Strategy and experience in testing of suspected counterfeit medicines at the Swedish OMCL using NMR and LC-MS-TOF

Torbjörn Arvidsson, Greisfwald, October 12, 2012

LÄKEMEDELSVERKET
MEDICAL PRODUCTS AGENCY

OMCL – Official Medicines Control Laboratories

Role:

independent testing of

- **medicines with suspected quality defect**
- **medicines in market surveillance programs**
- **suspected counterfeit and illegal medicines**

What are counterfeit medicines?

- **Counterfeit medicines are deliberately and fraudulently mislabeled with respect to identity or source: their quality is unpredictable as they may contain the wrong amount of active ingredients, wrong ingredients or no active ingredients.**

- **In all cases counterfeit medicines are manufactured in clandestine laboratories with no possibility of control.**

http://www.who.int/impact/en

Strategy for testing of suspected counterfeits

- Screening with LC-MS-ToF for identification
- Analysis with NMR to verify identity
- Assay by quantitative NMR

- Visual inspection, recording of size and weight of capsules, tablets or any other formulation
- Photos of all products are taken

Screening with LC-MS for identification

Sample preparation
Grind tablets or mix contents from 10 capsules

Extraction
10-20 mg sample
2 ml methanol, vortex 30 min

Dilute
If needed with dilute formic acid or methanol (1+10)

Filter
GHP Acrodisc 0.45 um

Analysis with LC-MS

Liquid Chromatography–Mass Spectrometry (LC-MS)

