IMMUNOCHEMICAL SCREENING FOR SYNTHETIC CANNABINOIDS IN WORKPLACE DRUG TESTING: AN ITALIAN EXPERIENCE

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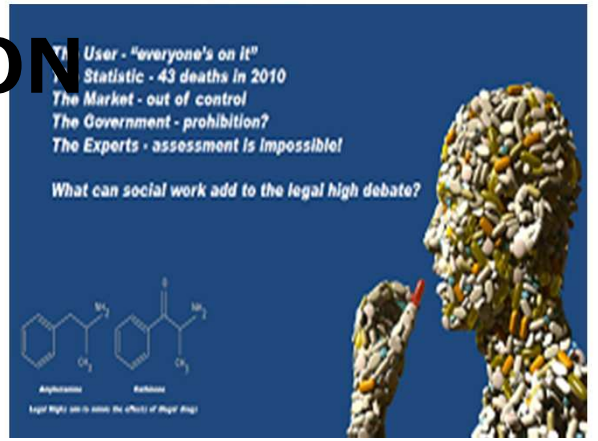
TREND IN ILLICIT MARKET

INTRODUCTION OF NEW PSYCHOACTIVE SUBSTANCES (NSP) *including Synthetic Cannabinoids*

INCREASE IN THE PRODUCTION AND MISUSE OF THE NPS *(not under international control)*



United Nations Office on Drugs and Crime
World Health Organization

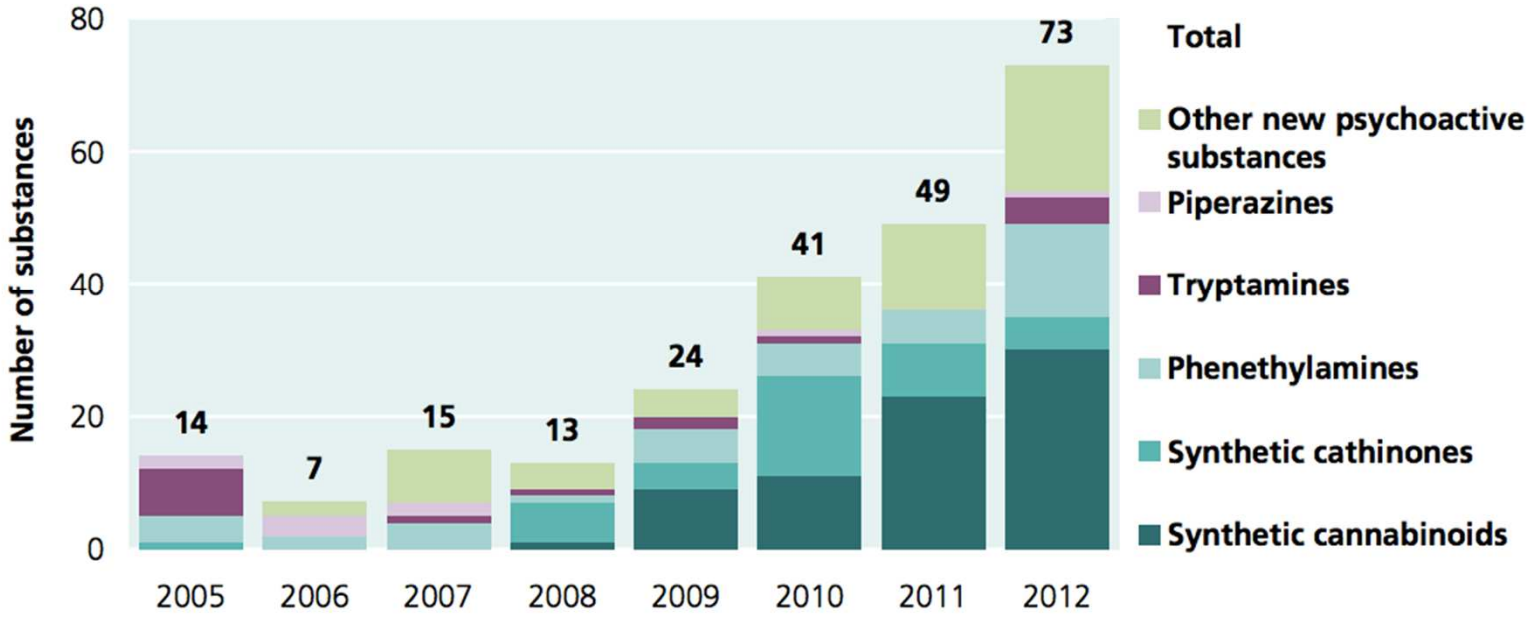
EXPERT CONSULTATION OF NPS
VIENNA, 9-11 december 2014

