Ambient ionization mass spectrometry applied to new psychoactive substance analysis

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Abstract
In the past decade a plethora of drugs with similar effects to controlled psychoactive drugs, like cannabis, amphetamine (amphetamine), or lysergic acid diethylamide, have been synthesized. These drugs can collectively be classified under the term new psychoactive substances (NPS) and are used for recreational purposes. The novelty of the substances, alongside the rapid rate of emergence and structural variability, makes their detection as well as their legal control highly challenging, increasing the demand for rapid and easy-to-use analytical techniques for their detection and identification. Therefore, interest in ambient ionization mass spectrometry applied to NPS has grown in recent years, which is largely because it is relatively fast and simple to use and has a low operating cost. This review aims to provide a critique of the suitability of current ambient ionization techniques for the analysis of NPS in the forensic and clinical toxicology fields. Consideration is given to analytical performance and ease of implementation, including ionization efficiency, selectivity, sensitivity, quantification, analyte chemistry, molecular coverage, validation, and practicality.

Keywords
ambient ionization, ambient mass spectrometry, new psychoactive substances, NPS

Abbreviations: Δ9-THC, Δ9-tetrahydrocannabionol; %RSD, % relative standard deviation; 2C-B, 2,5-dimethoxy-4-bromophenethylamine; 4-FA, 4-fluoroamphetamine; AIMS, ambient ionization mass spectrometry; ambient mass spectrometry; APCI, atmospheric pressure chemical ionization; API, atmospheric pressure ionization; APPI, atmospheric pressure photoionization; ASAP, atmospheric pressure solids analysis probe; BZP, 1-benzylpiperazhenine; CBS, coated blade spray; CID, collision-induced dissociation; CNS, central nervous system; CP compounds/series, cyclohexyl phenols; DAPPI, desorption atmospheric pressure photoionization; DART, direct analysis in real time; DBDI, dielectric barrier discharge ionization; DBS, dried blood spots; DESI, desorption electrospray ionization; DeSI, desorption sonic spray ionization; DSA, direct sample analysis; EASI, easy ambient sonic spray ionization; ELDI, electrospray-assisted laser desorption ionization; EMCDCA, European Monitoring Centre for Drugs and Drug Addiction; ESI, electrospray ionization; FAIMS, high field asymmetric waveform ion mobility spectrometry; FAPA, flowing atmospheric pressure afterglow; FCSI, filter cone spray ionization; GC, gas chromatography; HRMS, high-resolution mass spectrometry; IR, infrared; LAESI, laser-ablation electrospray ionization; LC, liquid chromatography; LDTD, laser diode thermal desorption; LLE, liquid–liquid extraction; LLOQ, lower limit of quantification; LOD, limit of detection; LSD, lysergic acid diethylamide; LTP, low-temperature plasma; m-CPP, 1-(3-chlorophenyl) piperazine; m/z, mass-to-charge ratio; MALDESI, matrix-assisted laser desorption electrospray ionization; MALDI, matrix-assisted laser desorption ionization; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDPV, 3,4-methylenedioxy-N-phenylpiperidine; MeOPP, 1-(4-fluorophenethylamine; 4-MPP, 1-(4-bromophenethylamine; 4-PVP, 1-(3-bromophenethylamine; 4-(3-trifluoromethylphenyl) piperazine; IR, infrared; LAESI, laser-ablation electrospray ionization; NMR, nuclear magnetic resonance; NPS, new psychoactive substances; PADI, plasma-assisted desorption ionization; PDP, phenacyclidine; PMMA, p-methoxyamphetamine; PSI, paper spray ionization; S/N, signal-to-noise ratio; SCRA, synthetic cannabinoid receptor agonist; SLE, solid–liquid extraction; SPE, solid-phase extraction; SPME, solid-phase micro-extraction; SWGDRUG, Scientific Working Group for the Analysis of Seized Drugs; TDM, therapeutic drug monitoring; TFMPP, 1-(3-trifluoromethylphenyl) piperazine; TIR, transition ion ratio; TOF, time-of-flight; TS, thread spray; UNODC, United Nations Office on Drugs and Crime; UV, ultraviolet; α-PVP, α-pyrrolidinopentiophenone.

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1 | INTRODUCTION: THE CHALLENGES OF NEW PSYCHOACTIVE SUBSTANCES

1.1  | Social and health challenge

New psychoactive substances (NPS) are made up of a diverse group of recreational drugs designed to mimic the pharmacological activity of established legally controlled drugs, like cannabis, amphetamine (amphetamine), cocaine, 3,4-methylenedioxyamphetamine (MDMA), or lysergic acid diethylamide (LSD). The term NPS includes a wide range of compounds, such as synthetic cannabinoids, hallucinogens, stimulants, or benzodiazepines (UNODC, 2020b).

These substances are not necessarily new inventions but they mostly derive from existing psychoactive compounds. The chemical structures of the original substances are constantly being modified, with slight changes that result in substances that fall outside of any effective legislation, creating uncertainty about their effects and toxicology (Abbate et al., 2018; UNODC, 2013). The modified compounds are traded as legal replacements for controlled drugs or sold misleadingly, as controlled drugs and fake medicines (Elliott et al., 2018). They are usually referred to as “designer drugs” or “legal highs”, however, they generally lack safety testing on animals/humans or quality-controlled profiles, and hence are not well understood in terms of possible health and social harms. The purity, composition, and potency are usually unknown, and the packaging is often mislabeled without side-effect warnings (Alcohol and Drug Foundation, 2020; Couto et al., 2018; Tettey & Crean, 2015). They have the potential for abuse and dependence (Elliott et al., 2018; Smith & Robert, 2014; WHO, 2020), alongside known and sometimes unexpected adverse effects including anxiety, paranoia, hallucinations, seizures, hyperthermia and renal and cardiac toxicity (EMCDDA, 2019b; Harris & Brown, 2013; Peacock et al., 2019; Rojek et al., 2014, 2012; Seely et al., 2012; UNODC, 2020a). Numerous cases of acute intoxications and deaths related to NPS intake have been reported in recent years (see Figure 1) (Elliott et al., 2018; EMCDDA & Europol, 2019; Office for National Statistics [ONS], 2019). Paradoxically, although widely distributed, many of them are labeled as “not for human consumption” and advertised as “research chemicals”, probably to circumvent legal controls (Couto et al., 2018).

1.2  | Legislative challenge

Every year, around 50–100 new substances appear in the market worldwide (UNODC, 2018, 2020a), making it highly diverse and dynamic. The ever-changing NPS trade makes the control of these drugs difficult (UNODC, 2018), nonetheless, attempts to regulate them have been put in place. For instance, at the global level, the Early Warning Advisory on NPS of the United Nations Office of Drug Control (UNODC) is an international drug control system that monitors these substances and serves as a platform for sharing knowledge and best practices. As of January 2020, the emergence of a cumulative total of 950 individual NPS have been reported to UNODC by 120 countries and territories (UNODC, 2020a, 2020b).

FIGURE 1  NPS-related deaths in England and Wales (2011–2018). Reported by the Office for National Statistics, August 2019 (ONS, 2019). NPS, new psychoactive substances [Color figure can be viewed at wileyonlinelibrary.com]
At a regional level, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) created the Early Warning System on NPS, which relies on international cooperation to monitor these compounds in Europe (EMCDDA, 2019a, 2020; UNODC, 2020b). Many countries have also adopted strategies to monitor NPS, be it through specific legislation or by inclusion in their existing drug monitoring systems. One notable example is the Psychoactive Substances Act 2016 in the United Kingdom, which establishes a blanket ban on the importation, production, or supply of most psychoactive substances not already covered by law (ONS, 2019; “Psychoactive Substances Act”, 2016).

Although these are good controls and the number of seizures of NPS seems to be stabilizing in recent years, the NPS problem is not likely to go away soon and the social threat remains (The Center for Forensic Science Research & Education [CFSRE], 2020; EMCDDA, 2019a, 2019b; UNODC, 2018). According to the Office for National Statistics, after the introduction of the Psychoactive Substances Act in 2016 in the United Kingdom, deaths involving NPS experienced a drastic drop in 2017 (numbers were reduced almost by half) but in 2018 numbers went back to 2016 levels (see Figure 1) (ONS, 2019; UNODC, 2020b).

Driven by this new and multiform phenomenon, the diffusion of NPS in the population is bringing along the evolution of the traditional approaches used to monitor drug abuse. Fast, simple, and cheap analytical methods are urgently needed to identify them and regulate them as soon as they hit the streets (Tettey & Crean, 2015).

1.3 Analytical challenge

The current toxicology workflow to monitor the use and abuse of drugs is shown in Figure 2, and it often involves the following steps: (a) sample collection, (b) sample preparation, (c) sample screening, (d) confirmatory analysis, (e) data analysis and verification and (f) reporting of the results (CFSRE, 2020; Waters corporation, 2007). The challenge that NPS poses to this workflow stems from their chemical variety, their rapid emergence and evolution, the absence of reference standards and the complex matrices in which they are found (Couto et al., 2018; Tettey & Crean, 2015).

The types of sample encountered in toxicology mainly come from forensic investigations, clinical intoxications, and seized drug analysis. Therefore, analytical methods for the accurate determination of NPS and their metabolites both in illicit preparations and in body fluids are required (CFSRE, 2020; Couto et al., 2018).

For the screening procedure, presumptive tests include color tests and immunoassays that are rapid and simple techniques suitable for on-site testing. However, rapid, preliminary testing methods for NPS are not fully

![Figure 2](https://example.com/figure2.png)

**Figure 2** The current toxicology workflow. The analytical approach involves (A) sample collection, (B) sample preparation, (C) screening, (D) confirmation, (E) data analysis, and (F) reporting of the results. GC, gas chromatography; HRMS, high-resolution mass spectrometry; LC, liquid chromatography; LLE, liquid-liquid extraction; MS, mass spectrometry; MS/MS, tandem mass spectrometry; SPE, solid-phase extraction. Created with BioRender.com [Color figure can be viewed at wileyonlinelibrary.com]
developed and have limited effectiveness across this structural class of molecules, analogs, and emerging variants (Musah et al., 2014). Immunoassays, despite being a high-throughput technique and still widely used by many workplace drug testing laboratories, typically have limited selectivity. Moreover, the availability of antibodies is limited, and their production takes time (Boyd & Sadrzadeh, 2019; Darwish, 2006; Jeffrey, 2004).

