

Analyse illegaler Arzneimittel: Eine Herausforderung für OMCLs (und die Massenspektroskopie)



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INHALT



- Einleitung
 - PharmMed – OMCL
 - Problem „Pharma Crime“
- Bekämpfung illegaler Arzneimittel
 - Warum illegale Arzneimittel analysieren?
 - Analysenstrategien
- 10 Fallstudien
- Zusammenfassung

- PharmMed
 - Österr. AM-Agentur
 - Zulassung, Inspektion, Pharmakovigilanz....
- **OMCL** (Official Medicines Control Laboratory)
 - **1) AM-Qualitätskontrollen**
 - Marktüberwachung (legale AM)
 - **2) Arzneibuch-Entwicklung**
 - Ph.Eur., ÖAB
 - **3) Pharma Crime, Enforcement**
 - **Analyse unbekannter / illegaler AM**



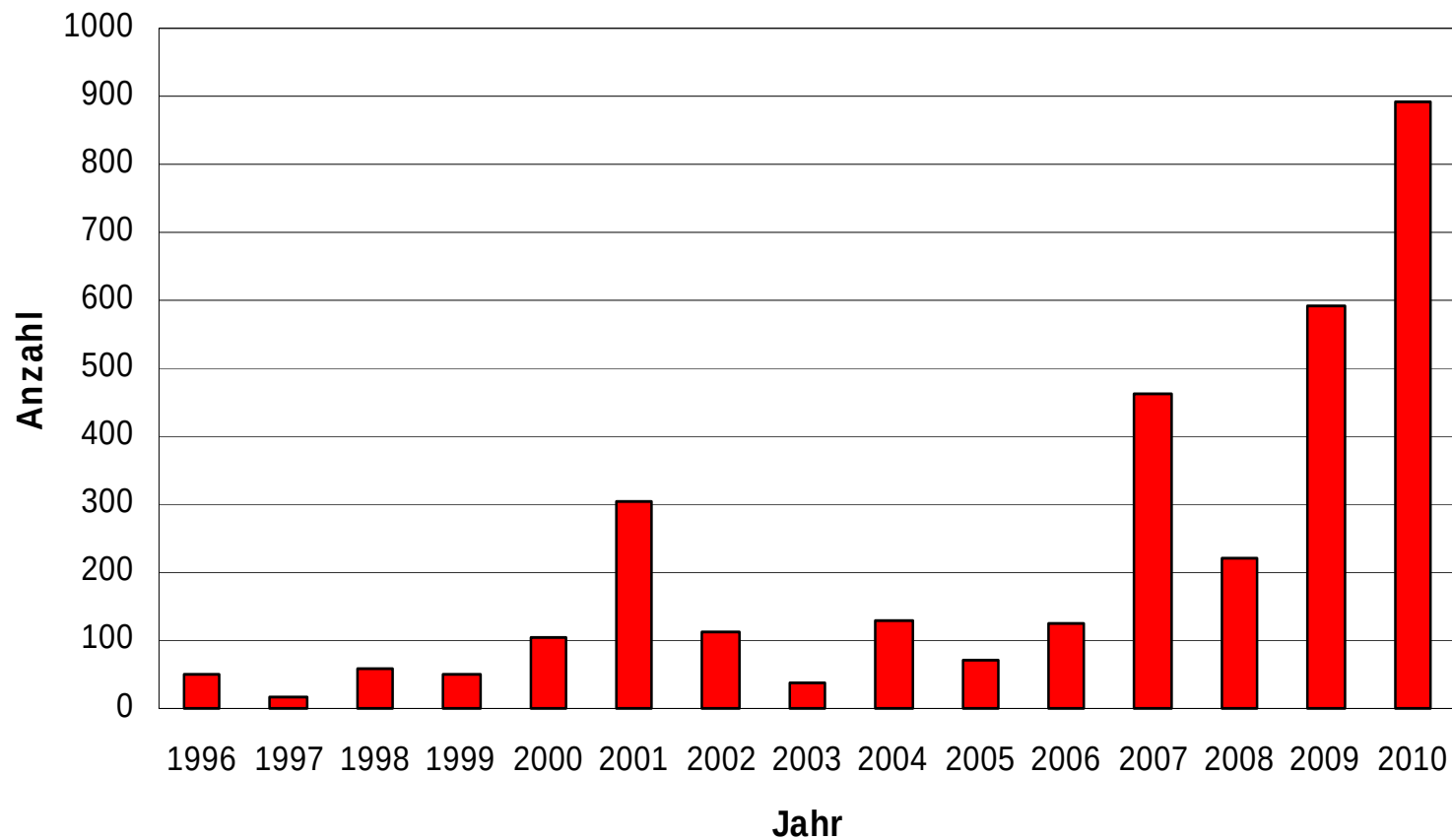
Problem „Pharma Crime“



- **Arzneimittel-Fälschungen (WHO)**
 - **ca. 15 % des AM-Weltmarktes**
 - Andere Produkte: ca. 7%
 - Profit >> Suchtgift-Kriminalität
 - **AT 2009: Nr. 1 bei Zollbeschlagnahmen (27.095 Pkg.)**
 - 95% der Internet-AM sind Fälschungen/Substandard
 - Internet-Apo in AT verboten (Rp.)
 - AM-Fälschungen am legalen Markt
 - **Africa 30 - 80% (700 000 fatalities) ⇔ Eur. < 1%**
 - Betroffene AM
 - Afrika: **überlebenswichtige AM**
 - Eur.: **Lifestyle Medikamente** (grauer – schwarzer Markt)

Situation in AT

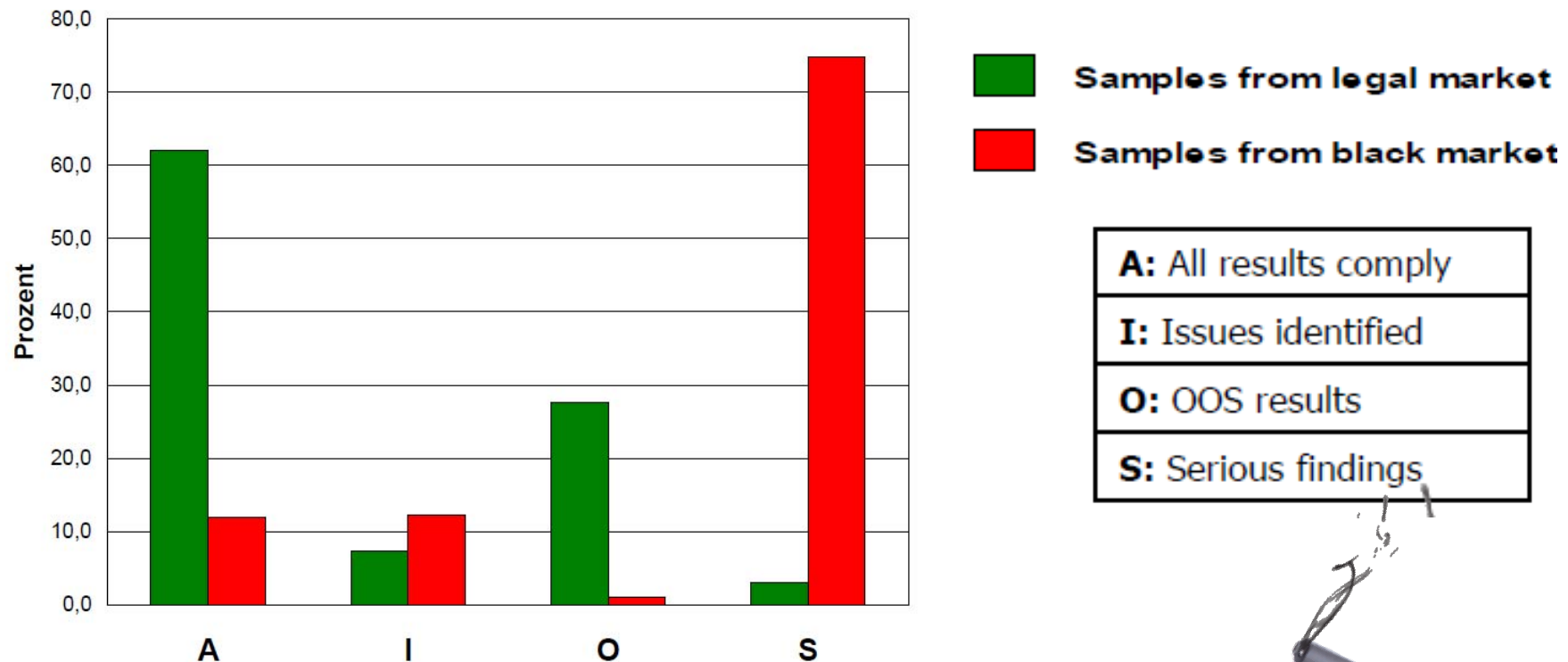
Analysierte illegale Arzneimittel (1996-2010)



