



Australian Government  
National Measurement Institute

# The Effective Characterisation of Novel Pure Substance Reference Materials while Meeting Users Needs in a Timely Fashion

Lindsey Mackay and Steve Davies  
National Measurement Institute, Australia

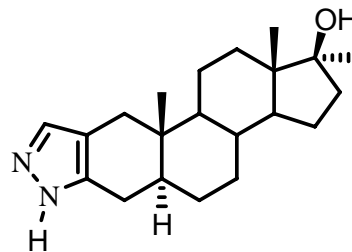
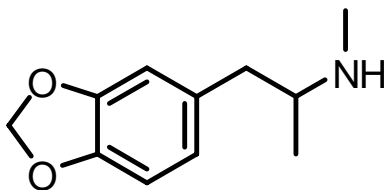
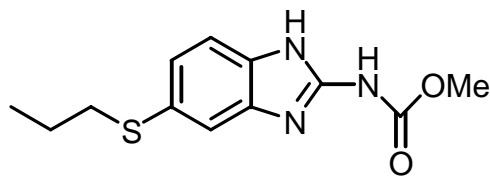


NMIA specialises in pure substance, organic reference materials in the areas of

- Illicit drugs
- Sports drug
- Agricultural and veterinary chemicals

Our biggest challenge is the continued need for new materials in the areas of

- New designer drugs
- New prohibited substances



# The Age of Designer Drugs

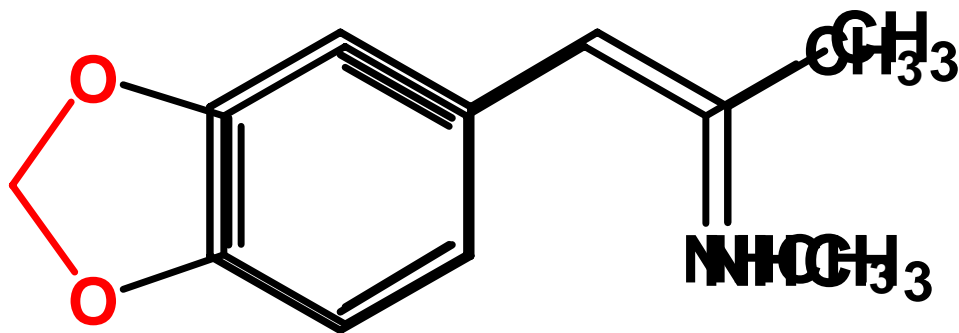
- Recent explosion in ‘Designer’ drugs
- Both Forensic and Drugs in Sport areas
- Specifically synthesised to avoid detection or to go beyond the boundaries of current legislation

# How do we Detect Designer Drugs?

- NMIA has a two significant programs aimed at:
  - **Forensics**
  - Within our forensic laboratory we endeavour to identify unknown compounds found in seizures
    - Mass spectral and NMR investigation to elucidate structure
- **Drugs in Sport**
- Research programs sourcing materials internationally that are available for abuse by athletes

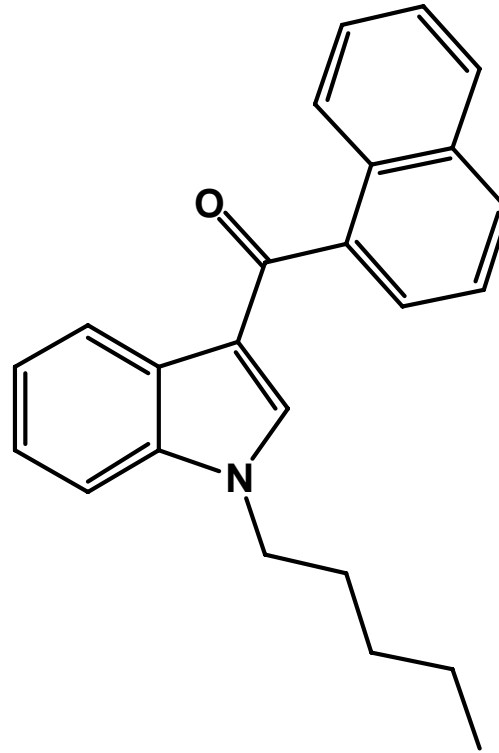
# What is a Designer Drug?

- Substance with modification in molecular structure designed to circumvent detection or legislation

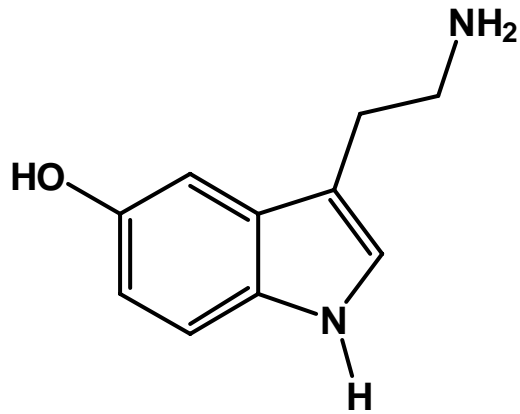


# New Forensic 'Designers' – what are they?

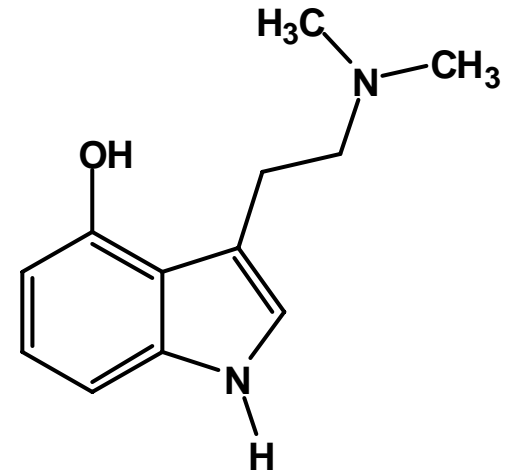
- Tryptamines
- Phenethylamines
- Piperazine derivatives
- Cathinone derivatives
- Synthetic cannabinoids
- 'Research chemicals'



