Direct and fast screening of new psychoactive substances using medical swabs and atmospheric solids analysis probe-triple quadrupole with data-dependent acquisition.

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Abstract

New psychoactive substances (NPS) have become a serious public health problem, as they are continuously changing their structures, modifying their potency and effects on humans, and therefore novel compounds are unceasingly appearing. One of the major challenges in forensic analysis, particularly related to the problematic of NPS, is the development of fast screening methodologies that allow the detection of a wide variety of compounds in a single analysis. In this study, a novel application of the atmospheric solids analysis probe (ASAP) using medical swabs has been developed. The swab-ASAP was coupled to a triple quadrupole mass analyzer working under data-dependent acquisition mode in order to perform a suspect screening of NPS in different types of samples as well as in surfaces. The compounds were automatically identified based on the observed fragmentation spectra using an in-house built MS/MS spectra library. The developed methodology was applied to the identification of psychoactive substances in research chemicals and herbal blends. The sensitivity of the method, as well as its applicability for surface analysis, was also assessed by identifying down to 1 µg of compound impregnated into a laboratory table. Another remarkable application was the identification of cathinones and synthetic cannabinoids in the fingers of potential consumers. Interestingly, our data showed that NPS could be identified in the fingers after being in contact with the product and even after cleaning their hands by shaking off with a cloth. The methodology proposed in this paper can be applied for routine analyses of NPS in different matrix samples without the need to establish a list of target compounds prior to analysis.

Keywords new psychoactive substances; medical swab; atmospheric solids analysis probe; data dependent acquisition; ambient ionization mass spectrometry.

Introduction

During 2018, 52 new psychoactive substances (NPS) were reported for the first time, around one compound each week [1, 2]. This trend has been observed during the last decade, and nowadays more than 700 different NPS are currently being monitored [1, 2]. The continuous rising of novel compounds increases the need of analytical methodologies that allow their fast analysis and identification.

Chromatography coupled to mass spectrometry is the most powerful analytical technique for the analysis of NPS [3] in a wide variety of matrices such as seizures, legal highs, and biological tissues and fluids [4]. In the last years, the development of ambient mass ionisation sources that allow the fast and direct analysis of samples, without any sample treatment, has posed a new promising scenario in forensic analysis [3]. Among the most commonly used for identification of psychoactive substances it can be highlighted the direct analysis in real time (DART) [5, 6], desorption electrospray [7], the recently developed swab touch spray [8], and the atmospheric solids analysis probe (ASAP), based on an atmospheric chemical ionisation (APCI) modified source, which have already proved its potential in toxicological analysis [9–11]. The ASAP source has demonstrated its applicability when coupled to high-resolution mass spectrometry (HRMS) but also to tandem mass spectrometry (MS/MS) with triple quadrupole [9, 12].

In this work, a rapid and efficient analytical methodology based on a modified ASAP-MS/MS system, has been developed for the suspect screening of a wide variety of NPS, and has been applied to different cases related to the consumption of these substances. The glass capillary was replaced by a medical swab in order to allow the determination of NPS in surfaces, including the fingers of a potential consumer. For suspect screening, a data-dependent acquisition (DDA) mode was used in the triple quadrupole instrument to obtain the fragmentation spectra of the compounds. The acquired product ion spectra

were then automatically searched in an in-house MS/MS spectra database for compound identification. The applicability of this methodology for tentative identification of selected NPS was tested in different matrices with emphasis on the sensitivity and reliability of the identification.

Materials and methods

Reagents and chemicals

Herbal blends, powders and pills were purchased in a local smart-shop and were previously analyzed by UHPLC-HRMS for compound identification [13]. Research chemicals had been provided by Energy Control and analyzed by UHPLC-HRMS and nuclear magnetic resonance for compound identification [14]. HPLC-grade methanol was purchased from Scharlau (Scharlab, Barcelona, Spain). Medical swabs were purchased from neoLab (neoLab Migge GmbH, Heidelberg, Germany).

Sample treatment

For direct analysis, a medical swab was placed on the ASAP probe and gently wiped in the sample or surface. 100 µL of methanol was added to the swab, and introduced into the ASAP holder for sample analysis.

