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The targeted analysis of New Psychoactive Substances in oral fluid through chromatographic-spectrometric methods: review of recent findings

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Abstract. – OBJECTIVE: The oral fluid was demonstrated as an effective matrix to assess drug consumption in forensic settings. Recently, the increasing number of intoxications related to New Psychoactive Substances raised the attention of the scientific community. To this concern, different analytical methods to detect and quantify NPS in oral fluids were developed and validated, most of them based on hyphenated techniques.

MATERIALS AND METHODS: A broad-ranging search was conducted on multidisciplinary research databases using "New Psychoactive Substances", "oral fluid", "toxicological analysis", "analytical method", "targeted method", "HPLC–MS/MS", "GC-MS", "GC-MS/MS" alone or in combination as search strings. All research articles published between 2017 and 2021 were considered.

RESULTS: Different chromatographic-spectrometric methods to detect and quantify the NPS in oral fluid were reported in the literature. The classes of NPS explored were synthetic cannabinoids, synthetic cathinones, new designer benzodiazepines, synthetic opioids, fentanyl analogues, tryptamines, and phenethylamines. The most used technique was HPLC–MS/MS due to the sensitivity and high throughput. The GC-MS technique was preferred for synthetic cannabinoids, anyway different HPLC–MS/MS methods were developed. Moreover, the LC-HRMS technique was applied for the development of an analytical assay to detect new synthetic opioids and fentanyl analogues.

CONCLUSIONS: The analytical interest on oral fluid as an effective matrix to assess drug exposure is increasing. The hyphenated techniques were demonstrated effective in the detection of NPS in oral fluids. The most suitable techniques are HPLC–MS/MS due to the sensitivity and the possibility to include different classes of substances in a single analytical run.

Key Words:
Oral fluid, New psychoactive substances, Chromatographic-spectrometric method, HPLC-MS/MS, GC-MS.

Introduction

Since the 1970s, oral fluid (OF) has been studied as a biological matrix alternative to blood to disclose current consumption of psychotropic drugs[1-4]. Some scholars[5] have documented its reliability in OF testing for psychoactive drugs in drug treatment, workplace drug testing, pain management and driving under the influence of drugs (DUID) programs. The transition of a drug from the blood or plasma to another fluid (or matrix) has been extensively studied. In general, drug passage into a fluid (or matrix) occurs by passive diffusion, being regulated by the physicochemical characteristics of the drug (such as molecular weight, molecular volume, dissociation constants, lipid solubility) and the pH of blood and OF, fraction bound to plasma proteins and salivary flow rate[3]. For a lipid-soluble compound, the ratio of total drug concentration (ionised and unionised) in a fluid which is separated from the plasma by a lipid barrier to that in plasma, may be expressed by a modified version of the Henderson-Hasselbalch equation[2]. According to that, weak bases unbound drugs, as the majority of classic drugs of abuse, will concentrate in fluids whose pH is lower than that of plasma[4]. Indeed, these drugs will achieve concentrations similar to those in plasma only in fluids with a pH approaching that of plasma.

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The fluid/plasma concentration ratio will be near unity for neutral drugs[2]. To this concern, an increasing interest of toxicologist to this matrix was registered, although the most important limitation is represented by the non-applicability of OF testing in some toxicological cases, such as post-mortem detection of drug to assess the cause of a suspected fatal intoxication[5]. A new psychoactive substance (NPS) is defined as "a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions"[6]. Since the beginning of new century, more than eight hundred new psychoactive substances have entered the illicit street and web markets, posing continuous new serious health threats for drug consumers[6]. In recent years, hundreds of NPS have been synthesized belonging to different structural and pharmacological class. The most abundant class is stimulants, followed by cannabinoids and opioids, including fentanyl analogues and new synthetic opioids. The harm from use of NPS is more noticeable at the individual level than at the aggregated population level, with the exception of NPS opioids, especially in North America where an epidemic of opioids is causing an alarming number of fatal intoxications[7]. In this scenario, the development and validation of proper analytical methods to detect and quantify the NPS in biological fluids may represent an essential tool to promptly individuate the cause of intoxication[8].

Materials and Methods

A literature search was performed on multidisciplinary research databases, such as PubMed, Scopus, Science Direct, Web of Science, and Research Gate, to identify relevant scientific publications from January 2017 to October 2021, through the following search strings and Medical Subject Headings terms: "New Psychoactive Substances", "oral fluid", "toxicological analysis", "analytical method", "targeted method", "HPLC-MS/MS", "GC-MS", "GC-MS/MS" alone or in combination. All research articles published between 2017 and 2021 were taken into account and independently reviewed by two of the co-authors in order to assess their suitability in the development of this review.

