Immunochromal Screening for Synthetic Cannabinoids in Workplace Drug Testing

Abstract

Background: synthetic cannabinoids (SC) have become a more recently abused cannabimimetic drugs; their abuse is an ongoing health issue worldwide. They are rarely detected in urine which is the most common matrix employed in workplace drug testing.

Methods: this technical note refers to the investigators experience with Randox (DOA V Synthetic Cannabinoids panel Biochip Array Technology) and Concateno (Drug Screen test) immunoassays for SC, 50 authentic and anonymous urine samples were collected and analyzed from workers. Drug free urine samples to which SC were added showed the expected results in term of the declared cross reactivity’s.

Results: Two urine samples obtained from workers showed positive results with Randox DOA V kit for phenyliperazines, the same urine specimens were negative with Concateno kit, which doesn’t include phenylpiperazines in the analytes panel. Additionally Concateno immunoassay found five positive samples for SC not revealed by Randox kit.

Conclusion: The current paper discusses problems to be addressed before a routine investigation for SC is conducted, because immunochromal techniques are really useful only when standards, metabolites and confirmation techniques are available and well standardized.

Keywords: Synthetic cannabinoids; Immunochromal screening; Workplace drug testing

Introduction

In the past few years, the European Union (EU) and many international research centers referred to the appearance of new psychoactive substances (NPS) - including Synthetic Cannabinoids (SC) - in the illicit market [1-3]. These compounds are synthetic drugs, also called “designer drugs”, with dangerous pharmacological and toxicological effects for humans as established by Weaver et al. in 2015 [4]. The high risk is related to the presence of unknown clinical effects, including acute toxic outcome. Many clinical cases demonstrated major effects on the psychophysical performances and state of consciousness [4-6].

They are marketed to avoid current European legislation as alternatives to cannabis, often labeled “not for human consumption” however, common routes of administration include inhalation and oral ingestion [4]. Before 2008, the use of products containing SC was restricted to a small number of experimental drug users [6] and the first SC drugs detected in herbal smoking mixtures in the European market were JWH-018 and JWH-073 [1, 6]. Since 2008, many different compounds appeared in the illicit market, and their analytical identification is still difficult for the wide variability of compounds, the unknown metabolites and pharmacokinetics [7, 8].

The classification of the SC, based on the chemical structures of the molecules, has been suggested by Howlett et al. [9] and Thakur et al. [10]; classical, non-classical, hybrid, aminoalkylindoles and eicosanoids.

The recent rise and widespread availability of many SC support the need for a urine screening, focused on the detection of these compounds [11, 12]. Methods using LC-MS/MS or high resolution
techniques for SC screening have been widely published [13]. However, these techniques are not always available for the routine analysis in all forensic laboratories, hence the employ of immunochromatographic screening should be helpful. Evaluation of SC use with specific drug screenings is necessary for clinical, forensic, drug treatment and workplace drug screening programs. The screening of workers employed in higher risk jobs does include drug testing analysis, with a restricted panel of the more common drugs of abuse. Workplace guidelines issued by the European Workplace Drug Testing Society (EWDTS) have defined the common drugs of abuse, their cut-off and which biological samples have to be used [14]. They don’t include the detection of the NPS, which are not under legal control in all European countries, although their increase would require a better evaluation.

Only a few forensic laboratories are equipped to identify the NPS with the immunochromatographic screening [15]. It is well known that immunoassay testing offers rapid separation of presumptive positive and negative specimens, prior to more costly and time-consuming chromatographic confirmation.

The most common commercially-available immunoassays for urinary SC tests in Europe are supplied by Concateno, Randox and Neogen. This technical note refers about experience with Randox (DOA V Synthetic Cannabinoids panel- Biochip Array Technology) and Concateno (Drug Screen test) immunoassays for SC, analyzing 50 authentic and anonymous urine samples collected from workers in the year 2013. No ethical approval was necessary for the experience.