# Tryptamines



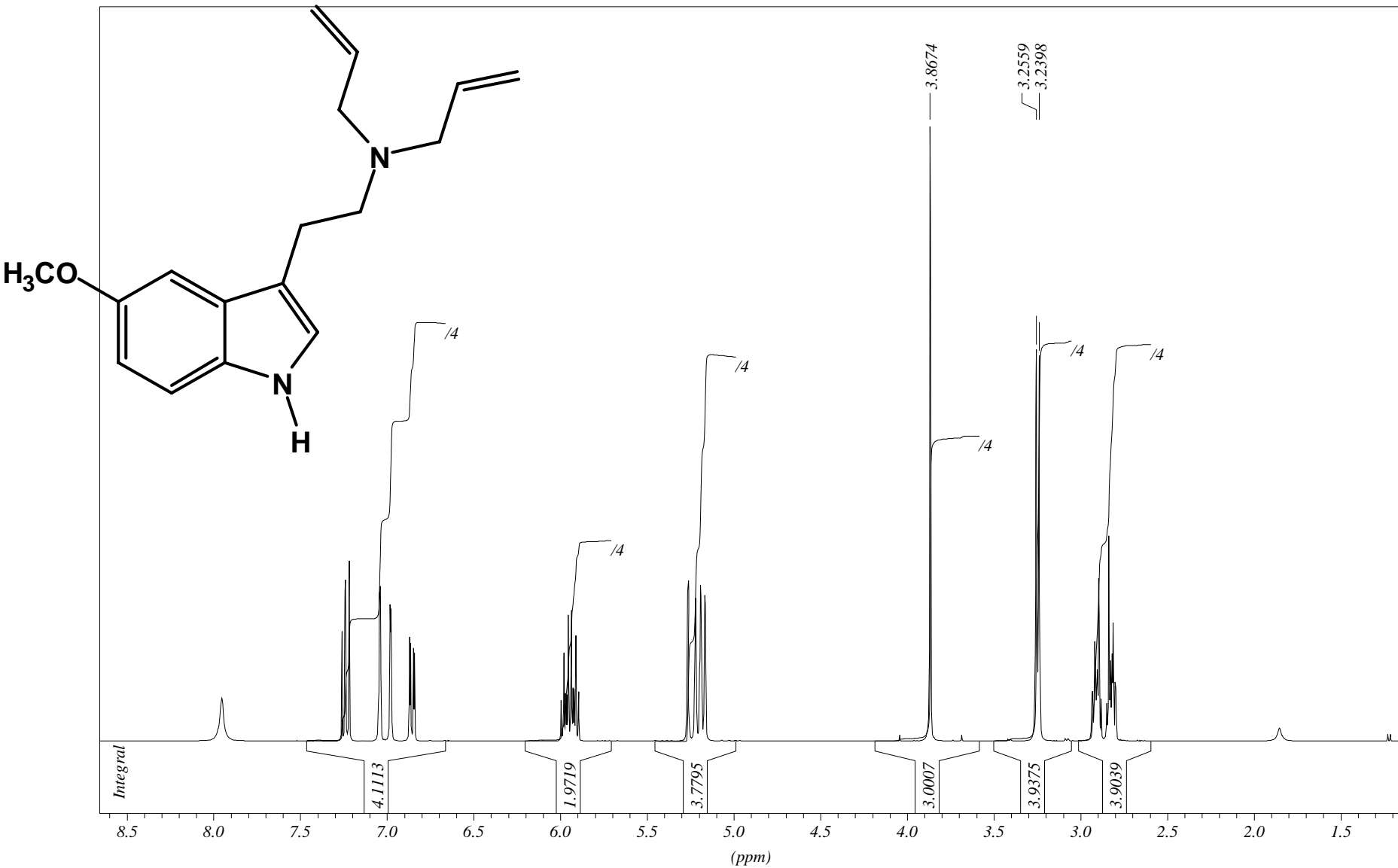
**Serotonin**



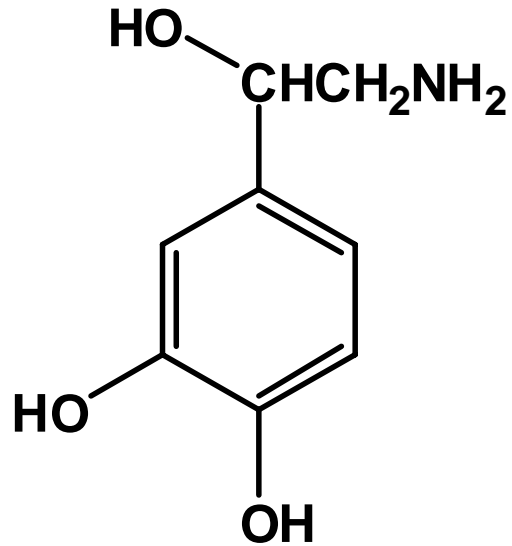
**Psilocin**



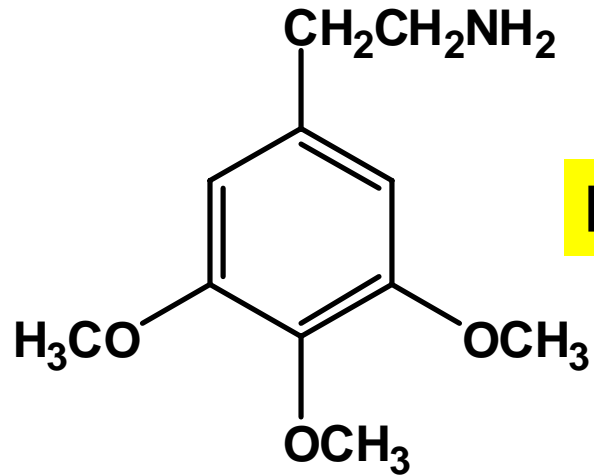
# 5'-Methoxy-N,N-diallyltryptamine



# Phenethylamines



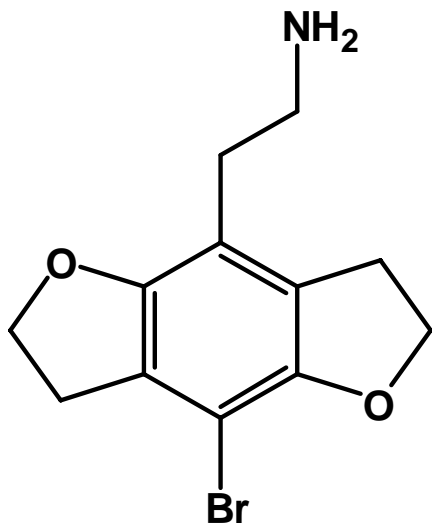
**Noradrenalin**



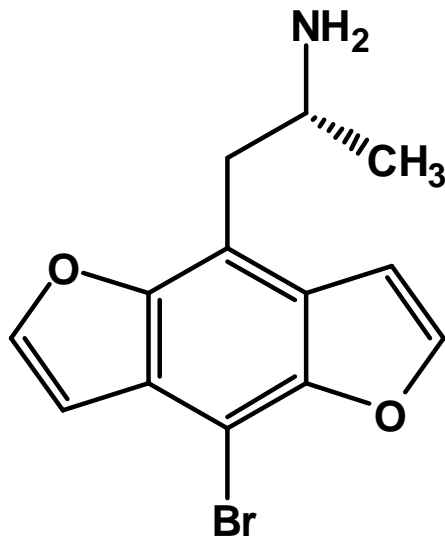
**Mescaline**



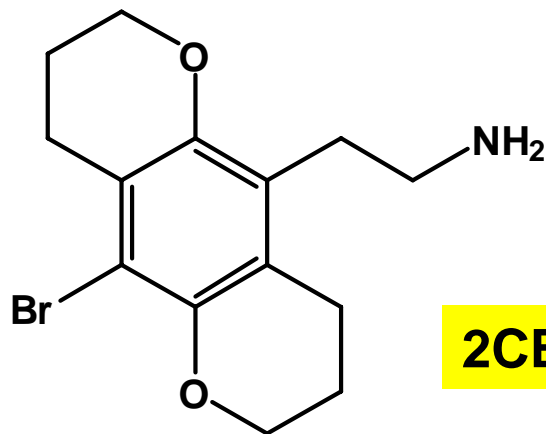
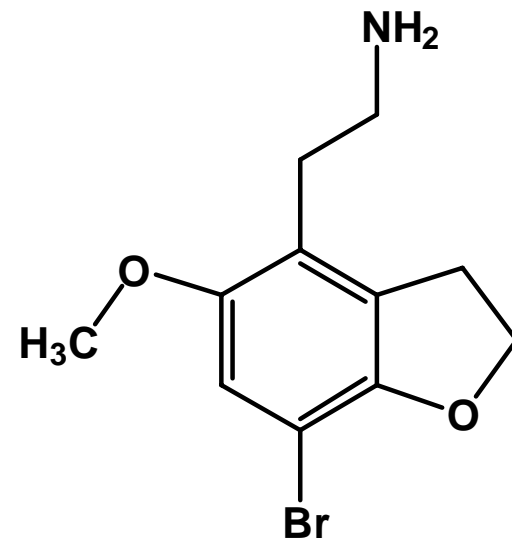
# The 'Fly' Compounds



**2C-B-FLY**

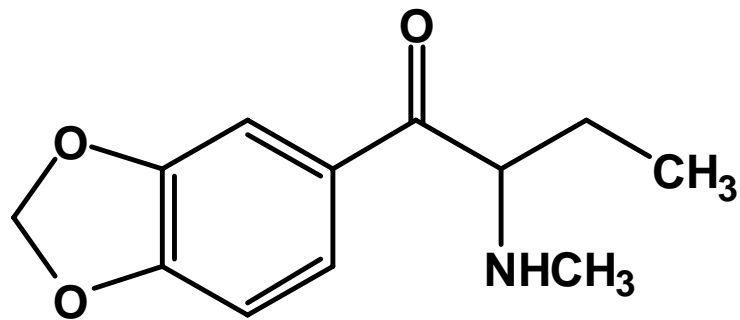
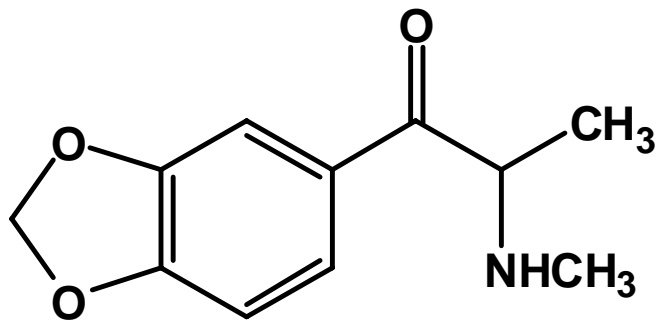
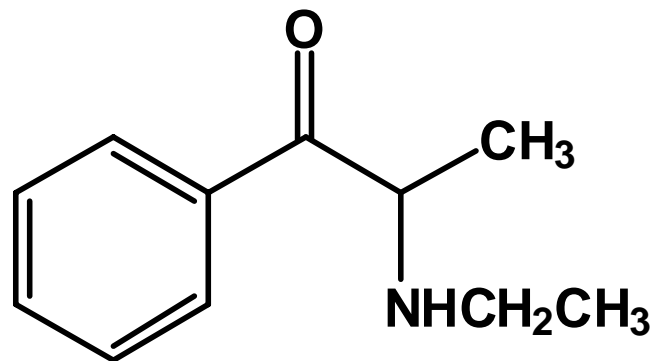
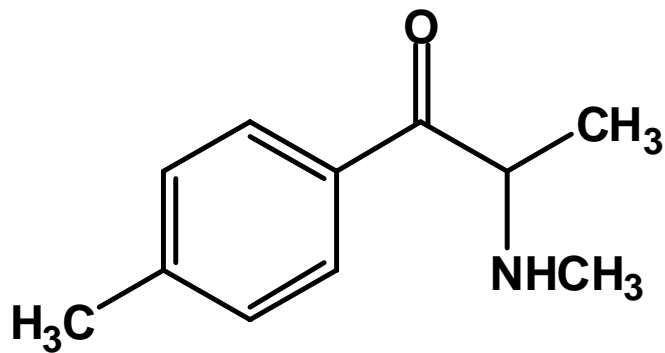


**Bromo-Dragon FLY**

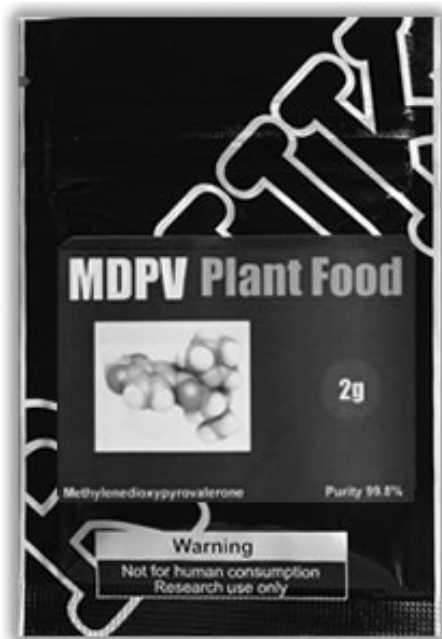
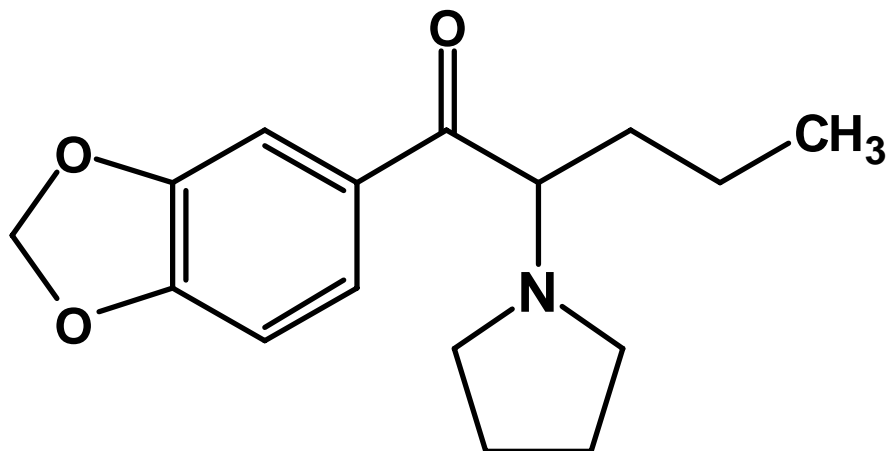