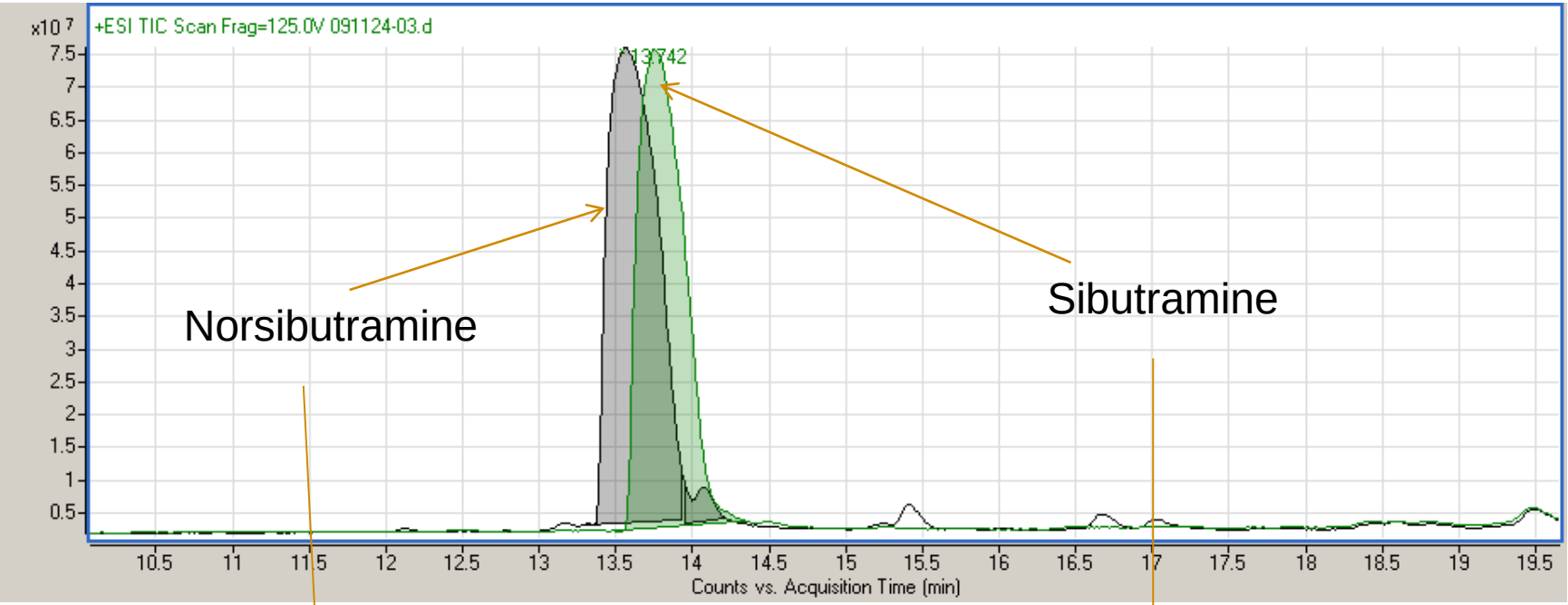
LC-MS-QTOF with DAD-detector

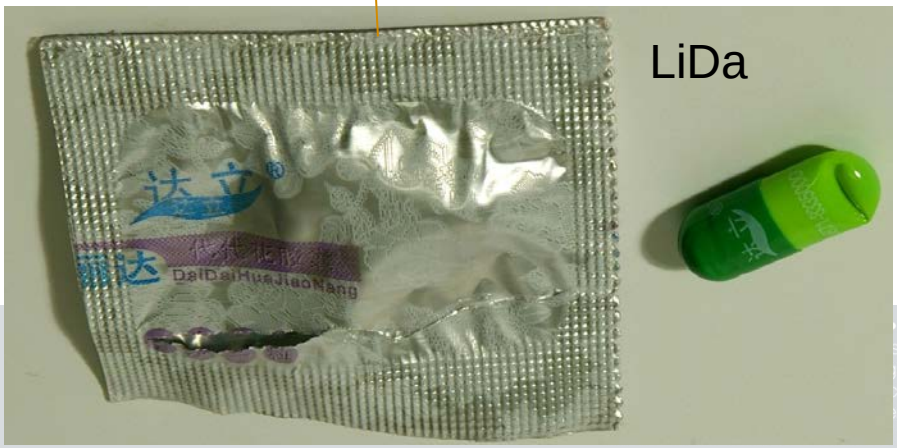
– In-house library containing the exact mass for about 4200 compounds, mostly pharmaceutical substances

Analysis with LC-MS

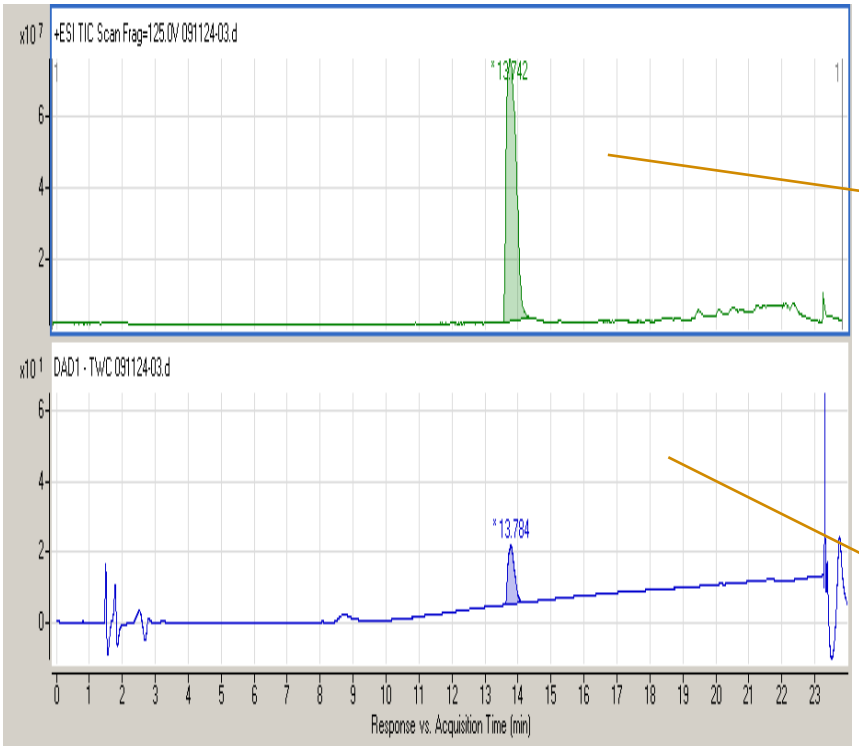
HPLC	1290 UHPLC system, Agilent Technologies
MS	6520 Q-TOF with dualESI ion source
Column	Zorbax Eclipse XDB-C18; 3.5 µm; 150x2.1 mm
Mobile phase	Gradient, 5-100% acetonitrile in 0.1% formic acid
Flow rate	0.25 ml/min
Column temp.	40°C
DAD-detector	210 – 500 nm
Data collection	0 – 25 min
Mass range,m/z	120 - 1100