New substances are particularly problematic for many screening methods based on liquid/gas chromatography (LC/GC) coupled with mass spectrometry (MS), since they often rely on library database searching for identification, and NPS are seldom included in standard databases (Borden et al., 2020; Monge & Fernandez, 2015). Despite giving the benefit of chromatographic separation of analyte mixtures, these techniques generally need some level of optimization to incorporate new analytes. Also, they can be time-consuming since they often require sample preparation before analysis, which complicates the identification of novel substances in a fast-evolving market of clandestine compounds. Furthermore, one single method developed for one or a group of NPS may not be suitable to a wider range of NPS; for instance, synthetic cathinones (and, to a larger extent, their metabolites) are usually polar and therefore poorly retained in reversed-phase LC, unlike the more lipophilic synthetic cannabinoids (Bylda et al., 2014; Couto et al., 2018).

LC coupled with MS is the accepted reference method to confirm the identity or presence of drugs of abuse in the various matrices, because of its high sensitivity, specificity, discriminating power, efficiency, broad analytical applicability, and ease of use. Both tandem mass spectrometry (MS/MS) (if high sensitivity is needed), or high-resolution/high mass accuracy mass spectrometry (HRMS) (if elemental composition is required), are used routinely by specialist laboratories and more and more by less specialized scientists. If quantification is required, LC-MS can provide highly accurate and reproducible measurements (Borden et al., 2020; Couto et al., 2018).

Nevertheless, there are many ways to manipulate a compound chemically to produce an analogue (and therefore an NPS), and obtaining a suitable reference material to be included in a spectral library is oftentimes a bottleneck in the drug confirmation process; once the drug is finally added to the library that drug may no longer be relevant in the streets and/or may have been replaced by a new analog (Tettey & Crean, 2015; UNODC, 2018).

Complex sample matrices, such as botanical mixtures, blood, or urine, contain other components that can co-elute in the LC-run and/or interfere with the ionization and detection of the analyte of interest. Moreover, most of the novel substances in the market are very potent and thus found in small amounts in biological fluids (sub ng/ml range) (Adamowicz et al., 2016; Drummer, 2019; Fels et al., 2020), emphasizing the need for sensitive, specific and reliable techniques. Therefore, lengthy extraction and enrichment procedures are typically performed before analysis (Borden et al., 2020; Bylda et al., 2014; Couto et al., 2018).

Finally, many of the NPS are closely related, sharing structural features and metabolites, needing chromatographic separation, high-resolution, high mass accuracy, and/or MS/MS to perform selected ion fragmentation experiments to distinguish between different analogs (Kennedy et al., 2016; Teunissen et al., 2017).

1.4 Analytical approach

For all of the reasons mentioned above, NPS pose a significant analytical challenge and the absence of efficient tools for rapid identification, monitoring, and control, calls for further investigation of faster, sensitive, and specific instrumentation for the analysis of these compounds. In recent years, the interest in new techniques that can reduce the analysis time and sample preparation steps has grown. Among them, ambient ionization MS (AIMS) techniques have drawn significant attention (Correa et al., 2016; Feider et al., 2019; Zhang et al., 2018). These are fast, direct forms of sample introduction in the MS, bypassing sample preparation and chromatographic steps and allowing surface analysis of native samples. Similarly to atmospheric pressure ionization (API), they operate at room pressure, however, the desorption and ionization of the analytes are performed outside the enclosure of the mass spectrometer and the ions enter the MS instrument directly (Monge & Fernandez, 2015). The increasing number of matrices in which NPS are found can be rapidly screened and a wider range of analytes can be tested without being restricted to a few drug classes associated with the classical preliminary testing methods (Borden et al., 2020; Boyd & Sadrzadeh, 2019; Couto et al., 2018).

The growing interest for AIMS techniques and their promising potential for toxicological screening of NPS, deserves to be further investigated. The aims of this study are to (a) provide a better understanding of the applicability of AIMS in the analytical toxicology field, and (b) review the available information critically and systematically regarding AIMS and NPS analysis. This review is intended to facilitate the wider use of AIMS that should help the creation of effective policies to protect citizens from the harms posed by unregulated and potentially harmful novel substances.
2 | METHODOLOGY

2.1 | Search strategy

A two-stage approach was adopted for this review; first, to gain a general understanding of the relevant scientific activity in AIMS applied to the field of toxicology, a simple search with restrictive criteria was undertaken in one database. Following this initial scoping exercise, a more focused, exhaustive search in various databases was performed to gain breadth and depth in AIMS and its applicability to NPS.

2.2 | Search in databases

For the general search, the Web of Science database (Clarivate Analytics, 2021) was used exclusively. The focused search used the following databases: Web of Science (Clarivate Analytics, 2021), Scopus (Elsevier, 2021), PubMed (National Library of Medicine, 2021), and Google Scholar (Google, 2021). The search terms were the following:

- General search: ambient mass spectrometry, ambient ionization mass spectrometry, toxicology.
- Focused search: new psychoactive substances, novel psychoactive substances, ambient mass spectrometry, ambient ionization mass spectrometry, synthetic cannabinoids, synthetic cathinones, phenethylamines, synthetic opioids, tryptamines, piperazines, designer benzodiazepines.

The nature of the search was by topic (title, abstract, and keywords) and the search regarding NPS analysis using AIMS was restricted to articles published from 2008 to November 2020. No report of NPS being analyzed with AIMS was found before 2008.

Inclusion criteria were “relevance of subject(s)/discipline”, that is, toxicology, drugs of abuse (DoA), analytical chemistry and “type of study”. For the focused search, only peer-reviewed primary studies with field data were included. For the general search, this was expanded to include review articles for a comprehensive overview.

Many publications were removed as they were not relevant because of their discipline, for example, physics, meteorology, atmospheric sciences, material science, engineering. Articles were screened based on their title, abstract, and then full text, depending on how well they conformed to the inclusion criteria. The results of this search now follow.

3 | AMBIENT MASS SPECTROMETRY IN THE FIELD OF ANALYTICAL TOXICOLOGY

Due to the polar and nonvolatile nature of many toxicologically relevant compounds, LC-MS methods are increasingly utilized in toxicology laboratories (Waters Corporation, 2007). Chromatography is excellent for its orthogonality to MS and analyte separation and, coupled with the speed, sensitivity, and specificity of modern MS, it has led to superior analytical performance compared with many other techniques (Borden et al., 2020; Brown et al., 2020). Also retention time is considered important by many authorities in matching an unknown against reference material (SWGTOX, 2013; US Department of Justice, 2011). Despite its merits, sample cleanup and chromatography are two key bottlenecks in drug analysis, especially when it comes to quickly evolving and novel substances such as NPS. First, chromatography can be inconvenient when many samples need to be analyzed (timewise and with regard to carryover) and the development of new methods or troubleshooting problems can be laborious. Second, before performing the analysis, suitable sample preparation procedures are required, and chromatographic conditions (typically reversed-phase LC for NPS analysis) must be tuned to obtain the appropriate resolution and selectivity, good compatibility with the MS detection and optimal analysis time (Bylda et al., 2014; Corso et al., 2010; Couto et al., 2018).

Some methods avoid the need for lengthy sample preparation, such as high-speed or parallel separations, online extraction systems, or dilute-and-inject. In the latter case, samples are diluted with an internal standard (IS) solution and directly injected into the LC-MS system. This method has shown good sensitivity and precision with the minimum use of consumables and the results are similar to existing, validated, solid-phase extraction (SPE) procedures using isotopically labeled IS. More sensitive systems, such as modern LC-MS/MS, make this strategy appealing since the sample can be diluted even more, thus minimizing matrix effects and eliminating the need for sample preparation techniques. Nevertheless, chromatography is still required and optimizing the experimental conditions takes time. Compatibility with this method depends on the matrix, LC conditions, limit of detection (LOD) needed, and instrument performance. Depending on the sample type or purity, contaminants can be introduced in the system, leading to blockage of the column or tubing. Also, sample availability is often limited, the analyte of interest is unknown and present in small amounts, thus it is difficult to know whether dilute-and-inject will be adequate. In addition, matrix
effects are rarely overcome by a simple dilution, leading to low reproducibility (Deventer et al., 2014).

In recent years, considerable attention has been devoted to direct sample introduction to the mass spectrometer, which can help shorten the analysis time. Direct introduction techniques have benefited from the development and evolution of atmospheric pressure ionization methods; the availability of MS sources that operate in the ambient environment has enabled the ionization of samples with minimal or no sample preparation by creating ions outside the vacuum of the instrument (Corso et al., 2010). The advantages that make AIMS techniques attractive compared with direct infusion into an atmospheric pressure ionization source are related to its minimum carryover, shorter analysis times, and high-throughput potential, as all parts in contact with the sample are usually disposable and there is no need for cleaning parts (Monge & Fernandez, 2015).

There are more than 80 ambient ionization techniques that have been explored; a discussion of all these techniques is not feasible here, therefore this review will focus on ambient methods that have appeared twice or more in the literature for applications of drug analysis related to NPS (highlighted in bold font). For the purpose of this review, the techniques discussed have been divided into three main classes depending on their working principles: (a) liquid extraction techniques, which are solvent-based, (b) plasma desorption techniques, encompassing principles that are plasma-based, and (c) others, including photoionization and laser desorption-based techniques, for example. In Table 1, some of the most established and commonly used AIMS techniques are listed, along with their acronyms, desorption and ionization mechanisms, year of introduction and the type of sample to which they are best suited. In Figure 3 the AIMS that will be discussed in this review are graphically represented.

Short fundamental descriptions of the technique are presented in this review. However, for detailed mechanistic insights, the reader is directed to a number of thorough reviews of ambient ionization that have been reported in the recent literature, including reviews on general aspects of ambient ionization (Borden et al., 2020; Feider et al., 2019; Frey et al., 2020; Huang et al., 2011; Nollet & Munjanja, 2019; Swiner et al., 2020; Zhang et al., 2018).

3.1 | Liquid extraction techniques

Liquid extraction techniques employ solvents to extract or desorb molecules from the sample surface (Feider et al., 2019). To generate ions, most of them use electrospray ionization (ESI) or similar ionization techniques and, because of that, they are suitable to analyze molecules with readily ionizable groups such as peptides, proteins, and phospholipids. This group includes spray-based techniques and substrate-based techniques, among others, which have been applied mainly to the fields of biomedical, forensic, and environmental sciences.

3.1.1 | Spray-based techniques

Spray-based techniques use charged aerosol droplets from a spray to desorb molecules from the sample surface, which are then analyzed by the mass spectrometer (Feider et al., 2019). This group includes techniques such as desorption electrospray ionization (DESI) and easy ambient sonic spray ionization (EASI), both extensively applied for analytical purposes in the field of toxicology (Borden et al., 2020).

