VYUŽITÍ RŮZNÝCH SKENOVACÍCH REŽIMŮ ORBITÁLNÍ PASTI NA MODELOVÉM METABOLITU JWH-018 K DOSAŽENÍ MINIMÁLNÍCH LOD A LOQ V PROBLEMATICE BIOANALÝZY SYNTETICKÝCH KANNABINOIDŮ

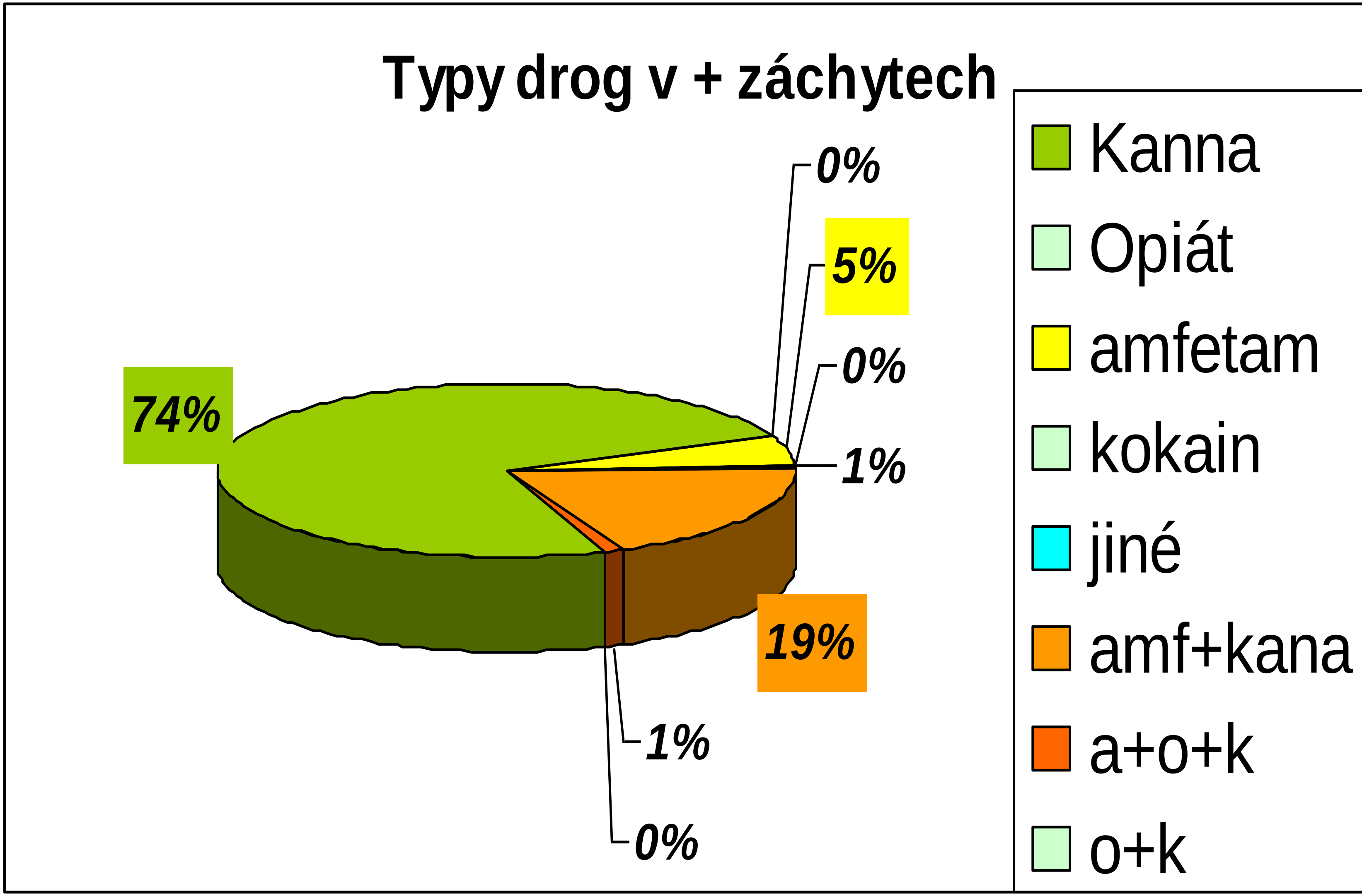
Voříšek V. (1,2), Oszvaldová S. (3), Šatínský D. (3)

1/ÚSTAV KLINICKÉ BIOCHEMIE A DIAGNOSTIKY FAKULTNÍ NEMOCNICE V HRADCI KRÁLOVÉ, Sokolská 581, 500 05 Hradec Králové

2/RADANAL s.r.o., Okružní 613, 530 03 Pardubice

*3/*FARMACEUTICKÁ FAKULTA UNIVERZITY KARLOVY V HRADCI KRÁLOVÉ, *Katedra analytické chemie, Akademika Heyrovského 1203/8, 500 05 Hradec Králové*

Use of different scanning modes of the orbital trap on the model metabolite JWH-018 to achieve minimal LOD and LOQ in the issue of bioanalysis of synthetic cannabinoids


Typy drog v + záchytech
0%
5%
0%
1%
74%
19%
1%
0%
Kanna
Opiát
amfetam
kokain
jiné
amf+kana
a+o+k
o+k

Rozdělení „nových drog“ z pohledu farmaceutické chemie

- kathinony
- psychedelické ethylaminy(IUPAC = ethanaminy), 2C řada
- psychedelické ethylaminy,deriváty (indolová řada) tryptaminu
- synthetické kannabinoidy
- deriváty benzofuranu
- arylcyklohexylaminy
- deriváty piperidinu
- piperaziny

Synthetické kannabinoidy, agonisté CB1 a CB2

- chemické CP a JWH smíšeny se sušenou herbou řady rostlin různého původu
- často však není chemické agens ve směsi přítomno nebo jen v malém množství
- euforie
- halucinace
- psychedelia

