Development and application of methodology for designer drugs

Determination of synthetic cannabinoids in urine by UPLC-MS/MS
Solfrid Hegstad

The challenge of new designer drugs by LC-Q-TOF
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Department of Clinical Pharmacology
27th of may 2014

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Determination of synthetic cannabinoids in urine by UPLC-MS/MS

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Synthetic cannabinoids-highly potent

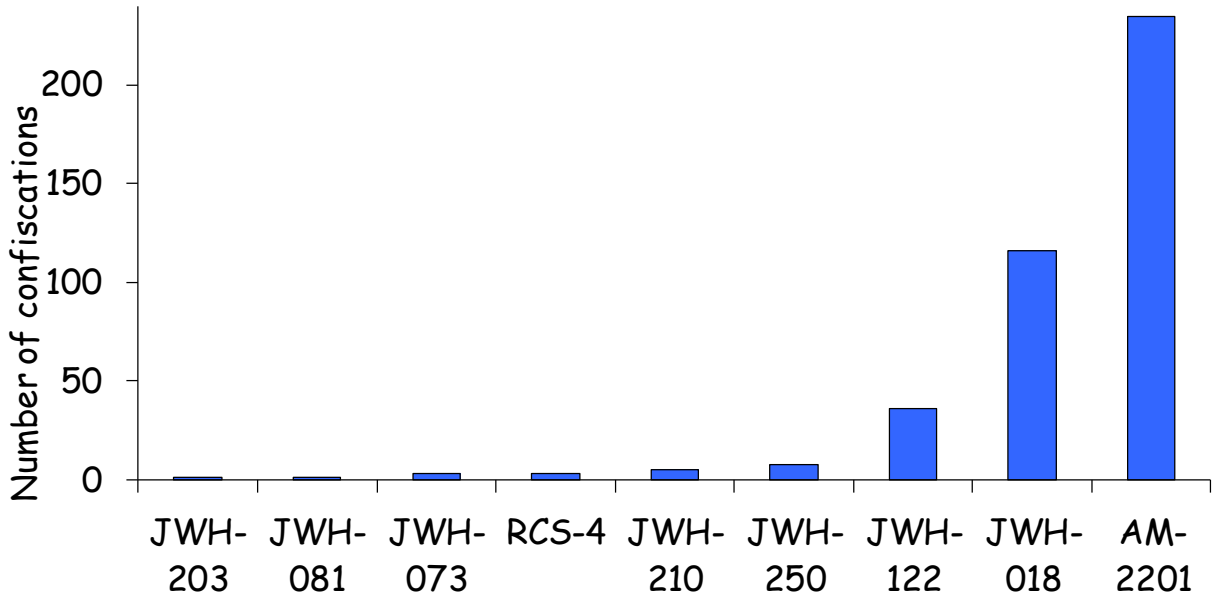


0.1 kg

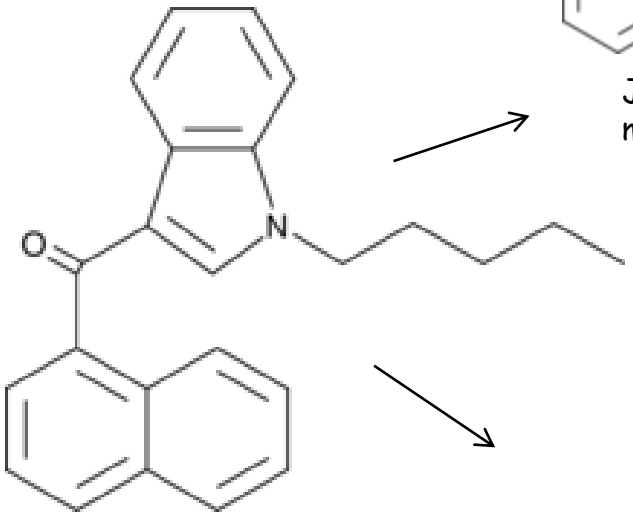
10 kg

Photo: Stig Jaarvik/NRK

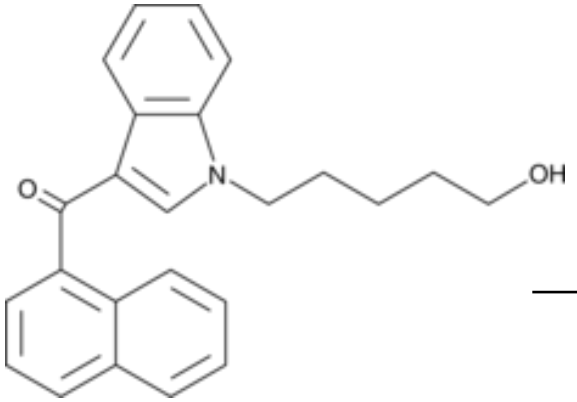
Confiscations conducted by Norwegian law enforcers, 2011-2012



