folkehelseinstituttet

FINDINGS OF NEW PSYCOACTIVE DRUGS IN FORENSIC SAMPLES

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- DNA analysis
- Family genetics
- Forensic pathology
- Toxicological analysis
- Expert witness statements
- Research examples: method development, SIDS, drugs of abuse epidemiology, neurobiology

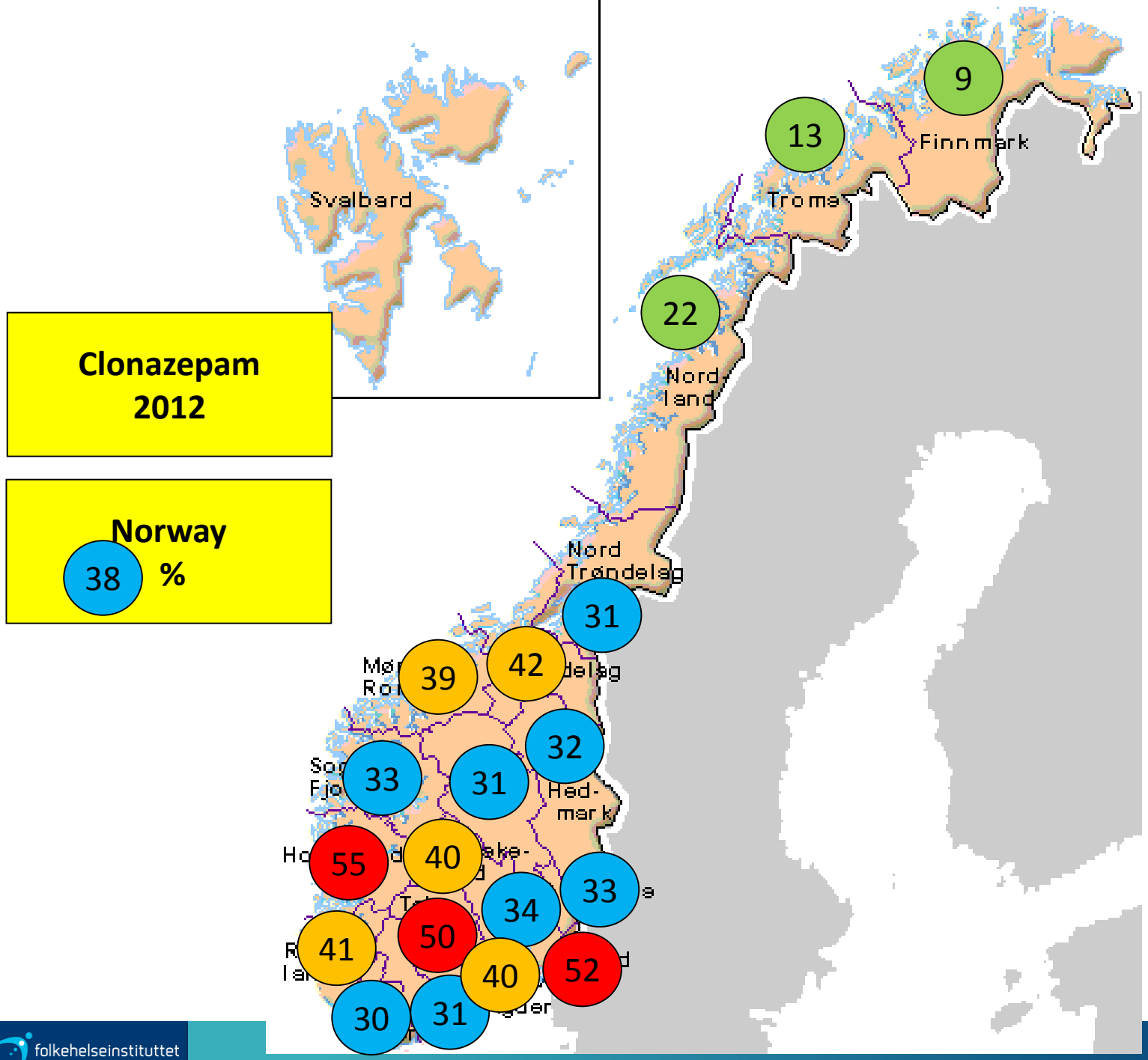
Forensic toxicological analysis

- Analysis of drugs of abuse and medicinal drugs
  - 12 – 24 000 prison cases
  - Driving under the influence cases approx. 3500 (alcohol), approx. 5000 (alc. + other substances)
  - Approx. 1600 autopsy cases
  - In adition some medicinal cases, work place testing, social services cases
- Immunoassay, LC-MS/MS, qTOF

Drug use: Global problem – regional differences

- Countries
  - Finland – very much buprenorphine, almost no heroin
  - Approx. 5 % of DUI cases positive for α-PVP
  - In Norway several deaths due to /with PMMA, in Sweden with 5-IT and o-desmethyl tramadol