Number of identified NPS in the European union increased
(from 14 in 2005 to 236 by the end of the past year)

EUROPE:
- cannabis the most commonly used of illicit substances
- continual increase in the introduction and use of NPS

EMCDDA : 236 NEW RECREATIONAL DRUGS REPORTED THROUGH THE "EARLY WARNING SYSTEM " (2005-2012)

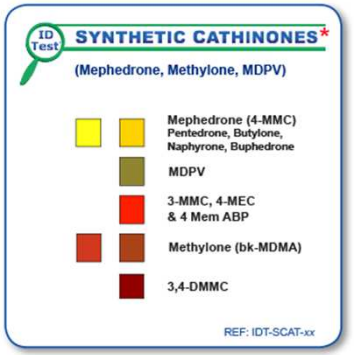
Fig. 9. **Number of new psychoactive substances newly reported annually through the European Monitoring Centre for Drugs and Drug Addiction's early warning system, 2005-2012**



Source: European Monitoring Centre for Drugs and Drug Addiction and European Police Office, *EU Drug Markets Report: A Strategic Analysis* (Luxembourg, Publications Office of the European Union, 2013).

NEW PSYCHOACTIVE SUBSTANCES

- **Synthetic cannabinoids**
- **Synthetic cathinones**
- **Phenethylamines** ***(as AMPH,MDMA..)***
- **Piperazines**
- **Ketamine**
- **Plant** ***as kratom, salvia divinorum, khat***

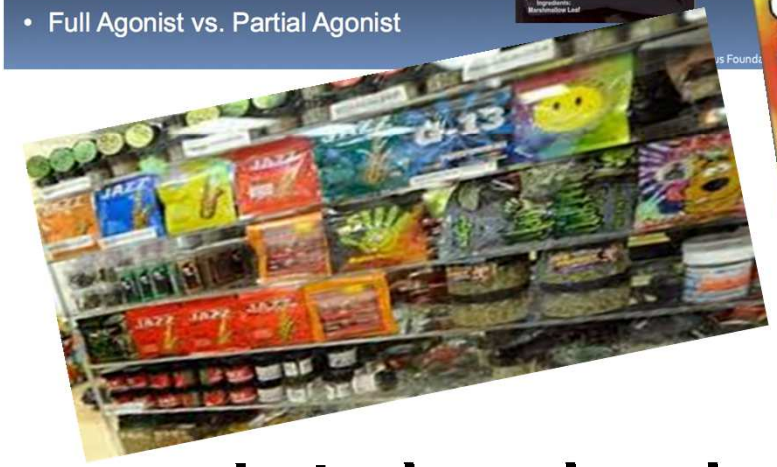


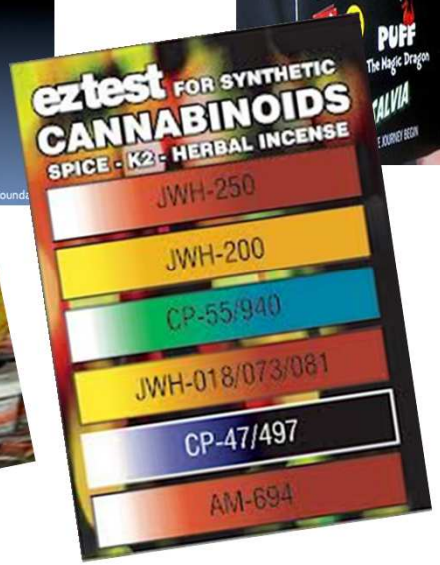
(According to UNODC classification)