Results

Synthetic Cannabinoids

Together with synthetic cathinones, synthetic cannabinoids (SC) are the class of NPS mostly abused to mimic and enhance the subjective effects of phytocannabinoids contained in the cannabis plant[5]. Three different SC: JWH-122, JWH-210, UR-144) have been identified in the consumers OF by last generation GC-MS screening method then quantified with their respective metabolites JWH-122 N-(4-OH), JWH-210 N-(4-OH), JWH-210 N-(5-OH) and UR-144 N-(5-OH) using UHPLC-HRMS[9].

In the same 2020, two investigation groups validated comprehensive analytical methods to measure a great number of SC in OF. The first one by Mulet et al[10] sought to screen 72 SC (among which 33 from JWH-family and 17 from PINACA-family) and metabolites in OF by LC-MS/MS using online solid phase extraction (SPE). The second one by da Cunha et al[11] screened 104 NPS, among which 23 SC in OF samples by UHPLC–MS/MS. Sorribes-Soriano et al[12] developed a GC-MS assay to detect of third generation synthetic cannabinoids (5F-ADB, MMB-CHMICA, THJ-2201, CUMYL-4CN-BINACA and MDMB-CHMCZCA) in OF. Extraction of OF samples by microextraction by packed sorbent (MEPS) was achieved. In 2019, Anzillotti et al[13] described a pilot study by solid-phase microextraction (SPME) coupled to gas chromatography/mass spectrometry (GC-MS) to determine synthetic and natural cannabinoids in OF. In the same year, a fast UHPLC–MS/MS method was presented by Malaca et al[14] for the simultaneous determination of 13 different psychoactive drugs, belonging to different chemical classes (6-monoacetylmorphine, morphine, codeine, cocaine, benzoylecgonine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, delta-9-tetrahydrocannabinol, cannabidiol, mephedrone, ketamine and 5F-AKB48 (5F-APINACA) in OF.

In 2018, Williams et al[15] reported a validated method to detect 19 SC in OF (AM2233, JWH-200, AB-005, AB-FUBINACA, AB-PINACA, AB-CHMINACA, AM2201, RCS-4, JWH-250, STS-135, JWH-73, XLR-11, JWH-251, JWH-18, JWH-122, JWH-19, UR-144, JWH-20 and AKB-48). A rapid LC–MS-MS analysis with minimal sample preparation was proposed and validated in accordance with National Association of Testing Authorities (NATA) guidelines. Rocchi et al[16]

determined 31 NPS standards, (synthetic cathinones, piperazines, phenethylamines, and synthetic cannabinoids) in OF without any real sample to prove methodology robustness by means of microextraction by packed sorbent followed by UHPLC-MS/MS. The SC included in this study were seventeen (AM-1220, JWH-200, AB-005, JWH-018 N-pentanoic acid, JWH-018 N-5-hydroxypentyl, WIN-55, XLR-11 N-4-hydroxypentyl, MAM-2201 N-pentanoic acid, JWH-073, UR-144 N-5-hydroxypentyl, JWH-250, MAM-2201, XLR-11, JWH-018, JWH-081, JWH-122, UR-144). OF samples clean-up as performed by using a MEPS syringe with a C18 sorbent inside the barrel-in-needle (BIN)[16].

Synthetic Cathinones

UHPLC-MS/MS has been the most used analytical technique for the determination of synthetic cathinones (SCath) in OF.

In 2017, Ares et al[17] applied an UHPLC-MS/MS method with sample microextraction by packed sorbent (MEPS) pre-treatment for the determination of SCath and classic drugs (opiates, cocaine) in OF from twelve patients on a methadone or buprenorphine/naloxone substitution therapy programme. Unfortunately, none of them resulted positive to SCs.

Similarly, Fernandez et al[18] applied their UPLC-MS/MS method for the quantification of drugs and SCath to 15 OF samples from patients on a detoxification programme and SCs were not detected.

In the same year, Williams et al[19] presented a new UHPLC-MS/MS method for the determination of "Bath Salts" in OF. Since the Australian Standard Procedures were not comprehensive of NPS and SCath may cross-react in drug screen test kits giving false-positive results for amphetamine, the authors developed and validated a specific method for NPS including cathinones. The method was designed for workplace drug testing and applied to pre-screened OF samples.

Similarly, Rocchi et al[16] in 2018 for the quantification of NPS, including 10 SCath, in OF. No authentic OF samples were tested but the method was developed to be used in a high throughput laboratory for confirmatory analyses.

