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Immunochemical Screening for Synthetic Cannabinoids in Workplace Drug Testing

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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 immunochemical techniques are really useful only when standards, metabolites and confirmation techniques are available and well standardized.

Keywords: Synthetic cannabinoids; Immunochemical screening; Workplace drug testing

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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 routinary analysis in all forensic laboratories, hence the employ of immunochemical 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 immunochemical 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 Anntrim, 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. Immunochemical 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, SCIII 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, Abbingdone, 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 30ng/ml. The test is based on the principle of competitive immunochemical 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-

Table 1a Randox DoAV kit technical specifications.

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
SCII-Synthetic Cannabinoids II Assay	JWH-018	0-200	0.87	3.69
SCIII-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	0-38	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

081 were added. The second sample was spiked with JWH-018 and JWH-018-N-4-hydroxypentyl. 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 reactivity's. Two urine samples obtained from workers showed positive results with Randox DOA

Table 1b Compounds producing positive results with Concateno kit.

COMPOUND	SENSITIVITY ng/mL
JWH-018 pentanoic acid	30
JWH-018–N-4-hydroxypentyl	200
JWH-081–N-5-hydroxypentyl	1000
AM-2201-N-4-hydroxypentyl	1000
RCS-4-N-5-carboxypentyl	250
JWH-073 butanoic acid	15
JWH-073–N-4-hydroxybutyl	300
JWH-200–N-6-hydroxyindole	300
JWH-250–N-5-hydroxyindole	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	No	N/A	JWH-018, JWH-073
Randox	Biochip array	Semi-quantitative	Urine	30'	54	Chemiluminescence	No	25 µl	JWH-018, JWH-398, JWH-250, Mephedrone HCl, 3',4'-Methylenedioxy-α-Pyrrolidinobutiophenone (MDPBP) 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-019, JWH-122, AM2201, AM694

Table 3 Cross reactivities of the three different immunoassays.

Compound	Neogen		Randox % CR				Concateno	
	1-50 (ng/mL)	%CR	SC1	SC2	SC3	SC4	ng/mL	%CR
JWH-018	0.98	100	100.0	100.0	100.0	0.7		
JWH-073-N-(4-hydroxybutyl) Metabolite	0.10	980	61.9	407.4	138.1	1.3	300	10
JWH-018 N-5-hydroxypentyl	0.13	754	227.0	415.4	227.1	0.9		
JWH-200	0.16	613	269.0	382.0	115.0	<1		
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	298.5	127.5	<1		
AM1220	0.21	467	34.3	327.7	238.6	0.4		
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

AM2201	0.28	350	225.7	101.7	219.1	<1		
JWH-022	0.42	233	53.2	80.1	69.6	<1		
JWH-018 N-(5-hydroxypentyl) ββ-D glucuronide	0.49	200	18.0	308.4	65.3	0.8		
AM-2201 N-(4-hydroxypentyl) Metabolite	0.59	166	71.7	260.4	68.4	0.6	1000	3
3-(1-naphthoyl)-1H-Indole	0.64	153						
JWH-018 6-hydroxyindole	0.78	126	13.6	36.9	62.7	<1		
AM694	0.90	109	28.5	13.5	3.1	<1		
JWH-019	1.0	94	89.0	50.0	82.0	<1		
MAM2201	1.1	88						
JWH-015	1.2	83	26.3	44.5	5.1	<1		
JWH-018 4-hydroxyindole	1.6	60	30.6	3.6	10.7	<1		
JWH-122	1.9	51	71.2	2.0	9.8	<1		
JWH-018 5-hydroxyindole	2.0	50	4.9	51.8	65.5	<1		
AM-2201 6-hydroxyindole	2.0	50	6.9	72.3	54.2	<1		
JWH-007	2.9	34	16.0	17.0	2.0	<1		
JWH-398	7.5	13	20.9	<1	5.6	0.2		
WIN 55,212-3 mesylate	9.2	11	<1	11.0	8.0	0.0		
JWH-081	16	6.1	44.2	<1	<1	0.9		
JWH-210	21	4.8	51.3	<1	1.4	<1		
JWH-250-N-(5-carboxypentyl) Metabolite	51	1.9						
JWH-250-N-(4-hydroxypentyl) Metabolite	82	1.2	1.0	0.6	<1	206.0		
JWH-250	188	0.5	1.5	<1	<1	100.0		
JWH-203	205	0.5	<1	<1	<1	59.0		
RCS-4	255	0.4	61.0	<1	<1	4.1		
RCS-8	365	0.3	<1	<1	<1	0.7		
JWH 081 N-(5-hydroxypentyl)			172.3	1.5	2.5	<1	1000	3
RCS-4 N-(5-carboxypentyl)			5.5	<1	<1	<1	250	12
JWH 200 6-hydroxyindole metabolite			73.7	540.4	146.1	<1	300	10
JWH-250-N-5-Hydroxyindole							300	10
Lamotrigine							50	60

- Neogen Cross reactivity is calculated as CR=IC50 of JWH018 standard/IC50 of Cross reactant × 100
- Concateno Cross reactivity is calculated as CR=Cut off concentration of 30 ng/mL/concentration of cross reactant to result in positive result × 100
Note the Concateno CR is an estimate as it is only a qualitative assay

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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