MOST WIDELY NPS USED IN EUROPE: Synthetic Cannabinoids

- cannabinoid receptor agonists which produce effects similar to those of Δ9-Tetrahydrocannabinol
- often laced with herbal products and sold as «SPICE» «K2» «KRONIC», etc

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction)- European Drug Report 2013

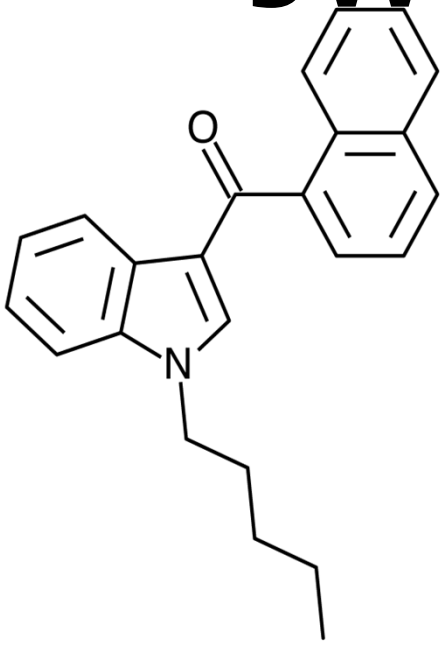


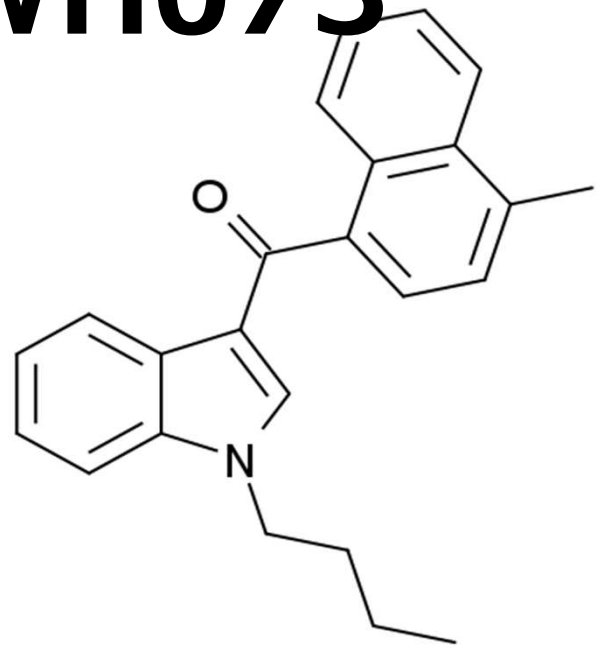


- **marketed as legal alternatives to cannabis, often labeled *"not for human consumption"***
- **common routes of administration inhalation, oral ingestion**

The first S.C. detected in herbal smoking mixture *(so called incense/room odorizes)*

JWH018 e JWH073





CLASSIFICATION OF S.C.

Classical cannabinoids

(THC, other constituents of cannabis; and their structurally related synthetic analogues)

Nonclassical cannabinoids

(cyclohexylphenols or 3-arylcyclohexanols)

Hybrid cannabinoids

(combinations of structural features of classical and non-classical cannabinoids)

Aminoalkylindoles : naphtoylindoles

(e. g. JWH-018, JWH-073, JWH-398, JWH-015, JWH-122, JWH-210, JWH-081, JWH-200, WIN-55,212); enylacetylindoles; naphthylmethylindoles and benzoylindoles

Eicosanoids

(endocannabinoids and their synthetic analogs)

Others, diarylpyrazoles, naphtoylpyrroles, naphthylmethylindenes or derivatives of naphthalene-1-yl-(4-pentyloxynaphthalen-1-yl) methanone

Howlett AC et al . Pharm.Rev. 2002 ,54, 161-202
Thakur GA et al . Mini Rev.Med.Chem. 2005, 5, 631-40