Instrumentation

Samples were analyzed using a Xevo TQ-S mass spectrometer (Waters Corp, Manchester, UK) with a triple quadrupole mass analyzer, equipped with an ASAP source (Waters Corp, Manchester, UK). The corona pin current was 2.0 µA in positive ionization mode, and the cone voltage 30 V. Source temperature was stablished at 150 ºC, and desolvation temperature 450 ºC. Nitrogen (Praxair, Valencia, Spain) was used as cone and desolvation gas at 150 and 800 L/h, respectively. MS/MS was operated in DDA acquisition mode. Survey MS scan data were acquired from *m/z* 170 to 450 with a scan time of 50 ms. For automatic MS/MS, the switch threshold was $5 \cdot 10^5$ counts/s, acquiring

data from *m/z* 60 to 450, with a scan time of 50 ms, using a 25 eV collision induced-dissociation (CID) energy (argon 99.995%; Praxair), an isolation window of 1 Da and an exclusion time of 10 s for the previously detected precursor ion. Only one *m/z* value was selected for MS/MS in each survey MS scan. The total run time was 1.5 min.

Data were acquired using MassLynx data station operation software (v4.1; Waters), and processed using MassLynx and MS Search (v2.0; NIST, USA) for automatic compound identification based on DDA MS/MS data. For compound identification, experimental MS/MS spectrum was directly processed with MassLynx, searching in the fragmentation spectra database generated in our laboratory. For automatic search, MS Search software must be installed together with MassLynx.

Results and discussion

Acquisition parameters optimization and building the spectra library

The ASAP source and the DDA acquisition parameters were carefully optimized for the use of swabs. Detailed information about the optimization, as well as the construction of the spectra library, can be found in the **electronic supplementary material**.

Application to blind samples and detection/identification of NPS in different surfaces

In order to demonstrate the applicability of the developed methodology for identifying the active compounds, different experiments were performed to assess selectivity and sensitivity of the swab-ASAP-MS/MS DDA.

Firstly, the identification of NPS present in blind research chemicals and legal highs samples was tested. The selected products included herbal blends, pills, crystal and powder samples, containing NPS of different families. **Figure 1** shows the identification of the synthetic cathinone butylone in a legal high sample named *Euforia*, purchased in a

local smartshop through its webpage [13]. The automatic MS/MS function shows the presence of a certain ion with a high response (**Figure 1A)**. When the MS/MS spectrum was searched in the database using the MS Search, only one compound presented a match higher than 800 (minimum value for considering a compound tentatively identified), as shown in **Figure 1B**.

The applicability of this approach was supported by the analysis of several research chemicals containing synthetic cathinones, synthetic cannabinoids, opioids or tryptamines. For example, the synthetic opioid U-47700 was tentatively identified in a powder sample by swab-ASAP-MS/MS DDA. In this case, the swab was wiped into the plastic bag that contained the product. The opioid was tentatively identified with a match of 859.

The high sensitivity observed when analyzing these products, encouraged us to perform sensitivity tests using the swab-ASAP. For this purpose, a small amount of compound was placed onto the laboratory table using a certain volume from a stock solution at high concentration (e.g., 10 µL from a 1 mg/mL stock solution). In order to simulate a real situation, the solvent was allowed to evaporate before applying the swab. In this experiment, 12 µg of the synthetic cannabinoid AMB-FUBINACA and 12 µg of the tryptamine 5-MeO-MiPT were satisfactorily identified, as it can be observed in **Figure 2A and 2B**. Moreover, the methodology allowed the identification of 1 µg of the synthetic cathinone 3,4-MDPV placed onto the laboratory table as shown in **Figure 2C**.

To complete the whole set of experiments, the swab-ASAP-MS/MS DDA analysis was applied to the detection and identification of NPS in fingers of potential consumers who had touched legal highs with their hands. Experiments consisted on the simulation of somebody snorting a powder sample or preparing a cigarette with an herbal blend, cleaning subsequently his hands by shaking off with a cloth. After cleaning their hands

no traces of powder or herb were observed, being apparently clean. However, the analysis of the finger surface by swab-ASAP-MS/MS DDA revealed the presence of intense peaks. Based on the observed fragmentation, it was possible to identify α-PVP after "snorting" (**Figure 3A**), as well as the synthetic cannabinoids XLR-11 and UR-144 after "preparing the cigarette" (**Figure 3B**).