In the case of DESI, analytes are extracted and ionized with the help of a voltage applied to generate an electrospray. It is one of the oldest AIMS techniques, first described in 2004 by Cooks’ team (Takáts et al., 2004). Two years later, EASI was reported by Haddad et al. (2006) under the name of “desorption sonic-spray ionization” (DeSSI); this technique employs a supersonic spray to generate ions, leading to droplets with mild ionization energy and favoring intact molecular species (Haddad et al., 2006). The overall source design simplicity of EASI and the absence of voltage are the reasons why it has been widely explored for on-site testing and can be used by minimally trained personnel (Teunissen et al., 2017). Both DESI and EASI have been most often used in chemical imaging technologies (Alberici et al., 2017; Eberlin et al., 2011; Lamont et al., 2018; Wiseman et al., 2008), although another interesting application is therapeutic drug monitoring (TDM). Direct analysis of dried blood spots (DBS) with an IS (added before spotting) demonstrated the detection of three model drugs (sitamaquine, terfenadine, and prazosin) using DESI-MS, showing good linearity of results within the range 10–10,000 ng/ml (Wiseman et al., 2010).

Spray-based extraction has been reported for the analysis of pure materials, such as tablets and blotter papers, and biological matrices, including blood, urine, or oral fluid. It has been used for various types of analyte, ranging from strongly polar analytes to biomacromolecules, as well as NPS such as synthetic cannabinoids and cathinone analogs (Bianchi et al., 2019; Cotte-Rodríguez et al., 2007; Eberlin et al., 2011; Kauppiila et al., 2007; Morelato et al., 2013; Romão et al., 2011, 2012; Takáts et al., 2005).

Despite being well suited for high-throughput screening and in situ forensic applications (Brown
| Technique                                      | Acronyms          | Desorption | Ionization                  | First report | Nature of the samples | Demonstrated analysis capability | References                      |
|-----------------------------------------------|-------------------|------------|------------------------------|--------------|------------------------|---------------------------------|---------------------------------|
| Atmospheric pressure solids analysis probe    | ASAP              | Thermal desorption | Corona discharge             | 2005         | S, L                   | Small molecules                 | McEwen et al. (2005)           |
| Coated blade spray                            | CBS               | Spray desorption | High voltage                 | 2014         | L                      | Small and large molecules       | Gómez-Ríos and Pawlisyn (2014) |
| Desorption atmospheric pressure photoionization | DAPPI            | Thermal desorption | Photoionization               | 2007         | S, L                   | Small and large molecules, imaging | Haapala et al. (2007)     |
| Desorption electrospray ionization            | DESI              | Spray desorption | Electrospray                 | 2004         | S, L                   | Small and large molecules, imaging | Takáts et al. (2004)         |
| Dielectric barrier discharge ionization        | DBDI              | Thermal desorption | Dielectric barrier discharge | 2007         | S, L, G                | Small molecules                 | Na et al. (2007)              |
| Direct analysis in real time                  | DART              | Thermal desorption | Corona discharge             | 2005         | S, L, G                | Small and large molecules, imaging | Cody et al. (2005)           |
| Direct sample analysis                        | DSA               | Thermal desorption | Atmospheric pressure chemical ionization | 2007         | S, L                   | Small molecules                 | Chen et al. (2007)           |
| Easy ambient sonic-spray ionization           | EASI              | Spray desorption | Sonic spray                  | 2006         | S, L                   | Small and large molecules, imaging | Haddad et al. (2006)         |
| Electrospray-assisted laser desorption ionization | ELDI           | Laser desorption | Electrospray                 | 2005         | S, L                   | Small and large molecules, imaging | Shiea et al. (2005)          |
| Laser-ablation electrospray ionization        | LAESI             | Laser desorption | Electrospray                 | 2007         | S, L                   | Small and large molecules, imaging | Nemes and Vertes (2007)       |
| Laser diode thermal desorption                | LDTD              | Thermal desorption | Corona discharge             | 2004         | S, L                   | Small molecules                 | Peng et al. (2004)           |
| Low-temperature plasma                        | LTP               | Thermal desorption | Corona discharge             | 2008         | S, L, G                | Small molecules                 | Harper et al. (2008)         |
| Matrix-assisted electrospray ionization       | MALDESI           | Laser ablation | Electrospray                 | 2006         | S, L                   | Small and large molecules, imaging | Sampson et al. (2006)        |
| Paper spray ionization                        | PSI                | Spray desorption | High voltage                 | 2010         | S, L                   | Small and large molecules       | Liu et al. (2010)            |
| Plasma-assisted desorption ionization         | PADI              | Thermal desorption | Glow discharge               | 2007         | S, L                   | Small molecules                 | Ratcliffe et al. (2007)      |
| Thread spray                                  | TS                 | Spray desorption | High voltage                 | 2018         | L                      | Small and large molecules       | Jackson et al. (2018)        |

Abbreviations: L, liquid; G, gaseous; S, solid.

*Small, ≤600 Da, large, >600 Da.
et al., 2020), these techniques have typically suffered from slow overall analysis time, low ionization efficiency, and poor analytical reproducibility, mainly due to the dependency on operating conditions (Bianchi et al., 2019; Haddad et al., 2006; Tillner et al., 2017; Vircks & Mulligan, 2012). Recent efforts have been directed at optimizing the source design and solvent choice to reduce spray-to-spray variability (Abbasi-Ghadi et al., 2015; Tillner et al., 2017). However, the effect of biological matrix components and adulterants in seized samples have been shown to impact precision and sensitivity greatly (Bianchi et al., 2019; Stojanovska et al., 2020).

**FIGURE 3** Ambient ionization mass spectrometry techniques. (A) Desorption electrospray ionization (DESI), (B) easy ambient sonic-spray ionization (EASI), (C) paper spray ionization (PSI), (D) direct analysis in real time (DART), (E) atmospheric pressure solids analysis probe (ASAP), (F) direct sample analysis (DSA), (G) dielectric barrier discharge ionization (DBDI), (H) low-temperature plasma ionization (LTP), (I) desorption atmospheric pressure photoionization (DAPPI), and (J) matrix-assisted laser desorption ionization (MALDESI). Reprinted with permission from Borden et al. (2020) John Wiley and Sons [Color figure can be viewed at wileyonlinelibrary.com]
et al., 2014; Suni et al., 2011), and more research is required to improve the suitability of spray-based extraction for quantification.

### 3.1.2 Substrate spray techniques

Substrate spray techniques generate ions directly from the sample or from the substrate in which the sample is found (Feider et al., 2019). Among them, paper spray ionization (PSI) has been the most widely used since its first appearance in 2010 (Liu et al., 2010) and has been gaining attention in recent research studies because of its wide range of applications and ease and speed of sampling, which can help reduce sample backlog in analytical laboratories.

In PSI, the liquid sample is spotted on a triangular-shaped piece of filter paper and allowed to dry. After that, spray solvent and voltage are applied to the paper and droplets with analyte charged species enter the mass spectrometer (Liu et al., 2010). Theoretically, extraction and separation of molecules occur in the substrate as the solvent, together with the analyte, migrate through; thus, interfering species that can be present in the matrix adhere to the paper, increasing sensitivity and reproducibility. However, the complexity of biological samples such as blood or urine has shown increased matrix effects. Moreover, paper tip angular position, paper properties, solvent choice, and spray stability seem to impact significantly the quality of the analysis (Bills et al., 2018; Birk et al., 2019; Takyi-Williams et al., 2020; Vega et al., 2016). To reduce variation and improve signal stability and sensitivity of analytes in complex samples, commercial paper spray cartridges can be used, and the tip or surface of paper substrates can be modified and functionalized, although reproducibility needs to be improved further (Bills et al., 2018; Costa et al., 2019; Kennedy et al., 2018; Salentijn et al., 2018). A commercial system is available and it has shown great sensitivity for a number of drugs in blood (Espy et al., 2014; Ren et al., 2019); given that PSI is steadily gaining interest, alternatives are likely to emerge (McBride et al., 2019; Riboni et al., 2019).

Aside from screening applications, PSI has precise quantitative potential by IS introduction into the liquid sample before deposition on the paper or onto the paper before sample introduction (Kennedy et al., 2018; Teunissen et al., 2017; Vandergrift et al., 2018). It is a robust and simple technique well suited to portability and coupling with an autosampler for high-throughput analysis. Furthermore, it has potential for use in in situ applications, TDM (through direct analysis of DBS spotted on the paper substrate, for example) and harm-reduction drug checking (Espy et al., 2014; Ma et al., 2015; Manicke et al., 2011; Vandergrift & Gill, 2019).

Small amounts of unprepared sample (<10 μl) containing both small and large molecules can be analyzed. This technique performs well in a wide range of matrices, including blood, urine and extracts from plant materials and powders in solution (Brown et al., 2020). A wide variety of NPS have already been analyzed with PSI, such as fentanyl analogs, synthetic cannabinoids, and cathinone derivatives (Carvalho et al., 2016; Kennedy et al., 2016; Teunissen et al., 2017; Vandergrift et al., 2018).

Other substrate-based techniques that have shown good sensitivity, ease of use and wide applicability include coated blade spray (CBS) and thread spray (TS) (Gómez-Ríos & Pawliszyn, 2014; Jackson et al., 2018). Although they have been used for drug analysis (Borden et al., 2020; Swiner et al., 2019), none of them have been applied to NPS to date.

### 3.2 Plasma desorption techniques

Plasma desorption techniques use a process of plasma discharge to desorb and ionize molecules and, unlike solvent-based techniques, they can be tuned for analysis of nonpolar compounds via electron-transfer ionization mechanisms (Feider et al., 2019). One of the most used techniques in this group is **direct analysis in real time** (DART), which was introduced by Cody and colleagues in 2005. The ionization process starts with a pin electron discharge that generates metastable gas atoms (e.g., helium, nitrogen) in a confined plasma source. These are directed to the sample to desorb and ionize the molecules (Cody et al., 2005). This ionization source has quantitative capabilities that depend on heating the gas to achieve increased desorption and sensitivity. However, the specificity of the methods reported is typically poor (mainly determined by type of discharge gas used, temperature of drift gas, and detector resolution) (Monge & Fernandez, 2015; Nie et al., 2016; Yu et al., 2009); thus it is primarily used as a qualitative analysis technique. Given its source simplicity and robustness, it is suited for portability and coupling with an autosampler (Brown et al., 2016; Yu et al., 2009).

DART has a wide range of forensic and clinical sampling applications, including small and large molecules, though the sensitivity is dependent on the volatility of the analyte. It excels at analyzing solid surfaces, and it is also adequate for biomatrix analysis (Brown et al., 2020). It has been applied to a variety of NPS, such
as synthetic cannabinoids and cathinone analogs, mainly in solid samples (Grange & Sovocool, 2011; Musah et al., 2012, 2014; Nie et al., 2016; Sisco et al., 2019). Validated DART-MS methods for the screening of drugs of abuse are in use by several crime laboratories, such as the Virginia Department of Forensic Sciences (Steiner & Larson, 2009) and the Harris County Institute of Forensic Science (2020).