Situation in AT



2010 Global Results



Illegal Medicines kill people - **Evidence??**

➤ Heparin/OSCS, aristolochia, glycol-sirups, nitrosofenfluramin.....

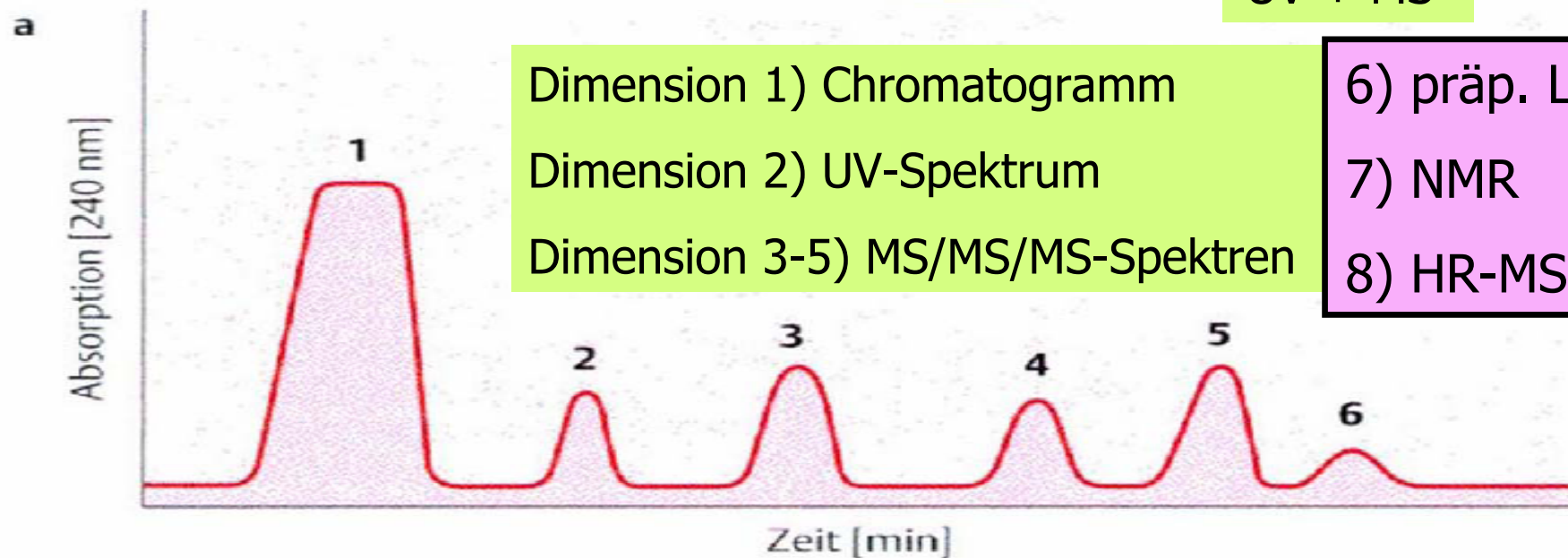
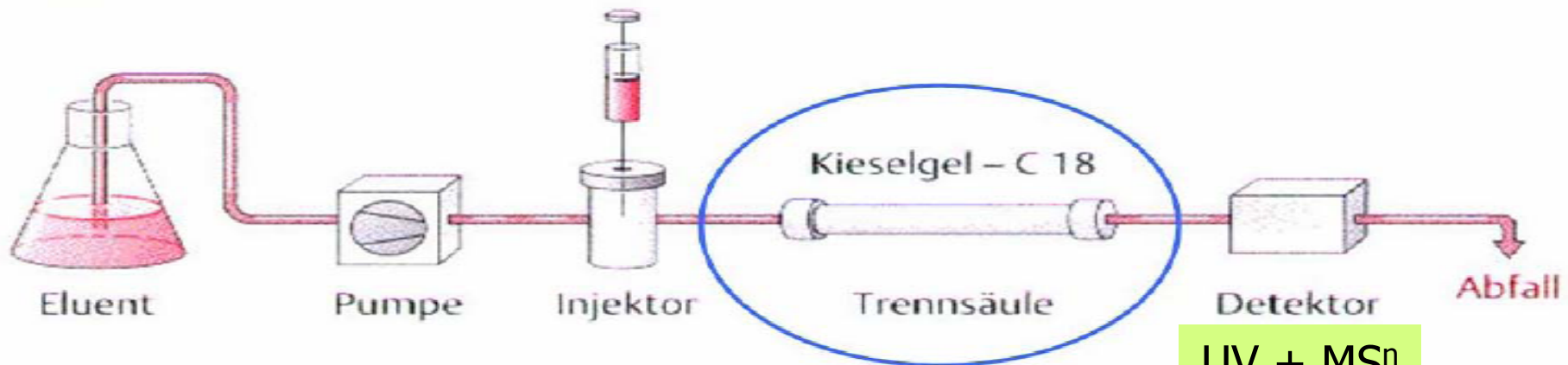


Analytical strategies: Legal Market

- CF check in OMCL
- A perfect counterfeit is implausibly
- Market surveillance programme:
 - **CF candidates: CF check against authentic**
 - Image analysis
 - Total composition (ATR-IR)
 - Tablet film
 - Tablet core
 - **No counterfeit in official AT supply chain**
 - Black market: predominantly counterfeits



Analytical strategies: Black Market



Dimension 1) Chromatogramm

Dimension 2) UV-Spektrum

Dimension 3-5) MS/MS/MS-Spektren

6) präp. LC

7) NMR

8) HR-MS

Analytical strategies: Black Market



- GC – EI – MS
 - General Screening (commercial + own libraries)
- LC – PDA – ESI/APCI Ion Trap MS
 - General Screening (own libraries)
- HPLC / UPLC: RRT + UV spectra
 - Specific test methods, e.g. PDE5-Inhibitors (UV-CF)
- Unknown Peak / API?
 - Preparative HPLC / Crystallisation
 - o $^1\text{H}/^{13}\text{C}$ -NMR, HR-MS, IR, titration, KF/LOD.....
- UPLC – HR-Q-TOF-MS started
 - Exact Mw $\Rightarrow$ elemental formula
 - Fragmentation $\Rightarrow$ identity

UPLC – HR-Q-TOF-MS started



1st Samples

1. Somatropin
2. Melanotan-1

Examples



- PDE5 Inhibitors: Viagra & Co.
- Heparin case
- Doping scandal
 - Anabolics
 - Growth hormones
- Legal Highs
 - Cannabinoids
 - Cathinones
- Conclusion

Viagra Counterfeit



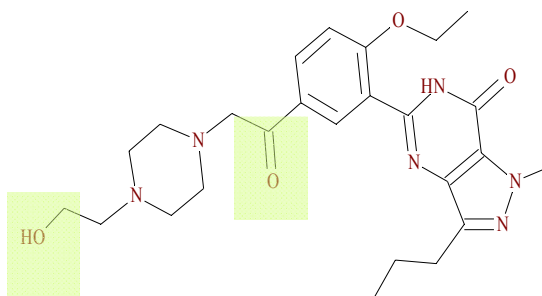
- **Viagra 100**
 - Confiscation by customs
 - AUS packaging nearly perfect
 - **Sildenafil**
 - **150 – 240** mg/tab.
 - or
 - **Tadalafil**
 - 21 mg/tab.