Also significantly, da Cunha et al[11] presented a screening method for the detection in OF of 104 NPS including nineteen SCs. Finally, with a comprehensive UHPLC–MS/MS method for the detection and quantification in OF and other matrices of 77 NPS 24 classic drugs and 18 related metabolites, Di Trana et al[20] included 16 different SCath (including 16 SCs) in their 2020 study.

Phenethylamines

One of the most recent methods, by da Cunha et al[11] in 2020, consisted of an LC-MS/MS-based targeted OF screening technique that covered a broad range of phenethylamines from NBOmes derivatives and from 2C family.

Accioni et al[21] developed an LC-MS/MS assay to quantify phenethylamines, such as amphetamine derivatives. Sample treatment and extraction of analytes were simultaneously achieved by applying supramolecular solvents (SUPRAS) tool and the efficacy of this approach[20]. LC-MS/MS was also applied to measure 4 fluoro-amphetamine (4-FA) in OF[22]. In 2018, Rocchi et al[16] developed and validated an UHPLC–MS/MS assay in OF for 31 NPS belonging to different chemical and toxicological classes comprising 2-CB. OF sample preparation was based on protein precipitation followed by clean-up utilizing microextraction by packed sorbent (MEPS). In 2017, Williams et al[15] provided another validated method for the rapid detection of 14 phenethylamines in OF (MDA, PMA, TMA, MDEA, MBDB, MTA, 2C-B, MDPV, DOB, DOET, 2C-T-7, 25C-NBOMe, 25B-NBOMe and 25T4-NBOMe).

Tryptamines

Between 2017 and 2021, only one analytical method has been reported for the determination of tryptamines in OF. The method described, was developed for NPS detection in OF samples of unknown consumers, comprising 7 different tryptamines. Two samples were positive for Acetyl-O-dimethyltryptamine (AcO-DMT)[23].

Designer Benzodiazepines

Currently, two different analytical methods have been set up and validated to determine the presence of designer benzodiazepines in OF. A number of designer benzodiazepines was included in the method: bentazepam, diclazepam, etizolam, flubromazepam, phenazepam and pyrazolam. Using a similar methodology, metizolam was detected in OF, hair, sweat and exhaled breath of a 54-year-old healthy man orally administered with 2 mg tablet of metizolam. OF was collected over 8 hours using the NeoSalTM (Neogen) device and analysed using UHPLC-MS/MS. Metizolam was detectable in saliva for 8 hours, with concentrations always lower than 1 ng/

mL[24]. The third method was used to assess the detectability of two designer benzodiazapine in OF in two different self-administration studies. Two of the authors ingested 0.25 mg and 6 mg of flunitrazolam and deschloroetizolam, respectively. OF was collected over 8 hours using the NeoSalTM (Neogen) device and analyzed using UHPLC-MS/MS[25,26].

Non-Fentanyl Derived Synthetic Opioid

Currently, little is known about analytical techniques for the detection of non-fentanyl derived novel synthetic opioids (NSO).

An LC-QTOF-MS assay was developed to detect 4 new synthetic opioids in OF and urine samples[27]. Another study[28] reports the development of an analytical method validated for the detection and quantification of 7 NSO. OF sample were collected as described above from 18 anonymous detainees, though no samples gave any positivity for NSO investigated.

Fentanyl and Analogues

5 analytical methods for fentanyl and its analogues have been reported in literature from 2017 to 2021. One analytical method for 9 fentanyl analogues, 1 metabolite and 4 novel opiates in OF was developed to perform the analysis of 30 heroin users' real samples. Moreover, OF samples were compared with urine to evaluate OF as alterative matrix in overdose cases[29]. Another method was developed for the screening on 14 fentanyl analogues in 20 post-mortem OF and blood samples using a LC-QTOF-MS[30]. In 2019, a rapid and simple quantitative touch spray-MS assay was developed to detect 30 drugs of abuse including fentanyl and fentanyl analogues in OF[31].

The sample of OF was collected from the infant to evaluate fentanyl neonatal exposure using a UHPL-MS/MS. Out of three samples collected in one sample was detected fentanyl and norfentanyl[32]. 17 fentanyl analogues were included in a method for NPS detection in OF of unknown consumers using UHPL-MS/MS, whereas only 1 out of the14 real OF samples collected resulted positive for carfetanil[5].

Conclusions

OF was demonstrated to be an effective matrix for assessing exposure to NPS of different classes, e.g., synthetic cathinones, fentanyl analogues, designer benzodiazepines. The best advantage is represented by the ease of sample collection through non-invasive techniques. Among the others, the chromatographic-spectrometric method is widely used as confirmatory method, especially HPLC-MS/MS due to its sensitivity, the possibility to include a wide number of different molecules and the high-throughput of methods. Moreover, the GC-MS technique finds an application especially for synthetic cannabinoids.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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