In 2005 the atmospheric pressure solids analysis probe (ASAP) was first reported. In this technique a probe is used to introduce the sample into a heated desolvation gas stream (from atmospheric pressure chemical ionization/ionization [APCI] probe) and thermally desorbed analytes are ionized by a corona discharge (McEwen et al., 2005). Typically, the probes are glass melting point capillary tubes or medical swabs (Fabregat-Safont et al., 2020); it allows the direct analysis of liquid or solid samples in seconds, although it is limited to volatile and semi-volatile compounds. The advantages of ASAP include some of the benefits of APCI (lessened matrix effects), high-throughput, and the fact that it can be easily interfaced with any commercial mass spectrometer and the sampling probe is inexpensive and disposable, eliminating carryover (Borden et al., 2020). ASAP has typically been used in material and tissue analysis, and literature related to drugs of abuse is not extensive; solid materials as well as biomatrices have been analyzed successfully, however, quantitative work is limited (Brown et al., 2020; Crevelin et al., 2016; Jagerdeo et al., 2015; McCullough & Hopley, 2021; McCullough et al., 2020). Synthetic cannabinoids, synthetic cathinones, and synthetic opioids have been identified with ASAP (Fabregat-Safont et al., 2020; Jagerdeo & Wriston, 2017).

In 2007 direct sample analysis (DSA) was first reported by Chen and colleagues; DSA uses heated nitrogen gas from an APCI source which is ionized by an electrical discharge and initiates the desorption and ionization process on the sample surface. Nitrogen ions ionize water molecules in the source and form water cluster ions that will ionize the analyte of interest. Although this technique may resemble DART, the ionization process differs; DART uses a Penning ionization technique to initiate the ionization process and relies upon the formation of metastable gas atoms to generate protonated water clusters, which will ionize the analyte of interest (Drury et al., 2018). Also, unlike DART which has an open source, DSA samples are introduced via a sample holder in a closed system, limiting the ambient air entering the housing and reducing chemical background noise (Chen et al., 2007).

The main applications of DSA involve high-throughput rapid screening of samples; it has been used for the analysis of seized tablets, powders, blotter papers and also biomatrices, like urine (Borden et al., 2020; Brown et al., 2020). In the NPS context, it has been widely used for the detection of phenethylamines, but also synthetic opioids and fentanyl analogs and, coupled with time-of-flight (TOF) mass spectrometry, demonstrated the identification of true unknowns without reference standards or libraries and with minimal matrix interference (Botch-Jones et al., 2016; Daughtery & Crowe, 2014; McGonigal et al., 2017; Moore et al., 2019).

Plasma-based techniques are generally capable of ionizing a wide variety of molecules, however, they can be somewhat limited to low and mid-polarity compounds, since they use gas-phase ionization, and sufficient analyte volatility is required. Moreover, the stream of heated gas may cause the fragmentation of labile compounds and/or thermal damage of the sample (Ayodeji et al., 2017; Chernetsova & Morlock, 2011; Newsome et al., 2018); high molecular weight compounds usually perform poorly and need thermal assistance or derivatization (Hajslova et al., 2011; Musah et al., 2012; Nagy et al., 2018; Yu et al., 2009). To approach this difficulty, unheated plasma techniques have been explored, such as low-temperature plasma (LTP), dielectric barrier discharge ionization (DBDI), and plasma-assisted desorption ionization (PADI) (Harper et al., 2008; Na et al., 2007; Ratcliffe et al., 2007).

### 3.3 Other techniques

Other ambient ionization sources include laser ablation techniques. These employ ultraviolet (UV) or infrared (IR) laser sources to promote sample desorption in combination with ESI ionization. The main advantage of laser beams is that they can be optically focused, which enables highly efficient desorption at superior spatial resolution (10–50 μm) and pulse frequencies (few ns) than spray- and plasma-based techniques (Feider et al., 2019; Lawal et al., 2019). Some of the techniques used for analytical work are matrix-assisted laser desorption electrospray ionization (MALDESI), laser diode thermal desorption (LDTD), laser ablation electrospray ionization (LAESI), and electrospray-assisted laser desorption ionization (ELDI), mainly applied to molecular imaging and the analysis of proteins, lipids and metabolites in biological tissue matrices (Deimler et al., 2014; Peng et al., 2004, 2010; Robichaud et al., 2014; Shrestha et al., 2010). ELDI is a matrix-less method suitable for water-rich samples, whereas MALDESI and LAESI require the pretreatment of the sample with a suitable matrix, limiting their in vivo imaging capabilities (Nemes & Vertes, 2007).
This group also includes techniques based on photoionization, such as **desorption atmospheric pressure photoionization (DAPPI)**. DAPPI achieves analyte ionization with the help of a photoionization lamp or indirectly via gas-phase interaction with dopant (solvent) molecules. A direct jet of hot, vaporized solvent is directed toward the surface of the sample and analytes are thermally desorbed. Depending on the dopant selection, both polar and nonpolar compounds can be analyzed effectively (Kauppila et al., 2004, 2008).

The technique DAPPI has been applied to drug analysis in various solid matrices, such as tablets, blotter papers, powders, and herbal products, and has shown to be a sensitive and effective tool for both qualitative and quantitative applications (Borden et al., 2020). However, the presence of dopant-like solvent is necessary for the ionization of the analytes and thermal desorption can limit the analytes amenable for this technique. Also, the photoionization lamp can sometimes be difficult to handle, expensive and the light output is typically low (Hamatsu Photonics, 2016; Kauppila et al., 2008, 2011; Mirabelli & Zenobi, 2018).

### 3.4 AIMS summary remarks

As AIMS techniques continue to be refined, their use will expand across a broad range of scientific disciplines. Improvements in their analytical performance are expected, including lowering the detection limits and enabling quantitative assays for compounds at trace levels or within complex sample matrices (Feider et al., 2019). Although selectivity and sensitivity are mainly a function of the type of MS, the efficiency of the ambient ionization source may have a substantial effect on them. For example, in DART, sensitivity depends on analyte volatility, basicity/acidity, fluid dynamic ion transfer effects as well as temperature gradient within the ionization region. In DESI, it depends on variables such as ion fugacity, ion-source geometry, and spray parameters that affect the dynamics of splashing mechanism resulting in changes in droplet size, charge, and analyte dissolution extent. While DART specificity is determined by the type of discharge gas used and temperature of the drift gas, DESI specificity is given by wet chemistry (extraction and reaction) between solvent solution and surface-bound analyte (Monge & Fernandez, 2015). Therefore, for the purpose of this review, these properties will be considered as related to the ionization source as well as the MS detector.

Most of the published work on applications using AIMS is related to DESI, DART and PSI, which are commercially available, as well as a special interest in AIMS-based MS imaging. These ionization sources have been widely applied to the fields of biomedicine, forensic and pharmaceuticals analysis, microbiology, and cancer pathology. Recently, integrated platforms have been developed combining multiple ambient ionization techniques into an all-in-one system with interchangeable sources (Jagerdeo & Wriston, 2017; Lawton et al., 2017). The advantage of these is that different sources can be operated individually but also simultaneously to analyze a wide variety of compounds.

### 4 APPLICATION OF AIMS TECHNIQUES TO NPS ANALYSIS

As mentioned earlier, synthetic designer drugs have been the cause of growing concern since the turn of the twenty-first century, and the most notorious include synthetic cannabinoid receptor agonists (SCRAs), cathinone derivatives (cathinones), synthetic opioids, and phenethylamine-like compounds (see Figures 4 and 5) (EMCDDA & Europol, 2019; Habala et al., 2016). The dynamic and fast-growing NPS market together with the high number of intoxications reported (EMCDDA, 2019b; UNODC, 2020a), are some of the reasons for them being frequently reported in analytical toxicology studies, including AIMS-related literature.

Since 2004, new herbal products containing potent cannabinoid receptor agonists continue to appear in the market. In 1988, the Δ9-tetrahydrocannabinol (Δ9-THC) synthetic analogue HU-210 was first synthesized in Israel by Raphael Mechoulam (Mechoulam et al., 1988). It has a potency of at least 100 times that of Δ9-THC and, because of its structural similarity to the natural plant cannabinoid, it is regarded as a classical cannabinoid (Seely et al., 2012). Nonclassical cannabinoids include cyclohexyl phenols (CP compounds), which were developed by the pharmaceutical industry as analgesics, and aminoalkylindoles (such as naphthoylindoles, phenylacetylindoles, and benzoylindoles), among others (Abbate et al., 2018). One of the best known SCRA is JWH-018, which is three times more potent than Δ9-THC and was developed as a test compound for therapeutic purposes by John W. Huffman together with other novel cannabinoids of the JWH series (abbreviated from the name of the inventor). Although the pharmacology of SCRAs in humans is not fully understood, studies have shown that these substances bear structural features that allow binding to cannabinoid receptors, acting as cannabimimetics. Furthermore, they are often laced into herbal products that are usually smoked or vaporized (Gwak, 2015; UNODC, 2013).
Cathinone is the principal active ingredient in the leaves of the khat plant (Catha edulis), which is the base compound from which many synthetic cathinones derive. Cathinone derivatives appeared in the market in the mid-2000s; methylone and mephedrone were first reported to the EMCDDA in 2005 and 2007, respectively. Other cathinone analogs identified are 3,4-methylenedioxypyrvalerone (MDPV), first synthesized in 1969, and α-pyrrolidinopentiophenone (α-PVP). These substances act on the central nervous system (CNS) and possess stimulant effects by mediating the actions of dopamine, norepinephrine, and serotonin neurotransmitters. Some synthetic cathinones have been explored for medical purposes, but overall, very few have been exploited clinically because of their abuse and dependence potential. They are often referred to as “bath salts” and labeled “not for human consumption” to circumvent local legislative bans. They are sold as powders, granules, or crystals that can resemble Epsom salts, and in pill form, which can be administered in many forms: ingested, injected, inhaled, snorted, and smoked (Gwak, 2015; UNODC, 2013).

Synthetic opioids are a chemically diverse group of substances that include very potent fentanyl analogs and derivatives of opiates. These CNS depressants...
Phenethylamines refer to a class of substance with psychoactive and stimulant effects, which include amphetamine, metamfetamine, and MDMA (UNODC, 2013). Variations on the natural phenethylamine mescaline led to synthetic compounds such as two-carbon phenethylamine homologues (2C series) and dimethoxy phenylisopropylamine (DO series). Other widely known compounds in this class include the NBOMe series, Bromo-Dragonfly, 4-fluoroamphetamine (4-FA), and p-methoxyamphetamine (PMMA). There is limited scientific literature on their mode of action, however, most of them are known to act as serotonergic agonists with hallucinogenic effects. They are commonly sold as powders, tablets, or blotter papers that are usually ingested and injected (Gwak, 2015; UNODC, 2013).