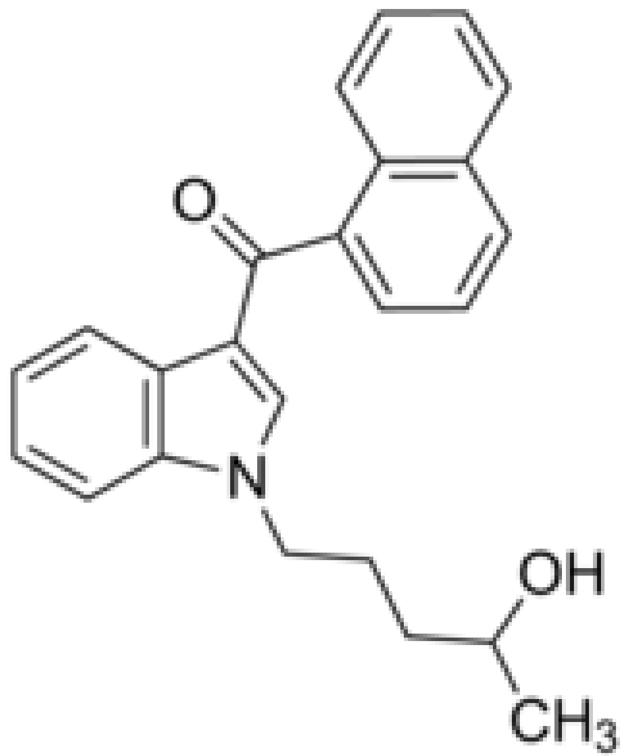
Background

- JWH-018 is historically the first synthetic cannabinoid with a direct and quantitatively clearly measurable effect on the endocannabinoid system developed by a group centered around Professor John William Huffman (Clemson University). Its nominal initials form the designation of all about 400 structures of this group. At the time of the present study, we had available its derivative N- (4-hydroxypentyl) and the labeled analog indole-D5.

[1] Mechoulam, R., Lander, N., Breuer, A. and Zahalka, J. 'Synthesis of the individual, pharmacologically distinct, enantiomers of a tetrahydrocannabinol derivative', *Tetrahedron: Asymmetry* 1.5 (1990): 315-18.
[2] United Nations Office on Drugs and Crime (UNODC), *Synthetic cannabinoids in herbal products*, Vienna, 2011: 5; see also Hudson, S. Ramsey, J. 'The emergence and analysis of synthetic cannabinoids', *Drug Testing and Analysis* 3 (2011): 466–478.
[3] John W. Huffman is a US chemist and a retired professor of organic chemistry at Clemson University in the United States, whose research led to the synthesis of non-cannabinoid cannabimimetrics in the 1990s. Dr Huffman's research group focussed on the synthesis of analogues and metabolites of THC with the aim to develop new pharmaceutical products for medical treatment.
[4] For more information see Hall, W. and Solowij, N., "Adverse effects of cannabis" *Lancet* 352 (Nov 1998): 1611-6; Ashton, C. H., "Adverse effects of cannabis and cannabinoids", *British Journal of Anaesthesia* 83 (1999): 637-49.
[5] H. Müller, et.al., "Panic attack after spice abusein patient with ADHD", *Pharmacopsychiatry* 43.4 (2010): 152-153; A. Mir, et.al., "Myocardial infarction associated with use of the synthetic cannabinoid K2", *Journal of Pediatrics* 128.6 (2011): 1622-1627; S. Every-Palmer, "Synthetic cannabinoid JWH-018 and psychosis: an explorative study", *Drug and Alcohol Dependence* 117 (2011): 152-157.
[6] C.Y. Lin, et.al., "Toxicity and metabolism of methylnaphthalenes: comparison with naphthalene and 1- Nitronaphthalene", *Toxicology* 260 (2009): 16-27.
[7] J. Lapoint, et.al., "Severe toxicity following synthetic cannabinoid ingestion", *Clinical Toxicology* (Philadelphia) 49 (2011): 760-64.
[8] I. Vardakou, C. Pistos and C.H. Spiliopoulou, "Spice drugs as a new trend: mode of action, identification and legislation", *Toxicology Letter* 197 (2010): 157-162.
[9] Ludger, E., et.al., "Synthetic cannabinoids in 'spice-like' herbal blends: first appearance of JWH-307 and recurrence of JWH-018 on the German market", *Forensic Science International* 222.1 (2012): 216-222.

JWH-018 4-Hydroxypentyl metabolite solution

100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®



Synthetic cannabinoids - definitions

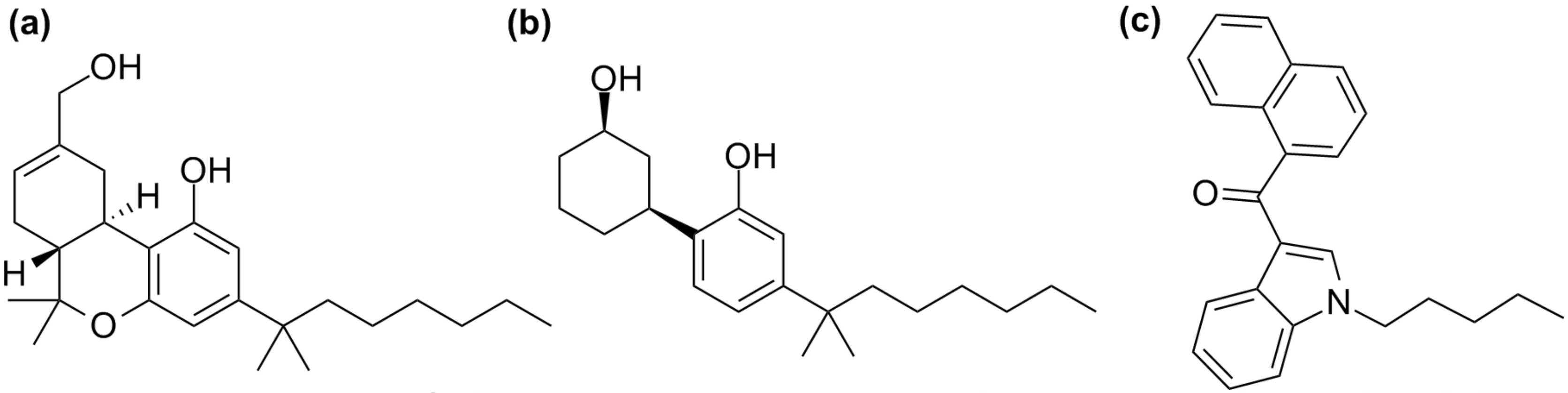


Figure 1 Depiction of the most common synthetic cannabinoids: (a) HU-210 (classical cannabinoid), (b) CP-47,497 (non-classical cannabinoid), (c) JWH-018 (aminoalkylindoles)

- The appearance of 'herbal highs' in the market is not a new phenomenon. Such products usually consisted of plant mixtures with little psychoactive effects. Since 2004, however, the composition of these herbal products seems to have substantially changed to include potent new psychoactive compounds known as synthetic cannabinoids.