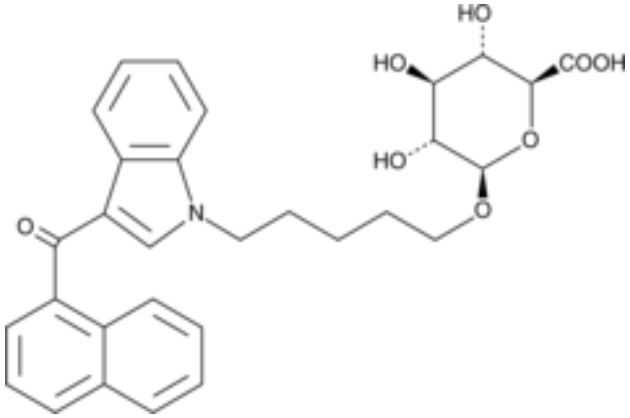
Source: Kripos



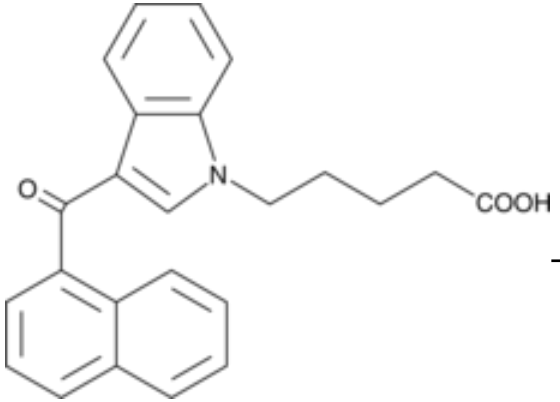
JWH 018



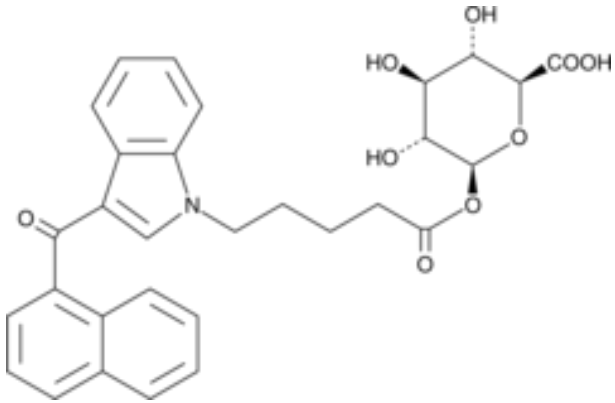
JWH 018 N-(5-hydroxypentyl) metabolite



JWH 018 N-(5-hydroxypentyl) β-D-Glucuronide

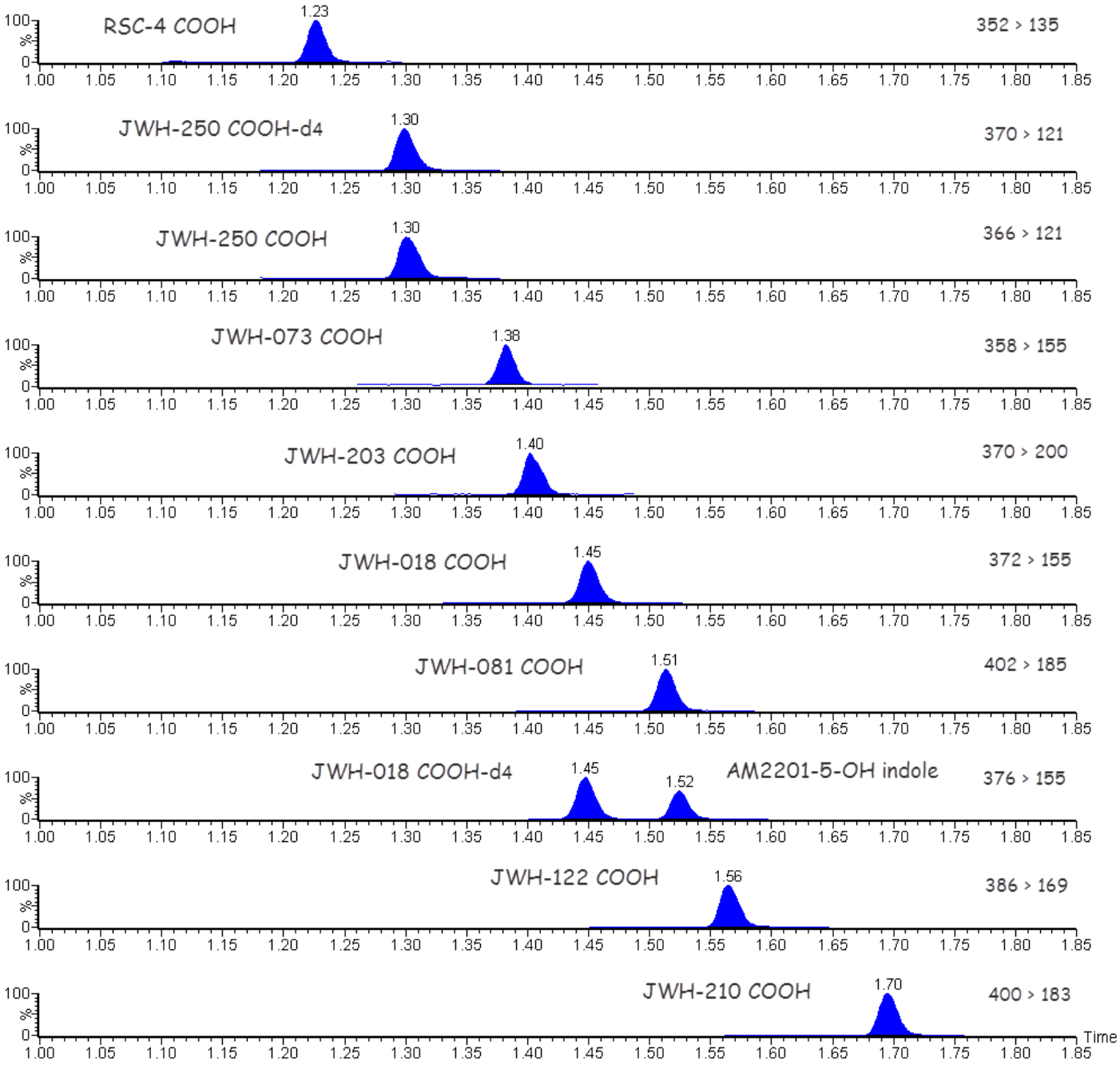


JWH 018 N-pentanoic acid metabolite



JWH 018 N-pentanoic acid β-D-Glucuronide

Method	
Pipetting robot Tecan Freedom EVO	100 µl urine, 1:10 methanol, buffer
	Enzymatic hydrolysis
Analytical column	HSS T3 2,1X100mm
Mobile phase	0.1 % HCOOH/ acetonitrile
Runtime	2.5 min
UPLC-MS-MS Acquity Xevo-TQ-S, Waters	ESI+
Internal standard	JWH-250 COOH-d_4, JWH-018 COOH-d_4


RSC-4 COOH
1.23
352 > 135
JWH-250 COOH-d4
1.30
370 > 121
JWH-250 COOH
1.30
366 > 121
JWH-073 COOH
1.38
358 > 155
JWH-203 COOH
1.40
370 > 200
JWH-018 COOH
1.45
372 > 155
JWH-081 COOH
1.51
402 > 185
JWH-018 COOH-d4
1.45
AM2201-5-OH indole
1.52
376 > 155
JWH-122 COOH
1.56
386 > 169
JWH-210 COOH
1.70
400 > 183
100
%
0
1.00 1.05 1.10 1.15 1.20 1.25 1.30 1.35 1.40 1.45 1.50 1.55 1.60 1.65 1.70 1.75 1.80 1.85
Time