- Difference in drug use for different regions


Clonazepam
2012
Norway
38 %
Svalbard
9
13
Finnmark
Troms
22
Nord-
land
Nord
Trøndelag
31
39
42
32
33
31
Hed-
mark
55
40
34
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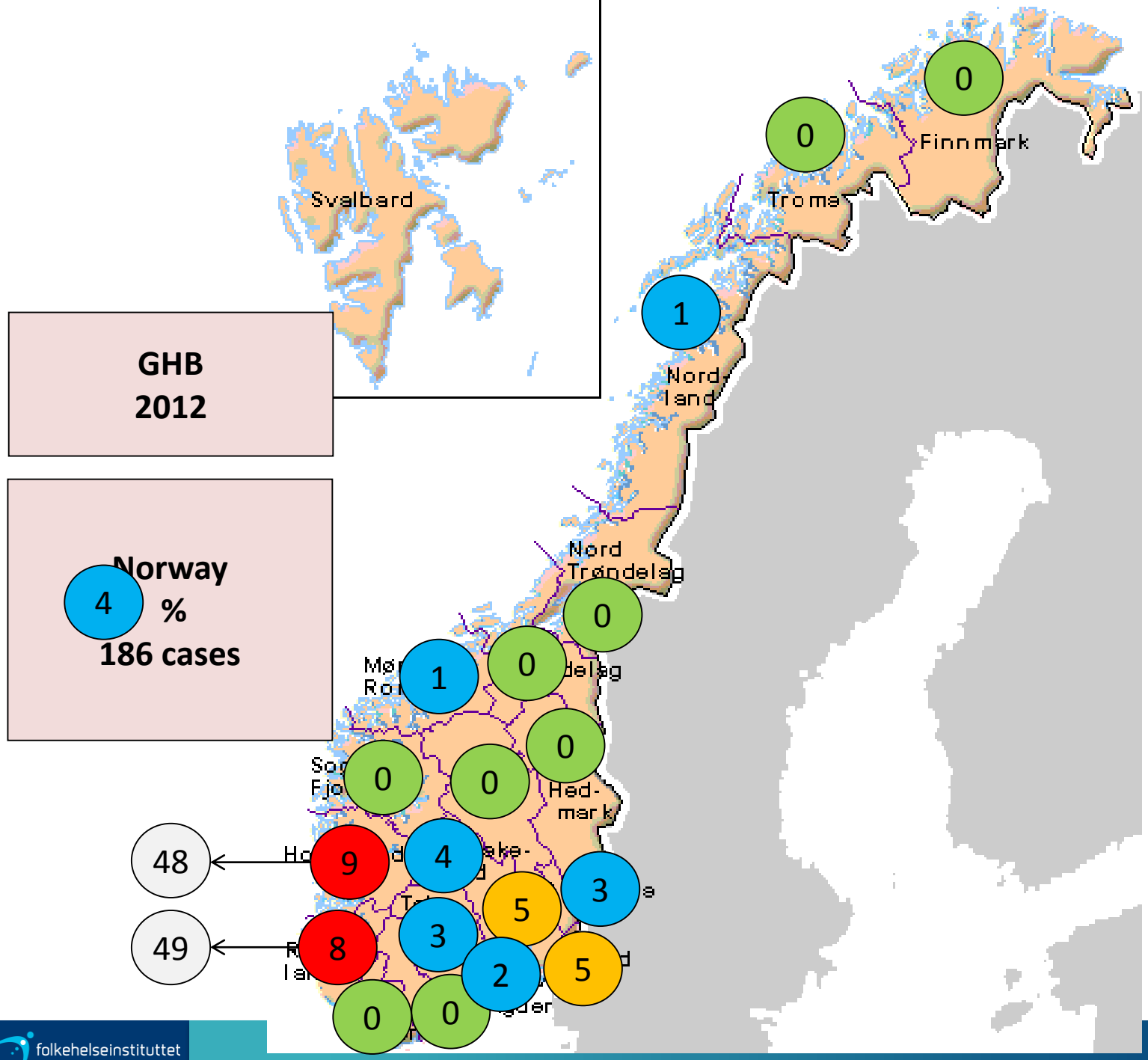
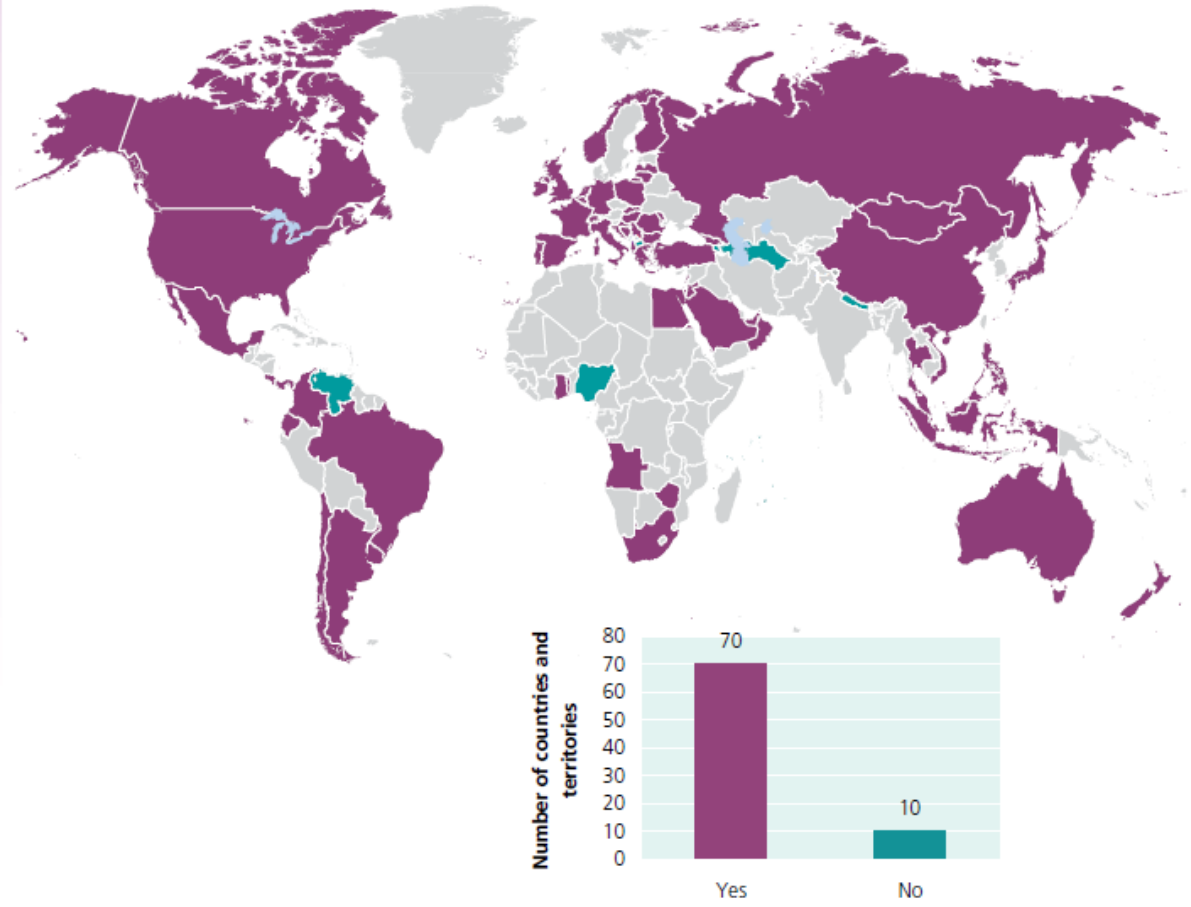
52
30
31


GHB
2012
Norway
4
%
186 cases
Svalbard
Finnmark
Troms
Nord
land
Nord
Trøndelag
Hed-
mark
48
49
0
1
4
9
5
3
8
2

NPS 2012

Map 1. Global emergence of new psychoactive substances up to July 2012



Source: United Nations Office on Drugs and Crime, *The Challenge of New Psychoactive Substances* (Vienna, March 2013).

UNODC
United Nations Office on Drugs and Crime

WORLD DRUG REPORT 2013

How do we find what is on the market?

