondary reference materials was applied to the analysis of several stimulants, including cathinones in seized materials, and exhibited the capacity to estimate the purity of the drug (Mesihää et al., 2020). One additional limitation to GC-IR is reference libraries (like for GC-MS) do not exist so laboratories may not be able to search their unknown IR spectra against other known drugs or NPS.

Another type of chromatographic technique reported in the analysis of synthetic cathinones is the ultrahigh-performance supercritical fluid chromatography (UHPSFC) (Carnes et al., 2017). This chromatographic technique employs mobile phases with reduced viscosities and columns packed with sub 2- and 3-μm particles, providing efficient and faster separations (Breitenbach et al., 2016; Carnes et al., 2017; Wang et al., 2017). In comparison to GC or LC, supercritical fluid chromatography usually offers faster separations and higher resolution (Pauk & Lemr, 2018). In addition, in UHPSFC, supercritical carbon dioxide combined to small amounts of organic solvents enable the direct injection of organic samples, leading to more sensitive analyses and enabling the analysis of a broad range of compounds (Fujito et al., 2017; González-Mariño et al., 2018). The use of CO_2 also provides more environmental-friendly and cost-effective analyses (Fujito et al., 2017; González-Mariño et al., 2018; Wang et al., 2017). The use of MS as detection system in UHPSFC also yields high sensitivity and selectivity (Fujito et al., 2017) UHPSFC with diode array-single quadrupole MS (UHPSFC-DAD-MS) was able to differentiate 28 positional isomers of synthetic cathinones out of 34 compounds, using certified reference standards. The authors suggest that a combined approach of UHPSFC and GC can provide a good discriminatory power for 32 of the 34 positional isomers of selected synthetic cathinones. However, buphedrone and ethcathinone were not separated using either of both techniques (Carnes et al., 2017). Another application of UHPSFC employing triple quadrupole MS detection showed ability to detect 11 synthetic cathinones, and using a BEH silica column the run time could be significantly reduced to 1.6 min for fast screening procedures (Pauk et al., 2015).

With regard to spectroscopy techniques, IMS is another instrumentation that has been employed in the analysis of synthetic cathinones. IMS offers easy operation, fast analyses, and high sensitivity (in range of nanogram to picogram), showing good potential for screening procedures (Armenta et al., 2015). It is also a portable instrumentation that can be deployed to different environments (Armenta et al., 2015). However, IMS exhibits moderate discriminatory power (Yanini et al., 2018). This technique has been proposed for the analysis of cathinones. A study with 11 synthetic cathinones and other NPS employed IMS with ^{63}Ni foil radioactive ionization source and limits of detection as low as 49, 35, and 20 pg were achieved for 4-MEC, methylone, and butylone, respectively (Armenta et al., 2015). In another study, other synthetic cathinones in seized materials were analyzed by IMS reaching limits of detection of 110 pg for N-ethylpentylone, 130 pg for 4-chloroethcathinone and 160 pg for alpha-PHP, for example (Yanini et al., 2018).

Another method recently reported is based on IR ion spectroscopy (IRIS), combining the mass selectivity and sensitivity of MS to the potential for structural characterization of IR spectroscopy, providing the IR spectrum of an ion, without requiring sample preparation (Kranenburg, Van Geenen, et al., 2020; Van Geenen et al., 2021). For example, IRIS has been successfully applied in the analysis of synthetic cathinones such as 3-MMC (Kranenburg, Van Geenen, et al., 2020). This technique has also been used in the study of fragmentation patterns of synthetic cathinones in

reference materials (Tyler Davidson et al., 2020). However, it has been reported that the analysis by IRIS can still be challenging for isomers (Van Geenen et al., 2021).