Analyte	Calibration range (ng/mL)	LOQ (ng/mL)	Theoretical conc. (ng/mL)	Between-assay n=10, (%CV)	Bias (%)	ME (%)
AM2201 5-OH indole	1.0-10	0.3	1.5	8	2	85
			2.5	3	8	
			8	5	5	85
JWH-018 *COOH*	0.3-10	0.1	0.45	6	-3	83
			2.5	5	1	
			8	3	-1	89
JWH-073 *COOH*	0.3-10	0.1	0.45	7	-4	69
			2.5	4	-2	
			8	4	-2	73
JWH-081 *COOH*	0.5-10	0.2	0.75	6	-3	76
			2.5	4	3	
			8	4	-0.3	78
JWH-122 *COOH*	0.3-10	0.1	0.45	9	-3	85
			2.5	3	3	
			8	4	-1	89
JWH-203 *COOH*	0.3-10	0.1	0.45	7	-3	70
			2.5	5	2	
			8	4	-1	76
JWH-210 *COOH*	0.3-10	0.1	0.45	8	-1	81
			2.5	3	4	
			8	3	-1	84
JWH-250 *COOH*	0.5-10	0.3	0.75	4	-6	84
			2.5	4	1	
			8	5	-1	85
RCS-4 *COOH*	0.5-10	0.2	0.75	9	-5	75
			2.5	4	2	
			8	3	-4	82

Application

- In routine since May 2013
- 1800 samples
- Only 30 positive with JWH-073 COOH and JWH-018 COOH
- From 7 different individuals

Screening SPE-LC-Q-TOF

- Solid phase extraction
  - Automation on Tecan
- Simple validation of selected compounds

The challenge of new designer drugs

- Health challenges
- Analytical challenges
- Juridical challenges

Analytical challenges

- What designer drugs are we looking for?
- Low concentrations / need very sensitive instruments
- Unknown urine metabolites
- No reference material

Scan for unknown designer drugs?



?

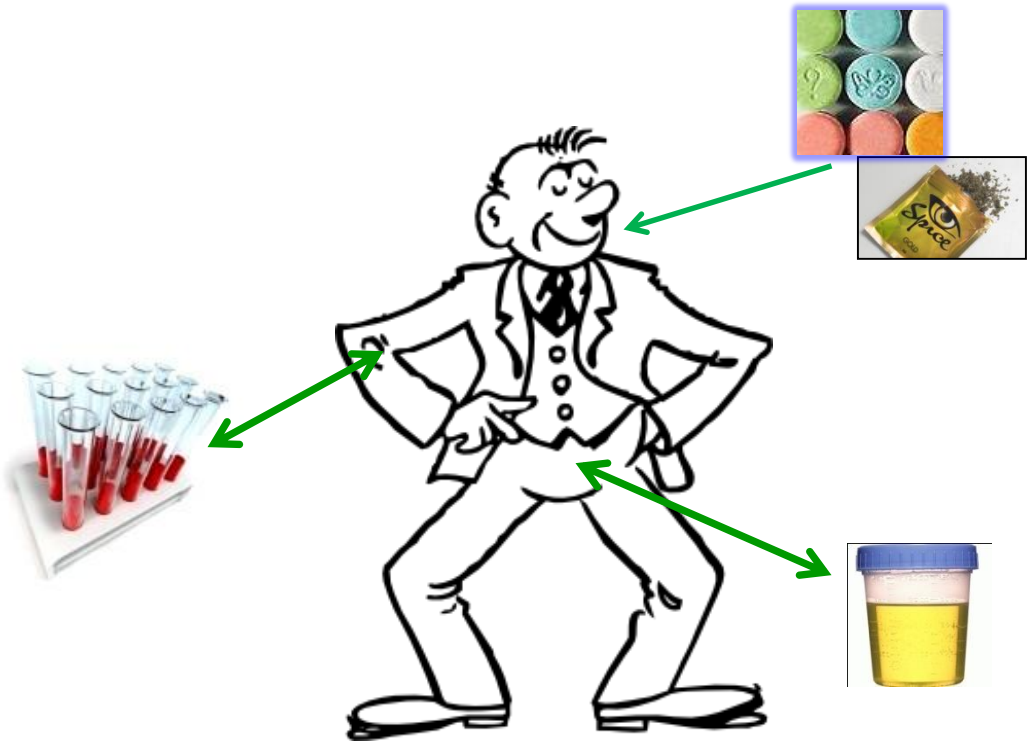


GCMS



LC-Q-TOF

Analysing-material on LC-Q-TOF



Confiscations

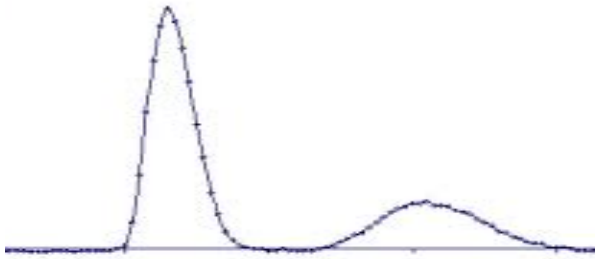
Forensic blood/serum

Forensic urine

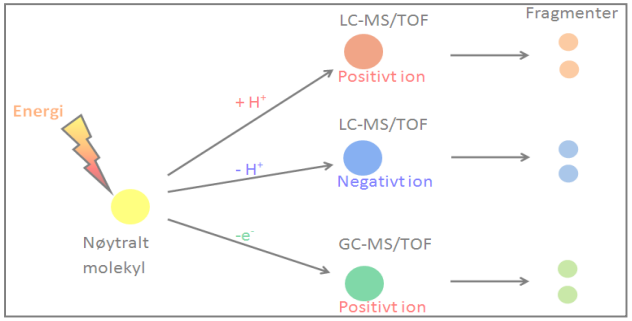
Special cases

How does LC-Q-TOF work?