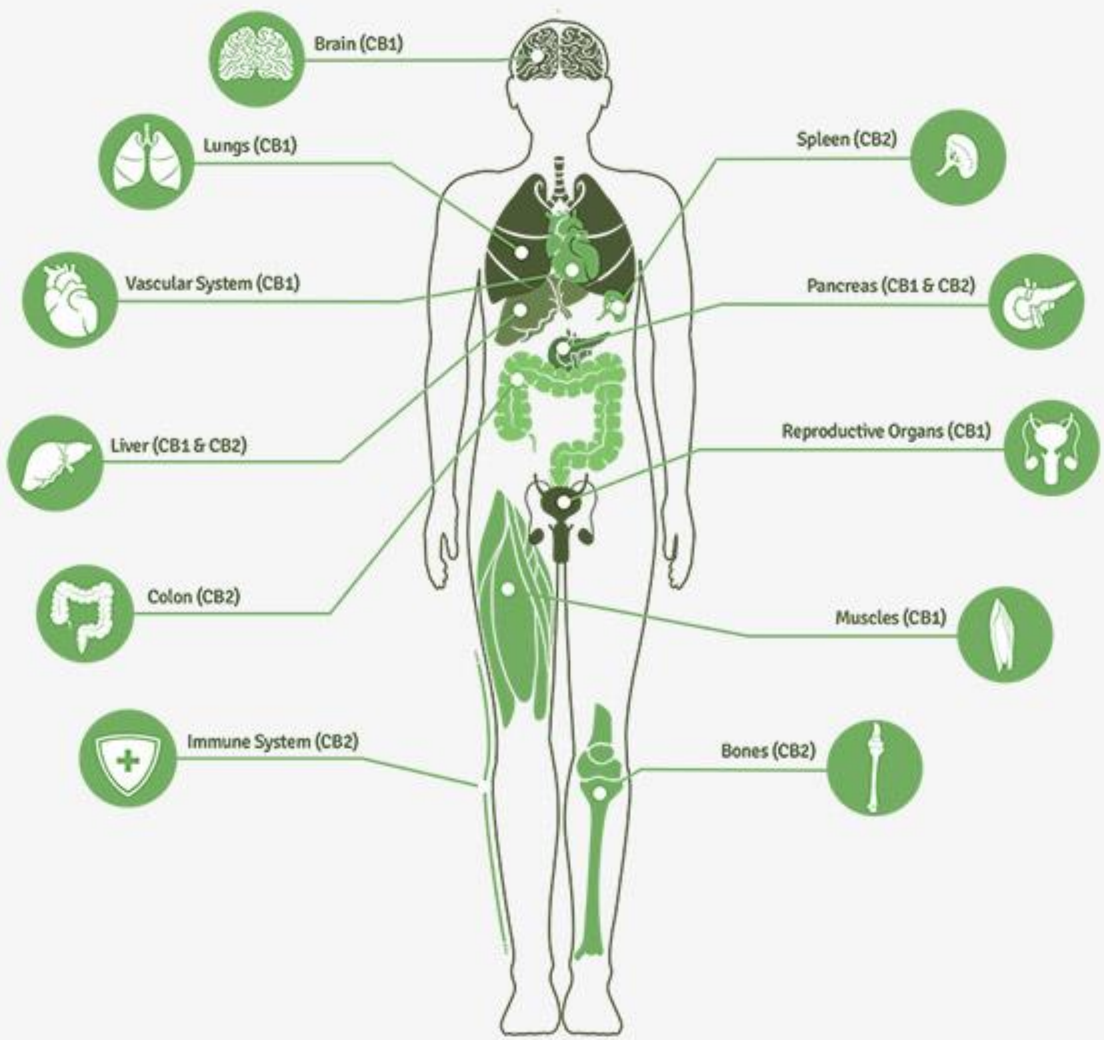
The synthetic cannabinoids fall into seven major structural groups:

- Naphthoylindoles (e.g. JWH-018, JWH-073 and JWH-398).
- Naphthylmethylindoles.
- Naphthoylpyrroles.
- Naphthylmethylindenes.
- Phenylacetylindoles (i.e. benzoylindoles, e.g. JWH-250).
- Cyclohexylphenols (e.g. CP 47,497 and homologues of CP 47,497).
- Classical cannabinoids (e.g. HU-210).

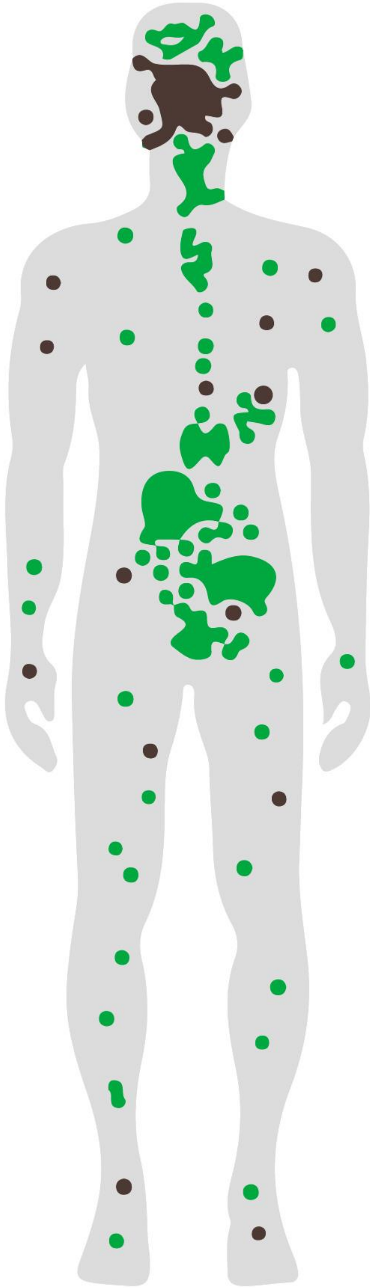
Original aims and therapeutic goals

- Research on the mechanism of cannabis activity dates back several decades when molecules with similar behaviour to Δ9-tetrahydrocannabinol (THC) were first examined.
- A synthetic analogue of THC, ‘HU-210’, was first synthesized in Israel in 1988 [1] and is considered to have a potency of at least **100 times** that of THC.
- Due to its similar chemical structure to THC, ‘HU-210’ is regarded as a ‘classical cannabinoid’ and has been found in synthetic cannabinoids sold in the United States and other countries.
- Another group of synthetic cannabinoids termed ‘non-classical’ include cyclohexylphenols or 3-arylcyclohexanols (‘CP’compounds). ‘CP’ compounds, were developed as potential analgesics by Pfizer, a pharmaceutical company, in the 1980s.