Other analytical platforms that have been gaining attention in forensic analyses of seized drugs are electrochemical techniques. Cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, and amperometry have also been drawing attention for NPS analysis due to the low cost (in relation to other platforms), minimal sample preparation, fast analyses (González-Hernández et al., 2021) and high sensitivity and selectivity (Dronova et al., 2016; González-Hernández et al., 2021; Schram et al., 2021; Smith et al., 2014). These techniques may also exhibit potential for portability and use outside the laboratory environment (Dronova et al., 2016; González-Hernández et al., 2021). However, certified reference materials may be usually required in method development and analysis. In the literature, there are applications of electrochemical techniques to the analysis of synthetic cathinones. A voltammetry-based method employing graphite screen-printed electrodes was developed and optimized for mephedrone and 4-MEC, reaching limits of detection of 11.80 and 11.60 μg/ml for mephedrone and 4-MEC, respectively, and showing good correlation with the findings obtained by a LC-based method (Smith et al., 2014). Another study reported square wave voltammetry on graphite screen-printed electrodes in the detection of mephedrone, ethcathinone, methylone, butylone, and 4-Cl-alpha-PVP, showing a good analytical performance in the detection of this class in seized materials (Schram et al., 2021).

Crystallographic techniques have also been reported. X-ray diffraction (XRD) is classified by the SWGDRUG as a "Category A" technique, able to provide structural information, more specifically crystallographic structural information (SWGDRUG, 2019). Therefore, XRD can provide structural information on crystalline substances (Maheux et al., 2016). One of the main reported advantages of this technique is the capacity to provide non-destructive analysis, preserving the sample, and to identify multiple components in a mixture, including cutting agents in the sample (Jurásek et al., 2019; Maheux et al., 2016). Moreover, this technique requires minimal sample preparation and is easily executed (Jurásek et al., 2019). However, instrumental libraries need to be created and updated for implementing the method in routine analyses (Jurásek et al., 2019). XRD was recently reported in the analysis of several synthetic cathinones, as a single technique or in combination with other techniques, the later especially for unknown samples (Jurásek et al., 2019; Kuś et al., 2019; Maheux et al., 2016; Nycz et al., 2011; Piorunska-Sedlak & Stypulkowska, 2020). For example, XRD was reported in the determination of two polymorphs of ethylone (Maheux et al., 2016).

Finally, other new approaches have also been developed and reported. An example is the development of a photoluminescent sensor using bovine serum albumin-stabilized gold nanoclusters probe (Yen et al., 2019). This sensor was developed toward 4-chloromethcathinone and in the presence of cathinones, the solution changed from red to dark blue, visible under UV light at 365 nm. This method presented specificity toward the class of cathinones, resulting in positive results for seized samples of *N*-ethylpentylone (ephylone), butylone, 4-Cl-*N,N*-dimethylcathinone and a mixture of dibutylone and 4-Cl-*N,N*-dimethylcathinone. The assay showed no reaction with other drugs such as amphetamine, MDMA, ketamine, cocaine, and others. This approach is practical and shows potential for implementation *in-loco* (Yen et al., 2019).

15 | COMBINATION OF MULTIPLE TECHNIQUES

As described in the previous sections, several non-instrumental and instrumental techniques are able to detect a synthetic cathinone in seized drugs. Some of these techniques rely on instrumental libraries and reference standards for comparative analysis whereas other techniques are effective in providing accurate mass and detailed structural information. The combination of multiple techniques is generally an effective approach and also recommended by SWGDRUG when analyzing illicit drugs in seized materials, especially when less discriminatory techniques are used (SWGDRUG, 2019). Table 2 summarizes literature reports combining multiple techniques in the identification of synthetic cathinones in seized materials.