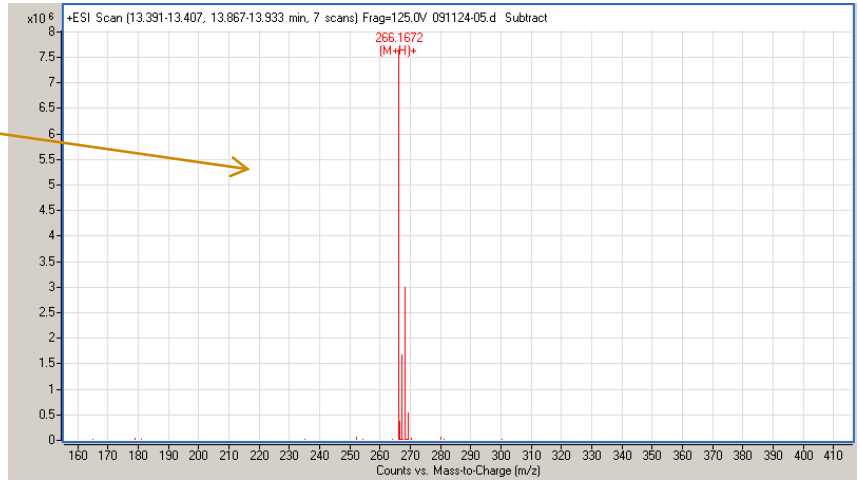
Slimming products

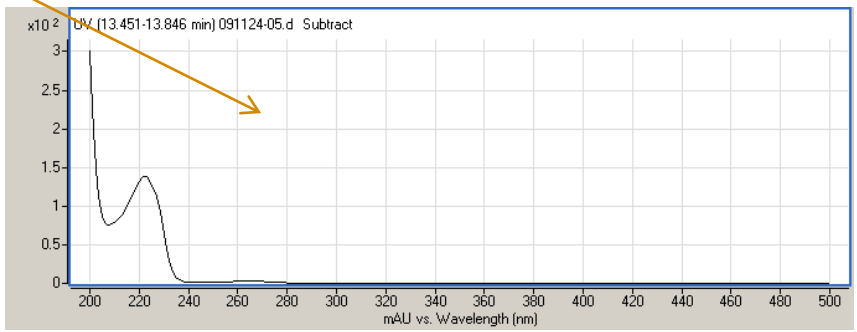




LiDa







LiDa

MS Formula Results: + Scan (13.391-13.407 ... min) Sub

m/z	Ion	Formula	Abundance
266.1672	(M+H)+	C16 H25 Cl N	7632477

Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match	DBE
☑	C16 H24 Cl N	C16 H25 Cl N	96.32		266.167	-0.58	99.81	88.82	98.36	5
☐	C13 H28 Cl N S	C13 H29 Cl N S	67.4		266.1704	12.06	43.21	80.8	99.69	0
☐	C14 H23 N3 S	C14 H24 N3 S	63.39		266.1685	5.35	84.76	0.18	96.5	5
☐	C17 H19 N3	C17 H20 N3	53.83		266.1652	-5.67	83.09	0.01	59.91	10
☐	C9 H23 N5 O2 S	C9 H24 N5 O2 S	49.96		266.1645	-9.96	56.4	0.16	96.85	1

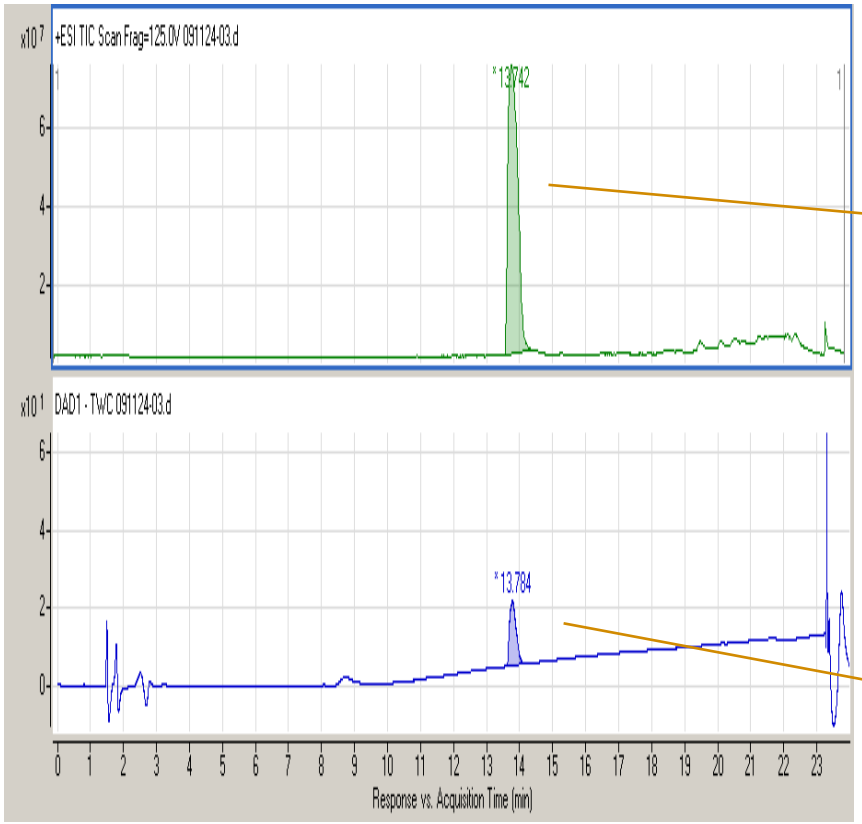
DB Search Results: + Scan (13.391-13.407 ... min) Sub