Compound name	Date and place of first identification	Group type
JWH-018	December 2008 (Germany, Japan)	Naphtoylindole
CP-47,497-C8	December 2008 (Germany, Japan)	Cyclohexylphenol
CP-47,497	December 2008 (Germany, Japan)	Cyclohexylphenol
JWH-073	January 2009 (Germany)	Naphtoylindole
HU-210	January/June 2009 (USA, UK)	Classical cannabinoid
JWH-250	October 2009 Germany)	Phenylacetylindole
JWH-398	October 2009 (UK)	Naphtoylindole
JWH-200	December 2009 (Russia)	Naphtoylindole
JWH-081	March 2010 (Germany)	Naphtoylindole
4-Methyl-JWH-073	April 2010 (Germany)	Naphtoylindole
RCS-4	May 2010 (Germany)	Benzoylindole
JWH-015	June 2010 (Austria)	Naphtoylindole
AM-694	July 2010 (Ireland)	Benzoylindole
JWH-122	July 2010 (Latvia)	Naphtoylindole
JWH-210	September 2010 (Germany)	Naphtoylindole
JWH-019	September 2010 (Germany)	Naphtoylindole
JWH-203	October 2010 (Germany)	Phenylacetylindole
WIN-48,098 (Pravadolin)	November 2010 (Germany)	Benzoylindole
JWH-007	November 2010 (Germany)	Naphtoylindole

Source- United Nations Office on Drugs and Crime - WORLD DRUG REPORT

EUROPEAN WORKPLACE DRUG TESTING
urine screening

- ACTUALLY A RESTRICTED PANEL *(for more common drugs of abuse)*

- WORKPLACE GUIDELINES defined the drugs of abuse, their cut-off, which biological samples have to be used

- The detection of the NPS was not included*(not under legal control in all European countries)*

- WIDESPREAD DIFFUSION WOULD SUGGEST A DEEP EVALUATION

COUNTRY	ENFORCEMENT DATE	CONTROLLED SUBSTANCES/REMARKS
AUSTRIA	2009	"spice" products classified as medicinal preparations
	2010	CP-47,497-C6/C7/C8/C9,JWH-018,HU-210,JWH-015/019/073/81/200/250
DENMARK	2010	CP-47,497-C6/C7/C8/C9, JWH-018/73, HU-210, JWH-250/398/200
ESTONIA	2009	CP-47,497-C6/C7/C8/C9, JWH-018/73, HU-210
FINLAND	not controlled	JWH-018/073/200/210, CP-47,497-C6/C7/C8/C9
		classified as medicinal preparations
FRANCE	2009	JWH-018, CP-47,497-C6/C7/C8/C9, HU-210
GERMANY	2009	emergency regulation JWH-018,CP-47, 497- C6/C7/C8/C9
	2010	permanent control and addition of JWH-019/073
	2011	JWH-015, JWH-081, JWH-200/250/122
IRELAND	2010	GENERIC APPROACH
ITALY	2010	JWH-018, JWH-073-JWH-122, JWH-250 8 TABLE I-SECTION B ITALIAN LAW N.309/90
JAPAN	2009	controlled as "designated substances" under the Pharmaceutical Affairs Law
		CP-47, 497-C7/C8, JWH-018, HU-210
	2010	JWH-073, JWH-250
LITHUANIA	2009	CP-47, 497-C6/C7/C8/C9, JWH-018, JWH-073, HU-210, JWH-250, JWH-398, JWH-200
LUXEMBOURG	2009	GENERIC APPROACH
NEW ZEALAND	not controlled	HU-210 may be regarded as an THC analog
POLAND	2009	JWH-018,Leonotis Leonurus,Nymphacea caerulea
ROMANIA	2010	CP-47, 497-C6/C7/C8/C9, JWH-018, JWH-073, JWH-250
RUSSIA	2009	CP-47,497-C6/C7/C8/C9,HU-210,
		JWH-007/018/073/081/098/122/149/166/175/176/184/185/192/193/194/195/196/197/198/199/200
SOUTH KOREA	2009	JWH-018, HU-210, CP-47, 497
SWEDEN	2009	CP-47, 497-C6/C7/C8/C9,JWH-018, JWH-073,HU-210
SWITZERLAND	2009	control of "spice herbal mixes"under food regulation (5 grams allowesd for personal use)
	2010	JWH-018,JWH-019, JWH-073, JWH-250, CP-47,497-C6/C7/C8/C9
UNITED KINGDOM	2009	GENERIC APPROACH
USA		HU-210 SCHEDULED AS AN ANALOG OF THC
	2010	DEA ANNOUNCEMENT TO EMERGENCY SCHEDULE JWH-018, JWH-073- CP-47, 497, CP-47, 497-C8, JWH-200