Each platform has its own benefits and limitations, as discussed in the previous sections, which need to be taken into account. The majority of these techniques (e.g. color tests, GC-MS, LC-MS, FTIR, and DART-MS) are able to detect a synthetic cathinone in the sample if this drug is already available in the library or if a reference standard is analyzed and compared to the sample. However, if an unknown synthetic cathinone not previously reported is suspected in seized materials, the determination is usually more complex because it depends on the interpretation of data obtained from multiple techniques, as the information provided by a technique can complement and/or support the information provided by another one. For example, as shown in Table 2, the combination of GC-MS, LC-HRMS, and NMR is an

TABLE 2 Examples reported in the literature of comprehensive analytical approaches combining several analytical platforms for the analysis of synthetic cathinones in seized materials

Synthetic cathinone	Type of seized material	Preparation	Combination of analytical techniques	References
4-MEC	Herbal material	Extraction with methanol, sonication, incubation, and filtration	UHPLC-DAD/UV-MS	Li and Lurie (2014)
Alpha-PVP, pentedrone, pentylone	Powder	Dissolution in methanol, sonication, and filtration		
4-Fluoro-PV9 and Alpha-PHP	Powder	For LC and GC: dissolution in acetonitrile/methanol, sonication and dilution with methanol For NMR: dissolution in $CDCl_3$ For FTIR: mixture with KBr and pressing to form pellets For TGA/DSC: no treatment For UV–Vis spectroscopy: dissolution in methanol	LC-MS LC-DAD Ion trap MS GC-MS NMR ($^1H/^{13}C$) TGA DSC UV–Vis spectroscopy	Majchrzak et al. (2016)
2-, 3- and 4-MMC	Synthesized in the laboratory and seized material	For GC-MS: dissolution in methanol For FTIR: mixture with KBr and pressing to form pellets For NMR: dissolution in $CDCl_3$ or DMSO-d_6	GC-MS FTIR NMR ($^1H/^{13}C$)	Power et al. (2011)
4F-PBP	Powder	For GC-MS: extraction with MeOH/$CHCl_3$ (50:50) For HRMS: dissolution in water and dilution with water/methanol/formic acid (50/50/1%) For NMR: dissolution in DMSO-d_6 (elucidation) or solution of maleic acid in D_2O (quantification)	GC-MS HRMS NMR (^1H, ^{13}C, APT, ^{19}F, COSY, HMBC and HSQC)	Gaspar et al. (2015)
bk-IVP	Powder	For GC-MS and GC-IR: dissolution in $CHCl_3$ For UV-Vis spectroscopy: dissolution in H_2SO_4 For NMR: dissolution in $CDCl_3$	NMR (^1H, ^{13}C, DEPT, gradient HSQC and gradient COSY) FTIR GC-MS GC-IR UV–Vis spectroscopy Color tests	Yovanovich et al. (2018)
Alpha-PVP	Plant	Extraction with ethanol, centrifugation and dilution with methanol (for GC-MS) and mobile phase (for LC-DAD)	GC-MS and LC-DAD	Byrska et al. (2017)

TABLE 2 (Continued)

Synthetic cathinone	Type of seized material	Preparation	Combination of analytical techniques	References
Ethylone	Synthesized and seized materials	N/A	FTIR Raman XRD GC-MS HRMS NMR (^{1}H, ^{13}C and solid-state ^{13}C CPMAS)	Maheux et al. (2016)
3-MMC	Powder	Dissolution and dilution with methanol	GC-MS GC-IR LC-HRMS	Frison et al. (2021)
Buphedrone	Powder	For GC-MS: dissolution with methanol For LC-DAD: dissolution in methanol/water, centrifugation and dilution with 85% orthophosphoric acid in water	GC-MS LC-DAD	Zuba et al. (2013)
Eutylone and *N*-ethylpentylone	Seized materials	For GC-MS: dissolution in methanol or ethanol For LC-HRMS: dissolution in methanol or ethanol and dilution with mobile phase	GC-MS LC-HRMS	Fiorentin et al. (2019)
Mephedrone, flephedrone, methylone, PPP, methylone, N-ethylcathinone, MDPV, and Bk-MBDB	Powder	For GC-MS: extraction with methanol, agitation, centrifugation and dilution with mepivacaine solution For LC-HRMS: dissolution in solution 20% methanol For NMR: dissolution in deuterated methanol	GC-MS HRMS NMR (^{1}H, COSY, NOESY, ^{13}C, DEPT135, HMQC, HMBC)	Reitzel et al. (2012)
3′,4′-Methylenedioxy-2,2-dibromobutyrophenone[a]	Powder	For GC-MS: dissolution in acetone For LC-HRMS: dissolution in solution methanol:10 mM ammoniumformate in water (80:20%, v/v) For NMR: dissolution in $CDCl_3$ For LC-DAD: dissolution in methanol	FTIR GC-MS LC-DAD LC-HRMS NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC, and HMBC) Elemental analysis	Armenta et al. (2020)
Eutylone, 3-MMC, *N*-ethylpentylone, and MMMP	Capsules	For GC-MS: dissolution/extraction in ethanol For ATR-FTIR: direct analysis	FTIR GC-MS	Johnson et al. (2020)