Adulteration: PDE5 analogues

- **Perfex-men**

- Food supplement
- **Hydroxyacetildenafil**: 87–100mg
- Active isolated for
 - o Identification
 - o Qualification (Assay)
 - o In-vitro pharmacology
- PDE-5 = sildenafil
- **PDE-6 >> sildenafil**



Side effects!!!

Sildenafil and its Patent

B. Venhuis, ILFCM + OMCL-NE



- > 1 000 compounds
- 2 clinical candidates → 1 new API
- Patent
 - Industry → intellectual property
 - Scientist → scientific publication
 - **Criminal → Goldmine:** analogues / designer drugs
- Why is it done?
 - **Avoids detection:** unknown, no data, no CRS
 - **Complicates prosecution:** medicinal + legal status?

HEPARIN - Case

- USA Anfang 2008
 - >(>) 100 Tote
 - Ca. 1.000 schwere Nebenwirkungen
 - Chargenrückrufe: 60%
- Ursache: OSCS-Zumischung
 - **Over Sulfated Chondroitin Sulfate**
 - Heparinartig, teilsynthetisch, billiger
 - Analytisch (fast) **nicht unterscheidbar**
 -    **Anaphyllatoxin**   



Baxter International

Chinesisches Heparin Herstellung aus Schweinedarm



API-Herstellung

- großteils in Asien
- **Heparin fast ausschließlich in China**

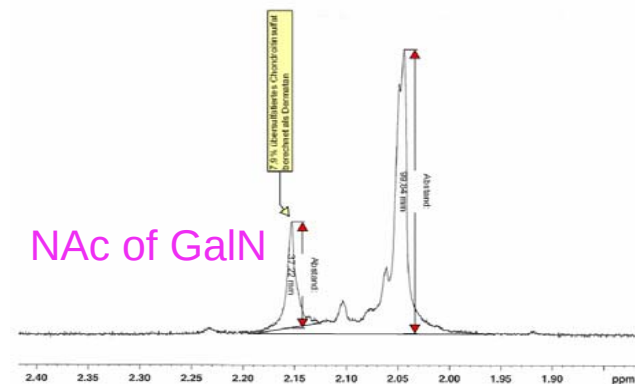


HEPARIN – Problem Marküberwachung in AT

- Ca. 400 Proben
 - **3 schwach kontaminierte Chargen** ausgetauscht (1-2% OSCS)
 - keine Zwischenfälle in AT
- **1. globale AM-Fälschung** in legaler Verteilerkette
 - Ph.Eur. Rapid Revision: 15.7.08
 - NMR.....



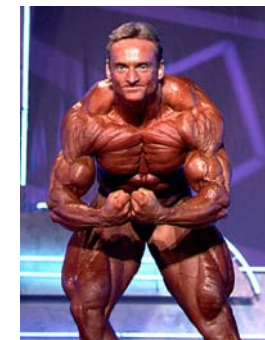
NMR: Pharmaziezentrum



Doping Scandal in AT



- Reasons.....
- Aug. 2008: anti doping act tightened
 - **Trade** ⇒ **5 y. arrest**
 - OMCL >> 1 000 Samples
 - 70 % anabolics + growth hormones
 - **95 % misused for leisure sports + joe six-pack**



Doping Scandal: „Anabolika-Hostien“



KURIER

Bäcker stellte Anabolika-Hostien her

Ein Waldviertler stellte Oblaten für Kraft und Potenz her. Weltweit wurden 30.000 Stück verkauft.



kommentieren

BOCKHORN Normalerweise langen Gläubige zu, wenn sie Priester ans Kirchenvolk verteilen. Besonders Kreative sind jedoch draufgekommen, dass Oblaten auch unter die Zunge gelegt ihre Wirkung zeigen. Keine spirituelle, dafür eine kräftigende - für Muskeln und Potenz. Das neu kreierte Backwerk wurde mit Anabolika, Hormonen und Stimulanzien versetzt und in die ganze Welt verkauft. Nicht irgendwo in einem Untergrundlabor im Ausland, sondern in einer kleinen Bäckerei im niederösterreichischen Waldviertel. Das brachten jetzt die Ermittlungen der Umweltgruppe des Landeskriminalamtes NÖ (Gruppe

Zwölf verschieden beschichtete Oblaten wurden hergestellt.

Friedl, Ermittlungsgruppe Anabolika) ans Tageslicht. Die Zerschlagung eines der größten internationalen Händlerringes für Anabolika ist um eine brisante Facette reicher.

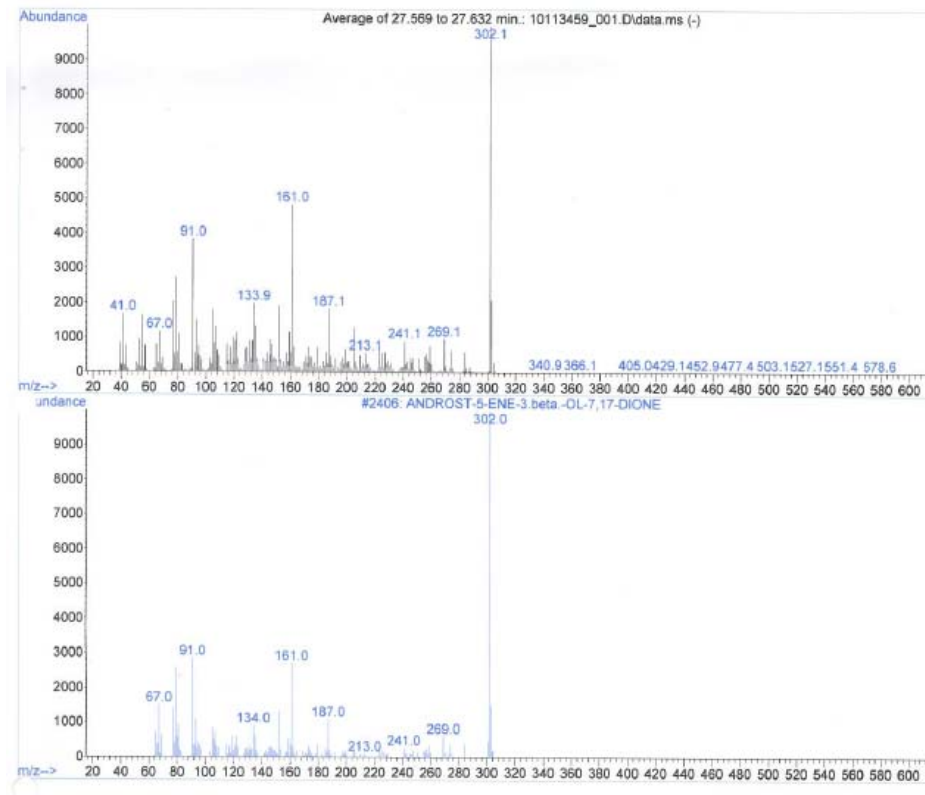
> 30 000 Anabolica Blades: illegal production in AT