FEW FORENSIC LABORATORIES EQUIPPED TO IDENTIFY S.C. WITH IMMUNOCHEMICAL SCREENING FOR the scarcity of the marketed kits

MOST COMMON KITS in EUROPE :

CONCATENO ***(92 Milton Park, Abbingdone, Oxfordshire, OX14 4RY,UK)***

RANDOX ***(Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Anntrim, BT29 4QY,UK)***

NEOGEN ***(Europe Ltd. The Dairy School, Auchincruive, Ayr, KA6 5HU Scotland , UK)***

KIT COMPARISON TABLE

	METHOD	QUALITATIVE/ QUANTITATIVE	MATRIX	ASSAY TIME	N. samples/ kit	DETECTION	SAMPLE DILUTION	SAMPLE VOLUME	MOLECULES DETECTED
Concateno	LATERAL FLOW IMMUNO ASSAY	QUALITATIVE	URINE	6'	25	AT A GLANCE	NO	N/A	JWH-018, JWH-073
Randox	BIOCHIP ARRAY TECHNOLOGY	SEMI-QUANTITATIVE	URINE	30'	54	CHEMILUMINESCENCE	NO	25 ml	JWH-018, JWH-398, JWH-250, MEPHEDRONE HCl, 3',4'-METHYLENEDIOXY-a-PYRROLIDINOBUTIOPHENONE (MDPBP) HCl, 1-BENZYLPIPERAZINE, 1-(3-CHLOROPHENYL) PIPERAZINE MONOHYDROCHLORIDE (m CPP), MESCALINE HCl, SALVORIN A
Neogen	ELISA	QUALITATIVE	URINE, BLOOD, SERUM	75'	96	ABSORBANCE	YES	20 ml	JWH-018, JWH-073, JWH-200, JWH-015, JWH-019, JWH-122, AM2201, AM694

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 glucuroni	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

Randox/ Concateno Drug Screen immunoassays for S.C.

50 authentic urine samples collected over one year in workplace drug testing context *(at -20 °C until the analysis)*

TO CHECK RANDOX SPECIFICITY:

- drug free urine samples added with SC standards (10 ng/ml)

JWH-251- JWH-073-JWH-019 (sample n.1)

JWH-018- JWH-122- JWH-073 butanoic acid (sample n.2)

JWH-018 pentanoic acid -JWH-081-N-5 hydroxypentyl (sample n.3)

JWH-073-5-hydroxyindole- JWH-250 (sample n.4)

TO CHECK CONCATENO SPECIFICITY:

- drug free urine samples added with SC standards (50 ng/ml)

JWH-073- JWH-081

JWH-018-JWH-018-4-hydroxypentyl

JWH-073 butanoic acid - JWH-18 pentanoic acid

RANDOX immunoassay

- Evidence Investigator Biochip Array Technology : a solid state device containing an array of discrete testing regions containing immobilized antibodies specific to different drugs of abuse (competitive chemiluminescent immunoassay)
- 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).

COMPOUND	CALIBRATION	ASSAY RANGE Ng/ml	SENSITIVITY Ng/ml	LIMIT OF DETECTION Ng/ml
SCI	JWH-018	0-200	1.47	3.67
SCII	JWH-018	0-200	0.87	3.69
SCIII	JWH-018	0-200	0.35	1.19
SCIV	JWH-250	0-100	0.31	1.17
BSI	MEPHEDRONE	0-38	0.08	0.18
BSII	MDPBP	0-1000	12.58	17.62
BZP	BENZYLPIPERAZINE	0-100	0.34	4.02
PNPI	m CPP	0-50	0.19	1.15
PNPII	m CPP	0-50	0.19	3.51
MESC	mescaline	0-250	0.65	4.07
SALVN	Salvinorin A	0-20	0.02	0.05