**2CB-Butterfly**

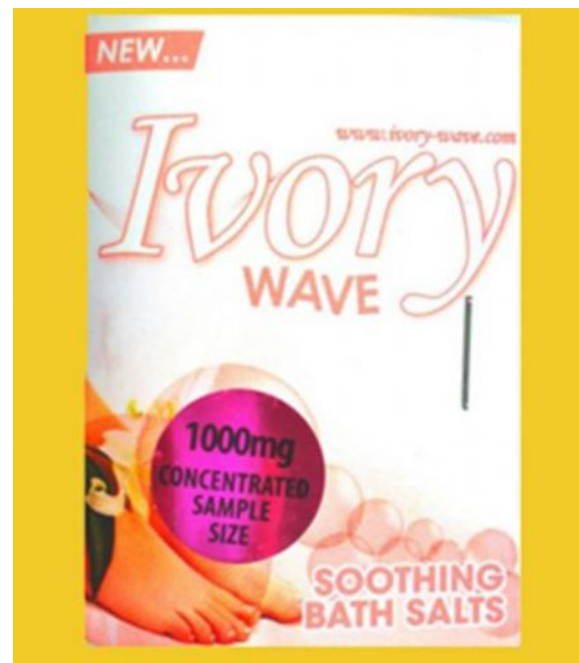
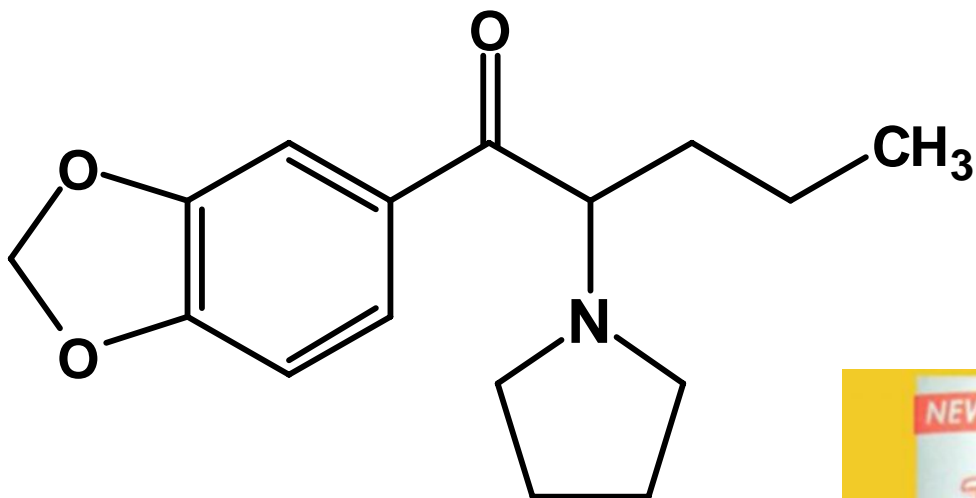
# The Cathinone Family



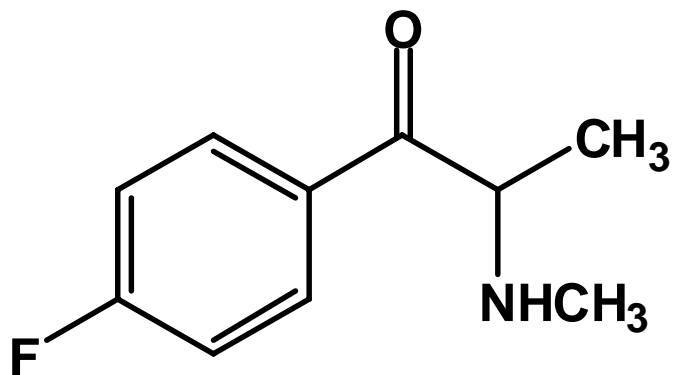
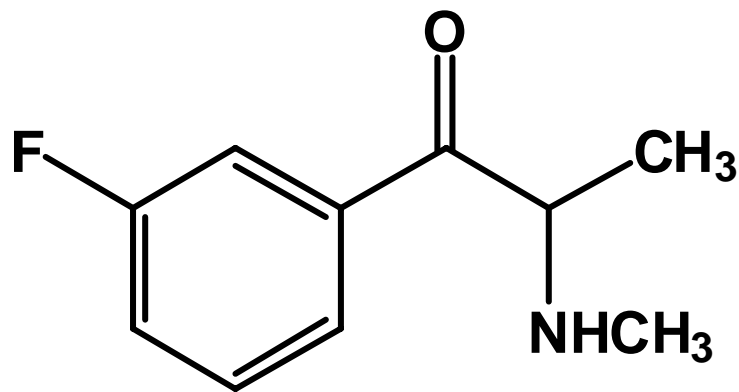
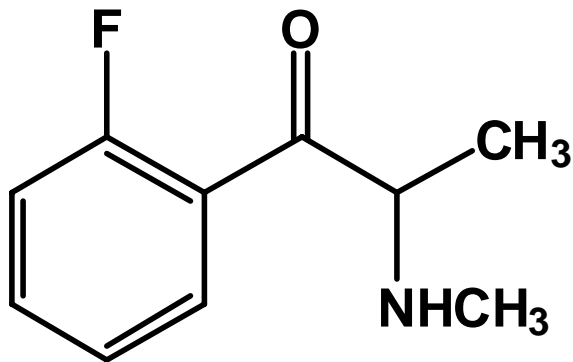
# MDPV 'Plant Food'!



# MDPV as Soothing Bath Salts



# Fluoromethcathinone Group



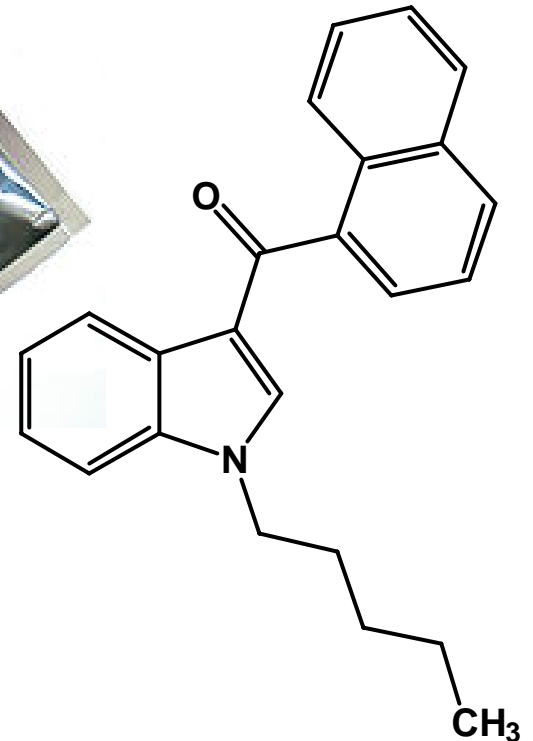
# The “Spice” Products – Herbal Highs

- In the mid-2000s, a new range of herbal smoking products appeared on the market - The ‘Spice’ range :

- Spice Diamond
- Spice Gold
- Spice Silver, etc



- Distributed by ‘Psyche Deli’
- Contents declared as a mixture of the usual herbal high materials
- “JWH” series



# Drugs in Sport

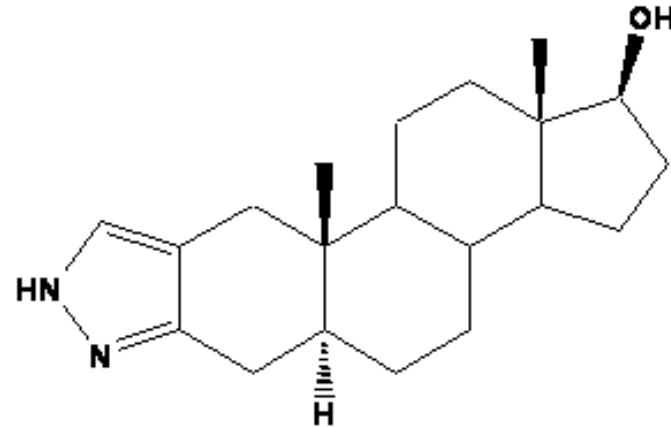
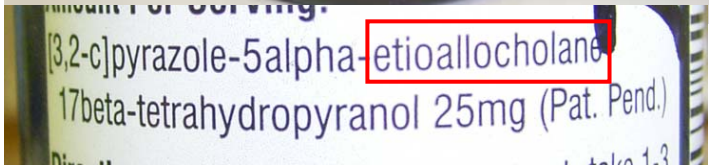
- Our program has sourced about 200 “supplements” over the last few years
  - Trawling body building sites on the web
  - Purchasing materials
  - Identifying the contents
  - Carrying out metabolism studies
  - Developing methods for the metabolites in urine/blood
  - Preparing reference materials where needed

# Sites

- Bodybuilding sites (bodybuilding.com)
- Amazon.com
  - supplements -184,114 products
  - sports supplements - 2,043 products
  - Many have no information on ingredients