(Continues)

TABLE 2 (Continued)

Synthetic cathinone	Type of seized material	Preparation	Combination of analytical techniques	References
Brephedrone	Capsules	For GC-MS: dissolution in methanol For NMR: dissolution in deuterated methanol	FTIR GC-MS LC-MS/MS NMR (^{1}H, ^{13}C, DEPT135 and HMBC)	Machado et al. (2017)
2-, 4-Chloroethcathinone, 4F-alpha-PHP, mephedrone, alpha-PVP, and *N*-ethylpentylone	Several materials	For GC-MS: dissolution in methanol	GC-MS LC-HRMS	Göl and Çok (2019)
23 Synthetic cathinones	Several materials	For GC-MS and LC-MS/MS: dissolution/extraction of pulverized materials or blotters with methanol For ATR-FTIR: direct analysis of pulverized materials or blotters	GC-MS LC-MS/MS FTIR	Machado et al. (2019)
3-MMC, methylone, butylone, 4-MEC, ethylone, methedrone, flephedrone, methoxetamine, MDPV, and pentedrone	Crystals and powders	For GC-MS: dissolution with methanol For LC-HRMS: dissolution and dilution with methanol For NMR: dissolution in $CDCl_3/CD_3OD$ (8:2 v/v)	GC-MS LC-HRMS ^{1}H-NMR	Strano Rossi et al. (2014)
Multiple synthetic cathinones	Several materials	For GC-MS: acid/base extraction For LC-HRMS: acid/base extraction and dilution with mobile phase For NMR: dilution in DMSO	GC-MS LC-HRMS NMR (^{1}H and COSY)	NPS Discovery (2021)

Abbreviations: 4F-PBP, 4′-fluoro-a-pyrrolidinobutyrophenone; 4-MEC, 4-methylethcathinone; 4-MMC, 4-methylmethcathinone; MMMP, 2-methyl-40-(methylthio)-2-morpholino-propiophenone; ATP, attached proton test; ATR-FTIR, attenuated total reflectance Fourier transform infrared spectroscopy; bk-IVP, 1-(2,3-dihydro-1H-inden-5-yl)-2-(ethylamino)pentan-1-one; Bk-MBDB, xxxxx; COSY, homonuclear correlation spectroscopy; CPMAS, cross polarization magic angle spinning nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; DMSO, dimethylsulfoxide; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; GC, gas chromatography; GC-IR, gas chromatography with infrared spectroscopy detection; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple-quantum correlation; HRMS, high-resolution mass spectrometry; HSQC, heteronuclear single-quantum coherence; LC, liquid chromatography; LC-DAD, liquid chromatography with photodiode array detection; LC-HRMS: liquid chromatography coupled to high-resolution mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MDPV, 3,4-methylenedioxypyrovalerone; MS, mass spectrometry; NMR, nuclear magnetic resonance; NOESY, nuclear overhauser effect spectroscopy; PPP, alpha-pyrrolidinopropiophenone; PVP, xxxxx; UV–Vis, ultraviolet–visible; TGA, thermogravimetric analysis; UHPLC-DAD/UV-MS, ultrahigh-performance liquid chromatography coupled to photodiode array-mass spectrometry; UV–Vis, ultraviolet–visible; XRD, X-ray diffraction.