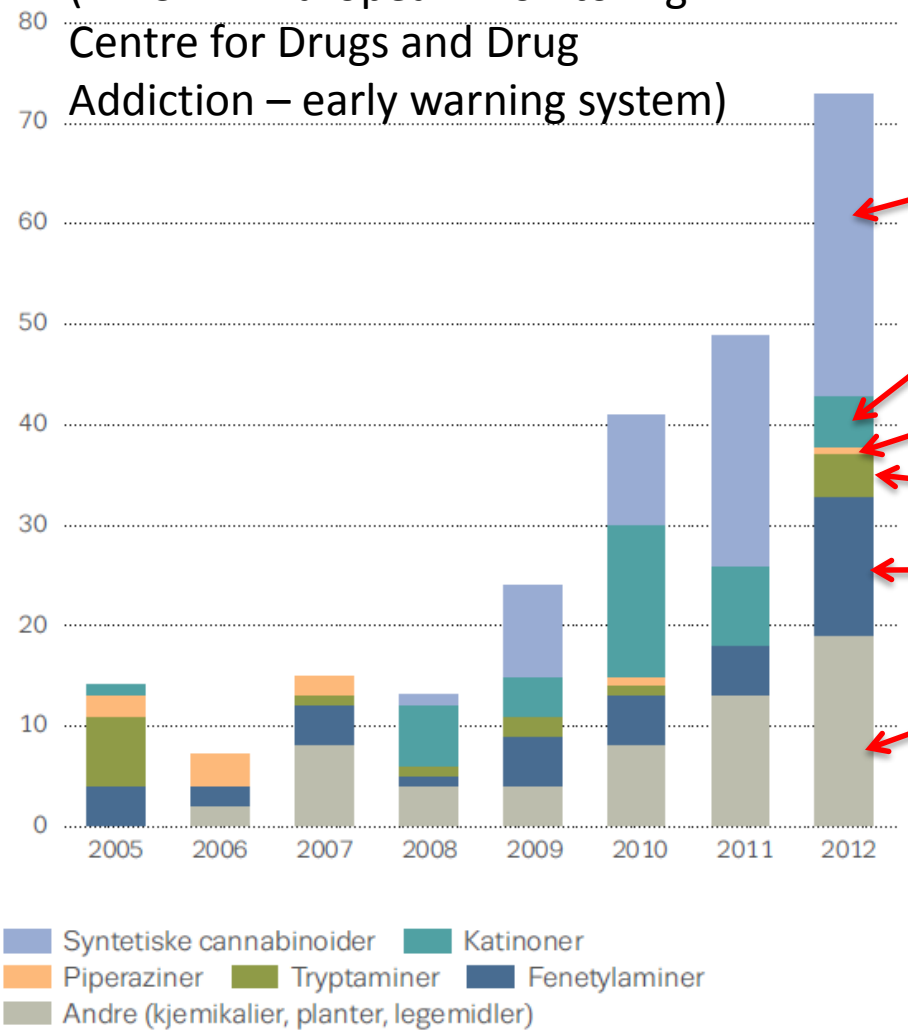
- Information from Kripos/Customs
- Hospitals/Poison information (Giftinformasjonen)
- Media
- Drug addicts, user forums
- Articles/case reports
- EMCDDA (European Monitoring Centre for Drugs and Drug Addiction)

FIGUR 1.14

The number of new substances on the market is increasing

Antall nye psykoaktive stoffer meldt til EUs system for tidlig varsling, etter hovedgruppe, 2005–2012

(EMCDDA European Monitoring Centre for Drugs and Drug Addiction – early warning system)



Synthetic cannabinoids

Cathinones

Piperazines

Tryptamines

Phenethylamines

Other
(chemicals, plants, medicines)

Generic drug list from 2013

- The (at that time) most common synthetic cannabinoids - 7 groups
- Cathinones
- Phenethylamines
- Tryptamines

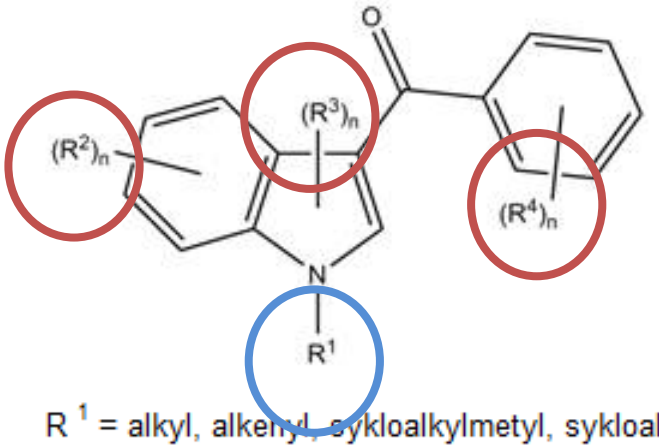
Generisk drug list – exceptions

Følgende grupper av stoffer regnes også som narkotika

1. *Benzoylindol-gruppe*

Enhver kjemisk forbindelse som strukturelt er avledet av 1*H*-indol-3-yl(fenyl)metanon ved substitusjon av indolringens 1-posisjon med en alkyl-, alkenyl-, sykloalkylmetyl-, sykloalkyletyl-, halogenalkyl- eller [2-(morfolino-4-yl)etyl]-gruppe (R^1), uansett om det er ytterligere substitusjon i benzen- eller indolringen (R^2, R^3, R^4).

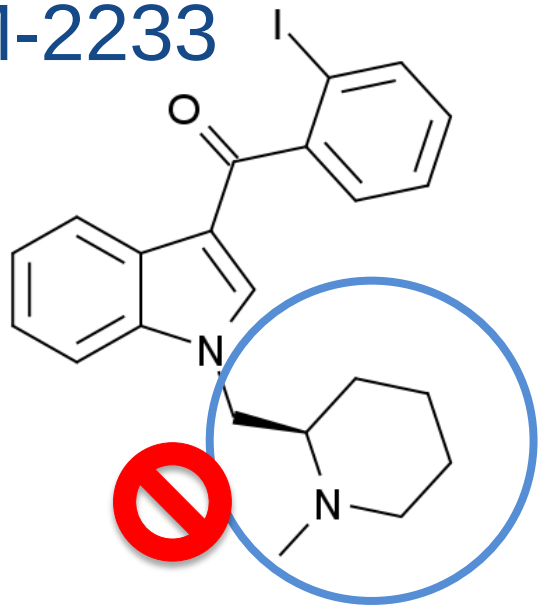
Generell struktur av benzoylindol-avledede kjemiske forbindelser:



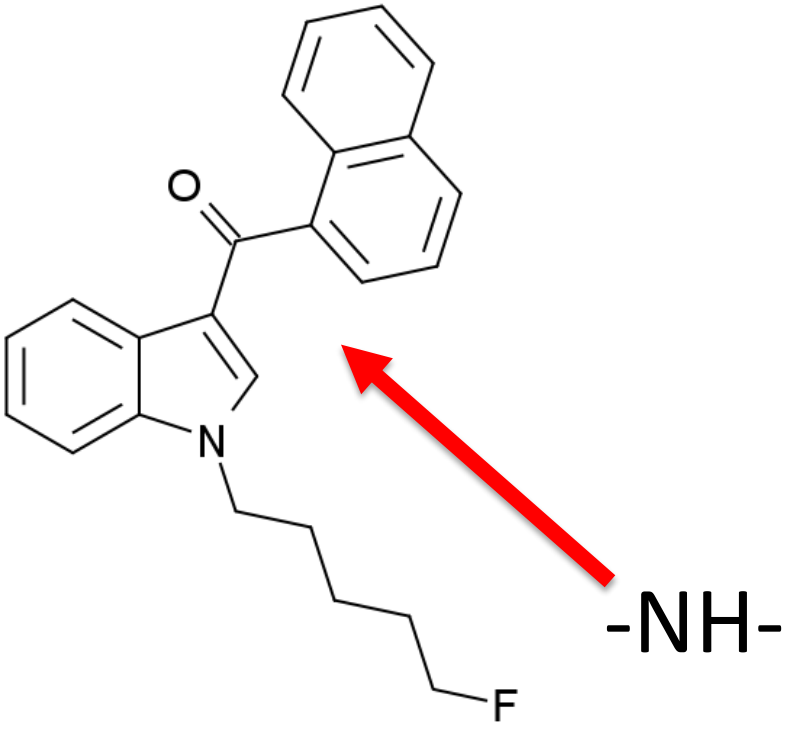
R^1 = alkyl, alkenyl, sykloalkylmetyl, sykloalkyletyl, halogenalkyl eller 2-(morfolin-4-yl)etyl.

R^2, R^3, R^4 = H (uten andre substituenter) eller en eller flere vilkårlige substituenter.