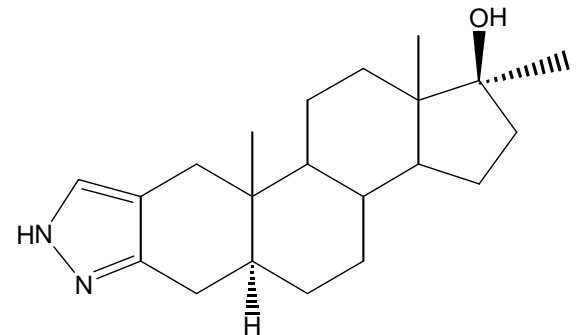


# Prostanazol

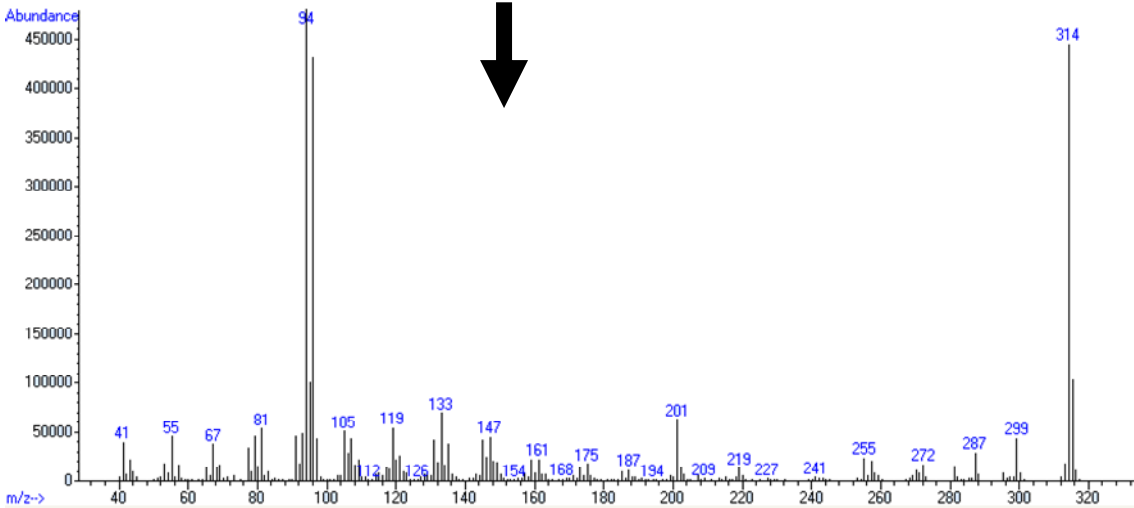


PROSTANAZOL

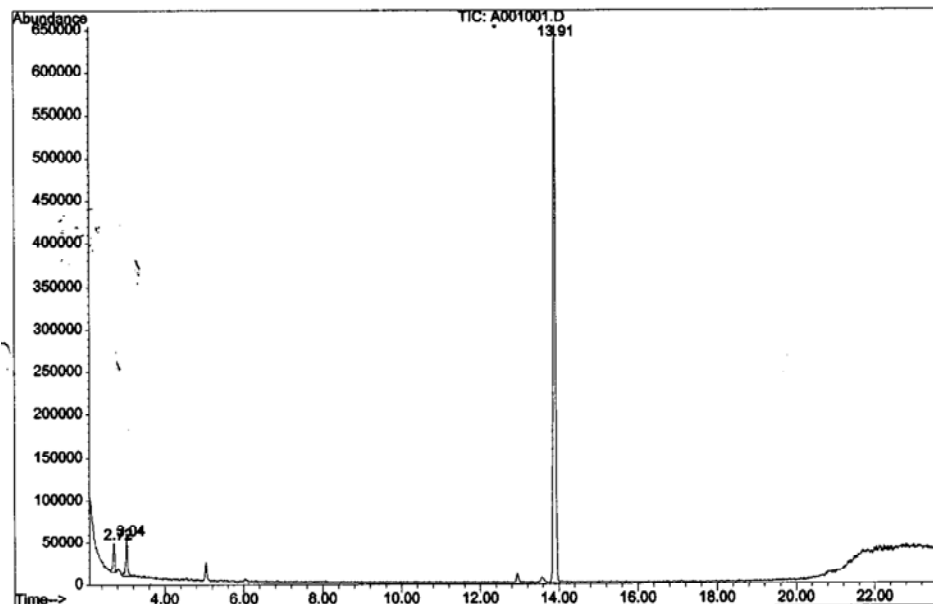
[3,2-c]pyrazole-5 $\alpha$ -**androstane**-17 $\alpha$ -methyl-17 $\beta$ -ol



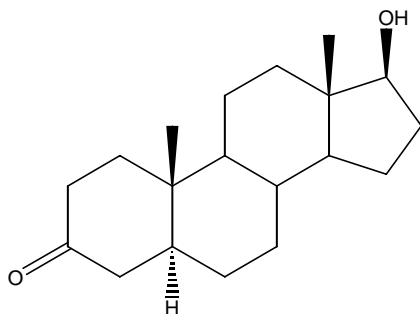
stanozolol



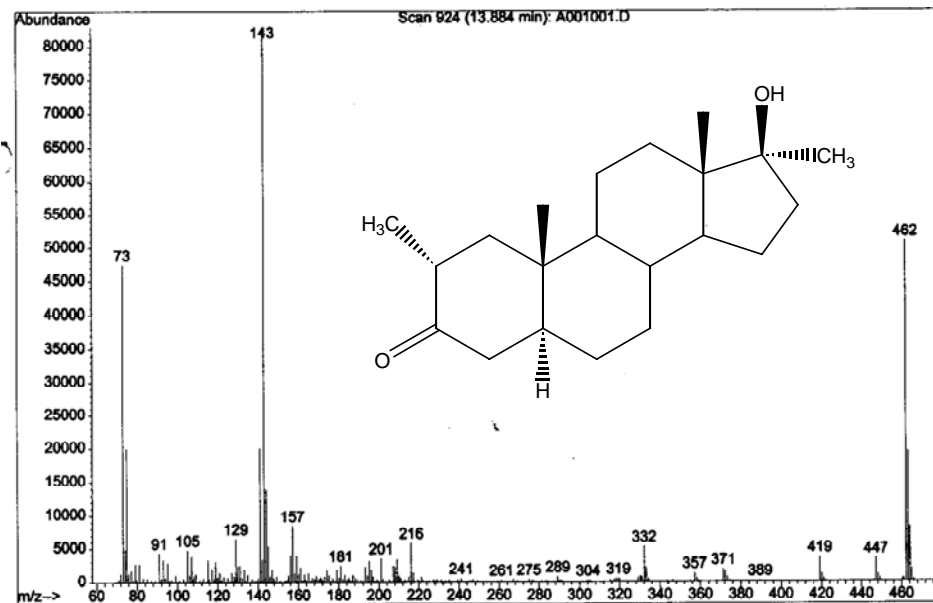
# Superdrol



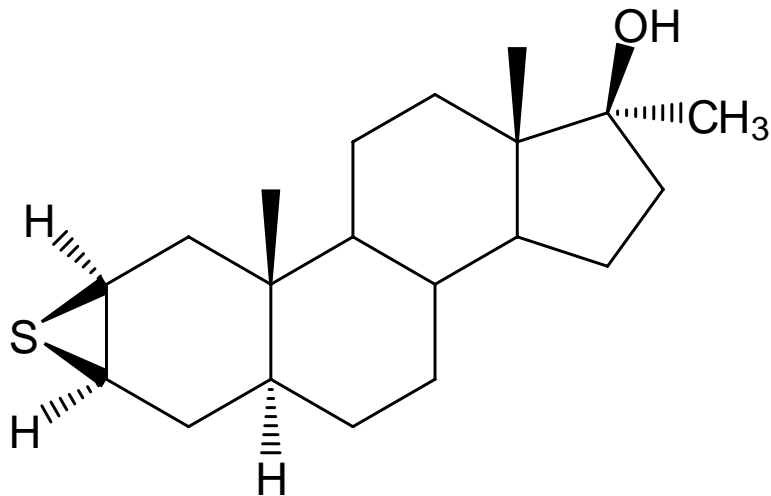
$2\alpha, 17\alpha$ -dimethyl- $5\alpha$ -androstane-3-one



Dihydrotestosterone (DHT)



# 2,3-Epithio-17 $\alpha$ -methyl-5 $\alpha$ -androst-17 $\beta$ -ol



# Australian Drug Analogue Legislation

- Section 314.4 (2) Border Controlled Drug
  - a stereoisomer; or
  - a structural isomer having the same constituent groups; or
  - an alkaloid;
  - a structural modification .....
  - otherwise a homologue, analogue, chemical derivative or substance substantially similar in chemical structure.

# World Anti-Doping Agency Prohibited List

- Similarly open with respect to analogues
- Bans anabolic agents
  - Lists 47 specific compounds and then adds “and other substances with a similar chemical structure or similar biological effect”
- Bans diuretics and stimulants ...”and other substances with similar chemical structure or biological effect”

# How do we Prepare Pure Calibrant Reference Materials to Meet these Needs?

- Need fit-for-purpose standards available in a timely manner
  - Rapid certification
  - Identity confirmation is essential
  - Purity values and measurement uncertainty that meet the requirements for prosecution

# Our Approach

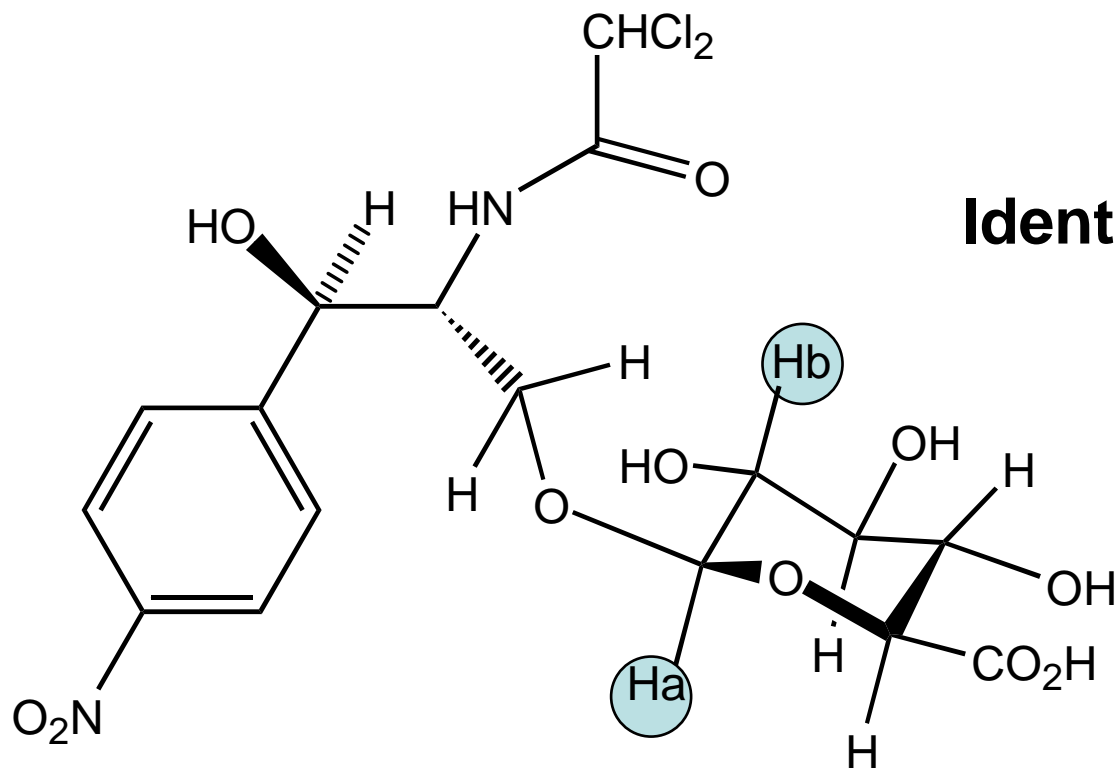
- Purify seizures or synthesise in-house
- Identity confirmation
- Certification of purity by an array of techniques to ensure cross checking of as much data as possible



# Establishing Identity

If available - comparison with reference spectra ( $^1\text{H}$  and  $^{13}\text{C}$  NMR, IR, MS) and m.p.