LC



Q



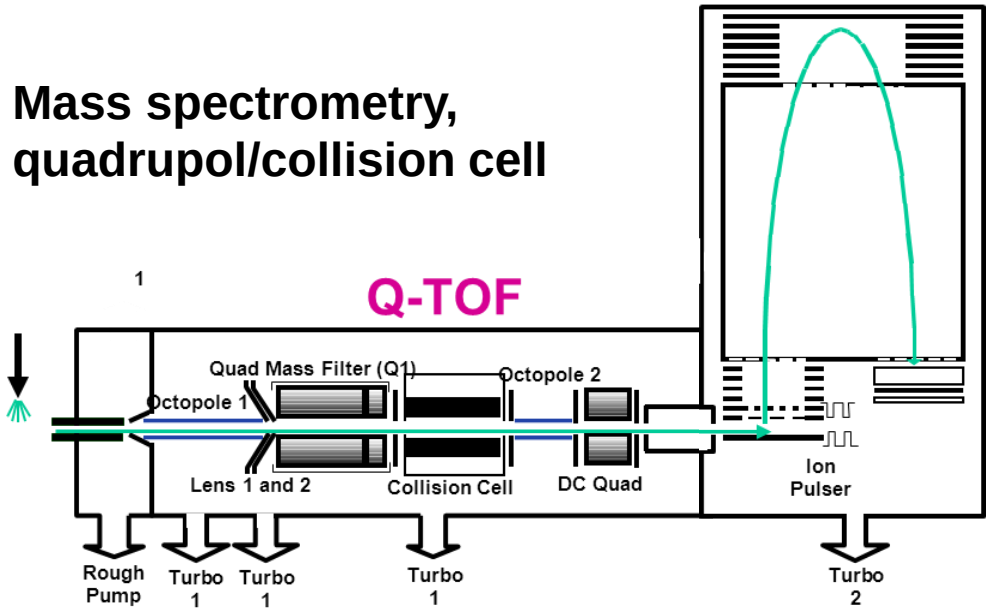
TOF



Liquid Chromatography



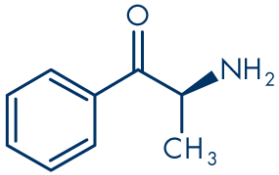
Mass spectrometry, quadrupol/collision cell



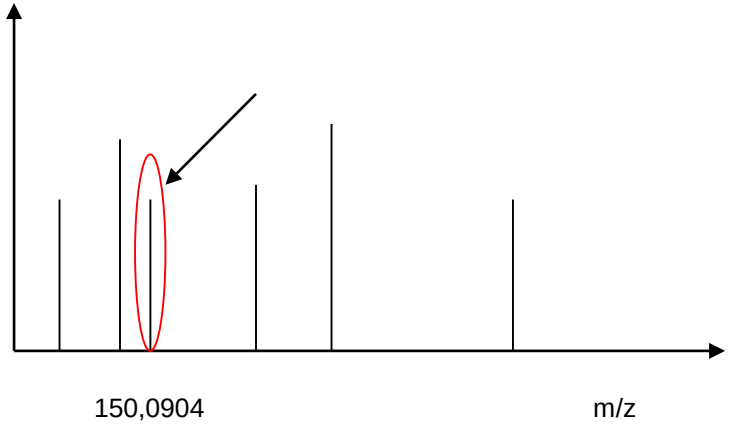
The flight time of ions are converted to accurate mass, $t = k\sqrt{m/z}$

One example:

- Cathinone MW 149 (nominal mass)
  MW 149.084060 (exact mass)







Catha edulis (khat)

Exact mass vs nominal mass?

- Exact, or calculated, mass of a molecule is the sum in the molecular formula of the most abundant isotop.
- Molecular formula $C_9H_{11}NO$

- Exact mass :

9xC	= 9x12	= 108
11xH	= 11x1.007825	= 11.086075
1xN	= 1x14.003074	= 14.003074
1xO	= 1x15.994915	= 15.994915
Sum		= <u>149.08406</u>

Nominal mass:

9x12 = 108

11x1 = 11

1x14 = 14

1x16 = 16

Sum = 149

Structural diversity, example: M = 149

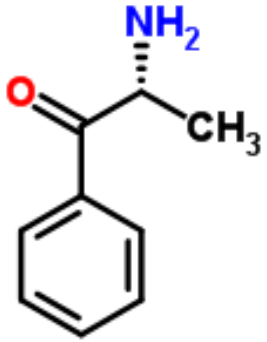
Number of isomers according to MOLGEN
(Molecular structure generation; http://molgen.de)

Included atoms:	Molecular formulas
C, H, N, O, S, P, F, Cl, Br:	120
C, H, N, O, S, P:	38
C, H, N, O:	27