1. Methandienon
2. Methyltestosteron
3. Clebuterol
4. Stanozolol

Doping Scandal: Anabolica Analogs

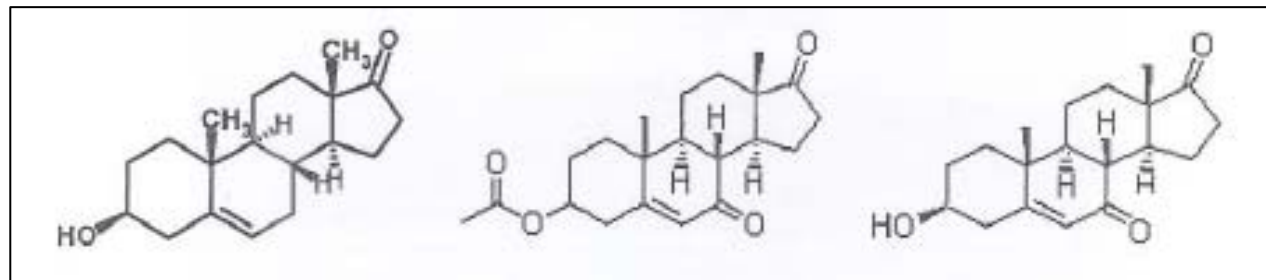


- **FINAJET** Highly Anabolic Oral Prep.
- GC-MS



Doping Scandal: Anabolica Analogs

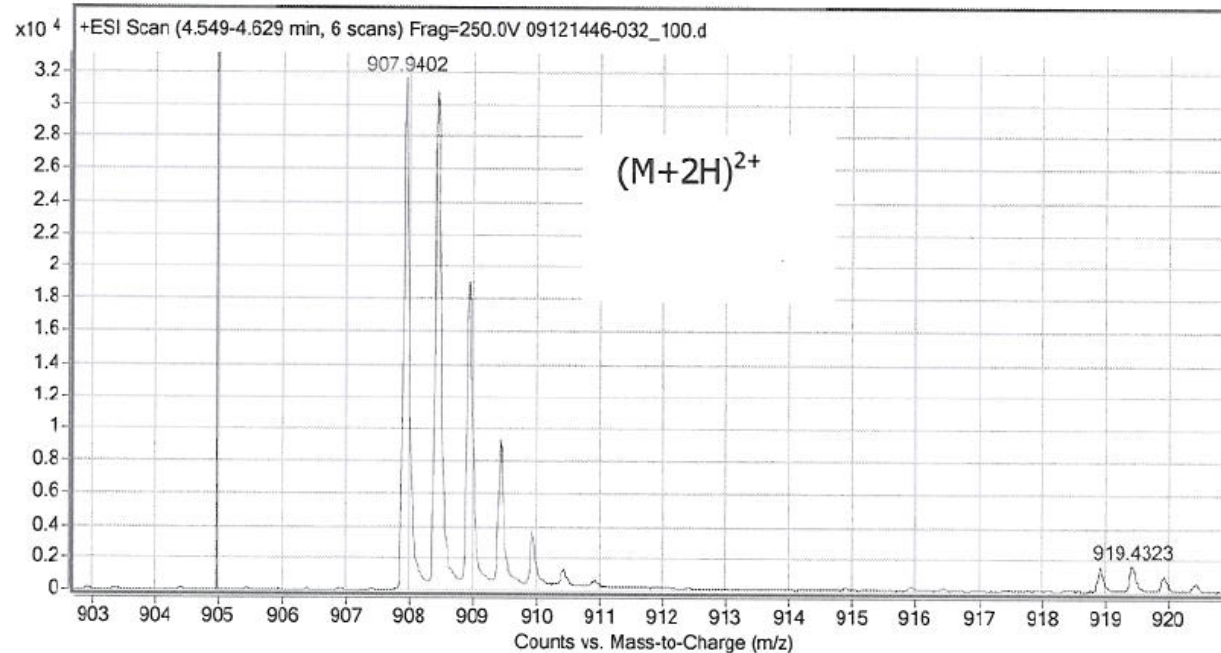
- **FINAJET** Highly Anabolic Oral Prep.
- GC-MS
 - ✓ **Andros-5-en-3- β -ol-7,17-dion**
 - ✓ **7,17-Dioxoandrost-5-en-3-yl-acetat**
 - ✓ Naringenin (Cytochrom-P-Inhibitor)
 - ✓ Diosgenin
- Prasteron-Analog
 - ✓ indirektes WADA-Verbot



Doping Scandal: Synthetic HGHs



- **HGH Fragment 176 – 191**
- **TOF-MS**



Doping Scandal: Synthetic HGHs



- **HGH Fragment 176 – 191**
- TOF-MS
 - ✓ $C_{78}H_{123}N_{23}O_{23}S_2$
 - ✓ **Tyr (Phe)** -Leu-Arg-Ile-Val-Gln-Cys-Arg-Ser-Val-Glu-Gly-Ser-Cys-Gly-Phe
- GH-RF-Analog
 - ✓ WADA-Verbotsliste

Legal Highs: Cannabinoids

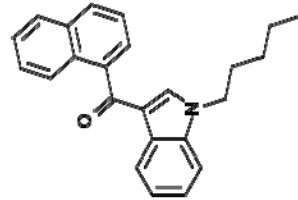
- **SPICE Case** (Nov. 2008)
 - Confiscated in a criminal case
 - Sold as Incenses
 - In **Headshops**



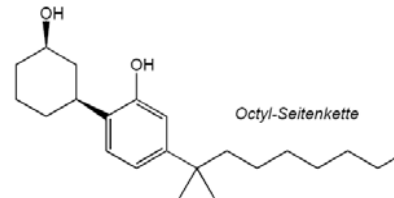
Legal Highs: Cannabinoids

- **SPICE:** mix of 2 synthetic cannabinoids

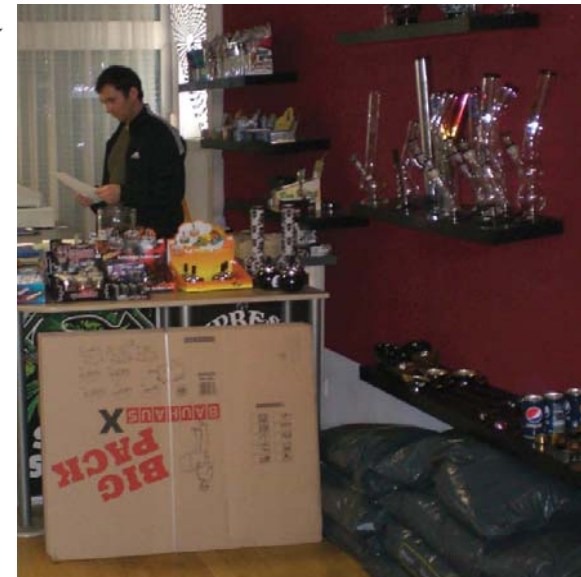
1. JWH-018



2. CP47.497-Homolog

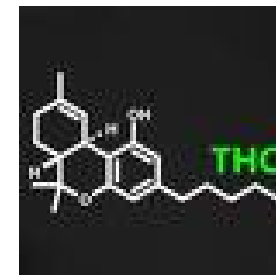


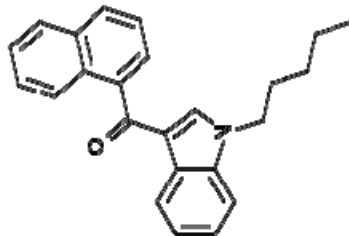
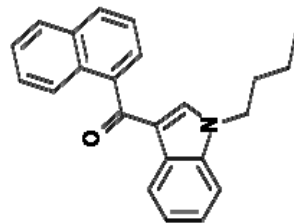
- **Ban** by order
- Confiscation from **headshops**
 - > 30 in AT
 - Approx. 10 000 € profit/week