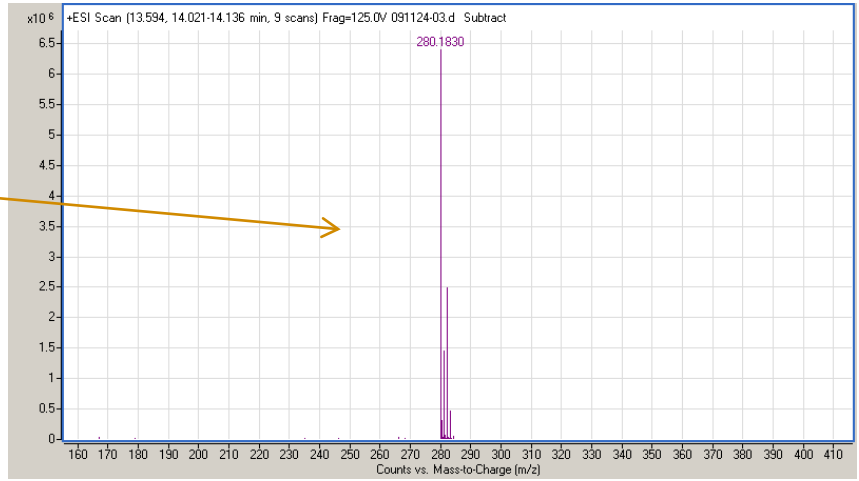
Database	m/z	Ion	Name	Ion Formula
LV_database...	266.1672	(M+H)+	Norsibutramine	C16 H25 Cl N

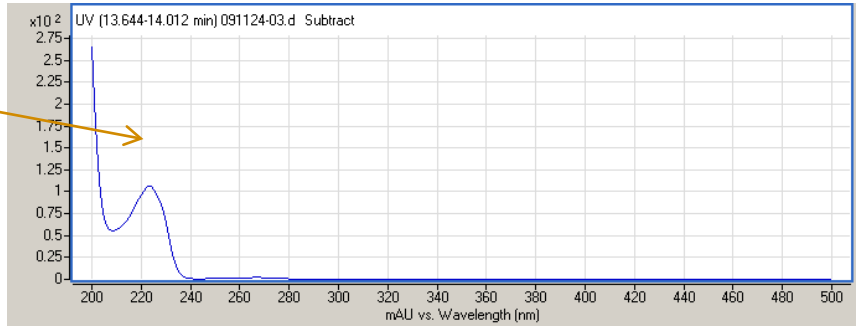
Best	Mass (DB)	Name	Formula	Score	Diff (ppm)	Mass Match	Abund Match	Spacing Match
☑	265.1597	Norsibutramine	C16 H24 Cl N	95.59	-0.75	99.67	87.88	96.67
☐	265.1579	Acridine, 3,6-bis(dim...	C17 H19 N3	48.47	-7.54	71.97	0.01	59.61
☐	265.1579	Mirtazapine	C17 H19 N3	48.47	-7.54	71.97	0.01	59.61
☐	265.1579	Antazoline	C17 H19 N3	48.47	-7.54	71.97	0.01	59.61

LiDa was found to contain 25 mg norsibutramine/capsule

Botanical slimming







Botanical slimming was found to contain 18 mg sibutramine/capsule

Nuclear magnetic resonance (NMR) spectroscopy

NMR instrument

- 300 MHz
- 600 MHz

Data base with 800 compounds:

- active pharmaceutical ingredients (API)
- excipient
- (residual) solvents

Analysis with NMR to verify identity

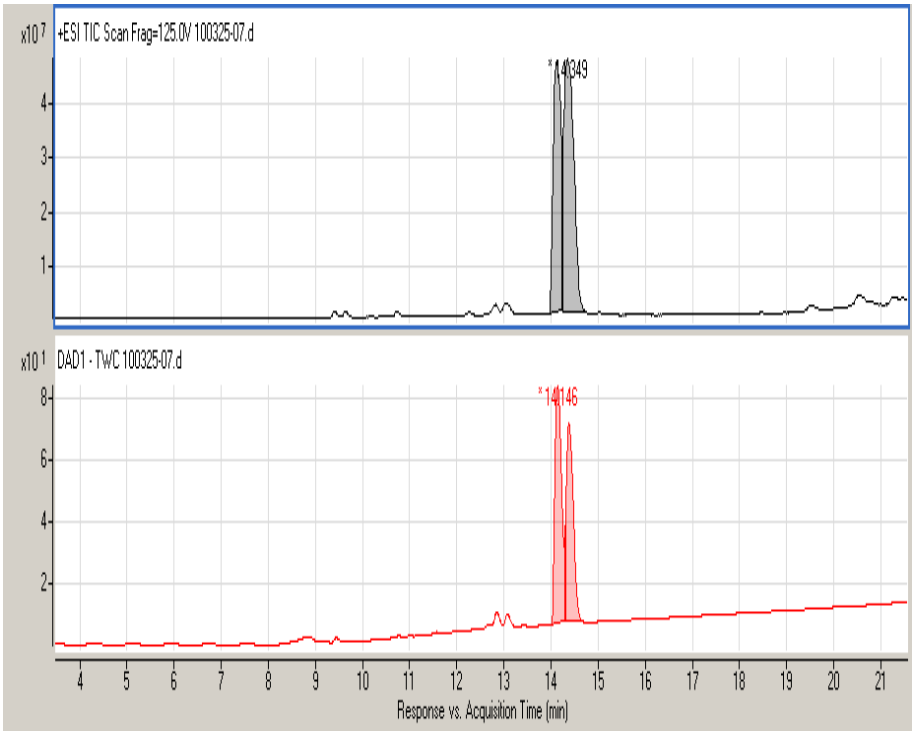
Sample preparation
Grind tablets or mix contents from 6 capsules

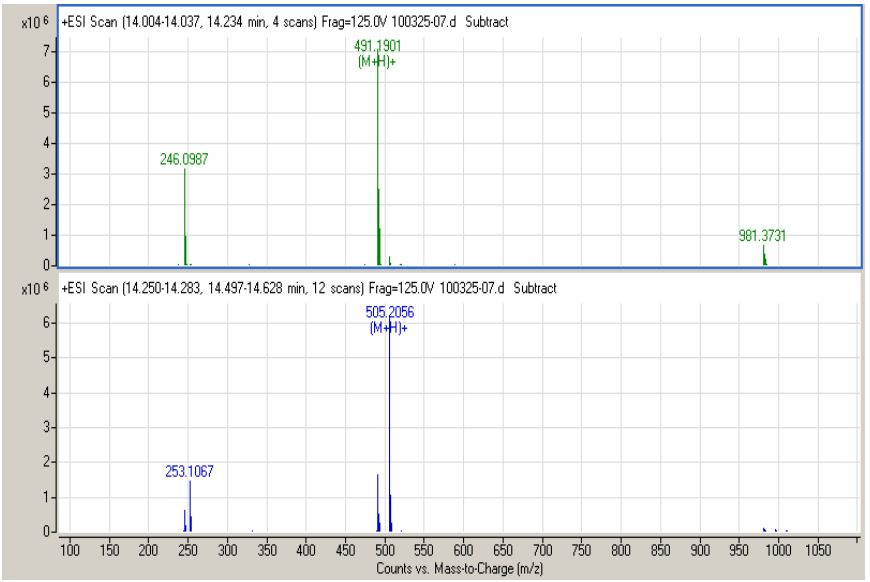
Mix
100 mg sample + 0.8 ml deuterated DMSO or acidic MeOH