New compounds need thorough in-house assessment



Identity - Regiochemistry?

- Stereochemistry?

●  $J = 8 \text{ Hz}$   
i.e  $\beta$  configuration

# Assessment of Purity

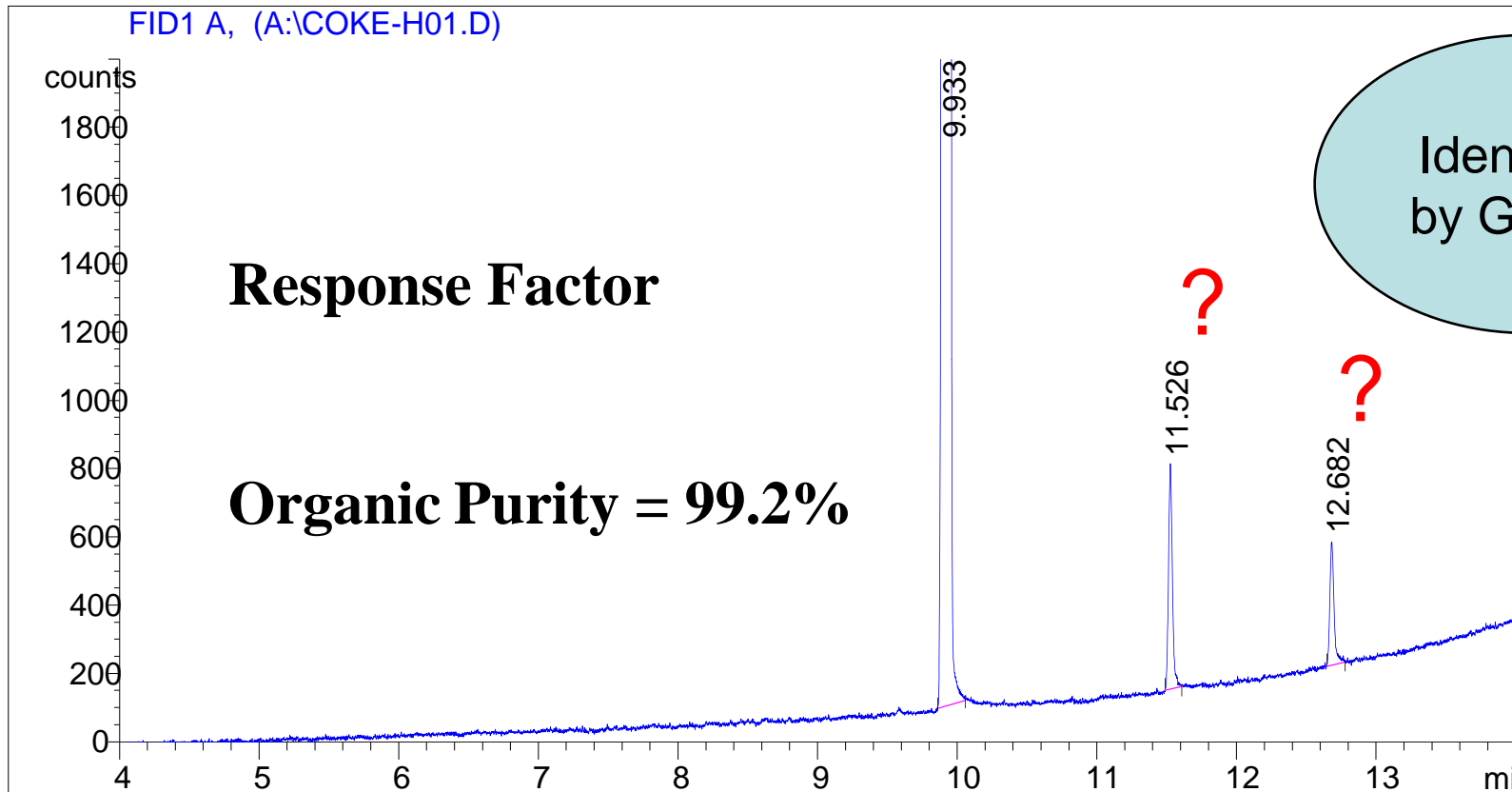
- Organic: Assay by GC-FID or HPLC-PDA/ELSD
- Volatile: Assay by TGA, Karl-Fischer
- Non-volatile: Assay by TGA (full combustion)

Cross check with  $^1\text{H}$  NMR, head space GC-MS and elemental analysis

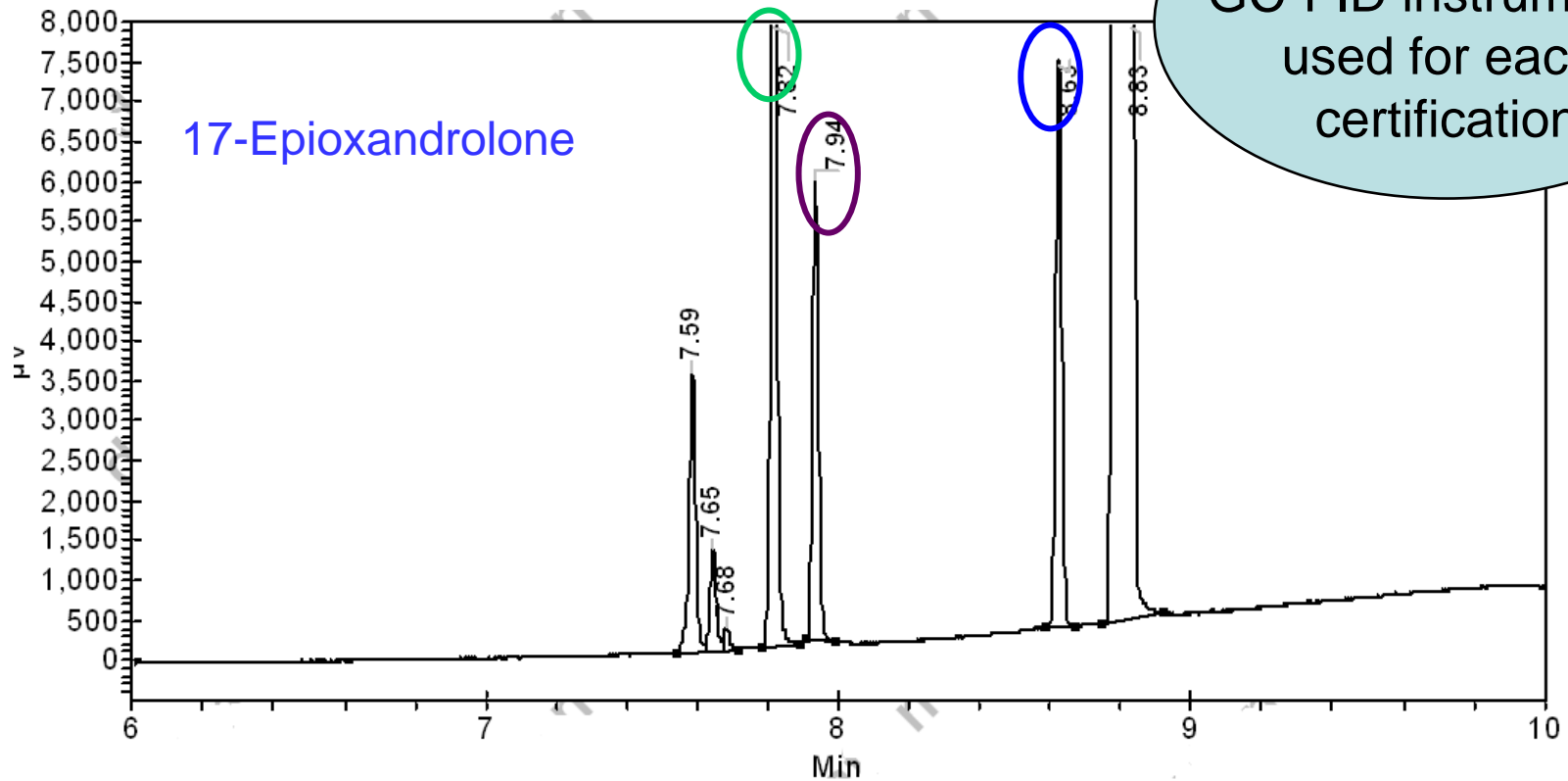
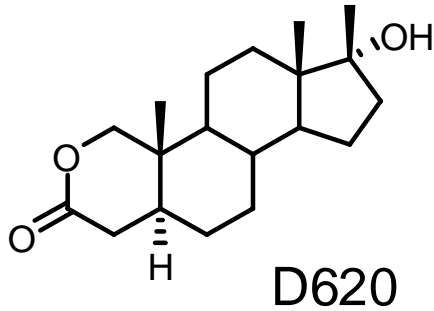
 Ultimate cross check QNMR

**Comprehensive characterisation not solely relying on the mass balance approach**

# Assessment of Organic Impurities by GC-FID



# Ensuring Minimal Bias in GC-FID



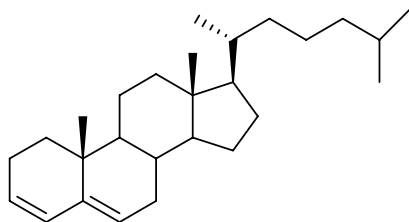
Three different GC-FID instruments used for each certification