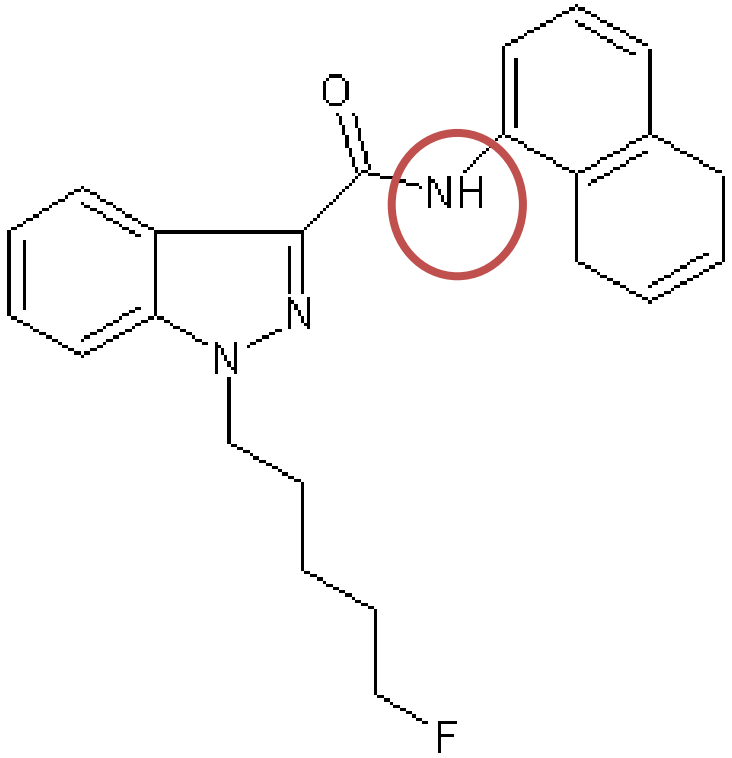
AM-2233



Generisk drug list – exceptions



AM-2201



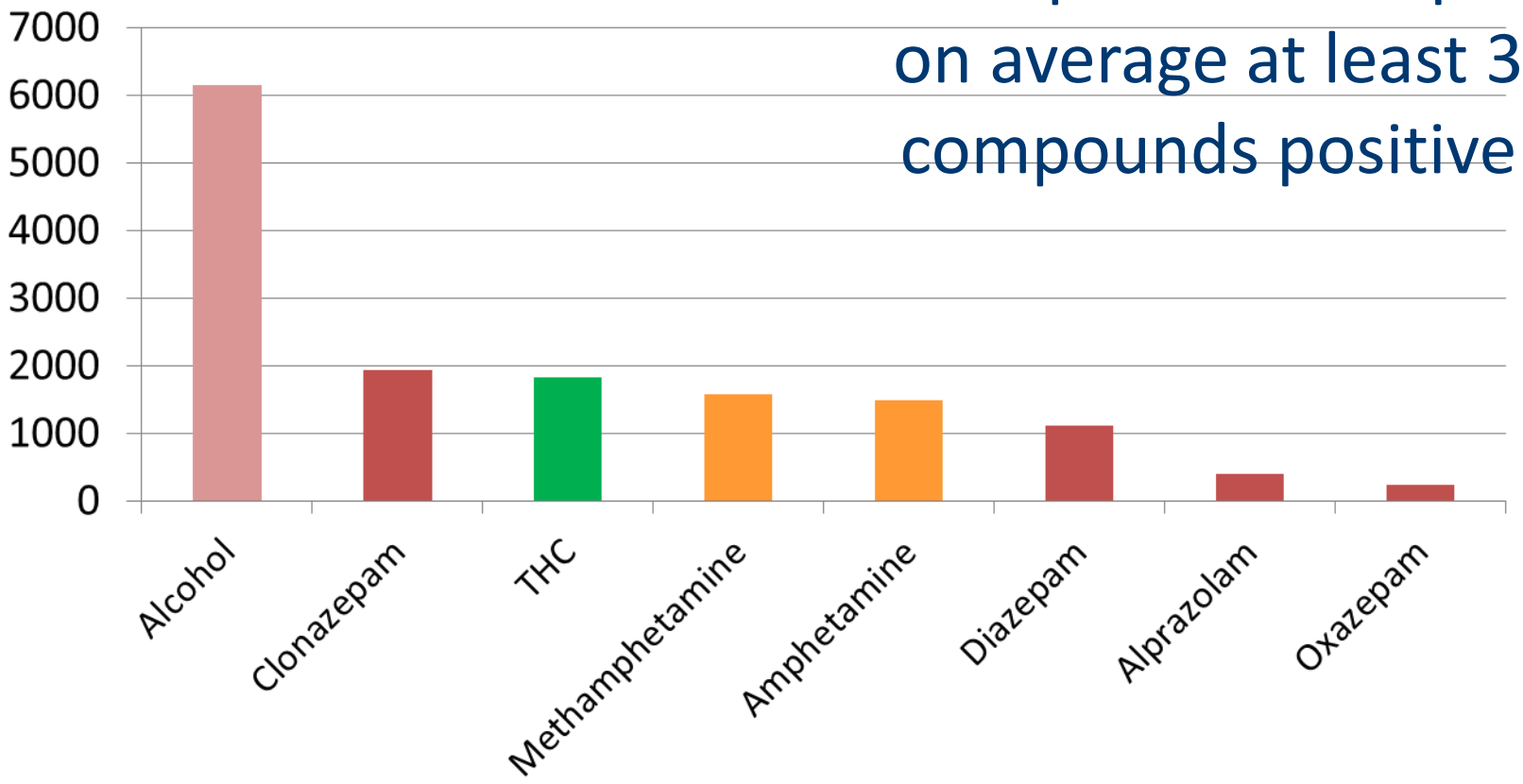
Routine samples

- Have a handful of NPS in the screening for all driving under the influence (DUI) and autopsy cases (AM-2201, UR-144, MDPV, 2CB, PMMA and some more)
- Some amphetamine type NPS included in the urine confirmation method (MDPV, 2CB, mephedrone, metkatinon etc)
- Immunoassay screening of syntethic cannabinoids
  - Kit #1 JWH-018, AM-2201 etc
  - Kit #2 UR-144, XLR-11

No kit for 4th generation synthetic cannabinoids (APINACA, PB-22 etc)