Lastly, other NPS classes are piperazines and phencyclidine-type substances. Piperazines are often referred to as “failed pharmaceuticals”, given that pharmaceutical companies developed some of them as potential therapeutic agents, but they never reached the market (King & Kicman, 2011). Most of these drugs are CNS stimulants, acting on dopamine, noradrenaline, and serotonin neurotransmitter systems in the brain; occasionally, they can also act as opioids (Gwak, 2015). Commonly abused are two types of piperazine derivates: benzylpiperazines and phenylpiperazines. These include 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP), and 1-(3-chlorophenyl) piperazine (m-CPP), usually available in the form of tablets or powders that are ingested.

Phencyclidine (PDP) derivates include substances like ketamine and its structural analogue, methoxetamine (MXE), which are misused as club drugs because of their hallucinogenic effects. MXE is generally sold as a free base and hydrochloride salt in powder, which can be administered nasally or orally (Gwak, 2015). Similar to ketamine, MXE inhibits dopamine reuptake acting on the glutamate and serotonin receptors, however, it has higher intensity effects and longer duration of action (Gwak, 2015).

As previously discussed in Section 1, legislation and control of these substances can be challenging because of international and national legislative gaps, lengthy regulatory processes for scheduling new compounds, and lack of rapid and direct identification methods. Being fast, high-throughput, and less prone to false positives compared with other screening methods, AIMS are a potential substitute to help screening, identifying, and hence regulating NPS more efficiently. The literature used in this review related to NPS analysis via AIMS is listed in Table 2, along with relevant data of the work published.

4.1 Ionization efficiency

The ionization efficiency of AIMS techniques is typically worse than that of traditional ionization sources like ESI and matrix-assisted laser desorption ionization (MALDI). This is simply because the ionization is performed outside the enclosure of the MS instrument; the configuration of the source, the distance between the ionization source and the MS inlet and the effect of atmospheric conditions potentially lead to reduced ionization efficiency, inefficient ion sampling, and less sensitive and reproducible measurements (Kuo et al., 2019).

The ionization efficiency of liquid extraction-based techniques is markedly affected by the sample substrate and the solvents used. The hydrophilic nature of the surface, the dielectric constant between the substrate and the spray, and the interaction types between the molecules of the analyte and the surface at the liquid-solid interface are the most critical factors that affect signal intensity and stability. By using substrates of high chemical inertness, solvent-surface and analyte-surface interactions can be reduced, allowing enhanced solubilization of analytes in the spray solvent, thus increasing the signal intensity (Bianchi et al., 2019).

In accordance with this, the signal of ambient ionization techniques such as DESI and PSI has been shown to be highly variable when analyzing phenethylamine-like compounds, but average and maximum signals obtained by PSI are significantly higher than those of DESI. This can be a consequence of the desorption mechanism of DESI, which requires the collection of secondary analyte ions liberated from the surface. On the other hand, PSI source flexibility makes it more susceptible to user error over the static nature of the DESI source (Lawton et al., 2017).

The efficiency of the PSI source has been studied by Bills and colleagues and by Vandergrift and his team.
| ID and/or Quant | Drug category | Time required for the analysis of one sample | Matrices | Validation criteria (typical range) | Application to real samples | References |
|----------------|---------------|---------------------------------------------|----------|-------------------------------------|--------------------------|------------|
| ASAP | Cathinones | <1 min | Solid and liquid form | – | Yes (n = 7) | Jagerdeo and Wriston (2017) |
| ID | Cathinones | – | Herbal blends, pills, powders, crystal samples, finger and solid surfaces | LOD: 1–12 μg of compound onto a surface | Yes (–) | Fabregat-Safont et al. (2020) |
| DAPPI | Phenethylamines | “Within seconds” | Tablets, blotter paper | – | Yes (–) | Kauppila et al. (2008) |
| ID | Cathinones | “Within seconds” | Herbal products, tablets, powder, oil | – | Yes (n = 27) | Kauppila et al. (2011) |
| DART | Cathinones | <1 min | Powder | LOD: 0.3–0.34 ng | Yes (n = 4) | Gwak and Almirall (2015) |
| ID and semi-quant | Cathinones | – | Solid form | No carryover observed. | No | Musah et al. (2014) |
| ID | Cathinones | Within 0.5 min | Solid form, herbal samples | LOD: 5–40 ng/ml | Yes (n = 3) | Nie et al. (2016) |

Good linearity $r^2 > 0.99$ over the range 10–200,000 ng/ml. Repeatability %RSD < 15% at 500 ng/ml. Recovery for phenethylamines and cathinones was within 84%–123%, and for SCRA 99.7%–180%. No matrix effect reported.
| ID and/or Quant | Drug category | Time required for the analysis of one sample | Matrices | Validation criteria (typical range) | Application to real samples | References |
|----------------|---------------|---------------------------------------------|----------|-----------------------------------|-----------------------------|------------|
| ID             | Cathinones    | “Few seconds”                               | Solid form | –                                 | No                          | Lesiak et al. (2013) |
| ID             | Other substances | –                                            | Urine Powder, pill | LOD: 500 ng/ml | Yes ($n = 5$) | Lesiak et al. (2014a) |
| ID             | SCRA          | –                                            | Herbal incense | (AM-2201 26–142 mg/g, JWH-210 4–38 mg/g) | Yes ($n = 6$) | Lesiak et al. (2014b) |
| ID             | Cathinones    | –                                            | Plant material, powder, pill | No matrix interference reported, and selectivity studies performed. | Yes (–) | Brown et al. (2016) |
| ID             | SCRA          | “Seconds”                                    | Herbal sample | –                                 | No                          | Musah et al. (2012) |
| ID             | SCRA          | 30 s                                         | Herbal sample, powder | LOD: 1000–2000 ng/ml | Yes ($n = 8$) | Habala et al. (2016) |
| ID             | Opioids       | –                                            | Packaging surface | –                                 | Yes ($n = 191$) | Sisco et al. (2019) |
| ID             | Cathinones    | <2 min                                       | Plant material, powder, pill, tablet | Intra- and inter-day precision were in the range of 0.00%–0.54% and 0.20%–1.14%, respectively. | Yes ($n = 50$) | Lian et al. (2017) |

**DESI**

| ID             | Phenethylamines | <1 min                                      | Solid form | LOD: 20–2800 ng/mm² of spot surface | Yes ($n = 1$) | Stojanovska et al. (2014) |
| ID             | Piperazines     |                                              |           | Intra-day and inter-day precision were <25% and <33%, respectively. Accuracy was within 10% | |
| ID             | Cathinones      | <10 s                                        | Powder, surface residues, fingerprint residue | LOD: low–to sub-ng | Yes (–) | Vircks and Mulligan (2012) |

(Continues)
| ID and/or Quant | Drug category | Time required for the analysis of one sample\(^a\) | Matrices | Validation criteria (typical range)\(^b\) | Application to real samples\(^c\) | References |
|----------------|---------------|---------------------------------------------|----------|-----------------------------------------|-----------------------------|------------|
| ID and quant   | Cathinones    | 15 min— including micro-extraction          | Oral fluid | LLOQ: 50-500 ng/ml | Yes (\(n = 40\)) | Bianchi et al. (2019) |
| SCRA           |               |                                             |           | Validated according to Guidance for Industry, Bioanalytical Method Validation (US Department of Health and Human Services & Food and Drug Administration, 2018). |                               |            |
| ID             | Phenethylamines | 30 s                                       | Surface residues | LOD: high ng range | No | Lawton et al. (2017) |
| DSA            |               |                                             |           | Validated according to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations (US Department of Justice, 2011). |                               |            |
| ID             | Phenethylamines | 15 s                                       | Blotter paper | (Phenethylamines: 50–800 μg in powder and blotter) | Yes (\(n = 1\)) | Botch-Jones et al. (2016) |
| ID             | Phenethylamines | 15 s                                       | Blotter paper | – | Yes (\(n = 2\)) | McGonigal et al. (2017) |
| ID             | Opioids       | 15 s                                        | Solid form | LOD: 100 ng/ml (fentanyl: 0.6–6.9 mg in counterfeit prescription pills and paraphernalia) | Yes (\(n = 81\)) | Moore et al. (2019) |
| EASI           |               |                                             |           | No carryover and no matrix interference observed. |                               |            |
| ID             | Piperazines    | 10 s                                        | Tablet    | 7.43 ng/tablet (22–80 mg of m-CPP per tablets) | Yes (\(n = 30\)) | Romão et al. (2011) |
| ID             | Phenethylamines | –                                           | Blotter paper | LOD: 1000 ng | Yes (\(n = 4\)) | de Morais et al. (2017) |
| ID and/or Quant | Drug category   | Time required for the analysis of one sample<sup>a</sup> | Matrices     | Validation criteria (typical range)<sup>b</sup>                                                                 | Application to real samples<sup>c</sup> | References                  |
|----------------|-----------------|----------------------------------------------------------|--------------|---------------------------------------------------------------------------------------------------------------|----------------------------------------|------------------------------|
| LTP ID         | Cathinones      | –                                                        | Powder       | LOD: 2–5 pg                                                                                                  | No                                     | Dalgleish et al. (2013)      |
|                |                 |                                                          |              | No sample carryover reported. Repeatability %RSD was 16%.                                                     |                                        |                              |
| ID and quant   | Cathinones      | –                                                        | Oral fluid   | LOD: 3.0–15.2 ng/ml                                                                                           | Yes (–)                                | Wang et al. (2018)           |
| SCRA           |                 |                                                          |              | Good linearity $r^2 > 0.99$ over the range 1–1000 ng/ml. Repeatability %RSD was <23% at 50 ng/ml. Recoveries are in the range 73.1%–126% at 50, 200, and 500 ng/ml. |                                        |                              |
| PSI ID         | Phenethylamines | 30 s                                                     | Surface residues | LOD: low to mid-ng range                                                                                     | No                                     | Lawton et al. (2017)         |
|                |                 |                                                          |              | Validated according to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations (US Department of Justice, 2011). |                                        |                              |
| ID and quant   | Opioids         | <1 min                                                   | Blotter paper | (15 μg per blotter or lower)                                                                               | Yes ($n = 6$)                          | Birk et al. (2019)           |
|                |                 |                                                          |              | LOQ: 0.5 ng/ml                                                                                               |                                        |                              |
|                |                 |                                                          |              | Good linearity $r^2 > 0.99$ over the range 0.5–50 ng/ml. Repeatability %RSD <15% at 10 ng/mL. Average %Bias was within 5%–48% at 2.5 ng/mL, and within 2%–4% at 50 ng/mL. Matrix effects reported. |                                        |                              |
| ID and/or Quant | Drug category     | Time required for the analysis of one sample<sup>a</sup> | Matrices                         | Validation criteria (typical range)<sup>b</sup>                                                                 | Application to real samples<sup>c</sup> | References                                      |
|-----------------|-------------------|------------------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------|----------------------------------------|-----------------------------------------------|
| ID              | SCRA              | <2 min                                               | Herbal incense, powder          | –                                                                                                               | Yes (n = 42)                           | Kennedy et al. (2016)                        |
|                 |                   |                                                     |                                 |                                                                    |                                        |                                               |
| ID and semi-quant | Opioids           | <5 min                                               | Solid form, diluted urine, analgesic slurry | LOD: 0.05–9.2 ng/ml Good linearity $r^2 > 0.99$ over the range 0.5–600 ng/ml. | Yes (n = 35)                           | Vandergrift et al. (2018)                    |
| ID and quant    | Cathinones        | 0.5 min                                              | Fingerprint                     | LOD: 25 pg/fingerprint LOQ: 50 pg/fingerprint Intra- and inter-day precision were within ±20%. Good linearity $r^2 > 0.99$ over the range 0.025–1.5 ng/fingerprint. No carryover observed, matrix effect was within ±13%. Stability and selectivity studied. | Yes (n = 6)                            | Czerwinska et al. (2020)                     |
| ID              | Opioids           | –                                                    | Surface residues, latent fingerprints, beverages, e-cigarette liquids | LOD: low to mid-ng range Matrix effects reported. | No                                      | Bruno et al. (2017)                         |
| ID              | SCRA              | 2 min                                                | Blood, urine, powder            | LOD: 0.86–1.4 ng/ml                                                                                             | No                                     | Bills et al. (2018)                         |
| ID              | SCRA              | –                                                    | Urine, oral fluid               | LOD: low ng/ml                                                                                                  | No                                     | Bills and Manicke (2020)                    |
| ID and quant    | SCRA              | –                                                    | Blotter paper, herbal medicine   | LOD: 0.17–1 ng/ml LOQ: 1.96–2.24 ng/ml                                                                       | Yes (n = 8)                            | Domingos et al. (2017)                      |
| ID and/or Quant | Drug category | Time required for the analysis of one sample | Matrices | Validation criteria (typical range) | Application to real samples | References |
|-----------------|---------------|-----------------------------------------------|----------|-----------------------------------|-----------------------------|------------|
| ID and quant    | Piperazines   |                                               |          | Good linearity $r^2 > 0.99$ in the range of 1–110 ng/ml. Matrix effects investigated. | No                           | Teunissen et al. (2017) |
| ID              | Phenethylamines | 1.3 min                                       | Whole blood | LOD: 15–50 ng/ml                   |                             |            |
|                 |               |                                               |          | Accuracy was within ±15% and average precision was better than 15%, and better than 20% at the LOQ. Good linearity $r^2 > 0.99$ over the various ranges assessed. Selectivity studies showed some interferences. |                             |            |
| ID              | Phenethylamines | 2 min                                         | Blotter paper | –                                 | Yes (n = 6)                  | Carvalho et al. (2016) |
| ID              | Cathinones    | –                                             | Tablet, blotter paper | –                                 | Yes (–)                      | Filho et al. (2020) |
| SCRA            | Phenethylamine |                                               |          |                                   |                             |            |
| Opioids         | SCRA          | 15 ms                                         | Surface residue, whole blood, and urine | LOD: 2 ng on a bench surface LOQ: 10–20 ng/ml in blood and urine Repeatability %RSD < 10% were achieved over the entire concentration range 5 to 1000 ng/ml and good linearity ($R^2 > 0.99$) was also obtained. | No                           | Ma et al. (2015) |