3 different types of column with different retention times

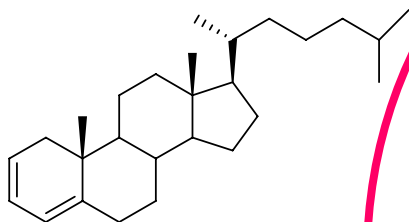
# HPLC for Organic Assessment – Greater Care Required

Photo Diode Array

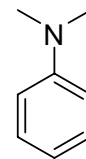
Peak area – f([analyte] and extinction coefficient)



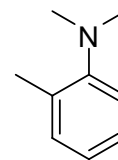
Cholesta-3,5-diene  
 $\lambda_{\max}$  235 nm  
 $\epsilon_{\max}$  19,000



Cholesta-2,4-diene  
 $\lambda_{\max}$  275 nm  
 $\epsilon_{\max}$  10,000

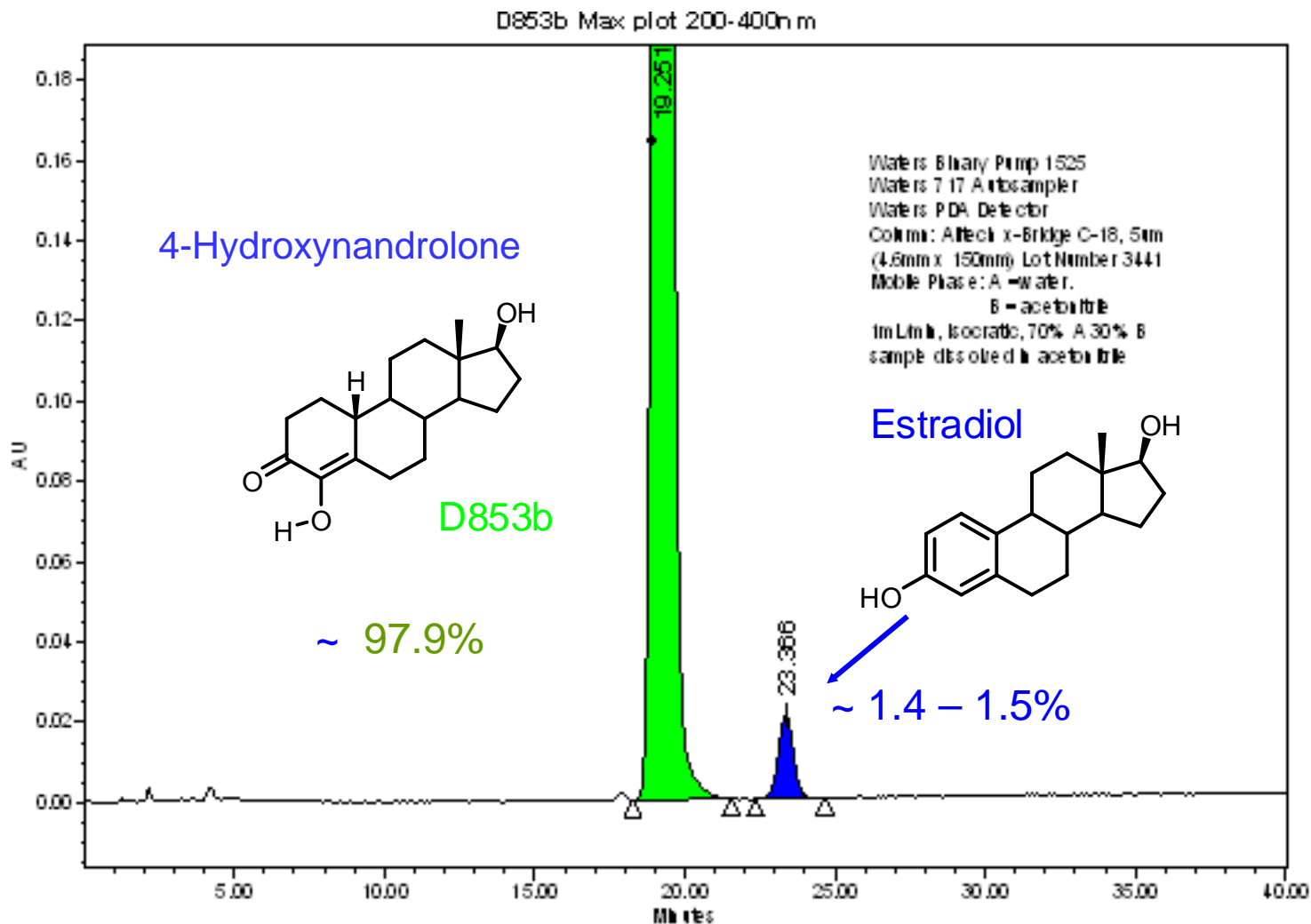


*N,N*-Dimethylaniline  
 $\lambda_{\max}$  251 nm  
 $\epsilon_{\max}$  15,500



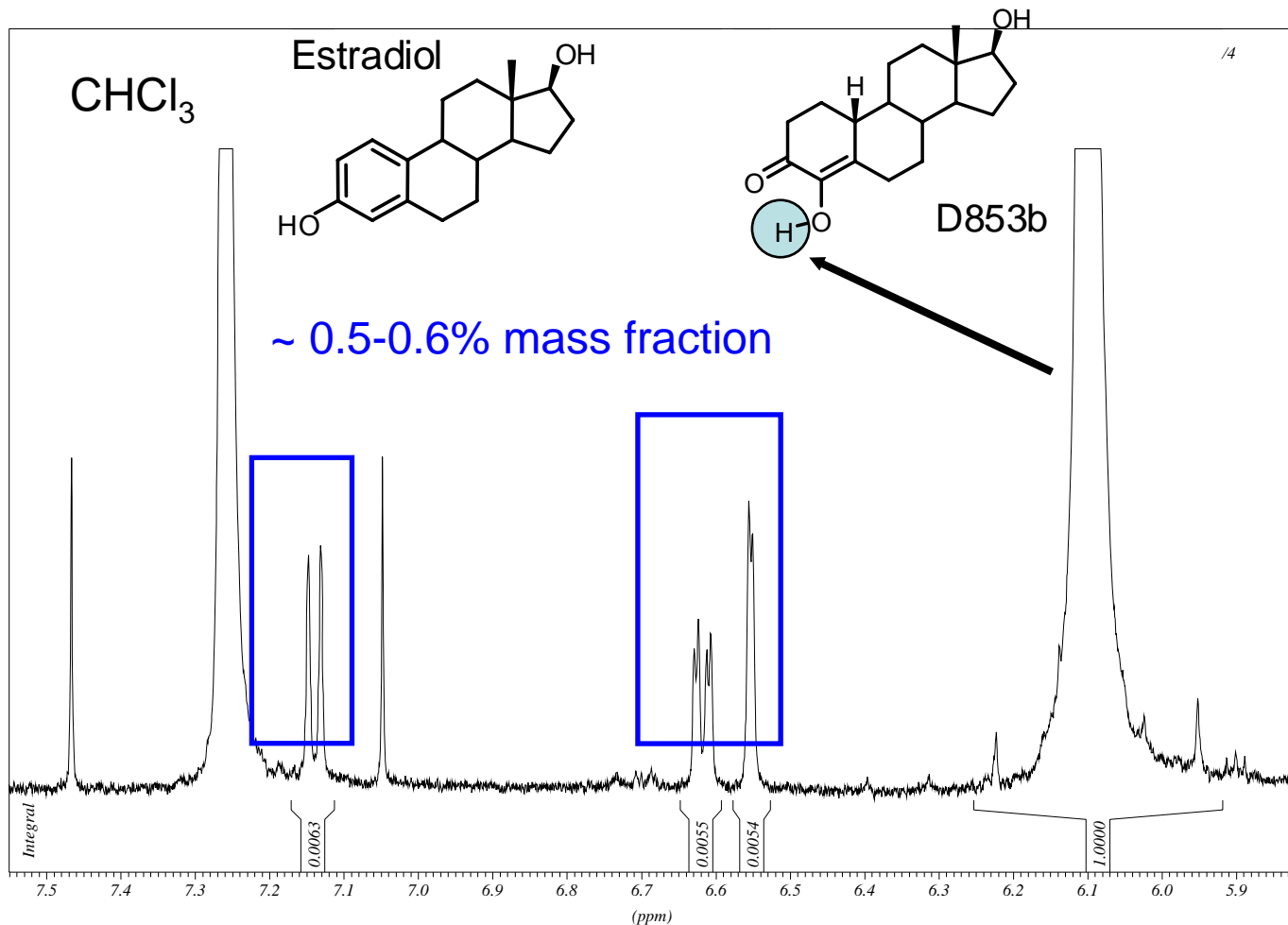
*2-Methyl-N,N*-dimethylaniline  
 $\lambda_{\max}$  248 nm  
 $\epsilon_{\max}$  6,360

# HPLC-PDA



Sample Name: D853b-1; Vial: 31; Injection: 1; Date Acquired: 5/20/2009 5:34:19 PM EST