Materials and Methods

Evidence Investigator Biochip Array Technology is used to perform simultaneous detection of multiple analytes from a single patient sample. The core of the technology is the Randox Biochip; a solid state device with array of discrete testing regions containing immobilized antibodies specific to different drugs of abuse compound classes. The Randox DOA V Urine kit (Randox laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, UK) used in this paper employs a competitive chemiluminescent immunoassay, where the drug in the specimen and drug labelled with horse radish peroxidase (HRP) are in direct competition for the antibody binding sites. Increased levels of drug in a specimen will lead to reduced binding of drug labelled with HRP and thus a reduction in the chemiluminescent signal emitted. The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a stored calibration curve. Immunochromatographic screening contains antibodies for mephedrone HCl (Bath Salts I assay- BSI), mescaline HCl (MESC), MDPV/MDPBP HCl (Bath Salts II-BSII), salvinorin A (SALVN), synthetic cannabinoids (SCI, SCII, SCIi and SCIV), benzylpiperazines (BZP), 1-(3-chlorophenyl) piperazine HCl (mCPP, PNPI and PNPII). Randox DOA V kit specifications provide sensitivity, limit of detection for each class of compound (Table 1a). Samples analysis has been performed as described in Randox DOA V kit insert. The Concateno Synthetic Cannabinoids Drug Screen Test (92 Milton Park, Abbingdon, Oxfordshire, OX14 4RY, UK) is intended for screening for the presence of cannabinoids in urine. It is a lateral flow immunoassay for the qualitative detection of SC metabolites in human urine, at a cut-off level of 30 ng/mL. The test is based on the principle of competitive immunochromatographic reaction between a chemically labeled drug and the drug or drug metabolites which may be present in the urine sample for the limited antibody binding sites. Compounds producing positive results, as Concateno specification, are reported in Table 1b. 50 authentic anonymous urine specimens positive for cannabinoids (obtained by the routinely immunoassay analysis for the common drugs of abuse) were analyzed for SC using Randox and Concateno technologies. Specimens were collected over one year from people submitted to workplace drug testing and stored at -20°C until the analysis. Furthermore, four drug free urine samples were spiked with SC certified reference standards available in the Forensic Laboratory (kindly obtained by the Department of Therapeutic Research and Medicines Evaluation-Drug Abuse and Doping Unit- Istituto Superiore di Sanità) at the final concentration of 10 ng/mL to check Randox kit. In the first sample JWH-251, JWH-073 and JWH-019 were added. The second sample was spiked with JWH-018, JWH-122, JWH-073 butanoic acid and the third with JWH-018 pentanoic acid and JWH-081-N-5 hydroxypentyl. In the last sample JWH-073-5-hydroxyindole, JWH-250 was added. Three drug free urine samples were spiked with the same SC standards at the final concentration of 50 ng/mL to check Concateno specificity; in particular in the first sample JWH-073 and JWH-

| COMPOUND                     | CALIBRATION       | ASSAY RANGE ng/mL | SENSITIVITY ng/mL | LIMIT OF DETECTION ng/mL |
|------------------------------|-------------------|-------------------|-------------------|--------------------------|
| SCI-Synthetic Cannabinoids I Assays | JWH-018          | 0-200             | 1.47              | 3.67                     |
| SCI-Synthetic Cannabinoids II Assay | JWH-018         | 0-200             | 0.87              | 3.69                     |
| SCIi-Synthetic Cannabinoids III Assay | JWH-018        | 0-200             | 0.35              | 1.19                     |
| SCIV-Synthetic Cannabinoids IV Assay | JWH-250          | 0-100             | 0.31              | 1.17                     |
| BSI-Bath Salts I Assay       | Mephedrone HCl   | 0-08              | 0.08              | 0.18                     |
| BSIi-Bath Salts II Assay     | MDPV/MDPBP HCl   | 0-1000            | 12.58             | 17.62                    |
| BZP-Benzylpiperazines        | 1-Benzylpiperazine | 0-100             | 0.34              | 4.02                     |
| PNPI-Phenylpiperazines Assay | 1-(3-chlorophenyl) piperazine HCl (mCPP) | 0-50              | 0.19              | 1.15                     |
| PNPIi-Phenylpiperazines Assay | 1-(3-chlorophenyl) piperazine HCl (m CPP) | 0-50              | 0.19              | 3.51                     |
| MESC-Mescaline Assay         | Mescaline HCl    | 0-250             | 0.65              | 4.07                     |
| SALVN-Salvinorin Assay       | Salvinorin A     | 0-20              | 0.02              | 0.05                     |

Table 1a Randox DoAV kit technical specifications.
081 were added. The second sample was spiked with JWH-018 and JWH-018-N-4-hydroxy pentyl. In the third sample JWH-073 butanoic acid and JWH-018 pentanoic acid were added.

**Results and Discussion**

To our knowledge the most common, commercially-available immunoassays for urinary SC tests are marketed by Concateno, Randox and Neogen. In their general characteristics referred by the manufacturers, are scheduled. All the tests are specific for urine matrix, but Neogen is able to analyze blood and serum too (Table 2). Randox has a dedicated kit for SC analysis on whole blood other than urine. Concateno identifies only SC, while Randox technology can identify much more molecules. Table 3 summarizes cross reactivities of the kits for SC only; their comparison reveals that Randox can identify many more molecules compared with Concateno.

A direct comparison between the different technologies is difficult due to high variability of the molecules and related metabolites. However, Randox system appears to be more sensitive than Concateno.