Abbreviations: cathinones, cathinone derivatives; ID, identification; LLOQ, lower limit of quantification; LOD, limit of detection; LOQ, limit of quantification; quant, quantification; $r^2$, coefficient of determination; %RSD, %relative standard deviation; SCRA, synthetic cannabinoid receptor agonist.

a Time not specified (–).
b No value reported (–).
c Number of samples not specified (–).
Paper properties, like pore size, the sharpness of the tip, or thickness, have shown to impact greatly on the ionization efficiency and recovery of fentanyl and SCRA substances; there was a consistent trade-off observed between ionization efficiency and recovery. Furthermore, the solvent choice influenced matrix effects to a greater degree than paper properties (Bills et al., 2018; Vandergrift et al., 2018).

In the case of sources that require heated gas, like DART, inefficient ionization comes from the fact that, in quantitative terms, air molecules are present in a higher number than metastable helium molecules, thus most of the excited helium is quenched half-way (Li et al., 2015). New designs have improved the angle and the distance between the DART source and the MS instrument; additionally, increasing the heating temperature can help enhance analyte desorption. Other sources like DSA, which are in a closed system, have reduced the influence of air molecules, which in turn increases reproducibility of the results (Drury et al., 2018).

The performance of ambient ionization, and particularly solvent-based sources, depends on many variables and still needs to be improved to achieve better reproducibility (Kuo et al., 2019; Teunissen et al., 2017). An interesting feature observed is the formation of multi-molecular ionized complexes, which can complicate the interpretation of results to nonexpert users and lead to total ion abundance being divided into several signals belonging to the same species. For instance, using PSI, dimers of SCRA have been identified by Carvalho and colleagues and Domingos and team (Carvalho et al., 2016; Domingos et al., 2017). Cluster, adduct, and dimer formation has been reported when analyzing designer drugs using a portable DART-MS instrument; this being mainly attributed to low desorption temperature and over-sampling (Brown et al., 2016).

4.2 Selectivity

Although AIMS techniques offer simplified and rapid analysis, this comes at the cost of reduced selectivity. The growing number of structurally similar NPS appearing in the recreational drug market increases the likelihood of obtaining false results, especially when isomers are present in the sample since they have the same molecular mass as the analyte of interest. For this reason, AIMS strongly relies on MS/MS and fragmentation experiments (e.g., collision-induced dissociation [CID]) for additional selectivity, leading to a high analytical dependency on the MS instrument and the experimental conditions used (Kennedy et al., 2016; Yanini et al., 2018).

Teunissen et al. (2017) performed various selectivity studies of eight amfetamine-like compounds via PSI-MS/MS. These included 3,4-methyleneoxyamphetamine (MDA) and PMMA, which have identical integer molecular masses (but different elemental compositions: MDA C_{10}H_{13}NO_2, PMMA C_{11}H_{17}NO = 179 Da) and share fragment ions of the same mass. In this case, it is critical to look for a diagnostic fragment to help distinguish them without compromising the limit of detection (Teunissen et al., 2017). Isobaric and closely related synthetic cathinones were analyzed via DART-MS by Lesiak et al. (2013), alone and in mixtures, and were differentiated using CID and high mass accuracy. The two cathinone isomers, isopentedrone and 3-methylethcathinone, were indistinguishable using high mass accuracy measurements alone, but a few unique and/or dominant peaks were detected for each under CID conditions (Lesiak et al., 2013).

Nevertheless, different compounds with the same m/z may show equal mass transitions, especially if the analyte consists of carbon, hydrogen, nitrogen, and oxygen elements only and its functional groups are common (Teunissen et al., 2017). In this case, the relative abundance of the transitions can be used to create transition ion ratios (TIR), which is the ratio of one fragment over another from the same precursor. For example, phenethylamine compounds that have been identified as potential false positives using PSI-MS/MS are 2-fluoroamphetamine (2-FA) and 3-fluoroamphetamine (3-FA) versus 4-FA, and these can be differentiated based on variations in TIR (Johansen & Hansen, 2012; Teunissen et al., 2017). The isomeric cannabinoids JWH-250, JWH-302, and JWH-201, which are difficult to distinguish by GC-MS alone, were found to have some common fragments with PSI-MS/MS, but different TIR that allowed the distinction of ortho- (JWH-250), meta- (JWH-302), and para-methoxy (JWH-201) position on the aromatic ring (J. Kennedy et al., 2016). However, this can be complicated with compounds that present low abundance transitions or a limited number of fragments, such as NBOMe derivatives (Carvalho et al., 2016). Botch-Jones et al. (2016) used DSA-MS to identify various NBOMe designer drugs and, in the absence of chromatography, the positional isomers 2T4-NBOMe and 2T7-NBOMe were indistinguishable. Fragmentation experiments were performed in an attempt to differentiate them using in-source CID, but there were only two fragments produced and the ratios between precursor and fragment ions for the two compounds could not be discerned (Botch-Jones et al., 2016).

One option that has been widely explored to improve AIMS selectivity is ion-mobility spectrometry (IMS), which adds another dimension of separation, although
some false positives may result from similar reduced mobilities between substances (Gwak, 2015; Gwak & Almirall, 2015; Lian et al., 2017). For example, Romão et al. (2011) reported the inability to differentiate m-CPP (meta-CPP), a polar pipеразine class drug, from its positional isomers (ortho-CPP and para-CPP) using EASI-MS; traveling wave IMS and nuclear magnetic resonance (NMR) measurements were required to distinguish them. The applicability of this complementary approach was also explored by Gwak and Almirall in 2015, using IMS as a presumptive test and DART-MS for rapid qualitative analysis (less than 1 min), achieving successful characterization of 35 NPS. Their data illustrate the benefits of this dual approach, which, while highlighting the narrower dynamic linear response of IMS with high analyte concentrations, it is certainly attractive for sub-nanogram detection of drugs of abuse (Gwak & Almirall, 2015). Alternatively, approaches combining IMS with AIMS-MS in a single instrument have been reported, though not yet with NPS (Tsai et al., 2018; Weston et al., 2005). This instrument allows for lower limits of detection and quantification to be achieved, improved selectivity and has potential for forensic analysis while avoiding sample reanalysis in two different instruments. A remarkable example is PSI coupled with high field asymmetric waveform ion mobility spectrometry (FAIMS) integrated with MS, which was used by Manicke and Belford for the separation of closely related opiate isomers: morphine, norcodeine, and hydrocodone (Manicke & Belford, 2015).

Lastly, NPS are oftentimes found in mixtures with other illicit drugs, impurities and/or cutting agents, and their use is frequently associated with polydrug use; these other components can interfere with the analysis and mask the presence of NPS, that further emphasizes the need for high sensitivity, selectivity, and resolution. For instance, Habala et al. (2016) identified up to three SCRAs together in real case samples using DART-MS; comparatively, synthetic cathinones, piperazines, and amphetamine-type substances in mixtures have been frequently reported (Kauppila et al., 2011; Lesiak et al., 2013; Musah et al., 2014; Stojanovska et al., 2014).