This approach has proved its potential for the rapid suspect screening of the compounds present in legal highs and research chemicals, as well as its applicability for detecting NPS in surfaces, such as the fingers of a potential consumer. Nevertheless, it must be continuously updated, including more and more fragmentation spectra for NPS, especially for novel compounds. The major handicap is, nowadays, the lack of on-line spectral libraries for NPS when using QqQ instruments, similarly to those available for HRMS [15]. Additionally, details about the use of this approach for the identification of isomeric compounds are also included in **electronic supplementary material.**

Conclusions

The developed methodology, based on swab-ASAP-MS/MS DDA, for the suspect screening of NPS in seizures and different surfaces in contact with a variety of products has demonstrated its applicability with high sensitivity and selectivity. It has allowed the identification of different families of NPS in several legal highs and research chemicals tested. The identification was fast, without the need of any sample treatment, and it was automatically performed by searching the acquired fragmentation spectra in an in-house built spectra database. The use of medical swabs was also tested for the analysis of different surfaces that were in contact with NPS, supporting the suitability of this approach for detecting low amounts of these compounds in a laboratory table, as well as in the fingers of a person who used legal highs. The methodology proposed in this work

should be continuously updated by including the fragmentation spectra of novel NPS. This would allow to build a wide spectra database that would notably facilitate the routine monitoring of the ever-changing NPS market. Additionally, future work will include the possibility of the use of swab-ASAP-MS/MS for quantification purposes, using calibration curves or isotope pattern deconvolution quantification for those compounds with isotopically-labelled standard available.

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Competing Interests

The authors declare that they have no conflict of interest.

Author contribution

D.F-S. and M.I. conceived the work. D.F-S., D.F-B. and M.M-P. performed sample treatment, instrumental analysis and data process. D.F-S., D.F-B and M.I. interpreted and discussed the results. F.H. and J.V.S. contributed with reagents and analytical tools. D.F-S and M.I. wrote the first draft of the manuscript. J.V.S, and F.H. provided useful comments and feedback for the manuscript.