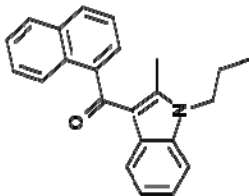
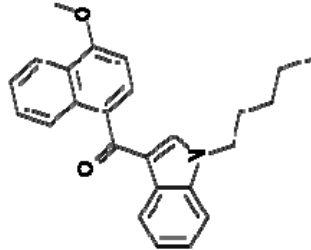
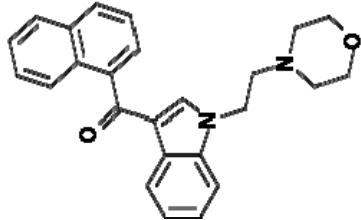
Synthetic Cannabinoids in AT

- Nov. 2008 - June 2011.....**ongoing**
- Total: **14 APIs** in approx. 130 samples

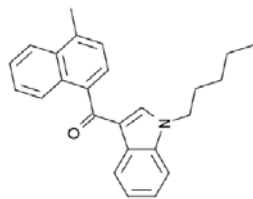
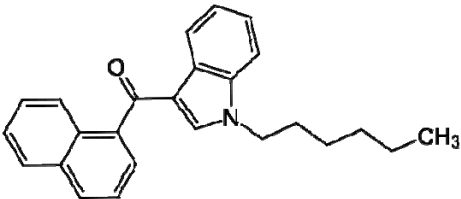
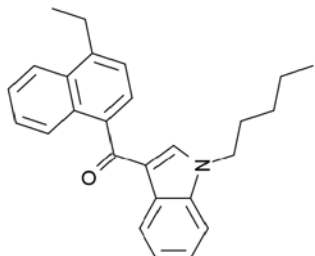


No.	1) Naphtoyl-Indols	Structure
1	JWH-018	
2	JWH-073	

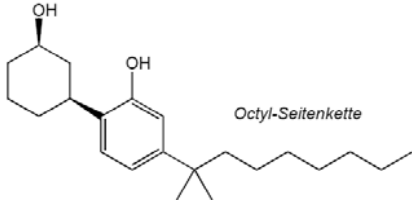
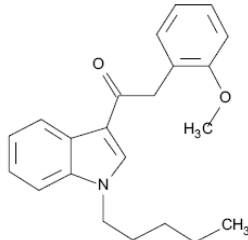
Synthetic Cannabinoids in AT

No.	1) Naphtoyl-Indols	Structure
3	JWH-15	
4	JWH-081	
5	JWH-200	

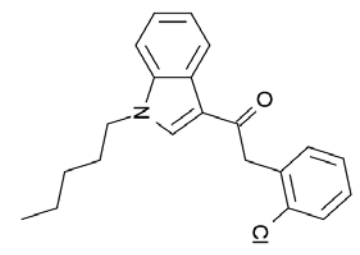
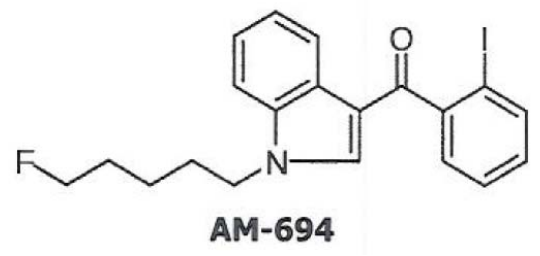
Synthetic Cannabinoids in AT

No.	1) Naphtoyl-Indols	Structure
6	JWH-122	
7	JWH-019	
8	JWH-210	

Synthetic Cannabinoids in AT

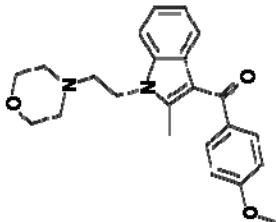
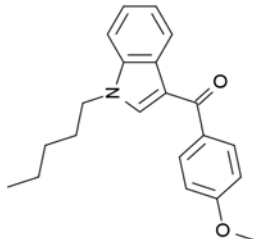
No.	2) Cyclohexyl-Phenols	Structure
9	CP47.497-C8-Homolog	
	3) Phenylacetyl-Indols	
10	JWH-250	

Synthetic Cannabinoids in AT

No.	3) Phenylacetyl-Indols	Structure
11	JWH-203	
	4) Benzoyl-Indols*	
12	AM-694	 AM-694

* Not (yet) covered by generic ban.

Synthetic Cannabinoids in AT

No.	4) Benzoyl-Indols	Structure
13	Pravadoline	
14	RCS-4	
15	??	??

Analytical Strategies: Cannabonoid AM-694

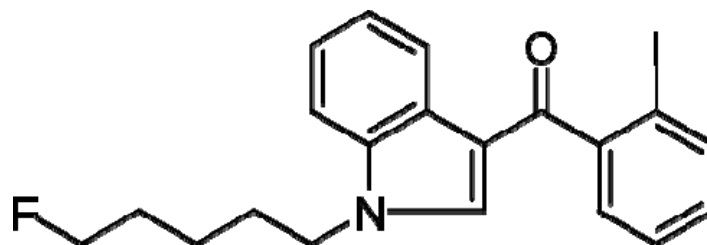
- **PULSE ULTRA**
- *Microscopy:*
 - 7/8 labelled herbs neg.
 - *Turnera diffusa*
 - Impregnated
- *Chem. Analysis:*
 - **AM-694:** 62.7mg/g



Analytical Strategies: Cannabonoid AM-694



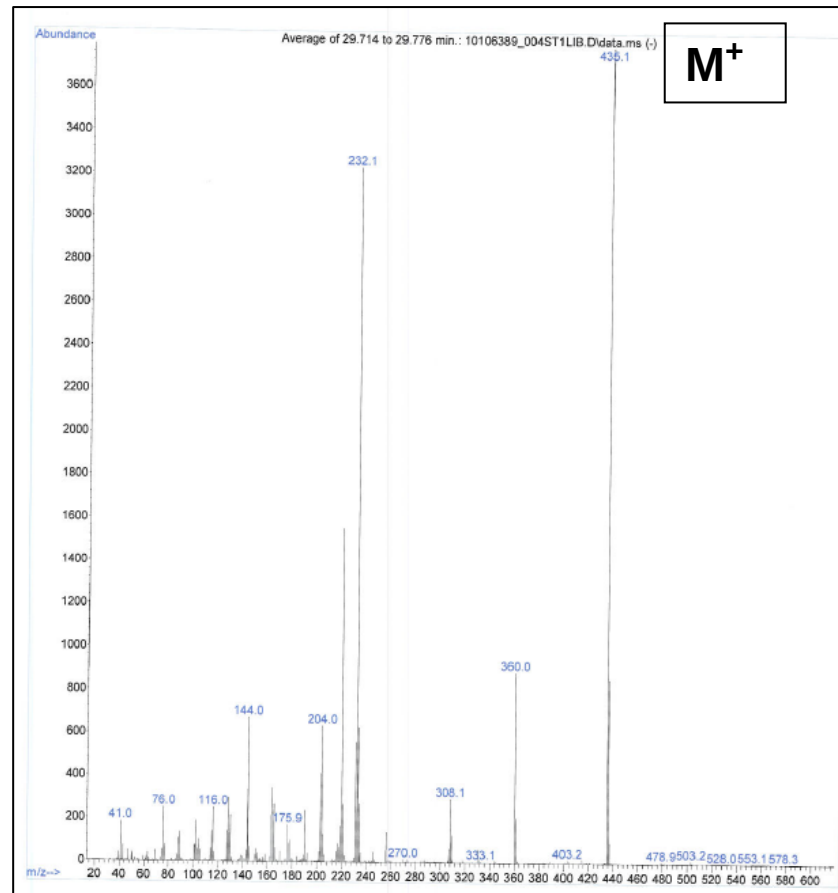
- AM-694: 62.7mg/g
 - 1-[(5-fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)-methanone
 - $C_{20}H_{19}FNO$
 - Mw 435
 - Potent cannabinoid, toxic metabolit fluoroacetate?