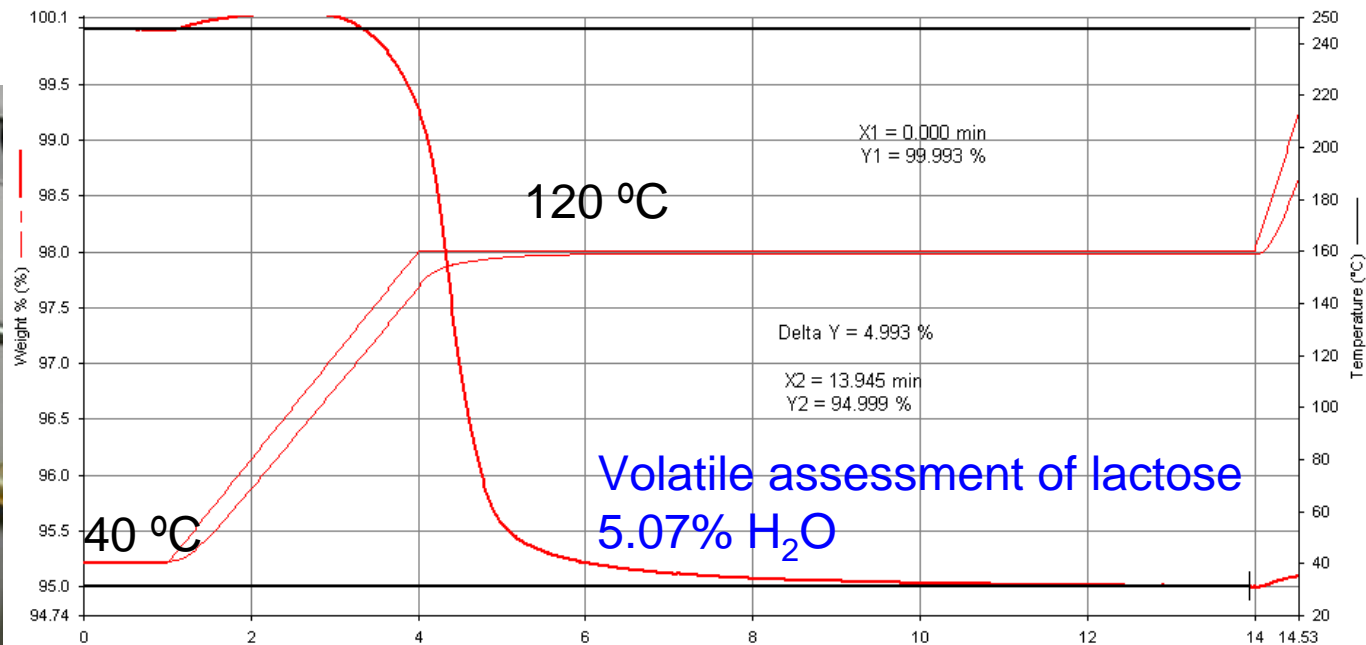
# Cross Check HPLC Result with $^1\text{H}$ NMR



$^1\text{H}$  NMR also detected 0.5% ethyl acetate

# Assessment of Volatiles

ThermoGravimetric Analysis - heat to 120°C and observe weight loss.



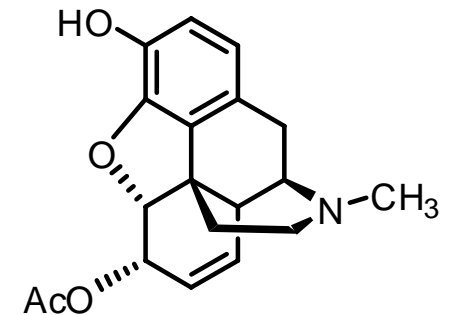
Results compared with elemental analysis,  
Karl Fischer analysis & <sup>1</sup>H NMR (occluded solvents etc).

# Volatile Content Determination Problems

TGA: < 0.2% volatiles at 120 °C, 140 °C  
and 160 °C.

But.....

Karl Fischer moisture analysis  
indicates ~ 0.5% H<sub>2</sub>O mass fraction.

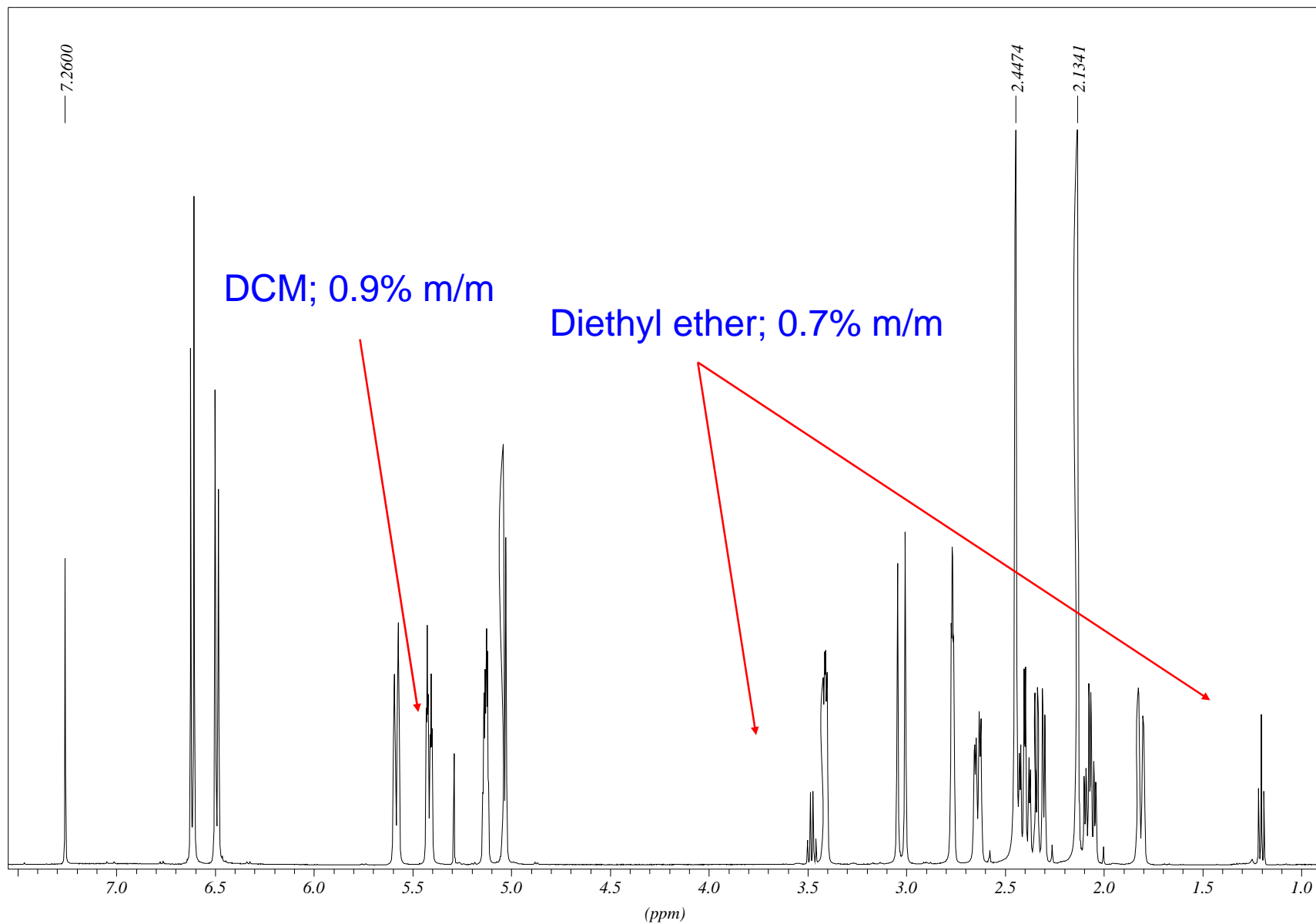


**6-Monoacetylmorphine**

**Underestimation of volatiles by TGA  
is commonly observed**

But that's not all.....