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Figures

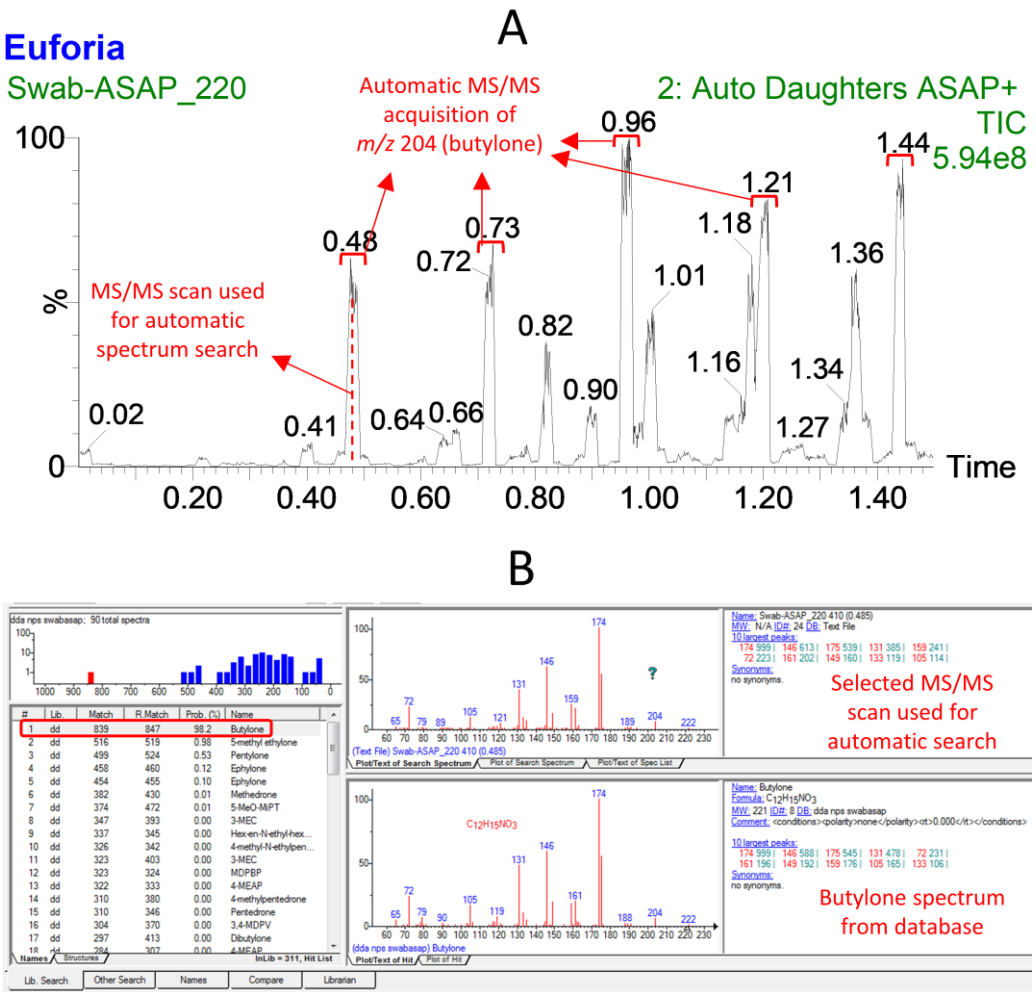

Figure 1. Identification of the synthetic cathinone butylone in a legal high sample. **A** Automatic MS/MS function generated during DDA analysis. **B** Compound identification using MS Search v2.0 and the in-house built database.