2 ppm = ± 0,0003

Formula	No. Isomers (MOLGEN)	Number Beilstein	number NIST	Exist	Accurate mass	M	M+1	M+2	M+3
$C_3H_3NO_6$	41580	0	0	NO	148,996038	100	3,89	1,29	0,05
$C_2H_3N_3O_5$	152977	0	0	NO	149,007271	100	3,51	1,08	0,04
$C_7H_3NO_3$	11449751	3	0	YES	149,011293	100	8,1	0,9	0,06
$CH_3N_5O_4$	99306	0	0	NO	149,018504	100	3,13	0,86	0,03
$C_6H_3N_3O_2$	50459744	2	0	YES	149,022526	100	7,72	0,67	0,04
$C_{11}H_3N$	53109027	0	0	YES	149,026548	100	12,31	0,69	0,02
$C_4H_7NO_5$	202072	10	0	NO	149,032421	100	4,99	1,13	0,05
$C_5H_3N_5O$	39760215	5	0	YES	149,033759	100	7,34	0,44	0,02
$C_3H_7N_3O_4$	822099	2	0	NO	149,043654	100	4,61	0,91	0,04
$C_4H_3N_7$	6505400	0	0	YES	149,044992	100	6,96	0,21	0
$C_8H_7NO_2$	48687255	265	13	YES	149,047676	100	9,2	0,79	0,05
$C_2H_7N_5O_3$	633408	0	0	NO	149,054887	100	4,23	0,69	0,03
$C_7H_7N_3O$	166085562	222	7	YES	149,058909	100	8,82	0,55	0,03
$CH_7N_7O_2$	121630	0	0	NO	149,066120	100	3,85	0,47	0,02
$C_5H_{11}NO_4$	121350	52	0	YES	149,068805	100	6,09	0,98	0,05
$C_6H_7N_5$	77737459	129	10	YES	149,070142	100	8,44	0,32	0,01
$C_4H_{11}N_3O_3$	473871	1	0	YES	149,080038	100	5,71	0,75	0,04
$C_9H_{11}NO$	**25 895 621**	**724**	**45**	YES	**149,084060**	**100**	**10,31**	**0,68**	**0,03**
$C_3H_{11}N_5O_2$	355574	0	0	NO	149,091271	100	5,33	0,53	0,02
$C_8H_{11}N_3$	49755227	182	5	YES	149,095293	100	9,93	0,44	0,01
$C_2H_{11}N_7O$	67609	0	0	NO	149,102504	100	4,95	0,31	0,01
$C_6H_{15}NO_3$	13539	23	2	YES	149,105188	100	7,2	0,84	0,05
$CH_{11}N_9$	2766	0	0	NO	149,113737	100	4,57	0,09	0
$C_5H_{15}N_3O_2$	44621	0	0	YES	149,116421	100	6,82	0,61	0,03
$C_{10}H_{15}N$	2569697	558	40	YES	149,120443	100	11,41	0,59	0,02
$C_4H_{15}N_5O$	26983	0	0	NO	149,127654	100	6,44	0,39	0,02

Examples of structural isomers of cathinone

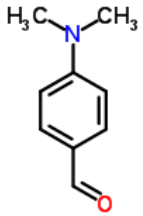
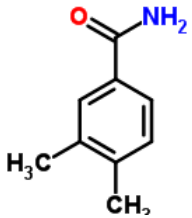
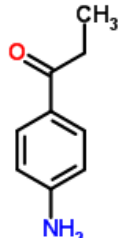
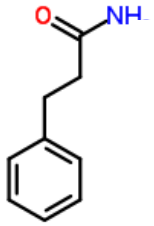
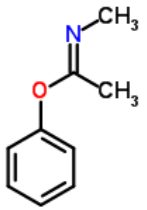


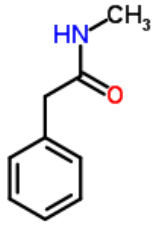
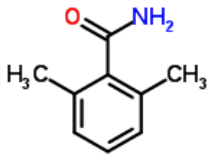
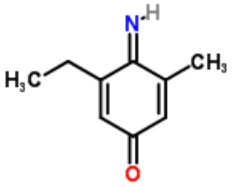
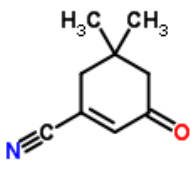
$C_9H_{11}NO$

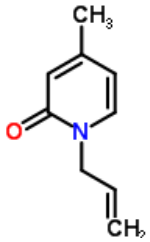
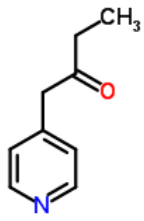
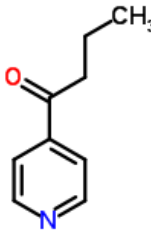
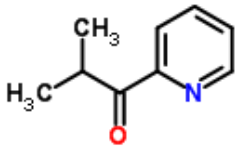
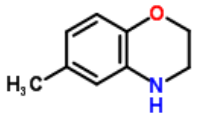
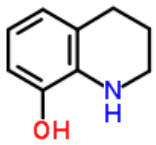
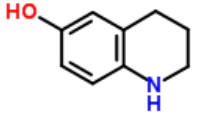
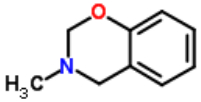
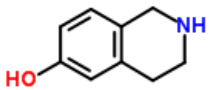
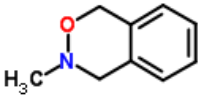
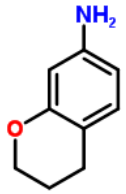
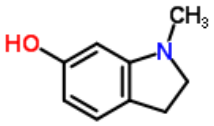
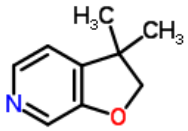
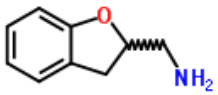
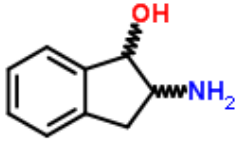
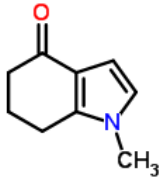
(www.chemspider.com, 829 hits)

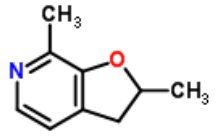
Altogether 25 895 621 structural isomers possible