THE BODY'S ENDOCANNABINOID SYSTEM



HUMAN ENDOCANNABINOID SYSTEM



CB1

CB1 Receptors Target

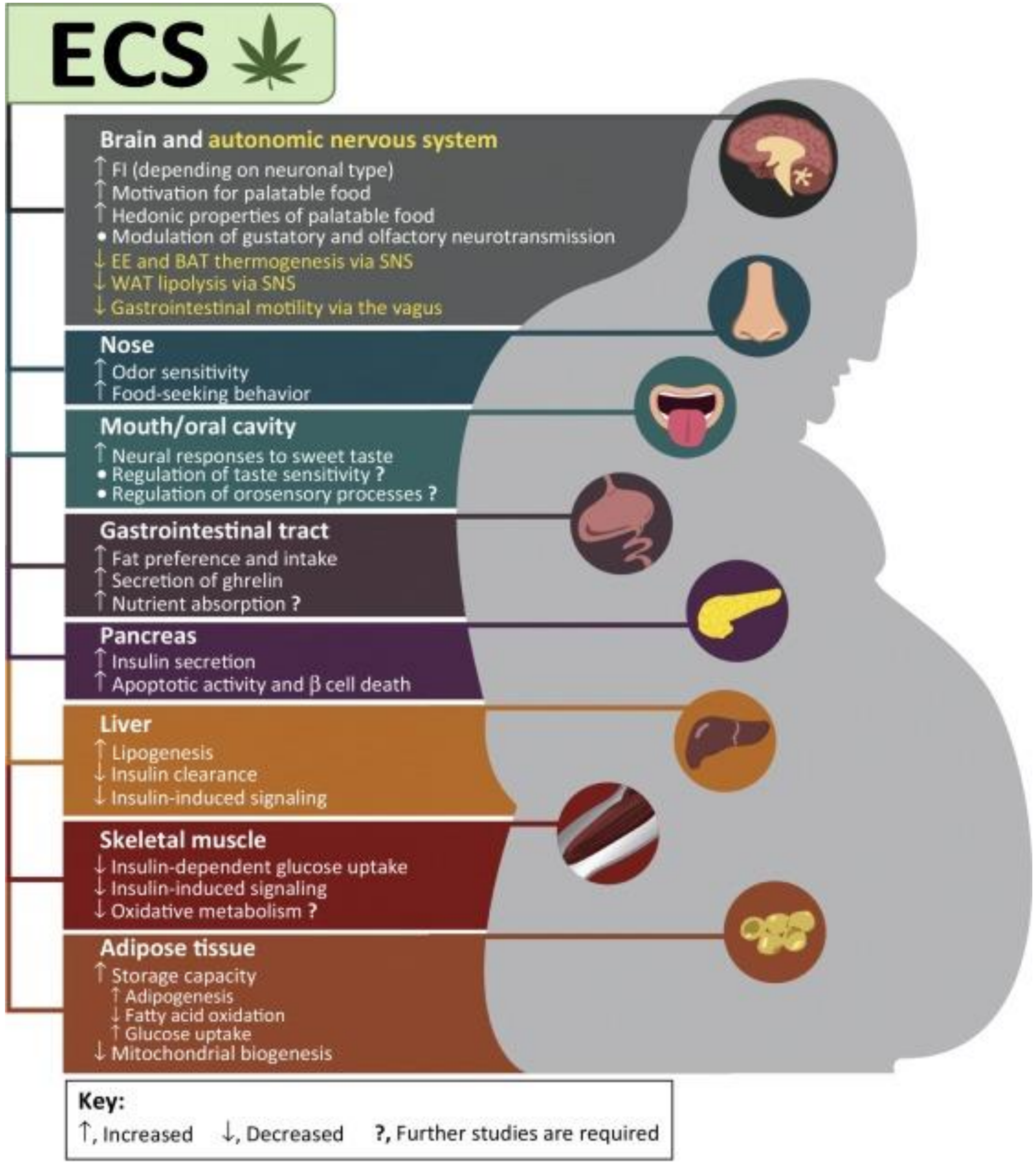
- Motor Activity
- Thinking
- Motor Co-ordination
- Appetite
- Short Term Memory
- Pain Perception
- Immune Cells

CB2

CB2 Receptors Are Much Broader Than CB1 And Influence Most Of The Body

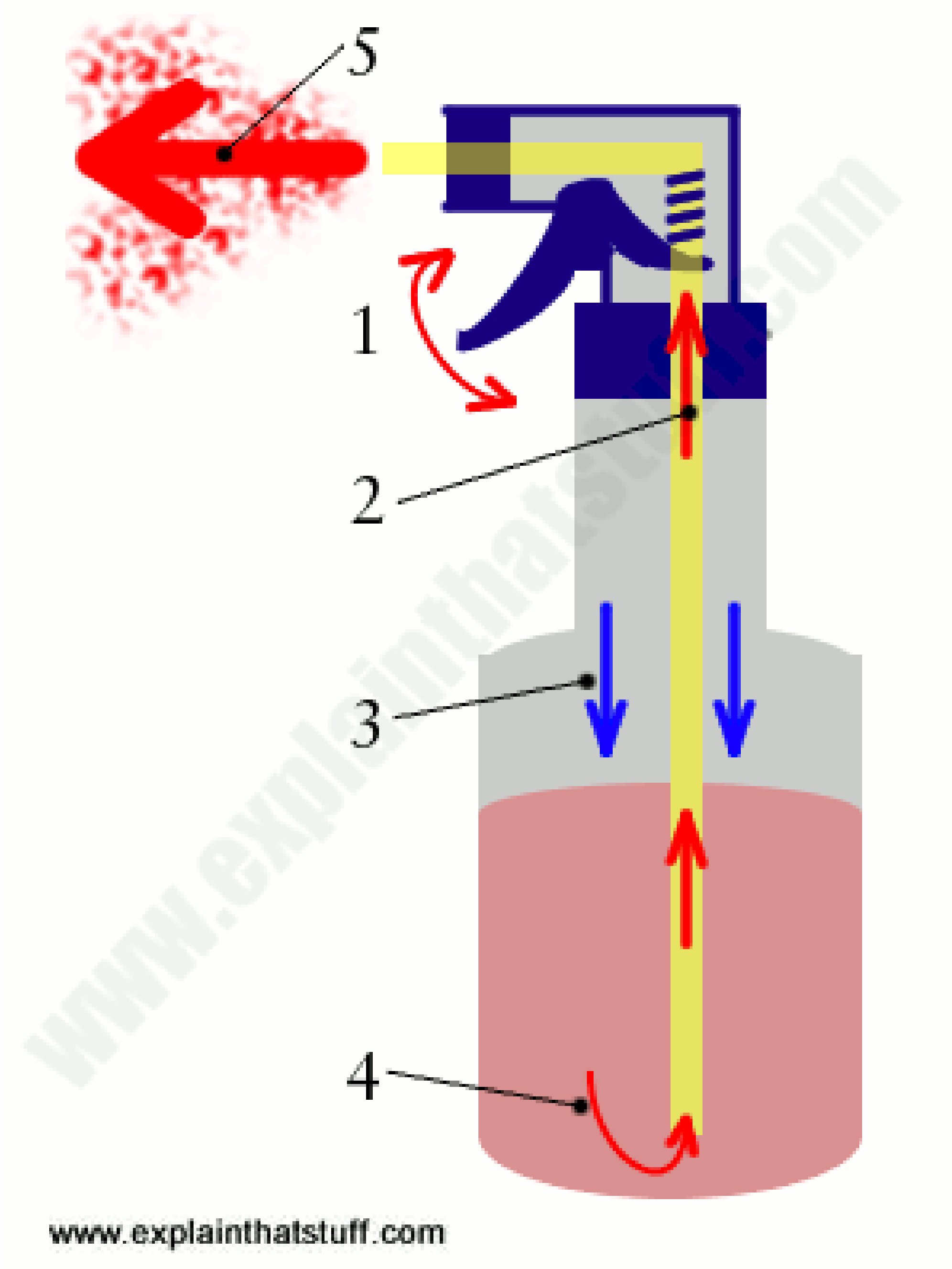
- Gut
- Kidneys
- Pancreas
- Adipose Tissue
- Skeletal Muscle
- Bone health
- Eyes
- Tumours
- Reproductive System
- Immune System
- Respiratory Tract
- Skin health
- CNS
- Cardiovascular System
- Liver