CONCATENO immunoassay

- CONCATENO synthetic Cannabinoids Drug Screen test : lateral flow immunoassay for the specific, qualitative detection of synthetic cannabinoids metabolites in human urine at a cut-off level of 30 ng/ml.
- TEST BASED ON principle of competitive immunochemical reaction
- Test contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a pad containing coloured antibody-colloidal gold conjugate.
- An internal procedural control is included in the test card

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

RESULTS OF IMMUNOCHEMICAL SCREENING

RANDOX: all urine samples negative for S.C.

two positive samples:

- ◊ BSII *(bath salts compounds including mephedrone HCl, methylone HCl,methedrone HCl,methcathinone HCl (> 30 ng/ml)*

  PNPII *(phenilpiperazine compounds > 7.5 ng/ml)*

- ◊ PNPI – PNP II *(>68 ng/ml)*

CONCATENO: five positive samples for S.C.

Drug free urine samples added with S.C. showed the expected results respect to the declared cross-reactivities

Urine samples positive with Randox were negative with CONCATENO

GC/MS ANALYSIS

- FOCUS GC coupled with DSQ operating in electron impact mode (70 eV)
- Equity 5 capillary column 30 m x 0.25 mm x 0.35 Um thickness
- Hydrolysis with HCl 90 °C for 60 min, alkalinization, liquid/liquid extraction with chloroform
- BSTFA derivatization
- Std. available: *JWH-251, JWH-251- JWH-073-JWH-019-JWH-018- JWH-122- JWH-073 butanoic acid -JWH-018 pentanoic acid -JWH-081-N-5 hydroxypentyl- JWH-073-5-hydroxyindole- JWH-250*
- L/L extraction of urine samples from acid and alkaline pH in order to detect the possible presence of interfering xenobiotics

SAMPLE	IMMUNOASSAY	GC/MS RESULTS
1 -	RANDOX	CAFFEINE
2	RANDOX	CAFFEINE
3-	CONCATENO	PROPOFOL-CARNEGINE-COCAINE METABOLITES
4-	CONCATENO	NEGATIVE
5-	CONCATENO	METHANONE
6-	CONCATENO	HYDROXYMETHYLCOLCHICINE- COCA INE METABOLITES
7-	CONCATENO	NIPACIDE

- BSI (bath salts)- PNPII (phenylpiperazines)
(positive results with RANDOX)
no confirmation GC/MS for unavailability of reference standards

- urine samples *(positive results with CONCATENO)* excluded the presence of the S.C . exploited *(JWH-251- JWH-073-JWH-019 -JWH-018- JWH-122- JWH-073 butanoic acid -JWH-018 pentanoic acid -JWH-081-N-5 hydroxypentyl JWH-073-5-hydroxyindole- JWH-250JWH-018-4-hydroxypentyl)*

- presence of the xenobiotics revealed by GC/MS *(caffeine, propofol, cocaine, carnegine, methanone, nipacide, hydroxymethylcolchicine)* could interfered with immunochemical results

NOT POSSIBLE TO EXCLUDE THE PRESENCE OF OTHER MOLECULES

CONCLUSION

- THIS PRELIMINARY STUDY UNDERLINE THE NECESSITY TO RESOLVE DIFFERENT PROBLEMS BEFORE THE ROUTINELY EMPLOY OF THE IMMUNOCHEMICAL KITS
- UTILITY OF IMMUNOCHEMICAL SCREENING FOR SYNTHETIC CANNABINOIDS FOR THE LABORATORIES THAT ARE ABLE TO CONFIRM THE PRELIMINARY RESULTS
- AVAILABILITY OF ALL REFERENCE STANDARDS
- UPDATING AND CIRCULATION OF INFORMATION BETWEEN THE DIFFERENT COUNTRIES
- SPECIAL ATTENTION BY THE EWDT SOCIETY TO THE DIFFERENT PROBLEMS RELATIVELY TO THE NEW PHENOMENON OF THE NEW PSYCHOACTIVE SUBSTANCES