Stereoisomers not included

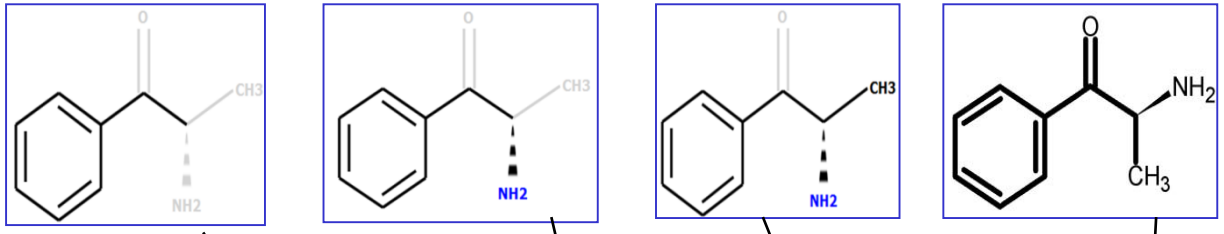





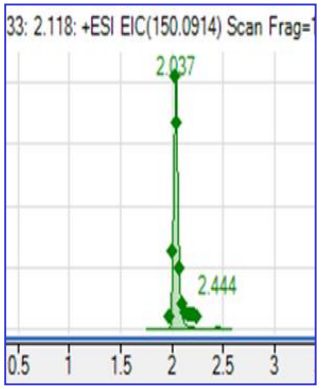


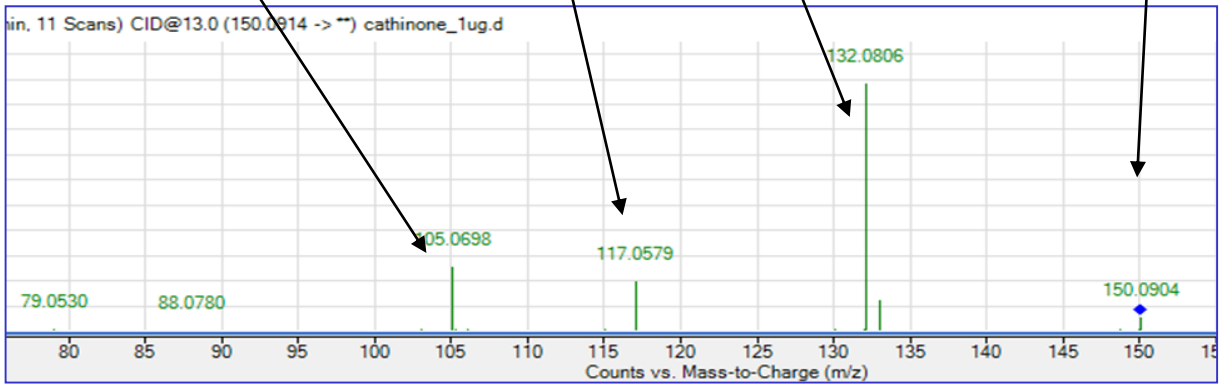
Collision cell fragments the parent ion, TOF give accurate mass of both parent- and daughter ions

Exact mass 149.084060



Retention time





The accurate masses of fragments and retention times are compared to database (exact mass of 9000 compounds) and library (MSMS spectra from 3000 injected reference material).

No hit→Chemspider→Correlator / Co-operation other labs. Retrospectively!

The landscape of designer drugs





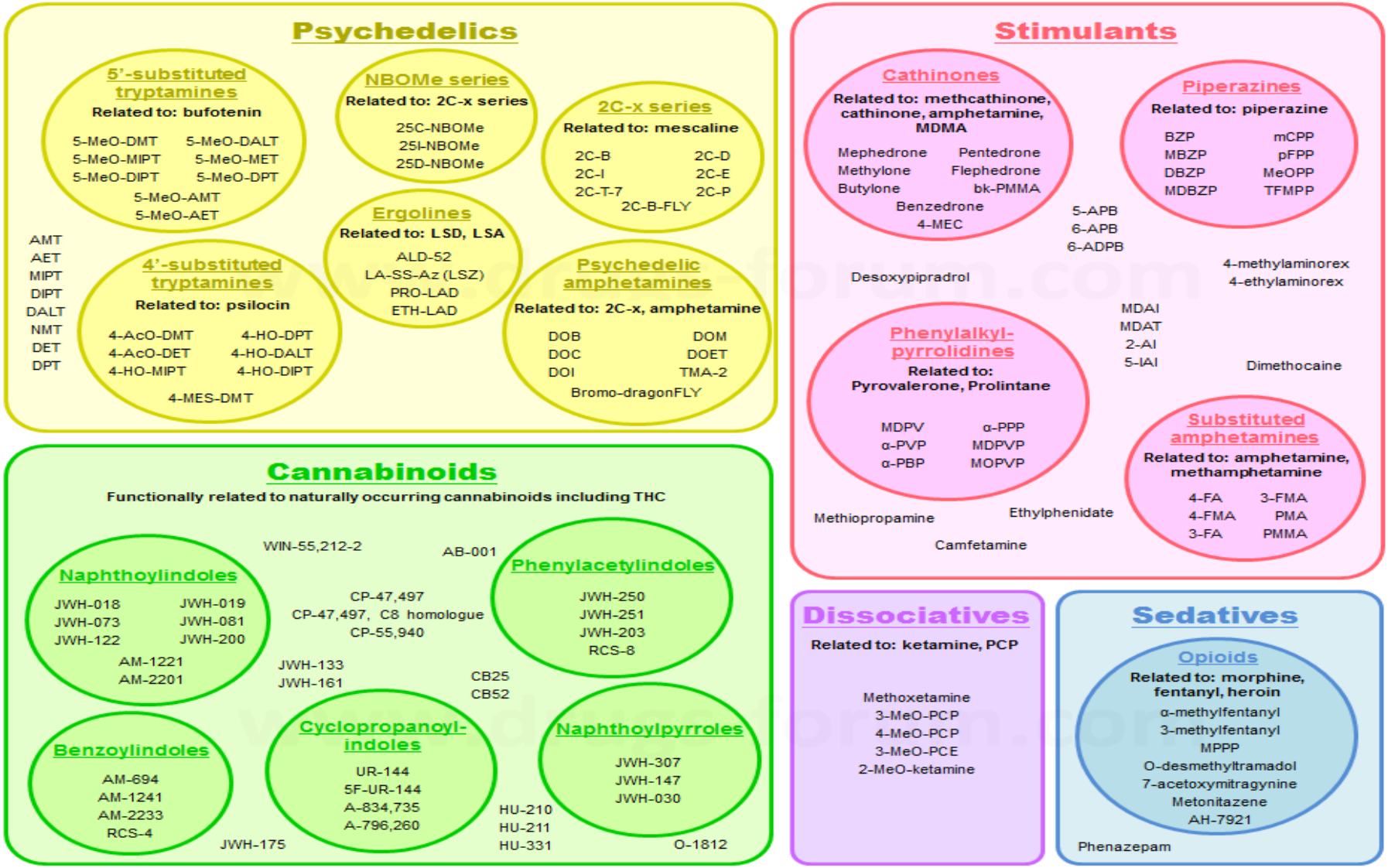








Sorted by effect



Major families and subfamilies of research chemicals,and some of their most prominent members.

http://www.drugs-forum.com/forum/showwiki.php?title=Category:Research_Chemicals#ixzz2LqP5MQt0