AMSTERDAM GENETICS



Trends in Endocrinology & Metabolism


Spice
DIAMOND



5
1
2
3
4
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Canavalia rosea, *Fabaceae (Asie, Afrika, Amerika)*

Nymphea caerulea, *Nympheaceae (Východní Afrika, povodí Nilu)*

Scutellaria nana, *Lamiaceae (Severní Amerika)*

Pedicularis densiflora, *Orobanchaceae* (*čes. zárazovité,* Kalifornie, Oregon)

Leonotis leonurus, *Lamiaceae (Jižní Afrika)*

Nelumbo lucifera, *Nympheaceae* (Indie, Indočína, Queensland AUS)

Used columns

- 1/ Phenyl Hexyl Kinetex®, 100 x 2.1 mm, 1,7 μm, Phenomenex, CA, USA (SCREEN)
- 2/ Luna Omega® Polar C18, 50 x 2.1 mm, 1.6 μm, Phenomenex, CA, USA (QUANT)
- 3/ Arion® Polar C18 UHPLC, 50 x 2.1 mm, 2.2 μm, Chromservis, CZ (QUANT)

Chromatography acquisition 1

- COLUMN 1

	Start	time	flow	gradient	%A	%B
1.	0,00	75	0,50	Step	98,00	2
2.	1,25	540	0,50	Ramp		100
3.	10,25	130	0,50	Step		100
4.	12,42	155	0,50	Step	98	2

Chromatography acquisition 2

- COLUMN 2

	start	time	flow	gradient	%A	%B
1.	0,00	30	0,60	Step	99,00	1
2.	0,50	940	0,60	Ramp		100
3.	16,17	50	0,60	Step		100
4.	17	70	0,60	Step	99	1

Chromatography acquisition 3

- COLUMN 3

	Start	Time	Flow	Gradient	%A	%B
1.	0,00	30	0,60	Step	99,00	1
2.	0,50	940	0,60	Ramp		100
3.	16,17	50	0,60	Step		100
4.	17	70	0,60	Step	99	1

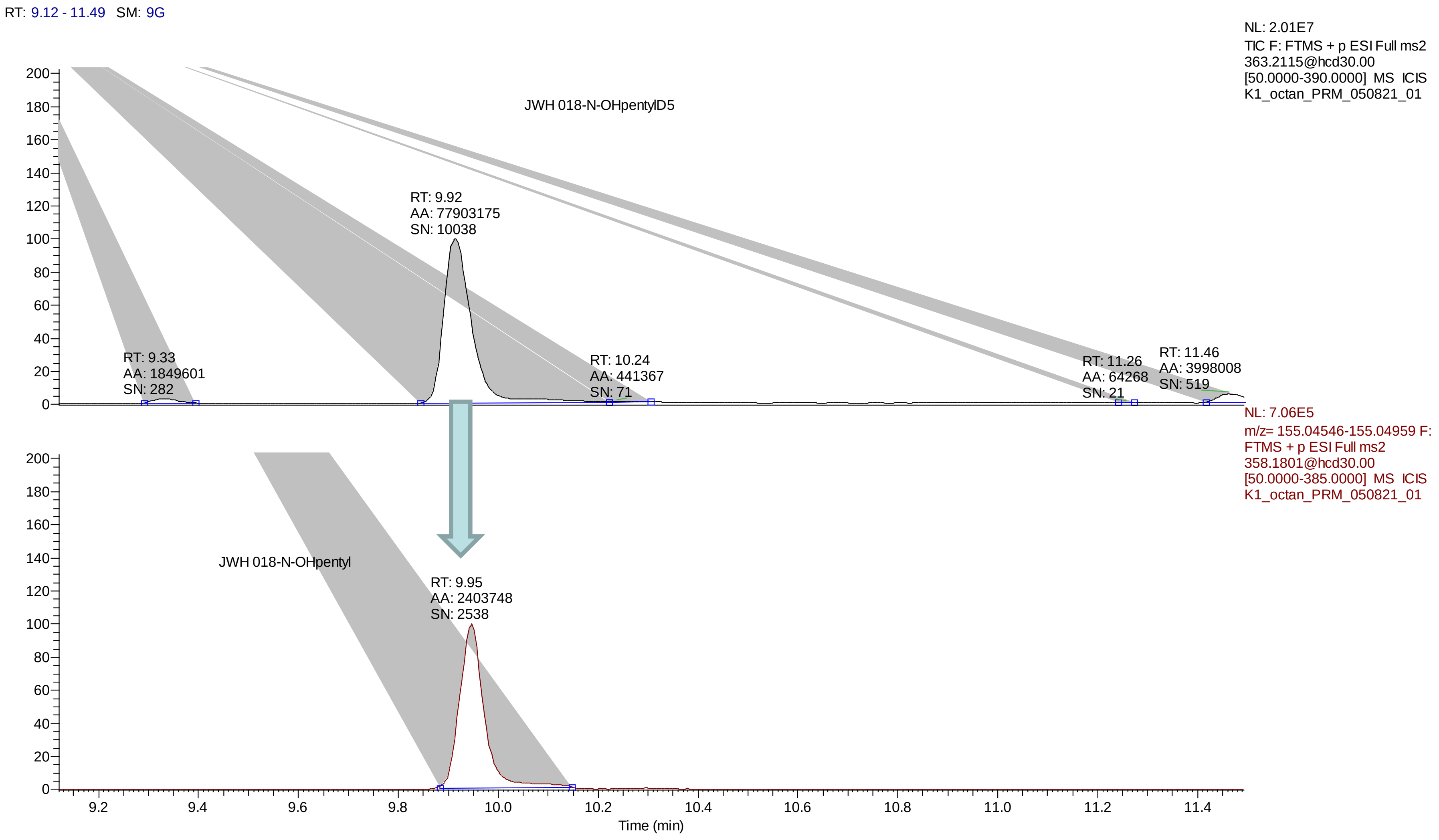
MS Parameters

- **Scan modes:** Full MS-AIF, PRM
- **Chrom peak width:** 6s.
- **Method duration:** 15,00 min
- **Resolution:** 70 000.
- **Scan range:** 90 – 625 m/z.
- **Autogain control(AGC targed):** 1e5.
- **H-ESI → Sheath Gas :** 45,
- → **Aux Gas:** 15.
- **Cone (spray) voltage:** 3,5 kV.
- **Capillary temperature:** 350 °C.
- **S-lens RF level:** 60,0.