DUI cases 2012

> 90% positive samples, on average at least 3 compounds positive



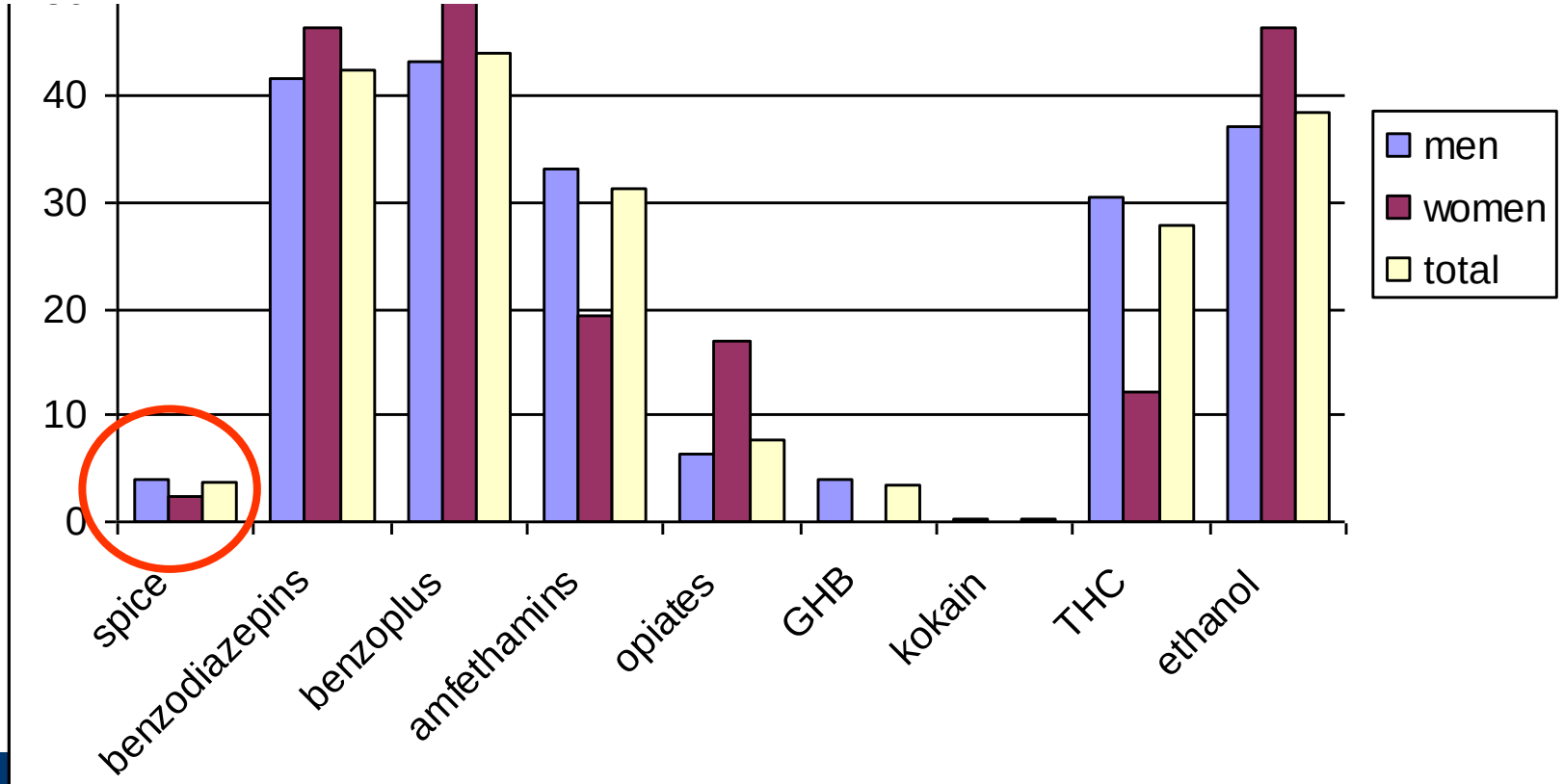
Alprazolam	Etylfenidat	Metaoxedrin (fenylefrin)	AM-2201
Bromazepam	Desomorfin	Fenylpropanolamin	AM-694
Diazepam	Harmin	1-fenyletylamin	AM-2233
Fenazepam	Metoxetamin	Efedrin	HU-210
Flunitrazepam	Xylazin	BDB	JWH-015
Klonazepam	2C-B	MDA	JWH-018
Lorazepam	2C-C	MBDB	JWH-019
N-desmetyldiazepam	2C-E	MDEA	JWH-073
Nitrazepam	2C-I	MDAI	JWH-081
Midazolam	2C-P	Fenmetrazin	JWH-122
Oxazepam	2C-T-2	DOB	JWH-200
Etizolam	2C-T-7	DOI	JWH-203
Karisoprodol	5-IT	2-DPMP	JWH-210
Meprobamat	m-CPP	2-fluoramfetamin	JWH-250
Zolpidem	1-benzylpiperazin	4-fluoramfetamin (PFA)	JWH-251
Zopiklon	pFPP (p-fluorofenylpiperazin)	4-metylamfetamin	MAM-2201
Pregabalin	TFMPP	4-fluormetamfetamin	RCS-4
Buprenorfin	alfa-PVP	3-fluormetamfetamin	RCS-4-C4
Etylmorfin	2,5I-NBOMe	4-metylmetamfetamin (4-MMA)	RCS-8
Fentanyl	2,5C-NBOMe	Homoamfetamin (3-amino-1-fenylbutan)	UR-144
Kodein	Salvinorin A	4-metyltioamfetamin	UR-144 degradant
Metadon	N,NdimetylMDA	PMA	URB-754
Morfin	DMAA	PMMA	WIN55,212-2
Oksykodon	tapentadol	Etylkatinon	XLR11
Tramadol	AH-7921	bk-MBDB (butylon)	XLR-11 degradant
Metylfenidat	Metiopropamin	Metylon (bk-MDMA)	5F-Apinaca
Ketamin	MDMA	bk-MDDMA	5F-PB-22
Amfetamin	MDPV	Katinon	AB-Fubinaca
Metamfetamin	6-ABP	Metkatinon	AB-Pinaca
Kokain	2,5-DMA	4-MEC	Apinaca (AKB48)
LSD	5-MeO-DMT	Pentedron	BB-22
THC	N-OH-MDA	2MMC	PB-22
GHB	alfa-Metyltryptamin	3-MMC	STS-135
GBL	Dimetyltryptamin (DMT)	Mefedron (4-MMC)	
	Bromo-Dragonfly		
Diclazepam			
Pyrazolam			
Flubromazepam			

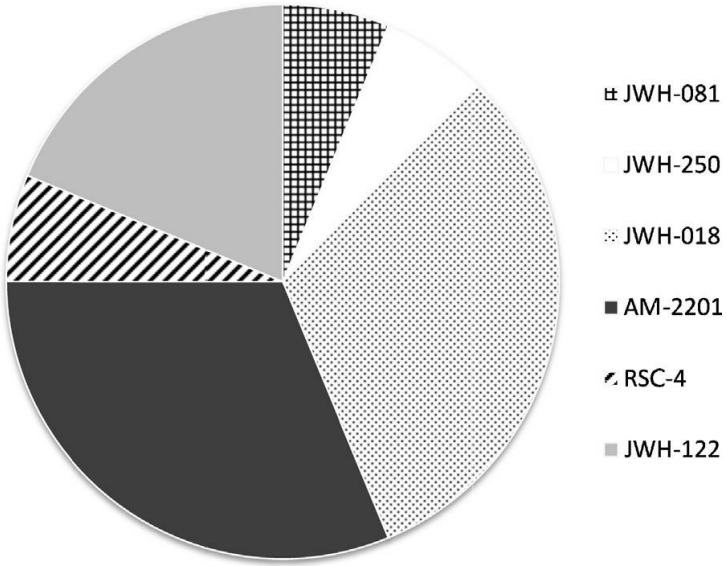
Driving under the influence cases

Results

3 weeks periode: synthetic cannabinoids was found in approx. 4% of the samples

In a later 3-weeks periode approx. 1%





In total 2,2% of cases in the combined period
JWH-018 and AM-2201 most prevalent

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Accident Analysis and Prevention

journal homepage: www.elsevier.com/locate/aap

Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period

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ABSTRACT

From early year 2000 different herbal products containing synthetic cannabinoids (SC) have appeared on the drug market all over the world, and new substances are frequently introduced. The prevalence of SC use in different populations is however still mainly unknown, also in Norway. This information is difficult to obtain, but studies of drivers suspected of driving under the influence of drugs (DUID), might provide important information.