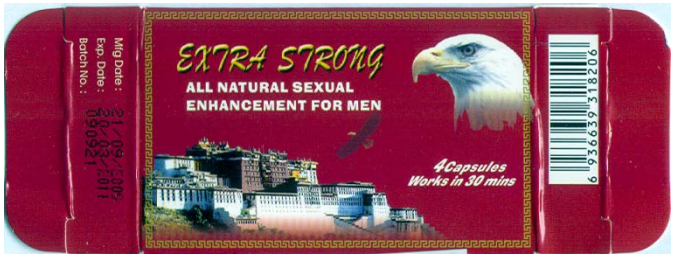
Extract for 15 minutes and **Centrifuge**

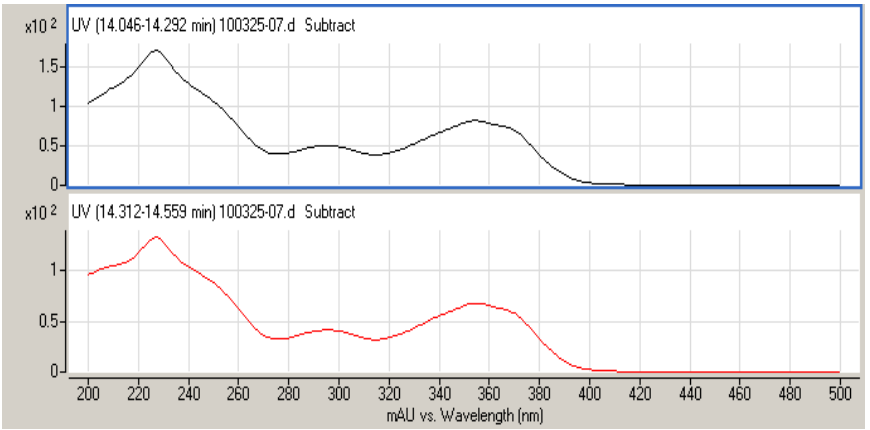
Analysis using a 600 MHz NMR-spectrometer. ^{1}H and ^{13}C NMR -spectra