Analytical Strategies: Cannabinoid AM-694



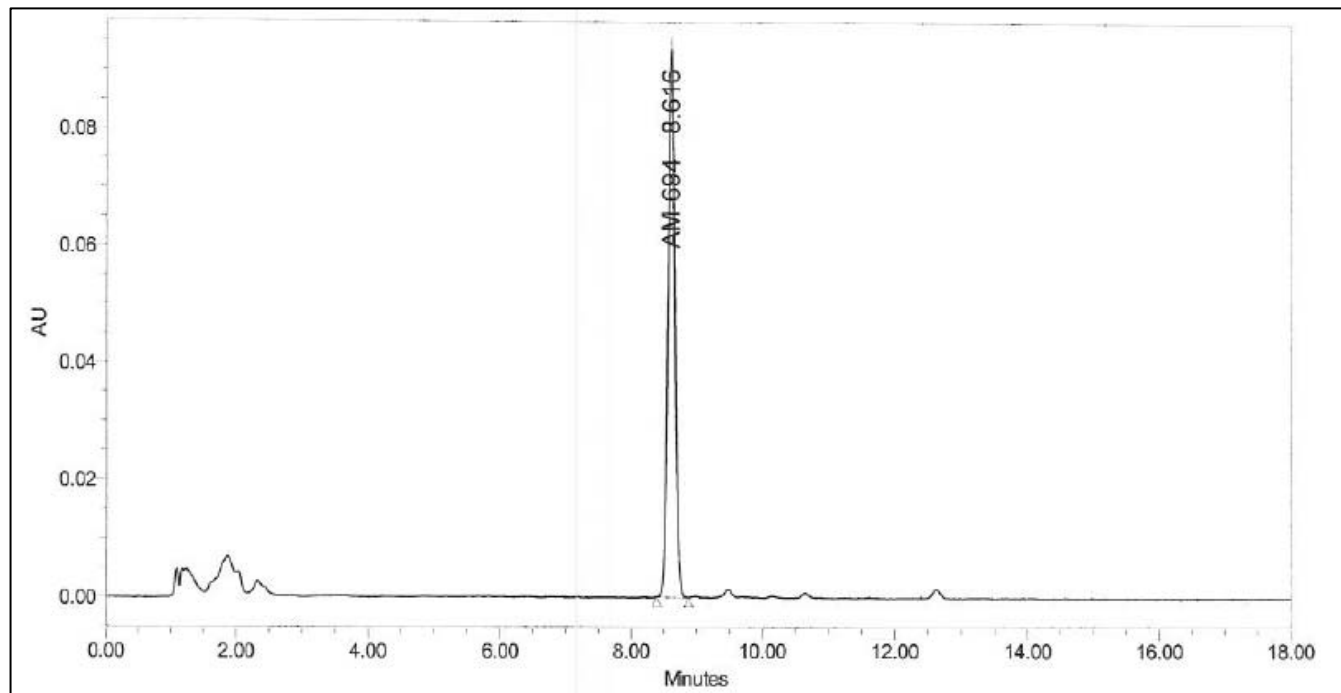
- GC-EI-MS of unknown peak AM-694 (??)



Analytical Strategies: **Cannabonoid AM-694**



- HPLC of sample in methanol



Analytical Strategies: Cannabonoid AM-694

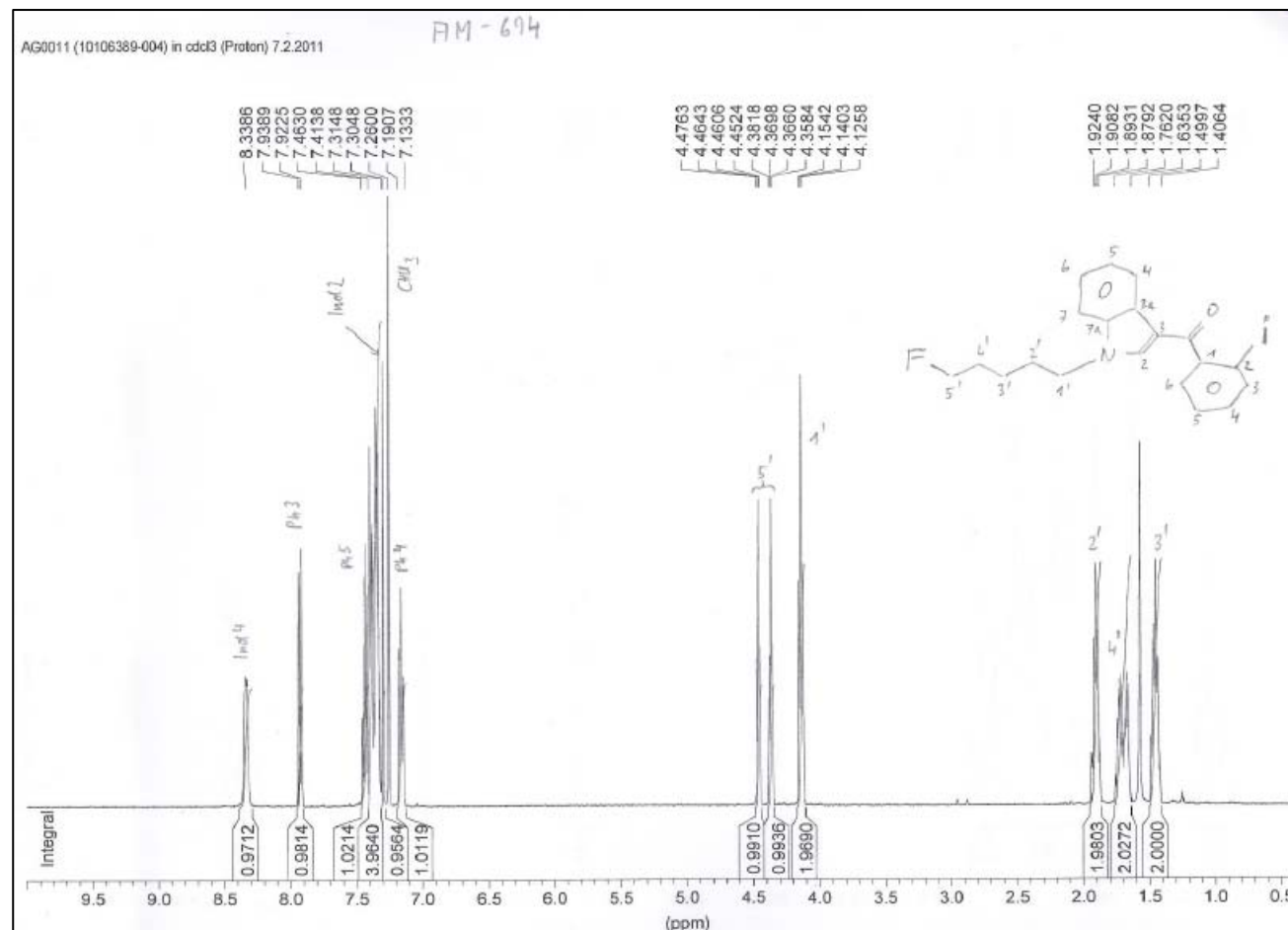
- **Preparative HPLC**

- Extraction with acetone, evaporation
- Sample solvents: acetone + DMF
- LC: WATERS 2695 + Fraction Collector
- Column: FORTIS C18, 10 μ m, 150 x 10 mm
- Injection volume: 200 μ l
- Mobile Phase:
 - 1st run ACN
 - 2nd run MEOH
- Flow rate: 3 ml/min
- **Oil: pure AM-694** (GC)
 - **Crystallisation:** starter
 - ethylmethyleketon + methanol