The aim of this study was to assess the prevalence of SC in drivers suspected of being under the influence of drugs in Norway, and investigate if SCs impair driving performance.

For two periods of three and four weeks all blood samples from drivers suspected of DUID in Norway were analyzed for the presence of 12 and 18 different SCs, respectively. A new ultra performance liquid chromatography tandem mass spectrometry method was developed.

A total of 726 cases were analyzed during our study period, and SCs were detected in 16 cases (2.2%) in total. The mean age of these drivers was 29.6 years. High concentrations of other psychoactive drugs were detected in all the blood samples where a SC was found. AM-2201 and JWH-018 were the most frequently detected SCs, each found in five cases. In addition RSC-4, JWH-122, JWH-081 and JWH-250 were detected. None of the drivers had reported using SCs prior to driving.

Despite the limited number of SCs investigated in this 7 week study period, a considerable percent of the cases were positive. Other psychoactive drugs of abuse were always found concomitant with the SCs, and the age of these drivers indicates that experienced drug users also ingest SCs. Since other drugs were found in all the samples, the psychomotor impairment caused by the SCs is difficult to estimate. Our study shows the importance of screening analyses of biological samples from different populations to assess the prevalence of drug use, since self-reporting might be encumbered with significant under-reporting.

1. Introduction

In the course of the past few years several types of synthetic cannabinoids (SC) have appeared on the drug marked word wide, as well as in Norway (Vardakou et al., 2010; Fattore and Fratta, 2011; Tuv et al., 2012). These compounds, known as e.g. "K2" and "Spice" are available on the Internet, often referred to as "legal highs" (Fattore and Fratta, 2011). To be able to keep up with the frequent appearance of new substances on the drug market, Norway has regulated groups of SCs under schedule 1 control (Slv, 2013) from February 14th 2013. Prior to this legislation, only eight SCs were registered as illicit drugs. The prevalence of use of these compounds in the drug abuser population is not known. It is in addition not known to what extent this population drives a car after intake of SCs and to what degree SCs impair driving skills and cause increased risk of traffic accidents.

Some studies have investigated the prevalence of SC use, both by surveys and analysing biological material. Winstock et al. investigated the population associated with the dance music scene in England, and 13% reported having used "Spice" (Winstock et al., 2011). Surveys conducted each year among households in England and Wales, revealed that the prevalence of Spice use was 0.4% among the age group 15–24 and 0.1% among the group 25–59 years (Smith and Flatley, 2011). The prevalence among college students from USA is reported to be 9% for use of "Spice and other smokable blends" (Hu et al., 2011b). Among athletes one study found SC in 2 out of 7500 urine samples tested (Moller et al., 2011) and in another study the prevalence of JWH-018, JWH-073 or any of their

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E-mail address: situ@fhi.no (S.S. Tuv).

du må opp i et par tusen for å få deg en vekt som måler nøyaktig i mg.

det virker som om du er relativt uerfaren med dette, jeg anbefaler deg å generelt lese m
og effektene av det, og eventuelle risikoer før du begir deg ut på noe potensielt dumt o

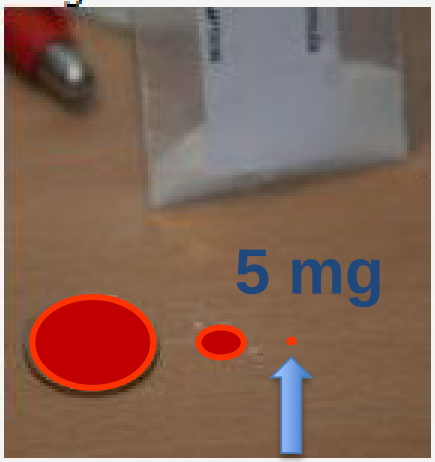
bare for å være sikker på at du ikke overdoser så til de grader, tok jeg et bilde av mitt..

1g:

2005

5mg:

5mg:



AM-2201 doses as low as 0.5 mg

Sensitive analysis necessary!

Death by NPS?

Synthetic cannabinoid effects

- Similar to cannabis, but stronger and lasts longer (very potent compounds), and more **poisonous**
- **-Psychological**:
  - Hallucinations, psychosis (long lasting!)
  - Anxiety, anxiety attack

Suicides
Accidents

- **Physical**:
  - Acting out
  - Cardiac arrest (teen-agers)
  - Cramps
  - Coma
  - Poisoning, death

Designer drugs (amphetamine type)

- **Psychological**: agitation, hallucinations, anxiety/panic, bisarre behaviour

Suicides
Accidents

- **Physical**: seizures, sirculation failure, extreme fever, organ failure, brain odema

  → many reports of poisonings

  → Deaths (mefedron, PMMA, 5-IT, MDPV, AMT, fluoramphetamines, methyl amphetamine, bromoDragonFLY ++)

En liten del av det 239 kilo store beslaget av metamfetamin som ble gjort av politiet i Sydney, Australia i mai måned. Det narkotiske stoffet, som også går under det engelske kallenavnet Ice (is), har nå dukket opp i en kopiert versjon kalt PMMA. Norge er det landet i Europa som er hardest rammet av det uforutsigbare dopets virkninger. FOTO: AFP

Kopi av amfetamin dreper flere i Norge

Folkehelseinstituttet advarer mot rusmiddel som selges som amfetamin, men som er dødelig.

TONE TVEØY STRØM-GUNDERSEN

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The PMMA epidemic in Norway: Comparison of fatal and non-fatal intoxications

Merete Vevelstad [a,*], Elisabeth Leere Øiestad [a], Gerrit Middelkoop [a], Inger Hasvold [a], Peer Lilleng [b], Gerd Jorunn M. Delaveris [a], Tormod Eggen [c], Jørg Mørland [a,d], Marianne Arnestad [a]

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- Extreme body temperature
- Acute respiratory problems
- Acute hearth failure
- Hallucinations
- Seizures
- Coma, multiple organ failure

PMMA

- Surprisingly large number of deaths (2010-13): 29 PMMA-related deaths
- Cause of death
  - 32% PMMA alone
- 36% PMMA and amphetamine/methamph/cocaine
- 21% PMMA and CSN depressants
- (7% other cause)

In the same time periode: identified in samples from 130 drivers suspected of DUI

Compounds found in blood at NIPH

JWH-018
AM-2201
JWH-081
JWH-122
JWH-250
RCS-4
MAM-2201
UR-144
UR-144 degradant
APINACA
5F-APINACA

mCPP
MDPV
Methylamphetamine
Fluoromethamphetamine
AH-7921
N,N-dimethyl MDA
3-MMC
Doxylamine
Homoamphetamin
Mitragynin
Metoxetamine

Etizolam
Diclazepam
LSD
Ketamine
Mephedrone
PMA
PMMA
α-PVP

Urine samples

- LC-MS/MS confirmation based on immunoassay screening
- Mainly findings of JWH-018 pentanoic acid

What to analyse in urine?