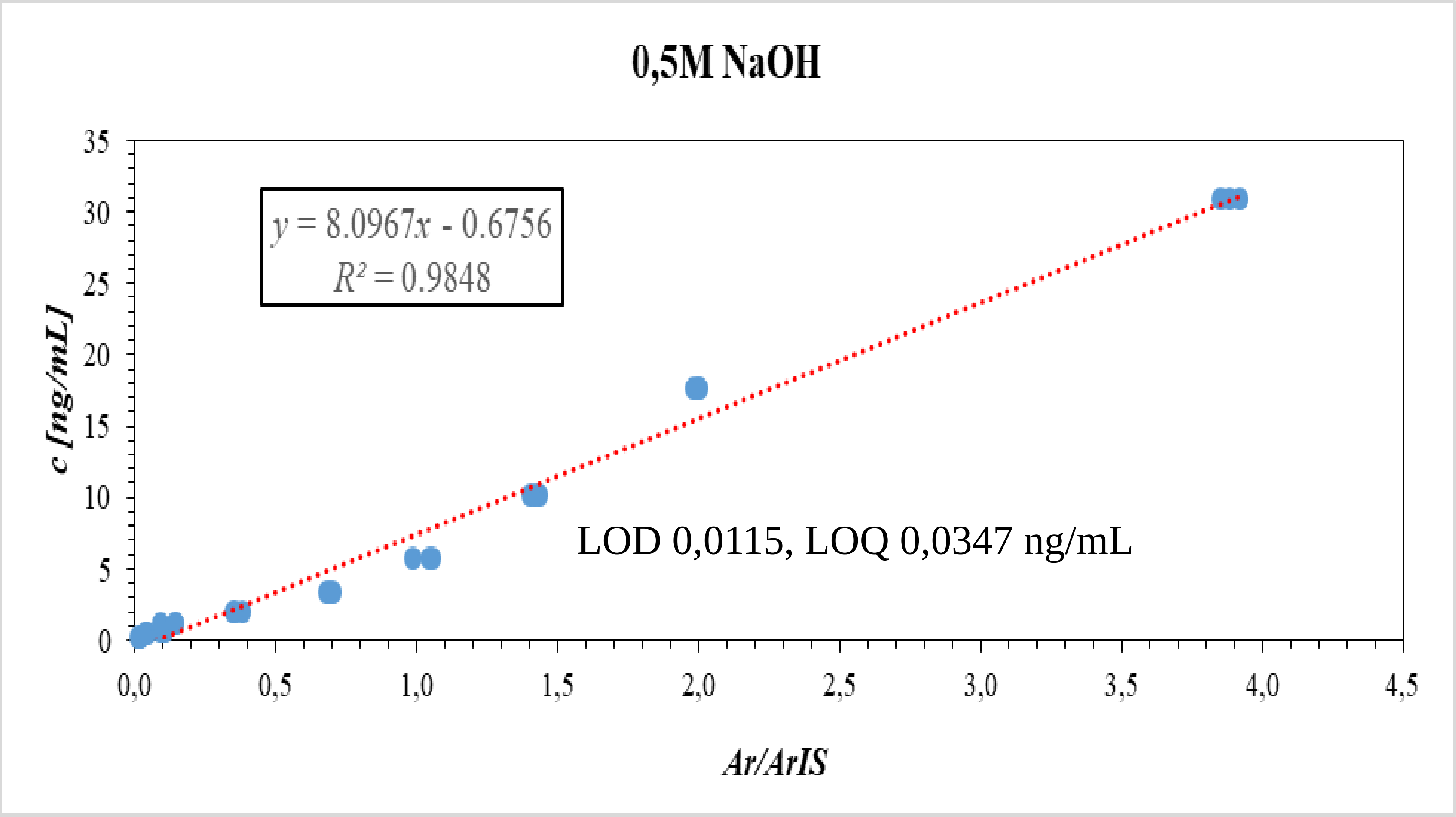
Calibration Range

	JWH-018
Calibrator	S035 (JWH-018 4-hydroxypentyl metabolite)
	spiked concentration
IDNumber	ng/mL
K0	0
K1	**0,20**
K2	0,35
K3	0,61
K4	1,07
K5	1,88
K6	3,28
K7	5,74
K8	10,05
K9	17,59
K10	30,79