4.3 Type of chemistry

The chemical diversity in the NPS family hampers the development of a unique and selective AIMS screening method for all NPS classes. The physicochemical properties, such as lipophilicity and formation of cationic or anionic precursor ions, define the set of compounds compatible with a given method. In general, ESI-based techniques are more effective with polar analytes whereas chemical ionization-based techniques yield better results with lower polarity compounds (Feider et al., 2019; Huang et al., 2011; McCullough & Hopley, 2021; Monge & Fernandez, 2015). For example, polar substances such as cathinones, containing an additional polar β-keto-group on the side chain of the phenethylamine structure, would be better suited for AIMS techniques like DESI rather than plasma-based techniques (Dalgleish et al., 2013). The opposite would be for small low polarity substances, like some SCRAs. However, this needs to be further investigated.

Interestingly, the hydrophilic nature of paper substrate in PSI can compromise the desorption of polar analytes depending on the paper-analyte interaction strength (e.g., hydrogen bonding and van der Waals forces), which directly interfere with sample ionization. On the other hand, low-polarity analytes interact less with the cellulosic paper, which favors their elution while improving analyte ionization. Chemical modifications of the paper surface can help improve the efficiency of PSI and increase analyte-specific sensitivity (Damon et al., 2016; Filho et al., 2020; Rossini et al., 2020).

The technique DAPPI has demonstrated good results with compounds covering a wide range of polarities, including SCRAs, phenethylamines, cathinones, steroids, and piperazines. Its sensitivity is like that of DESI when analyzing polar analytes and superior when analyzing neutral and non-polar analytes (Haapala et al., 2007; Kauppila et al., 2011; Luosujärvi et al., 2010).

Other thermal desorption-based sources seem to be restricted to low-polarity and volatile compounds; DART, ASAP and, to a larger extent, LTP (since it uses lower temperature), are primarily small-molecule techniques, enabling effective ionization of compounds with molecular weights generally not exceeding one kDa (Hajslova et al., 2011). For instance, Wang et al. (2018) analyzed phenethylamines, SCRAs, synthetic cathinones, and piperazines via LTP-MS; higher molecular weight compounds, like SCRAs, were reported to perform poorly without thermal assistance (Wang et al., 2018). To increase the mass range of these ionization sources, increasing the temperature or the volatility of the analytes by derivatization has been shown to help. As an example, Musah et al. (2012) found that the SCRA AM-251 (555.2 g/mol), which is of considerably higher mass than JWH-015 (327.4 g/mol), required higher temperature (+50°C) than the rest of the SCRAs analyzed for its detection with DART-MS (Musah et al., 2012). Also, Dalgleish et al. (2013) achieved increased selectivity of mephedrone and methylone using an LTP source by derivatization with trifluoroacetic anhydride, however, sensitivity was notably diminished (Dalgleish et al., 2013).
In addition to polarity characteristics, nitrogenous species like NBOMes, synthetic cathinones, and some SCRAs (JWH series) are excellent Lewis bases (low pKb) and are easily identified in positive ionization mode, whereas other SCRA substances such as HU-211 and the CP series have been successfully analyzed in negative mode because of the hydroxyl group (Bianchi et al., 2019; Domingos et al., 2017). This implies that analysis for unknown samples should be performed in both positive and negative modes, which can be a potential disadvantage compared with LC-MS.

Switching polarity creates complexities with some ambient ionization sources, especially those which do not employ voltage. EASI is one such example: although it is possible to switch polarities by applying potential in the path of the sonic spray, it requires two independent power sources (Chubatyi et al., 2012). On the other hand, with DART it can be done simply by changing the polarity of the disc electrode and grid (Cody et al., 2005).

Some reports regarding PSI-MS applied to pharmaceuticals and drugs describe the development of two different methods for positive and negative modes, implying that samples should be re-analyzed (McKenna et al., 2017, 2018) (provided that the sample volume is sufficient). Alternatively, the implementation of a dual source has been explored with DESI-MS to avoid the need for repeating the analysis (Williams et al., 2006). Nevertheless, literature on NPS analysis using AIMS has not revealed this concern so far.

### 4.4 | Sensitivity

AIMS have been mostly applied to surfaces or solid materials since biological matrices are more complex. Biomatrices contain other constituents that are found in much larger amounts than xenobiotics; these background species can generate chemical noise, reducing the signal-to-noise ratio (S/N), and can interfere with analyte signal (i.e., cause ion suppression or enhancement), thus compromising analytical sensitivity and reproducibility. These effects have mostly been observed with sources that allow ionization of molecules covering a wide range of molecular weights, like DESI, DART, and DAPPI (Birk et al., 2019; Jackson et al., 2010; Kuo et al., 2019; Lesiak et al., 2014a; Suni et al., 2011; Wang et al., 2018).

Techniques that use atmospheric pressure photoionization (APPI) and APCI sources, like DAPPI and DSA respectively, usually have fewer matrix effects than ESI-based techniques. The reason for this is that in APCI, the analytes are first transferred into the gas phase and then chemically ionized; in APPI, the use of a specific dopant and photons for ionization increases selectivity since matrix components are often not ionized this way (Zhou et al., 2017).

Composition complexity of solid materials, such as non-homogeneity of herbal samples, tablets, and powders, have also been shown to create interferences that can obscure NPS presence, and high analyte concentrations, which can be found in confiscated drug samples, can lead to instrument contamination and memory effects (Habala et al., 2016; Kauppila et al., 2011; Stojanovska et al., 2014). For example, when analyzing powder samples using DART, particulates can be swept up in the gas stream and contaminate the MS inlet (Brown et al., 2016).

On the other hand, in the PSI technique, powders are dissolved and spotted onto the substrate, and proteins and salts from blood and urine matrices bind well to the cellulosic paper, which prevents clogging at the spray tip and reduces background noise, allowing for LODs in the low picogram range to be achieved (Kennedy et al., 2018; Ma et al., 2015; Vandergrift et al., 2018). Nevertheless, a few matrix effects using PSI have also been reported and, as previously mentioned, the solvent choice has a greater impact on matrix effects than paper properties (Bills & Manicke, 2020; Bills et al., 2018; Bruno et al., 2017; Filho et al., 2020). As an example, Teunissen et al. (2017) observed up to 48% of interference in the lower limit of quantification (LLOQ) of amphetamine-like compounds in whole blood using PSI-MS; visual inspection revealed that these samples were extremely viscous and full of clots, potentially causing the interference (Teunissen et al., 2017). Also, Birk et al. (2019) reported matrix effects while using blotter papers as PSI substrate (Birk et al., 2019).

Many designer drugs require LODs well below the ng/ml range due to their high potency (UNODC, 2019). Without previous separation or a clean-up step, these may increase because of poor analyte recovery and ion suppression. Most AIMS techniques demonstrated detection of NPS down to the low ng/ml range, though this is sometimes difficult to estimate since LODs are not always reported (Teunissen et al., 2017; Vandergrift & Gill, 2019; Vandergrift et al., 2018; Vircks & Mulligan, 2012). The analysis of cathinones and SCRA in oral fluid using LTP-MS has shown LODs 10-100 times greater than with LC-MS/MS (Wang et al., 2018), and five to six times higher when using PSI-MS to analyze methadone in fingerprints (Czerwinska et al., 2020).

To reduce the matrix effects and achieve lower detection limits, sample pretreatment steps such as SPE, liquid–liquid extraction (LLE), solid-phase microextraction (SPME), and/or preconcentration, can be incorporated, however, this addition will lengthen the analysis time. Also, modifications of the porous substrate
in PSI (e.g., treating the paper substrate with various coatings) can help lower the LOD further (Bills et al., 2018).

Solid-liquid extraction (SLE) has been the most used sample preparation technique in combination with AIMS for the analysis of NPS (Botch-Jones et al., 2016; Habala et al., 2016; McGonigal et al., 2017). An example is by Nie et al. (2016), who reported using SLE on herbal samples before performing DART-MS analysis of cathinones, phenethylamines, and SCRA, reaching LODs as low as 5 ng/ml (Nie et al., 2016).

In addition to matrix complexity, sample volume obtained for toxicological analysis can be limited, further compromising the sensitivity. Bianchi et al. (2019) showed that the direct analysis of oral fluid using DESI-MS was not feasible because of the strong matrix effects, which prevented the successful detection of the analytes of interest (SCRA, cathinones). Because of the small amount of oral fluid obtained, micro-extraction by packed sorbent (MEPS) was chosen for sample clean-up and enrichment, and LLOQs within 50–500 ng/ml were achieved. This integrated extraction step considerably lengthened the analysis time (15 min) (Bianchi et al., 2019). Alternatively, techniques using a sample probe, such as the capillary tube in ASAP, allow sampling of tiny amounts of solid and liquid samples that may not be visible to the naked eye but still yield good results for identification. Although ASAP has shown LODs in the low ng/ml range for other drugs of abuse in urine, in the case of NPS the only LOD reported is in the low microgram range for compounds impregnated on a solid surface (Fabregat-Safont et al., 2020).

Another issue associated with sensitivity is the wide variability in composition and dosage of street samples, making it difficult to estimate the LODs required. NPS are often sold as unregulated complex mixtures and it is challenging to determine the actual dosages of these products as the concentration of ingredients across samples is inconsistent (Bills et al., 2018; Lesiak et al., 2014b).

4.5 | Quantification

Quantitative analysis with AIMS has shown successful results from biofluids and solid surfaces, mostly coupled with sample pretreatment procedures, and HRMS and MS/MS for increased selectivity and sensitivity (Kennedy et al., 2010, 2018). Nevertheless, matrix effects and inadequate precision increase the variability of analytical performance, limiting quantitative capabilities. The impact of matrix effects and signal fluctuations can be diminished using an IS, however, depending on the nature of the sample, the IS addition can become a significant challenge (Kuo et al., 2019; McCullough & Hopley, 2021). For instance, plant matrices can show complex mass spectral profiles, and the application of IS with a pipette can lead to nonhomogeneous distribution. An alternative option can be to spray the sample with an IS solution (Nie et al., 2016).

Most of the quantitative studies have been performed in biomatrices using PSI because of the ease of IS addition (Czerwinska et al., 2020; Domingos et al., 2017; Ma et al., 2015). For example, Kennedy et al. (2018) quantified fentanyl derivatives in real urine samples, in less than 1 min and using 10 μl of the sample and achieved LLOQs of 0.5 ng/ml and LODs as low as 2 pg/ml. The IS was added to the paper before analysis (Kennedy et al., 2018). Other sources, such as LTP and DESI, have been used to quantify cathinones and SCRAs in oral fluid, and LLOQs in the ng/ml range have been achieved (Bianchi et al., 2019; Wang et al., 2018). However, LTP-MS presented modest repeatability, and even though this was better with DESI-MS, the latter included a MEPS procedure that significantly increased the analysis time (15 min) and LLOQs were high (50–500 ng/ml).