Extra Strong, LC-MS

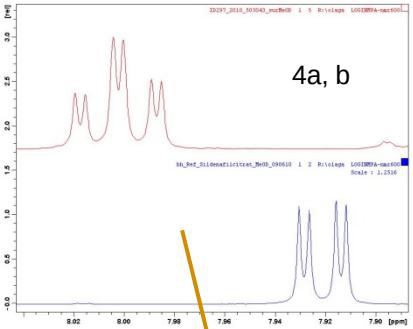


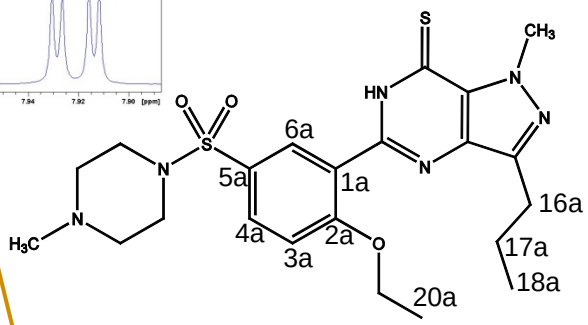




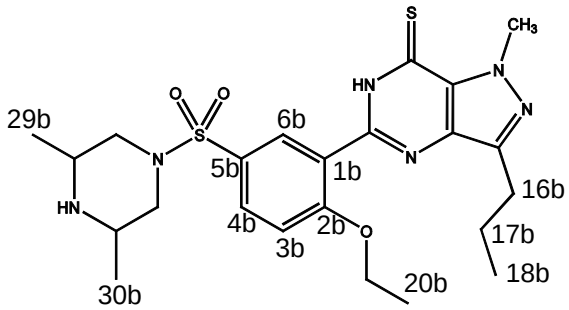


Extra Strong, NMR



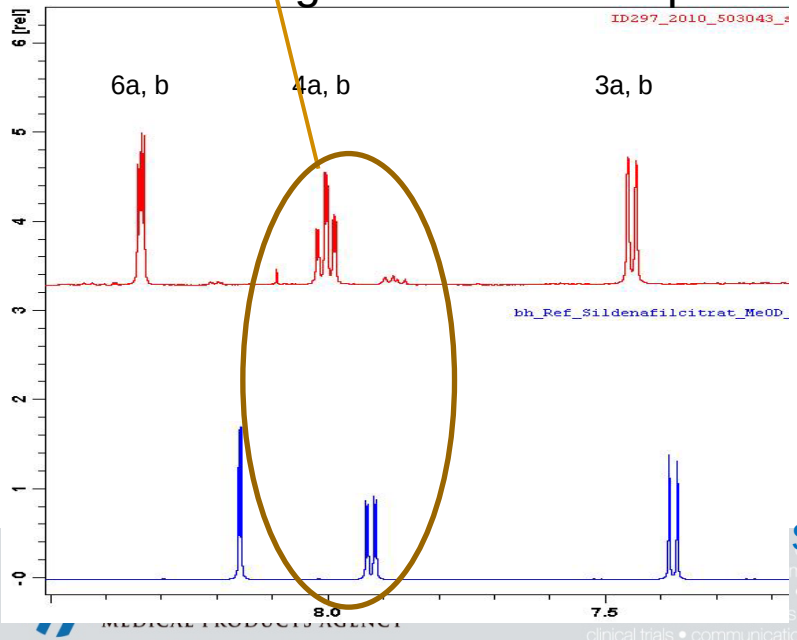


Thiosildenafil



Dimethylthiosildenafil

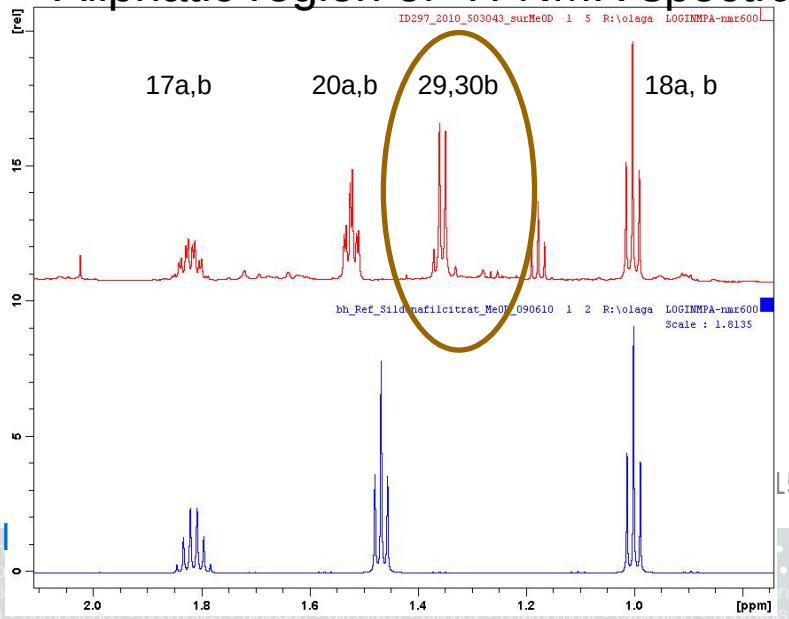
Aromatic region of ^{1}H-NMR spectra



Sample

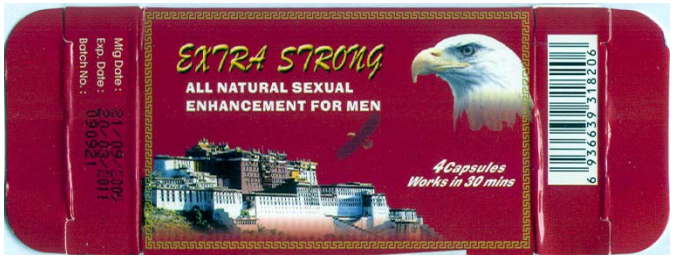
Sildenafil

Aliphatic region of ^{1}H-NMR spectra



Quantitative NMR

- **Extra Strong contained 23 mg tiosildenafil and 29 mg dimethyltiosildenafil per capsule**



Assay by quantitative NMR

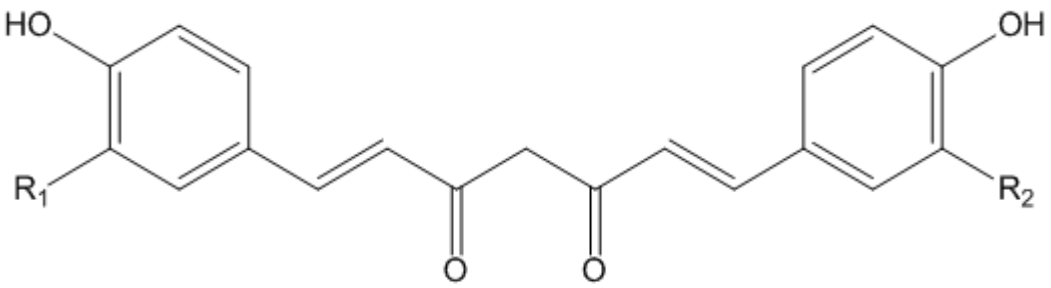
- **Quick - Answer in half a day**
- **General - No need for ”specific” internal standard**
- **Reliable - Comparable to GC, HPLC, titration**
- **Selective**

Hepatic adverse effects?