Analytical Strategies: Cannabinoid AM-694

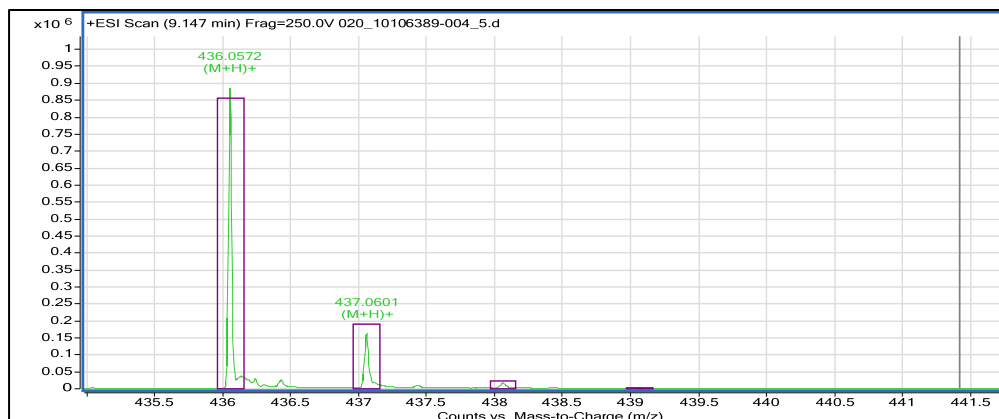
- **^1H -NMR**
- **^{13}C -NMR**
- Advantage
 - Identity
 - Assay



Analytical Strategies: Cannabonoid AM-694



- **HR-TOF-MS**
 - Accurate Mw
 - Mol. formula

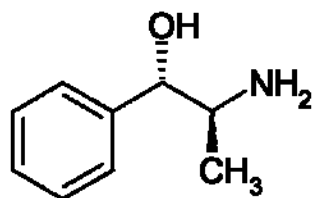


MS Formula results: +Scan(9.147 min)										
m/z	Ion	Formula	Abundance							
436.0572	(M+H)+	C ₂₀ H ₂₀ FINO	887162.8							
Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match	
☒	C ₂₀ H ₁₉ FINO	C ₂₀ H ₂₀ FINO	96.14		436.0568	-0.83	99.34	88.16	99.32	
☐	ClFH ₁₆ IN ₁₇	ClFH ₁₇ IN ₁₇	59.37		436.0565	-1.72	97.19	0	54.96	
☐	C ₁₄ H ₂₄ ClFIN ₀₃	C ₁₄ H ₂₅ ClFIN...	56.45		436.0546	-5.87	71.86	0.04	93.3	
☐	C ₉ H ₁₉ FIN ₇ O ₄	C ₉ H ₂₀ FIN ₇ O ₄	76.16		436.06	6.48	66.81	74.15	97.25	
☐	FH ₁₅ IN ₁₅ O ₄	FH ₁₆ IN ₁₅ O ₄	41.62		436.0533	-9	45.98	18.41	60.75	
☐	C ₁₀ H ₁₅ FIN ₁₁	C ₁₀ H ₁₆ FIN ₁₁	68		436.0613	9.55	41.68	90.53	93.62	
☐	C ₁₅ H ₁₉ FIN ₃ O ₃	C ₁₅ H ₂₀ FIN ₃ O ₃	70.2		436.0528	-10.08	37.74	99.64	99.77	

- **AM-694 hydrochloride CRS ready (identity + assay)**

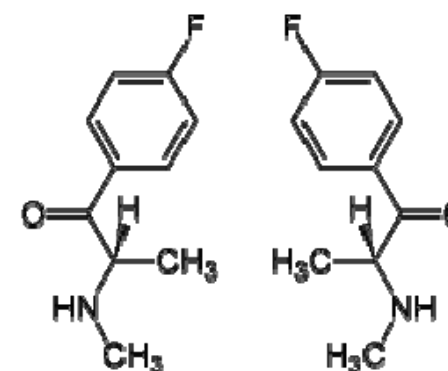
Cathinones

- Cathin + Catha edulis



- Spring 2009: **Bath salt „Buzz powder“**

- Flephedrone / 4-FMC
- 1st cathin derivate in AT

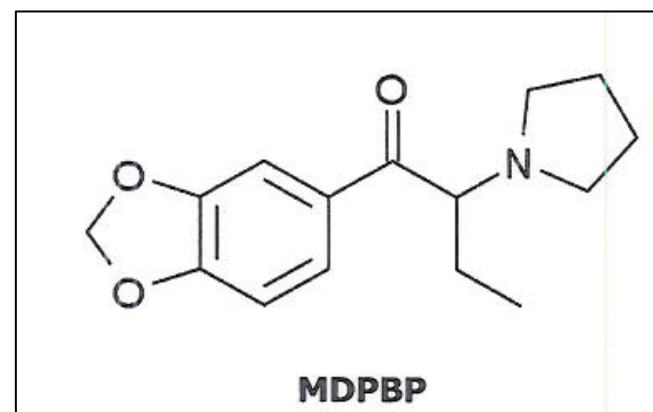
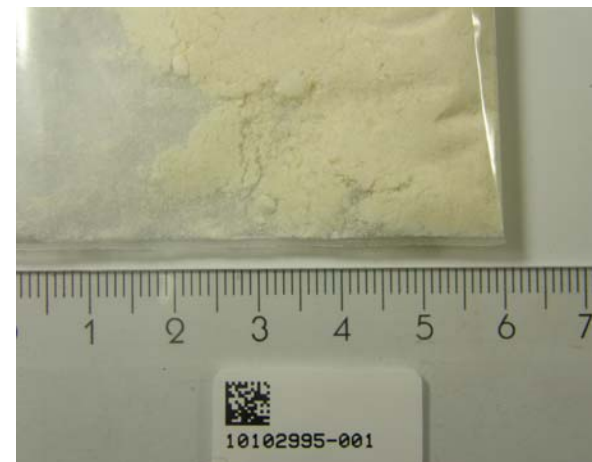


Analytical Strategies:

Cathinon MDPBP

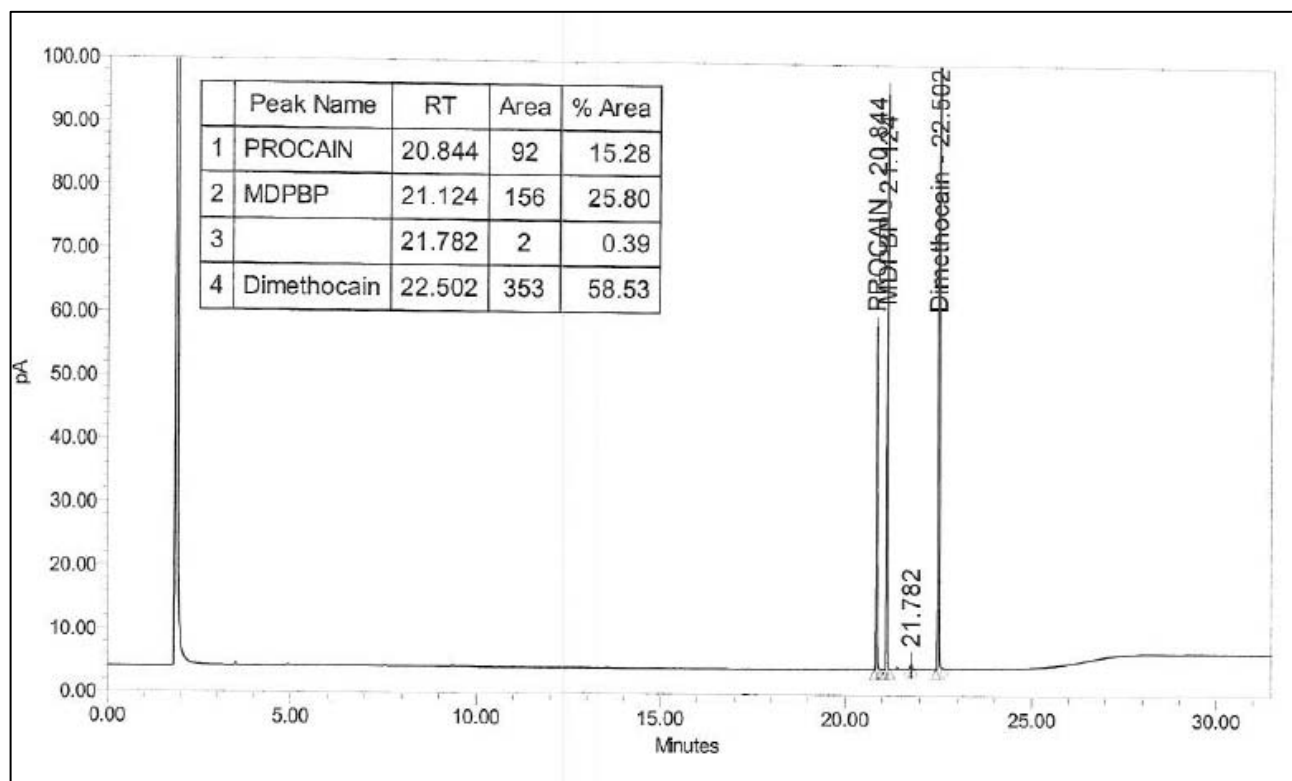


- **Unknown white yellowish powder**
- *Analysis result: Hydrochlorides of*
 1. Dimethocain: 493.5 mg/g
 2. Procain: 142.7 mg/g
 3. **MDPBP**: 239.2 mg/g
 - 3',4'-Methylenedioxy- α -pyrrolidino-butiophenon
 - $C_{15}H_{19}NO_3$
 - Mw 261
 - Stimulant, amphetamin type



Analytical Strategies: Cathinon MDPBP

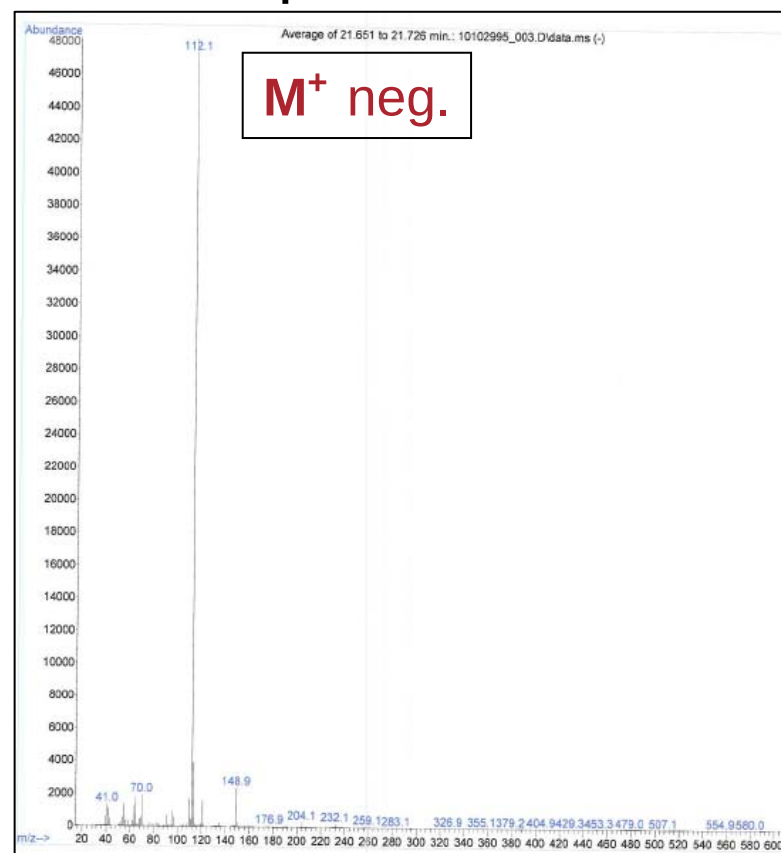
- GC-FID of unknown sample: 25.8% MDPBP



Analytical Strategies: Cathinon MDPBP



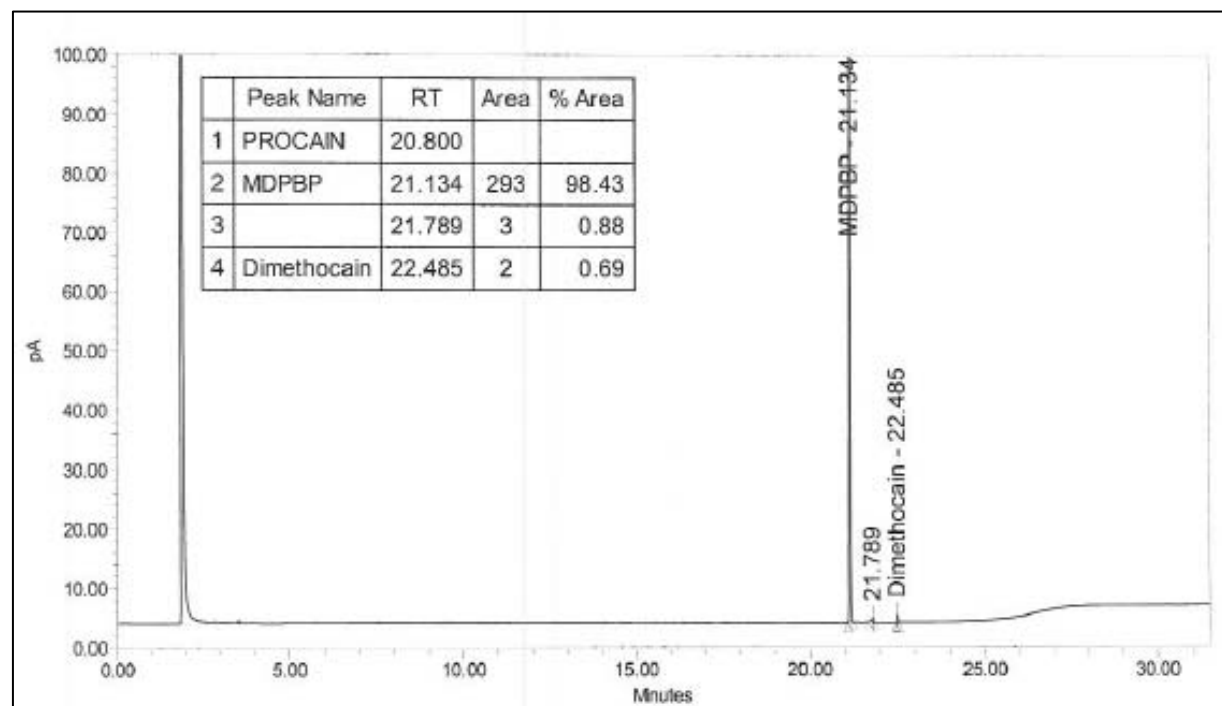
- GC-EI-MS of unknown peak



Analytical Strategies: Cathinon MDPBP



- **Fractionated Crystallisation**
 - Ethanol / n-heptan / fridge → white crystals
- GC-FID of 1st precipitate → 98.4% MDPBP
- NMR
- HR-MS
- pot. Titration



Conclusion



- Analytical progress is powerful (eg MS)
- New illegal APIs
 - Isolation for structural elucidation (NMR)
 - Synthesis....
 - Cooperation with academia
- Challenges for future
 - **Pharmacological assessment?**
 - **Legal instruments?**
 - **MEDICRIME Convention ante portas**



Thanks



- Auditorium
- OMCL-Team
- Uni Wien, Dept. Med. Chem. for NMR