Although little fragmentation is reported with AIMS, it is worth mentioning that labile compounds, such as some cannabinoids or acyl glucuronide metabolites can undergo thermal- and photo-degradation, and can also decompose in the source region, resulting in an inflated concentration of the parent drug and affecting quantification results (Bills & Manicke, 2020; Kennedy et al., 2016).

4.6 | Molecular coverage, dynamic range, and true unknowns

In analytical laboratories, there is a need to widen the range of detectable compounds as much as possible, especially in the screening phase. In this context, the demand for multi-analyte methodologies is continuously growing, but several requirements must be considered for the successful development of these.

Depending on the application, a wide dynamic range should be covered by the analytical method; expected concentrations may vary depending on the analyte and the nature of the sample (bulk or biological analysis) (Scholz et al., 2020). Therefore, the use of an isotopically-labeled IS is important to ensure quantification over a good dynamic range or to give a reference for the absence of ion suppression when screening for the presence of a particular analyte in a complex matrix. In the case of NPS analysis using AIMS, the dynamic range has been proven to stretch up to three to five orders of magnitude; in
earlier literature, the method’s detection has mostly focused on the lower end of the concentration range, which is of high interest when dealing with NPS in biological matrices (Bianchi et al., 2019; Nie et al., 2016; Nollet & Munjanja, 2019; Wang et al., 2018).

The absence of parent compounds and/or the unavailability of standards/reference spectra will eventually affect laboratories’ turn-around times. This can be tackled by using MS databases to maximize the size of the reference library, including NPS; nevertheless, the determination of true unknown compounds must be achieved by other means.

The combination of AIMS with HRMS allows for nontargeted analysis and formula prediction; fragmentation experiments in silico of the candidate structure can be matched using combinatorial approaches to the observed MS/MS spectra. However, fragmentation patterns are very dependent on the MS/MS conditions used, leading to inter-instrument variations (Lawton et al., 2017).

Moreover, relying exclusively on fragmentation patterns for unambiguous identification can lead to false negatives, as can be the case with structural isomers that fragment similarly. Most mass spectrometers are technically limited to m/z > 50; using smaller fragments diagnostic of substituents might require extending the m/z range to less than 50. For example, ion trap instrumentation can produce a low number of compound-specific fragments as a result of resonant excitation CID, and low mass fragment ions are inefficiently trapped (Lawton et al., 2017).

Determination of a true unknown was reported by Moore et al. (2019) when analyzing 81 seized drug samples via DSA-HRMS. In one case, no controlled substance was identified with GC-MS but there was an unknown peak in the chromatogram whose mass spectrum could not be located at the time in any spectral library accessible to the testing laboratory. The spectrum contained several ions that had previously been recognized as characteristic of fentanyl-type compounds, the chemical formula was predicted with high mass accuracy HRMS and the candidate compound furanyl fentanyl was identified. When the reference material became available, it was purchased, and the identification was confirmed by GC-MS (Moore et al., 2019).

4.7 Validation

The lack of full validation guidelines applied to AIMS methods hampers the introduction of these techniques for routine forensic casework. A few publications have validated methods according to the criteria for Bioanalytical Method Validation of the US Food and Drug Administration (FDA) (US Department of Health and Human Services, & Food and Drug Administration, 2018) or have followed the recommendations of the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (US Department of Justice, 2011).

Repeatability/precision (%RSD or CV%), linearity, and LODs are the variables reported most frequently, along with carryover, matrix interference, and LOQs. However, in most cases, only a few variables are mentioned and there is no clear report of how these were obtained. Lastly, the application to real samples is often reported, though the sample size is sometimes limited (<10).

In general, although LODs are remarkably low, high fluctuations of the absolute signal intensities among the repeated measurements are frequently observed (Kuo et al., 2019). The variability in results is one that causes major concern to the scientific community; some averaging of data is necessary for quantitation, and the precision, which can be as low as a few %RSD, is usually in the range 15%–20% (Cooks et al., 2006).

Reproducibility is affected by the flexible design of ambient sources, the surrounding environment which can affect the sampling (e.g., humidity, temperature, air flows) and irregular sampling surfaces or sample properties, which can affect the desorption process (Lawton et al., 2017). DART and PSI sampling have been shown to be successfully automated in an attempt to increase reproducibility (Bills et al., 2018; Gwak & Almirall, 2015; Habala et al., 2016; Kennedy et al., 2016). Also, matrix interference, as previously discussed, has been widely reported to affect precision and accuracy.

Carryover and recovery are rarely an issue with AIMS since samples are in contact with disposable elements and there is little or no sample extraction. For example, the single use of the paper substrate in PSI highly reduces the risk of carryover (Teunissen et al., 2017). However, memory effects that arise from rapidly introducing samples with analytes in a widely varying concentration range have been reported (Monge & Fernandez, 2015).

The ionization source with better overall performance, versatility, and acceptable validation criteria according to SWGDRUG recommendations, is PSI, although DART has also shown good performance when analyzing NPS. However, there is limited literature on other sources’ application to NPS, and further research would be required to evaluate their performance better.

LTP has shown limited molecular coverage and low ionization efficiency, DESI LODs are highly compromised by matrix effects, and DAPPI, ASAP, DSA, and EASI have only been applied to solid samples and sensitivity data is limited. Also, other AIMS techniques such
as flowing atmospheric pressure afterglow (FAPA), filter cone spray ionization (FCSI), desorption nano-electrospray ionization (nanoDESI), and LDTD, have been applied to NPS, but literature is very scarce, thus it has not been included for the purpose of this review.

4.8 | Practicality

The highlights of the AIMS applicability to NPS include the fast-analytical time, the simplicity, and the high-throughput capabilities through automation (up to hundreds h⁻¹), which makes it very appealing for routine sample screening. Even though the instrument cost can be expensive, the operation cost is lower than with LC or GC, substantially reducing solvent consumption and disposal, and eliminating chromatography maintenance costs. For instance, Mulligan et al. (2018) estimated up to 30% of savings per sample with a validated portable PSI-MS over the current forensic laboratory evidence processing for illicit drug analysis, including various NPS (Mulligan et al., 2018).

The use of AIMS can also lead to savings of substantial resources in terms of both time and cost over color tests and immunoassay disposable and consumable substances) was performed with PSI-MS over the current forensic laboratory evidence processing for illicit drug analysis, including various NPS (Mulligan et al., 2018).

The majority of AIMS techniques are minimally destructive. Although part of the sample may not be recoverable (~10 μl of liquid or 0.5–5 mg of solid, as reported in NPS studies, are needed but not lost in the analysis), usually most of the sample has its chemical features preserved, which would be beneficial in clinical and forensic toxicology where samples may need to be reanalyzed. Also, applicability to a wide variety of sample types is remarkable. DART, DAPPI, EASI, LTP, DESI, and DSA have demonstrated good applicability to solid materials and surfaces, whereas PSI has been successfully applied to analyze both solid materials and bio-samples like urine, oral fluid and blood.

The fact that the ion source does not require to be under vacuum allows the instrument to minimize power consumption on the pumps and be more readily downsized, making AIMS suitable to be made portable and field operable. Robustness and selectivity of portable and miniature MS instruments with ambient ionization sources have been demonstrated with PSI, DESI, and DART for the analysis of various NPS in solid form, including cathinones and SCRA, highlighting their potential use for rapid on-site screening, harm reduction strategies and road-side testing (Brown et al., 2016; Brown et al., 2020; Vircks & Mulligan, 2012). However, development of smaller vacuum systems is still challenging; miniature MS generally suffer from limited pumping capacity (thus, higher operating pressures) leading to reduced ion transfer efficiency and low S/N, which may result in lack of ion detection (Kuo et al., 2019).

Care must be taken with the ambient conditions, such as air flow or humidity, and systematic errors associated with user and instrument. AIMS are soft-ionization techniques, simple and user-friendly, though results with nontrained personnel have shown low reproducibility with PSI-MS (Lawton et al., 2017). To reduce systematic error, instrument operating conditions must be kept consistent throughout the experiment and the MS instruments should be kept clean (Kuo et al., 2019).

Because of its overall analytical performance, low cost, simplicity, and wide range of applications, PSI-MS has shown to be a great option for the rapid screening of NPS in a large array of toxicological samples, including pure materials and biomatrices. On the other hand, the excellent performance of the DART source with solid surfaces puts it in a good position for drug seizure analysis, harm-reduction strategies, and drug testing at music festivals, to name but a few examples. Both techniques are commercially available, in benchtop and portable format, enhancing their reproducibility.

5 | Conclusion

A number of scientific papers have been published describing the identification and characterization of NPS using AIMS, often together with analytical methods for their determination, although only a few studies have been directed to true unknowns. Many of the available NPS have yet to be characterized by the conventional analytical techniques and limited data are available in literature or databases.

The applicability of AIMS to NPS analysis has been demonstrated in numerous proof-of-concept studies, showing good sensitivity, selectivity, accuracy, and precision levels suited for rapid screening and semi-quantitative analysis. However, fully validated and implemented methods are thus far not well established.
Chromatography-based techniques remain the method of choice since validated protocols for them are generally accepted and associated with decades of experience. The shift from these traditional, fully consolidated, techniques will take time and strict quality criteria are required for AIMS to be used in routine forensic or clinical casework.

The overall performance of AIMS techniques applied to NPS is limited by (a) ionization efficiency, which is typically worse than that of traditional sources such as ESI and MALDI, (b) ion suppression, due to other interfering components present in the sample, (c) poor selectivity, as a result of the lack of sample preparation and chromatographic separation, (d) inability to distinguish isomeric or isobaric molecules, and (e) low reproducibility, mostly affected by sampling geometry and surface, sample properties and surrounding environment (Kuo et al., 2019).

Nevertheless, AIMS are promising techniques to reduce analysis time, which is of utmost importance when it comes to rapidly evolving substances. Well suited for fast screening and high-throughput analysis, they could replace nonselective color tests and immunoassays, and time-consuming sample preparation and chromatography-based methods. Modern analytical laboratories experience the pressure of workload, turn-around time, and cost per sample; thus, it is expected that efforts to mitigate AIMS limitations will increase. Future directions currently explored include coupling with IMS to improve selectivity, developing fully automatic platforms and moving toward on-site tests and portability.

Amongst the numerous AIMS techniques that have been applied to NPS, PSI and DART stand out and have shown good analytical results. Their performance is appropriate for the analysis of solid and biological samples, though matrix effects, selectivity, and reproducibility are issues that require more investigation, and confirmation of the results should be performed. With further research directed at (a) understanding the effect of sample complexity on the analyte of interest, (b) the introduction of IS to the sample, and (c) improvements to the instrument design, AIMS shows potential as an alternative technique providing minimal to no sample preparation and fast analysis time in screening applications. These perspectives can be taken as recommendations when considering future research.

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