Sorted by chemical properties

S. L. Hill & S. H. L. Thomas



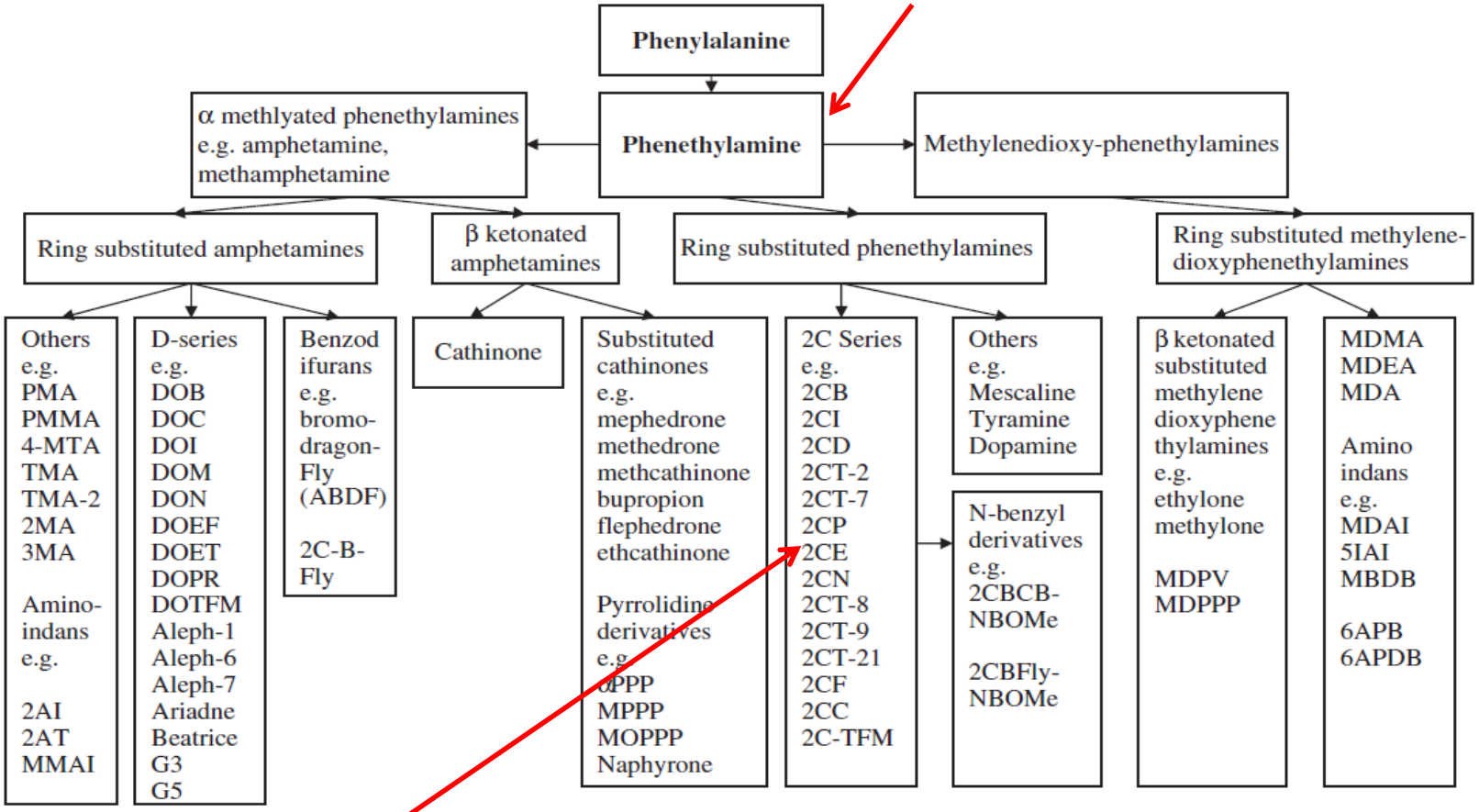
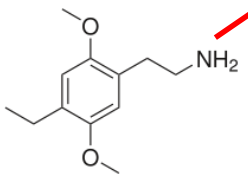
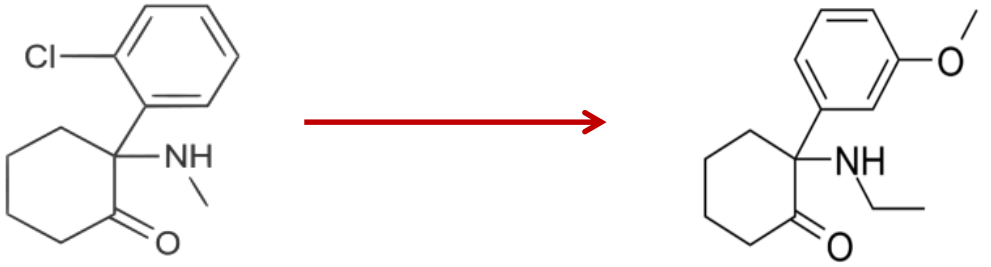


Fig. 4. Classification of the phenthylamines.

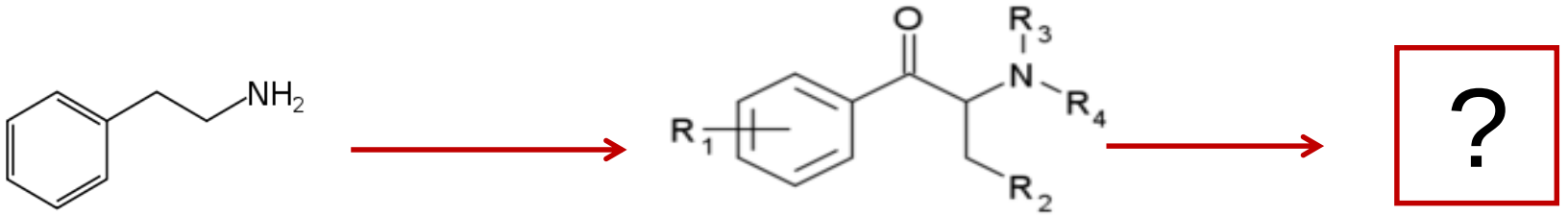


How make an illicit drug “legal”?



Ketamine

Methoxetamine



Phenethylamine

β ketonated amphetamines

This is not Chiron!