[a]Intermediate or precursor of cathinones.

effective approach to elucidate the structure of a new synthetic cathinone. MS-based techniques can provide the fragmentation profile of a synthetic cathinone, suggesting the atoms, groups, and bonds composing that structure. NMR is useful in providing the data about the chemical composition of the molecule and structural patterns, with regard to carbon and hydrogen atoms and their vicinities. Other spectroscopic techniques (such as FTIR and Raman) can indicate the presence of specific functional groups in the molecule. On the contrary, less discriminatory methods, such as color tests and TLC, would be less effective in providing structural data of a new drug. Therefore, if available, combining multiple adequate techniques may provide a solid knowledge on the structure of an unknown synthetic cathinone. In this context, it is important to consider the availability of reference materials, instrumental libraries, and other analytical platforms to obtain additional data, depending on the approach adopted in the investigation. In forensic laboratories, it is possible that not all the needed instrumentations are available. For example, NMR instrumentations are not commonly found in forensic laboratories but are essential for structural elucidation of new synthetic cathinones. Nevertheless, several forensic laboratories have partnered with research centers or universities, fostering research collaborations that are highly positive and important in the analysis of emerging synthetic cathinones. Moreover, computer-based methods have been proposed and might be an alternative tool when reference materials or analytical instrumentations are not available (Tcharkhetian et al., 2021).

16 | CONCLUSION

Synthetic cathinones remain prevalent on the NPS market worldwide, according to statistics provided by many national and international agencies, and seized materials are important forensic specimens for monitoring the emergence of this class of synthetic drugs for dissemination. A comprehensive review of the literature on this topic reveals that many analytical techniques are applicable for preliminary identification and/or confirmation of a synthetic cathinone in a seized drug material. Several of these techniques have been traditionally employed by forensic laboratories for "traditional" illicit drugs testing, such as color tests, GC-MS, LC-MS/MS, and FTIR. However, other techniques seem to be emerging in routine synthetic cathinones testing of seized drug materials, such as HRMS, NMR, DART-MS, and GC-IR. Therefore, with the analysis of synthetic cathinones in seized drug materials, it has been demonstrated that "conventional" analytical methods (normally adopted in routine forensic chemistry laboratories) are applicable and can be used for a wide range of compounds within this class. On the other hand, several studies have shown that there is still the field for further research and development, exploring new techniques and analytical tools.

In general, it is important to highlight that each analytical technique and method has its own features, advantages, and limitations. It is well known that some techniques are more discriminatory than others, for example, GC-MS and LC-HRMS in comparison to color tests. Some techniques are not able to discriminate isomers and additional testing by differing techniques may be required, which is particularly important for some synthetic cathinones that exhibit different effects, legal status, and/or toxicity. Another issue is that some techniques may require analytical databases and/or certified reference materials to be run simultaneously with the suspect sample. Although there are some approaches for analysis without requiring certified reference materials, it is important to consider that standards might not be commercially available yet, especially for recently emergent cathinones. Other aspects that need to be considered when selecting the technique include the need for sample preparation, instrumental costs, and analysis time. Moreover, if new analytical approaches are used, the methodology may need to be validated in order to implement in routine analyses and methods should be updated as new drugs emerge on the market and thus in forensic casework.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Eduardo G. de Campos: Conceptualization (lead); project administration (lead); writing – original draft (lead); writing – review and editing (equal). **Alex J. Krotulski:** Conceptualization (equal); writing – review and editing (equal). **Bruno S. De Martinis:** Writing – review and editing (equal). **José Luiz Costa:** Writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study

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