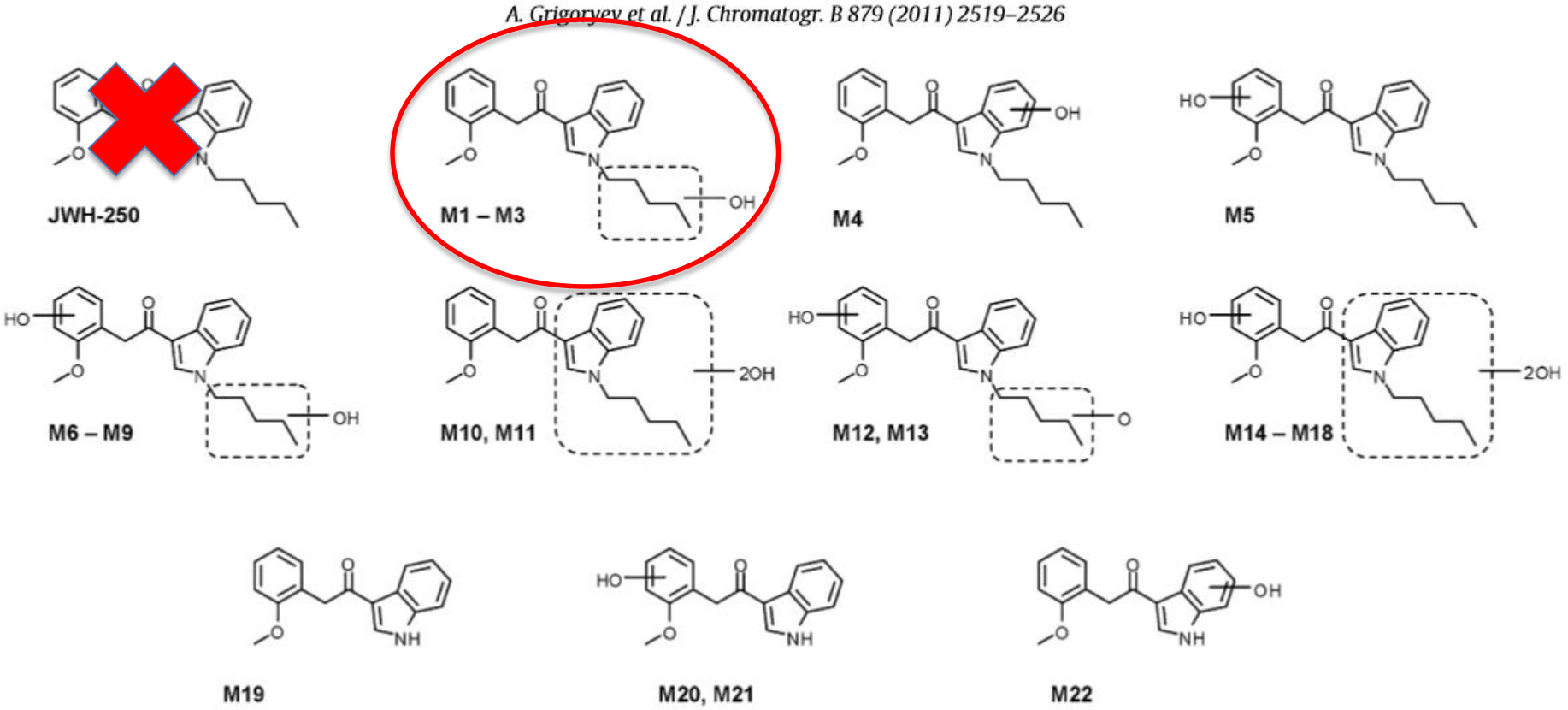


Fig. 1. Proposed structures of the identified JWH-250 urinary metabolites.

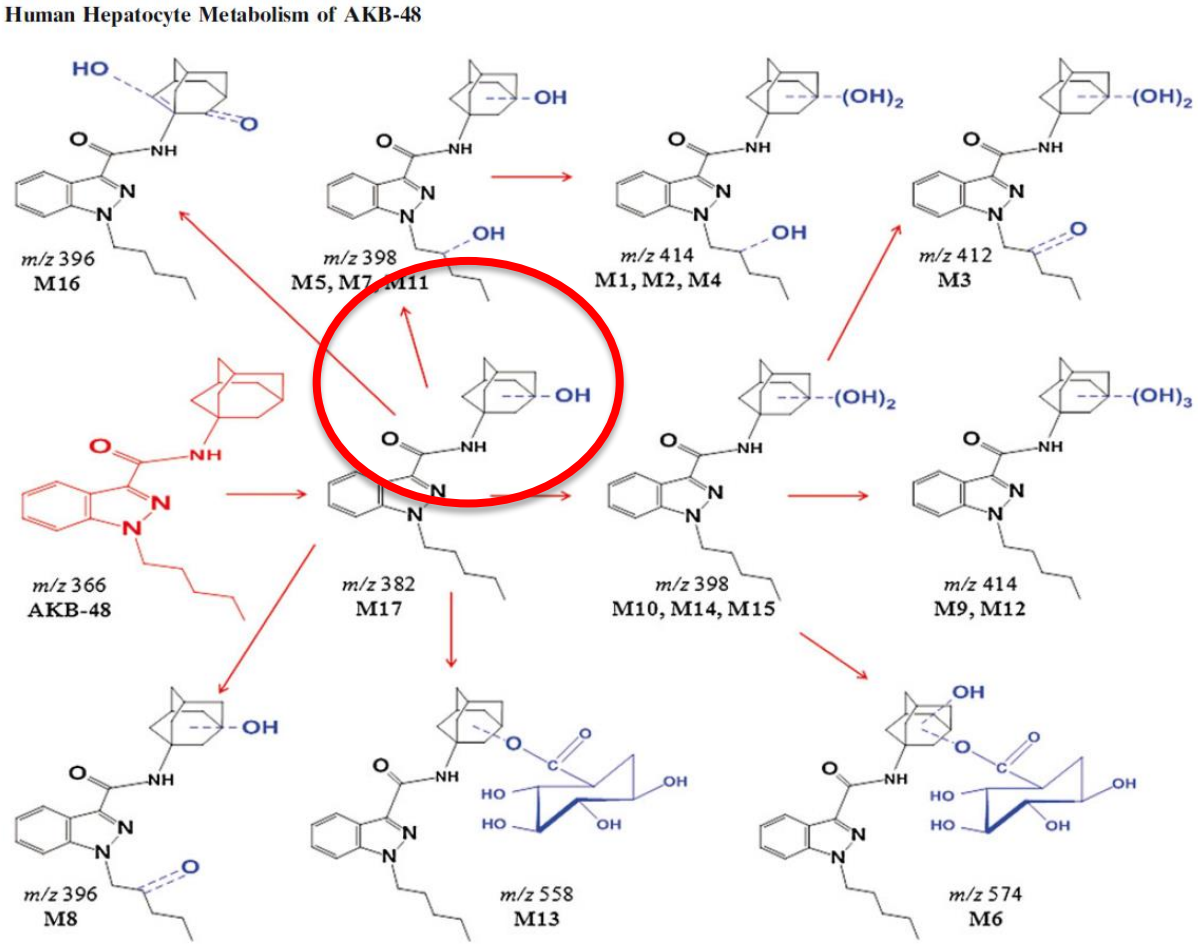
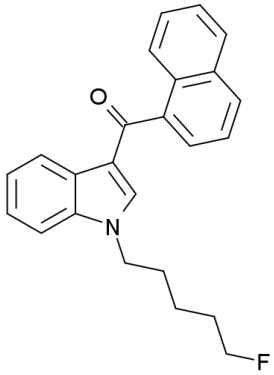