”Chemists” in clandestine laboratories



-Most of it made outside Europe in low cost countries,

-Intermediary Europe for mixing / packing /distribution

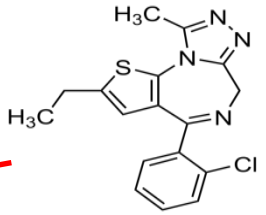


Case 1

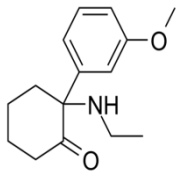
- Bought as etizolam, methoxetamine, UR 144
- Analyzed as etizolam, methoxetamine, JWH 210
- The leaves had been sprayed with JWH 210 (yellow powder), not UR144



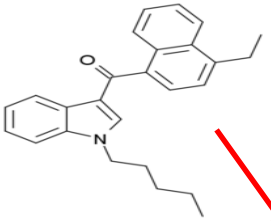




Etizolam

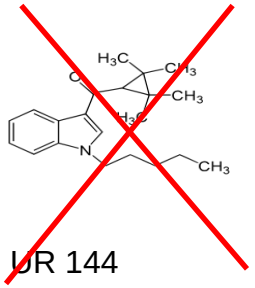


Methoxetamin



JWH 210



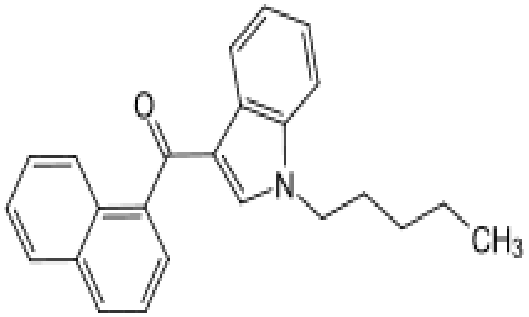


UR 144

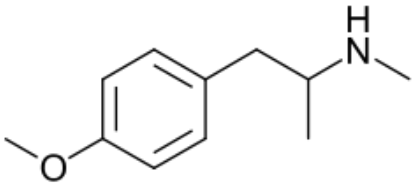
Case 2
Synthetic cannabinoids

- Inmates at a drug rehabilitation clinic were apparently under the influence of a drug, but their urine screenings were negative.
- Confiscation showed JWH 018





Case 3



Users bought drug as amphetamine

- Not expected result, injected more
- Reaction:”crossing eyes, bodily disaste, ”blackout” for 24 hours
- Drug was PMMA, not amphetamine

Parametoksymetamfetamin



En mann kjøpte dette hvite pulveret som ifølge selgeren var «meget god amfetamin». Han injiserte litt av stoffet, men det forventede kicket uteble, og han injiserte derfor litt til. Da opplevde han øyenmuskelkramper («øynene beveget seg i kryss») og et intenst kroppslig ubehag («kløe, indre uro, hjerteklapp og svetting»), deretter en slags «blackout» som varte i over et døgn. Fire andre personer injiserte det samme pulveret, med liknende erfaringer. Vi mottok pulveret til analyse og fant at det besto av parametoksymetamfetamin (PMMA).

PMMA er et sentralstimulerende middel, strukturelt beslektet med amfetamin og metylendioksymetamfetamin (MDMA, «ecstasy»), men billigere å produsere. Stoffet er i omløp i Norge, som pulver og tabletter. Det ser ut som og selges som amfetamin eller ecstasy. De fysiske effektene av PMMA – som takykardi og varmefølelse – likner dem brukerne kan oppleve etter inntak av amfetamin og MDMA.

Imidlertid er de psykiske effektene – som eufori og forsterkede sanseinntrykk – mindre uttalte. Dessverre kan fravær av de psykiske effektene lede brukerne – som tror de har inntatt svakt MDMA eller amfetamin – til å innta mer. I verste fall kan dette føre til potensielt dødelige forgiftninger, med hjerterytmeforstyrrelser, hjerneslag, hypertermi, kramper og/eller organsvikt.

Norge er et av de hardest rammede landene i Europa når det gjelder PMMA-dødsfall, med over 20 registrerte dødsfall siden sommeren 2010. Den utbredte bruken av amfetamin i Norge antas å være noe av forklaringen.

Andreas Austgulen Westin
andreas.westin@legemidler.no
Wenche Rødseth Brede
Avdeling for klinisk farmakologi
St. Olavs Hospital

Andreas Austgulen Westin (f. 1977) er lege i spesialisering ved Avdeling for klinisk farmakologi.
Ingen oppgitte interessekonflikter.

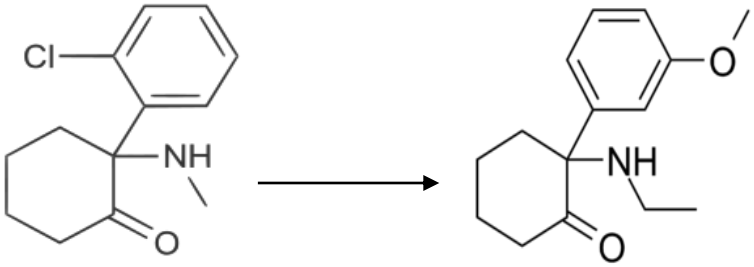
Wenche Rødseth Brede (f. 1951) er m.sc. og spesialbioingeniør ved Avdeling for klinisk farmakologi.
Ingen oppgitte interessekonflikter.

Mottatt 25.8. 2011 og godkjent 1.9. 2011. Medisinsk redaktør Jon Amund Kyte.

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Ettertrykk forbudt. Lastet ned fra *www.tidsskriftet.no* 29.5.2013

Case 4





Ketamine **Methoxetamine**

- A client bought white tablets thought to be ketamine.
- Became catatonic, restless, tachycardic, confused.
- Drug was methoxetamine.

- LC-Q-TOF in designer drug-search
  - High specificity / accurate mass for all compounds, vital for identification for new designerdrugs
  - High sensitivy / detects low concentrations
  - Compare to updated database/library
  - Use data retrospectively
  - Co-operate with other labs /new drugs, spectra-info

- Still challenges
  - Constant flow of new drugs!
  - Not updated database/library fast enough
  - Low concentrations/unknown urine metabolites
  - No reference material

Thank you for listening!



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