Drug free urine samples to which SC were had the expected results in term of the declared cross reaction’s. Two urine samples obtained from workers showed positive results with Randox DOA

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### Table 1b Compounds producing positive results with Concateno kit.

| COMPOUND | SENSITIVITY ng/mL |
|----------|------------------|
| JWH-018 pentanoic acid | 30 |
| JWH-018-N-4-hydroxy pentyl | 200 |
| JWH-081-N-5-hydroxy pentyl | 1000 |
| AM-2201-N-4-hydroxy pentyl | 1000 |
| RCS-4-N-5-carboxy pentyl | 250 |
| JWH-073 butanoic acid | 15 |
| JWH-073-N-4-hydroxy butyl | 300 |
| JWH-200-N-6-hydroxy indole | 300 |
| JWH-250-N-5-hydroxy indole | 300 |
| Lamotrigine | 50 |

### Table 2 Concateno, Randox and Neogen Kits main characteristics.

| Technology | Qualitative/quantitative | Matrix | Assay time | N. samples/kit | Detection | Sample dilution | Sample Volume | Molecules detected |
|------------|---------------------------|--------|------------|---------------|-----------|----------------|---------------|-------------------|
| Concateno  | Lateral flow | Qualitative | Urine | 6’ | 25 | At a glance | N/A | JWH-018, JWH-073 |
| Randox     | Biochip array | Semi-quantitative | Urine | 30’ | 54 | Chemiluminescence | 25 μl | JWH-018, JWH-398, JWH-250, Mephedrone HCl, 3',4'-Methylenedioxy-α-Pyrrolidinobutophenone (MDPB) HCl, 1-Benzylpiperazine, 1-(3-Chlorophenyl) Piperazine monohydrochloride (mCPP), Mescaline HCl, Salvinorin A |
| Neogen     | ELISA | Qualitative | Urine, blood, serum | 75’ | 96 | Absorbance | Yes | 20 μl | JWH-018, JWH-073, JWH-200, JWH-015, JWH-199, JWH-122, AM2201, AM694 |

### Table 3 Cross reactivities of the three different immunoassays.

| Compound | Neogen | Randox % CR | Concateno |
|----------|--------|-------------|-----------|
| JWH-018  | 0.98   | 100         | 100.0     |
| JWH-073-N-(4-hydroxybutyl) Metabolite | 0.10 | 980        | 61.9 | 407.4 | 138.1 | 1.3 | 300 | 10 |
| JWH-018 N-5-hydroxy pentyl | 0.13 | 754 | 227.0 | 415.4 | 227.1 | 0.9 | 30 | 10 |
| JWH-200  | 0.16   | 613         | 269.0     |
| JWH-018-N-pentanoic acid | 0.16 | 613 | 39.2 | 231.3 | 58.7 | <1 | 30 | 100 |
| AM2232   | 0.16   | 613         |           |
| JWH-073  | 0.20   | 490         | 116.1     |
| AM1220   | 0.21   | 467         | 34.3      |
| JWH-073 N-butanoic acid | 0.23 | 426 | 11.0 | 207.4 | 12.1 | <1 | 15 | 200 |
| (±) JWH-018-N-(4-hydroxypentyl) Metabolite | 0.25 | 392 | 77.7 | 295.6 | 126.8 | <5 | 200 | 15 |
V kit; the first one was positive for BSII (>30 ng/mL) and PNPII (>7.5 ng/mL), the second was positive for PNPI (>68 ng/mL) and PNPII (>68 ng/mL). The same urine specimens were negative with the Concateno kit, which doesn’t include phenylpiperazines in the analytes panel. Additionally the Concateno immunoassay found five positive samples for SC, which was not revealed by Randox kit.

The current paper discusses problems to be addressed before a routine investigation is conducted, because immunochemical techniques are only useful when standards, metabolites and confirmation techniques are available and well standardized. This is a very important aspect for the interpretation of the immunochemical results. The aim of this experimentation was also to note and underline the suggestions of EWDTS guidelines [14] that included also SC analyses in its last version.

Finally the authors want to emphasize the advice of the United Nations Office on Drugs and Crime - UNODC - [6] that promotes the collection, updating and sharing of scientific, epidemiological, forensic and toxicological information within specialists.

**Conclusion**

The number of abusers of SC has increased remarkably worldwide however there is an underestimation of the phenomenon. They are rarely detected in urine which is the most common matrix employed in different context as workplace drug testing.

The paper discloses problems to be underlined before the routine investigation, because immunochemical techniques are really useful when standards, metabolites and confirmation techniques are available and well standardized. Finally the aim of this experimentation was also to remember and underline the suggestions of EWDTS guidelines that included also SC analysis in the last version.
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