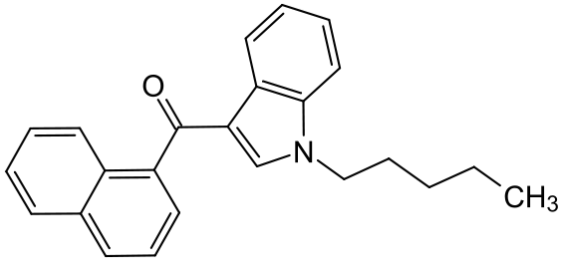
Fig. 8. Proposed metabolic pathways of AKB-48 in humans (*m/z* values correspond to protonated molecules)

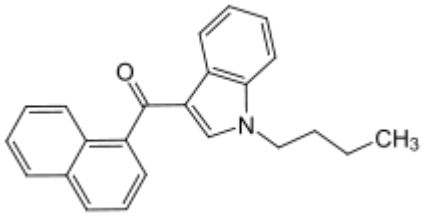
Gandhi AS, Zhu M, Pang S, Wohlfarth A, Scheidweiler KB, Liu HF, Huestis MA. First Characterization of AKB-48 Metabolism, a Novel Synthetic Cannabinoid, Using Human Hepatocytes and High-Resolution Mass Spectrometry. AAPS J 2013;15:1091-8.

Similar compounds can give the same metabolites

- AM-2201 JWH-018 JWH-073





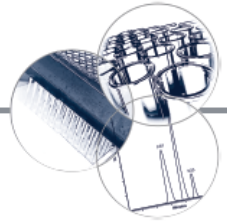


Example metabolite ratio

AM2201 metabolitt	RCS-4 N-(5-hydroksy-pentyl) metabolitt	JWH-073 N-(4-hydroksy-butyl) metabolitt	JWH-073 N-butansyre metabolitt	JWH-018 N-(5-hydroksy-pentyl) metabolitt	JWH-018 N-pentansyre metabolitt	JWH-122 N-(5-hydroksy-pentyl) metabolitt
3,1		0,31	11,4	52,7	72,6	
		0,01	3,6	39,7	6,2	
		0,05	1,6	18,3	2,3	
6,6		0,38	2,4	42,2	65,3	0,05
0,19		0,03	0,24	2,9	2,3	

Oral fluid samples

- Alternative when easy sampling is important
- No same gender issues
- Find the substances ingested
  - easier to change according to changing drug markets
- Shorter detection times



Screening of synthetic cannabinoids in preserved oral fluid by UPLC–MS/MS

Background: The abuse of a rapidly changing range of synthetic cannabinoids is increasing worldwide. Oral fluid, which contains the parent compounds and is easily collected, could be a good alternative medium for drug screening for synthetic cannabinoids. **Results:** A method for screening of 18 synthetic cannabinoids in preserved oral fluid collected with the Intercept® collection device, using UPLC–MS/MS, was validated. Limits of quantification ranged from 0.2 to 2 ng/ml in oral fluid. In several real cases, AM-2201 and/or JWH-018 were found. **Conclusion:** The presented method allowed rapid and sensitive screening of synthetic cannabinoids in preserved oral fluid collected with the Intercept collection device.

Synthetic cannabinoids (SC) often called 'spice' or 'legal highs' have become an increasing drug problem worldwide. The market is rapidly changing; for example, more than 20 new SC were registered in the European early warning system in 2011 [1]. Although the bulk of seizures in Norway are of minor size compared with cannabis (~10 kg and 1% of the number of seizures according to the Norwegian police drug statistics [101]), even minor amounts of SC equal very many user doses due to the very high potency of the compounds. If the amounts of SC seized in 2011 are converted to user doses, the amount is approximately 50% of the seized user doses for cannabis. Eight SC were put on the Norwegian Drug List in 2011 [102]. The use of SC is still a minor problem compared with, for example, the use of opiates, amphetamines and cannabis; however, the use of SC by very young users [2], using the internet as their main source of supply, seems to be increasing. The same seems to be the case for persons trying to avoid regular drug testing schemes such as, for example, prison inmates [KOLLANES JO, CORRECTIONAL SERVICES, REGION WEST, PERS. COMM.].

For the analysis of SC the primary focus has been on designing methods for blood/serum and urine [3–16], and only a few methods for **oral fluid** (OF) have, to our knowledge, been published [17–19]. One of the problems with developing urinary methods is that SC are excreted almost solely as metabolites, precluding detection of the parent compound [11,13,16]. Preferential screening of SC in serum or whole blood has therefore been suggested [7]. At least for the aminoalkylindole type SC the monohydroxylated metabolites have been identified as the most abundant [12], and identification of metabolites based on findings in pairs of serum and urine samples can be possible [20]. However, quantification is not possible before the corresponding reference substances have been synthesized. In addition, it seems that several substances can give the same urinary metabolites, making the identification of the actual substance ingested difficult [14,16]. If the metabolites are known and commercially available a urine method might still be more advantageous as the concentrations in urine are higher, and the detection times will be longer.

In OF, just as in blood/serum, the parent compounds themselves can be found [18], therefore OF is, possibly, a very beneficial matrix for SC testing, where the need for rapid change in repertoire according to trends in drug use is necessary. In cases with possible negative sanctions it is important to be able to supervise the collection process to avoid samples being adulterated or exchanged with clean samples. This is easily achieved for OF samples, and in addition, sample collection does not require medical personnel, which is a benefit compared with blood sample collection. The need for special facilities or collectors with the same gender as the person being tested are not necessary, as is the case with monitored urine sampling. OF has evolved as an alternate drug testing matrix routinely being used for many applications such as roadside testing, workplace testing and drug screening for pain patients and people in opiate maintenance programs [21–25]. The fact that the excretion of cannabis into OF is very low, and findings of tetrahydrocannabinol (THC) in OF are mainly due to residual THC in the mucosa [26] must, however, be kept in mind. The same

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FUTURE SCIENCE part of fsg

Project with prison inmates

- Samples after suspicion (n=45)
- 20% positive
- JWH-018 and AM-2201

- Information about intake mostly not given
- One case admission of intake 3 days earlier

Oral fluid samples 2014

- 224 oral fluid samples from prisons analyzed
- Large repertoire
- 23% positive
- 3,6% for NPS (2CE, JWH-018)

Other projects

- Night club patrons
  - Cooperation with SIRUS

- Acute medicine samples
  - Cooperation with Ullevål hospital ER
    and Oslo emergency ward (legevakten)
    (Spin-off of the Euro-Den project)

- ETORA - Evaluering av Toksisitet ved Rusmiddel Analyse
  - Cooperation with the Poison Information Centre (Giftinformasjonen)

Summary

- NPS can be found in all types of forensic cases
- Can be determined in blood, urine and oral fluid
- Sensitive analysis required
- Difficult to keep the repertoire updated