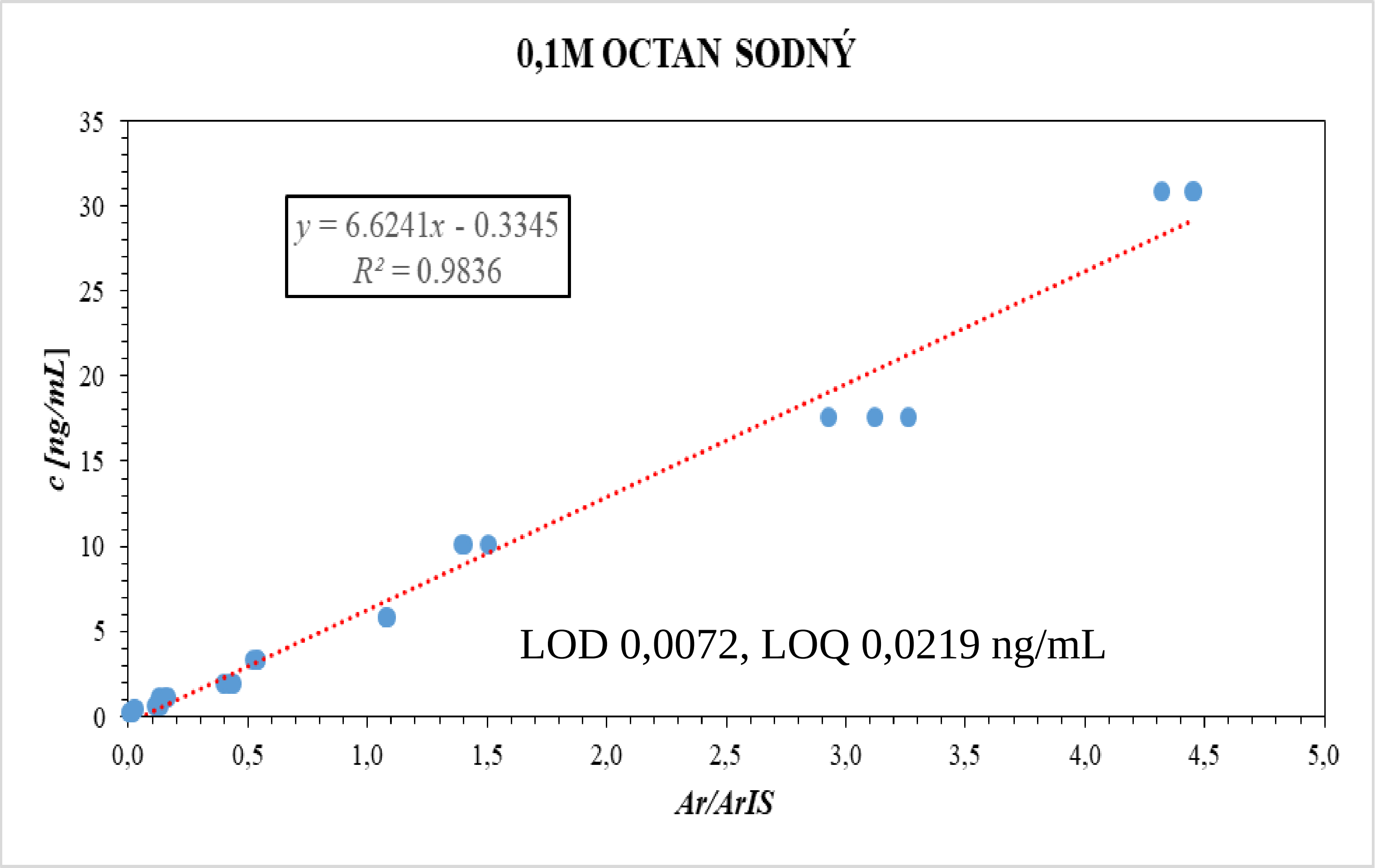
K1 Calibrator /PRM



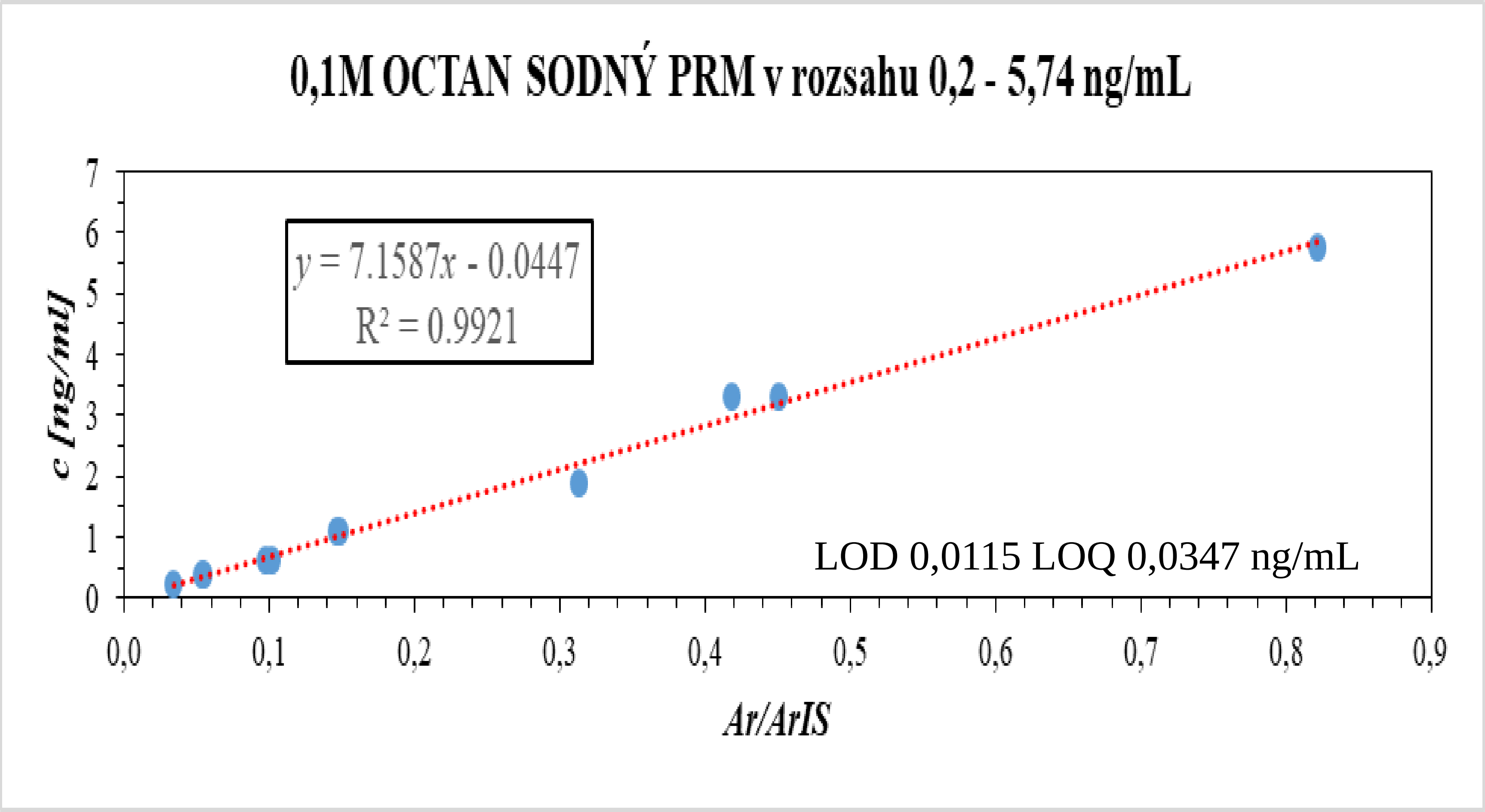
The Influence of extraction conditions in variable pH/NaOH, ARION