# $^1\text{H}$ NMR to the Rescue



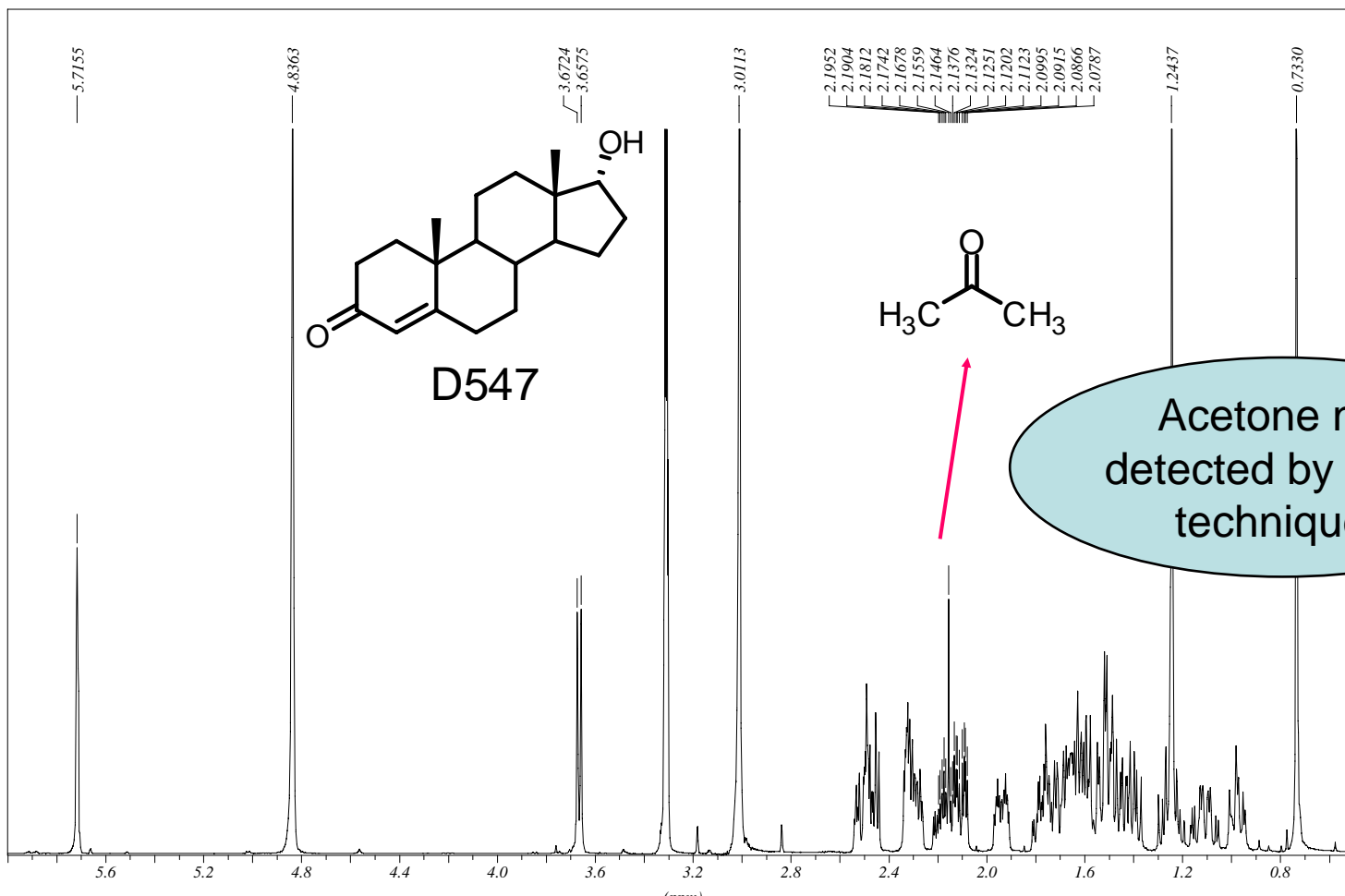
# Epitestosterone: Another Case Study



TGA: ~ 0% volatiles at 120 °C

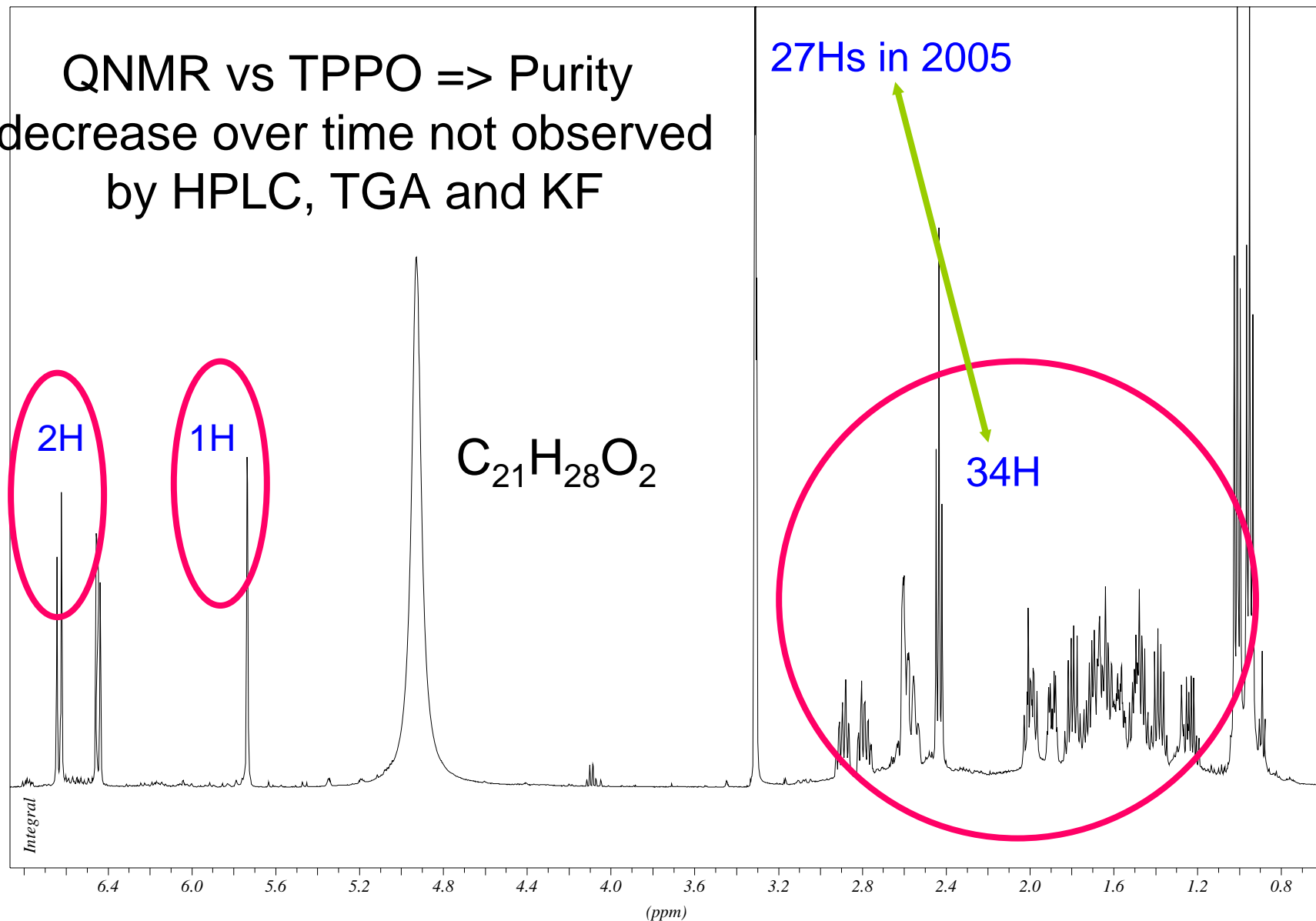


Water analysis by KF shows 0.4% H<sub>2</sub>O mass fraction



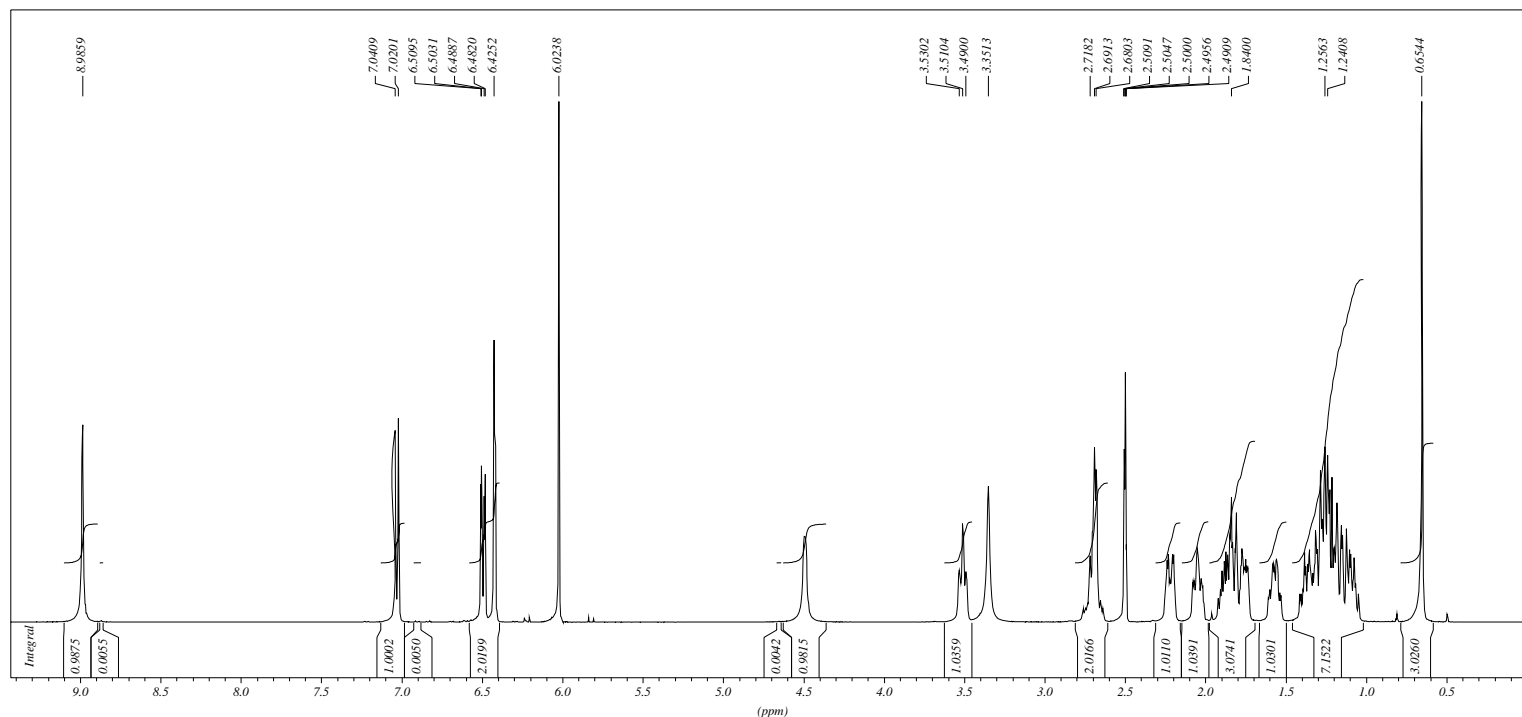
# Polymerisation?

QNMR vs TPPO => Purity  
decrease over time not observed  
by HPLC, TGA and KF



# $^1\text{H}$ QNMR Analysis

- Every compound is subject to QNMR
- Certified 5 very high purity materials to use as internal standards
- Compare multiple signals
- Completely independent cross check of absolute purity



# Certification

- We no longer rely on a simple mass balance purity approach
  - We use a range of techniques to cross check for bias in each component
- Techniques like NMR provide enormous structural and purity information in a rapid way
- Each material is subject to full homogeneity assessment
- Use 20 years of experience in producing forensic and drugs in sport reference materials to estimate a stability uncertainty in the first year
  - Have over 150 drugs in sport pure materials and over 150 forensic pure materials in our catalogue

# Designer forensic drugs seen at NMIA in 2009/2010

- 2-fluoroamphetamine
- 4-fluoroamphetamine
- 2-fluoromethylamphetamine
- 4-fluoromethylamphetamine
- 1-(3-trifluoromethylphenyl)-piperazine
- 1-(3-chlorophenyl)-piperazine
- 1-(4-fluorophenyl)-piperazine
- Naphyrone
- JWH-018
- *N*-ethylcathinone
- 4'-methylmethcathinone
- 3'-fluoromethcathinone
- 4'-methoxymethcathinone
- 3',4'-methylenedioxy-methcathinone
- 3',4'-methylenedioxypropylvalerone
- Butylone
- 5-methoxy-*N,N*-diallyltryptamine

# Designer forensic drugs seen at NMIA in 2009/2010

- 2-fluoroamphetamine
- 4-fluoroamphetamine
- 2-fluoromethylamphetamine
- 4-fluoromethylamphetamine
- 1-(3-trifluoromethylphenyl)-piperazine
- 1-(3-chlorophenyl)-piperazine
- 1-(4-fluorophenyl)-piperazine
- Naphyrone
- JWH-018
- *N*-ethylcathinone
- 4'-methylmethcathinone
- **3'-fluoromethcathinone**
- 4'-methoxymethcathinone
- 3',4'-methylenedioxy-methcathinone
- 3',4'-methylenedioxypropylvalerone
- **Butylone**
- 5-methoxy-*N,N*-diallyltryptamine

# Conclusions

- Huge challenges exist to meet customer needs
- Produce materials of appropriate quality for legal proceedings in a timely manner
  - Certificates must withstand legal scrutiny by defence experts
  - No point having the perfect reference material long after its actually needed