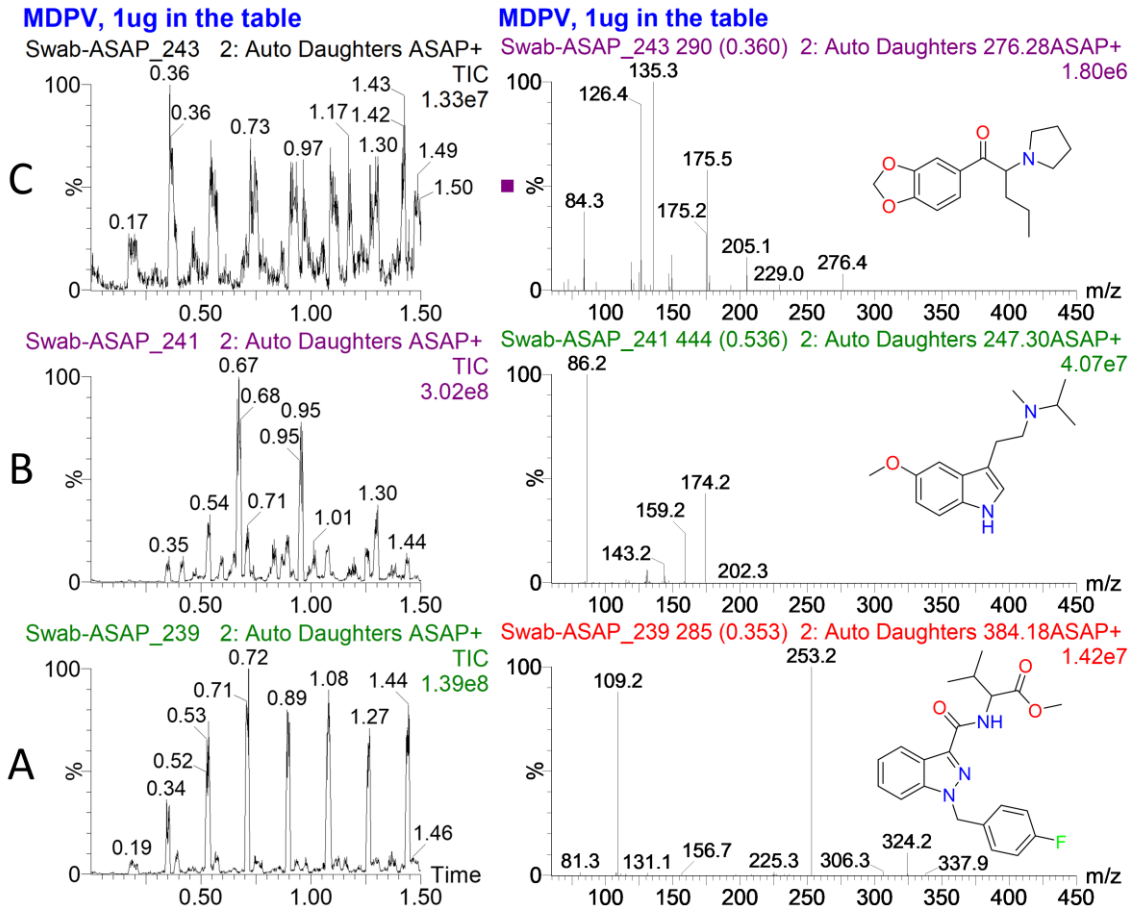

Figure 2. Analysis of NPS placed in the laboratory table. **A** 12 µg of the synthetic cannabinoid AMB-FUBINACA. **B** 12 µg of the tryptamine 5-MeO-MiPT. **C** 1 µg of the cathinone MDPV.

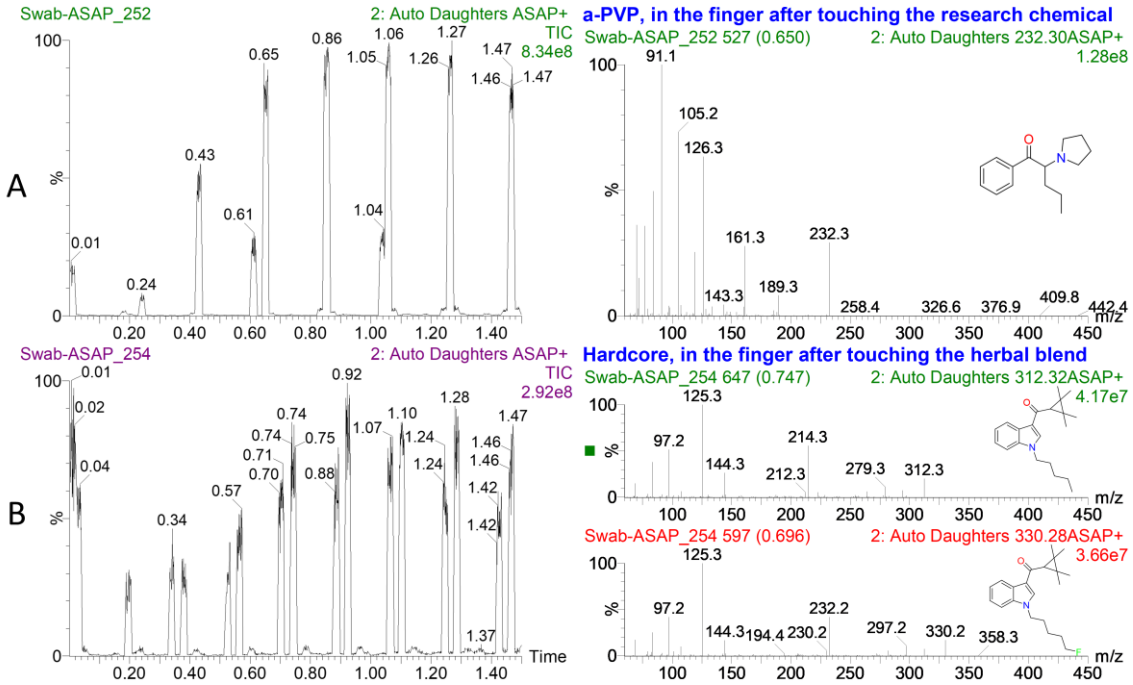

Figure 3. Analysis of NPS in the finger of a volunteer who had touched different products and had cleaned his hands by shaking off with a cloth. **A** Identification of the cathinone α-PVP after being in contact with a powder sample. **B** Identification of the cannabinoids UR-144 and XLR-11 (halogenated compound) after being in contact with an herbal blend sample.