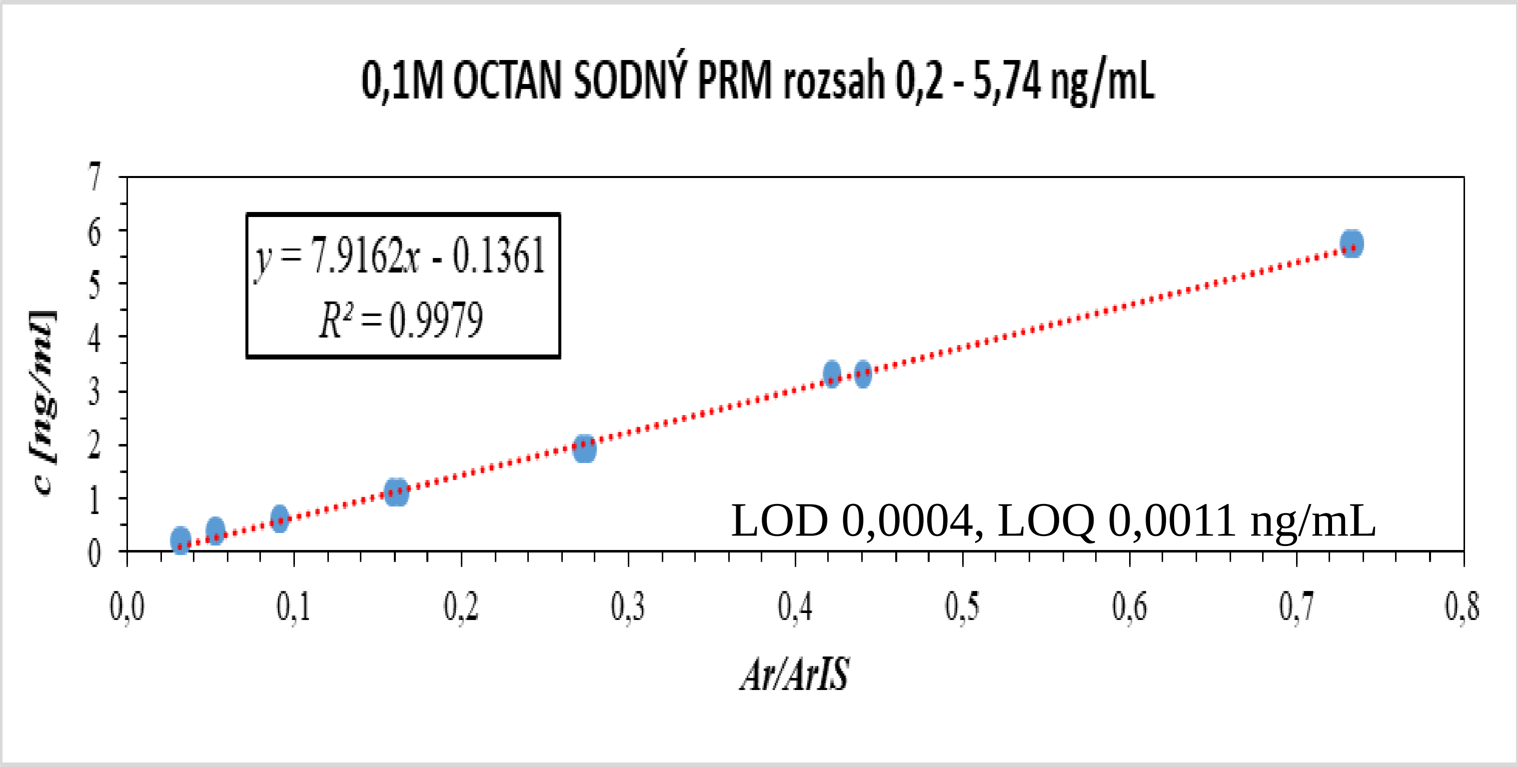
The Influence of extraction conditions in variable pH/sodium acetate, ARION



Low Calibration Range of JWH-018 N-(4-hydroxypentyl) /PRM /0,2 – 5,74 ng/Ml, Arion® Polar C18 UHPLC



Low Calibration Range of JWH-018 N-(4-hydroxypentyl) /PRM /0,2 – 5,74 ng/Ml, Luna Omega® Polar C18



LOD, LOG, R^2 /Arion polar C18 UHPLC

PRM scan				
Column	ARION POLAR C18 UHPLC			
	0,2 - 30,79 ng/mL	**0,2 - 5,74 ng/mL**		
LOD (ng/mL)	**0,0072**	**0,0079**		
LOQ (ng/mL)	**0,0219**	**0,0242**		
R^2	**0,9867**	**0,9921**		

LOD, LOG, R^2 / LUNA omega polar C18

PRM scan				
Column	LUNA OMEGA POLAR C18			
	0,2 - 30,79 ng/mL	0,2 - 5,74 ng/mL		
LOD (ng/mL)	0,0003	0,0004		
LOQ (ng/mL)	0,0010	0,0011		
R^2	0,9866	0,9979		


Syva® RapidTest THC
Date
ID
C
THC
S
DADE BEHRING
nezobrazená barevná linie v „C“
nezobrazená barevná linie v „C“
pozadí není čistě bílé
pozadí není čistě bílé
pozadí není čistě bílé
NEPLATNÉ VÝSLEDKY
PLATNÉ VÝSLEDKY
THC NEGATIVNÍ
barevná linie v „C“, ostrá nebo mlžná linie v THC, čisté bílé pozadí
THC POZITIVNÍ
barevná linie v „C“, čisté bílé pozadí
THC NEGATIVNÍ
barevná linie v „C“, ostrá nebo mlžná linie v THC, čisté bílé pozadí

RANDOX

Whole Blood Panel

a-PVP (Flakka)	Cannabinoids (THC)	Opiate
AB-PINACA	Carfentanil	Oxycodone
Amphetamine	EtG	Tramadol
Barbiturates	Fentanyl	TCA
Benzodiazepines	Methadone	UR 144 / XLR II
BZG/Cocaine	Methamphetamine	JWH-018
Buprenorphine	Phencyclidine (PCP)	6-MAM

Urine Panel

a-PVP	Buprenorphine	Opiates
AB-PINACA	Cannabinoids (THC)	Oxycodone
Amphetamine	Creatinine	Tramadol
Barbiturates	EtG	TCA
Benzodiazepines I (Oxazepam)	Fentanyl	UR 144 / XLR II
Benzodiazepines II (Lorazepam)	Methadone	JWH-018
BZG/Cocaine	Methamphetamine	6-MAM

Oral Fluid Panel

a-PVP (Flakka)	Cannabinoids (THC)	Oxycodone
Amphetamine	Fentanyl	PCP
Barbiturates	Ketamine	Tramadol
Benzodiazepines I (Oxazepam)	LSD	UR 144 / XLR II
Benzodiazepines II (Lorazepam)	Methadone	JWH-018
BZG/Cocaine	Methamphetamine	6-MAM
Buprenorphine	Opiate	-

Acknowledgements

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- 2. Pon D. and Fenyvesi I.S.: A Validated Method for the Detection and Quantitation of Synthetic Cannabinoids in Whole Blood and Urine, and its Application to Postmortem Cases in Johannesburg, South Africa. S Afr. J.Chem, 71:24-29, 2018