Fortodol

Declaration of contents:

- Curcuma longa (extract 20:1) 400 mg
- dl-phenylalanine 50 mg



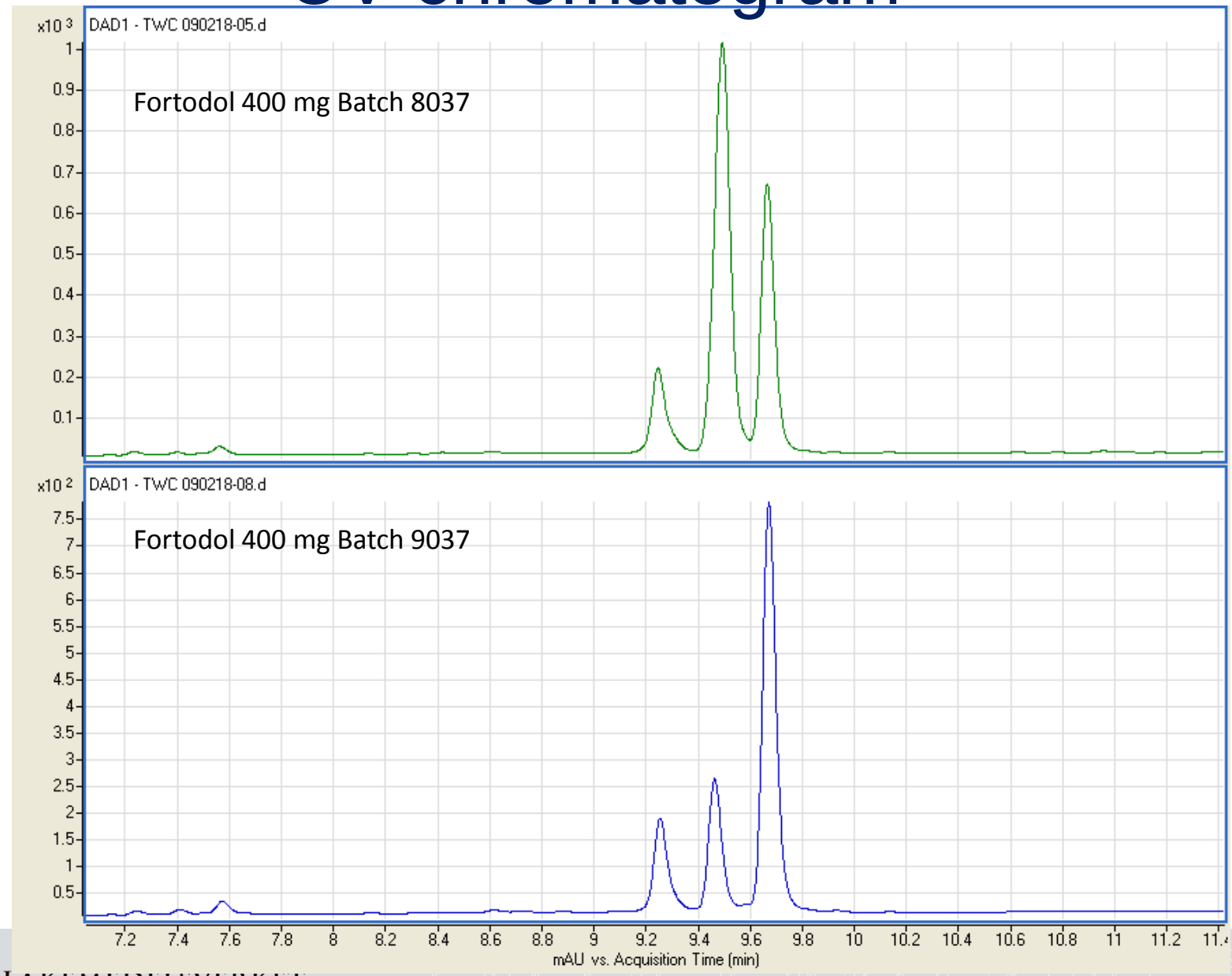
R_1 and R_2 = H and/or OCH_3

Fortodol

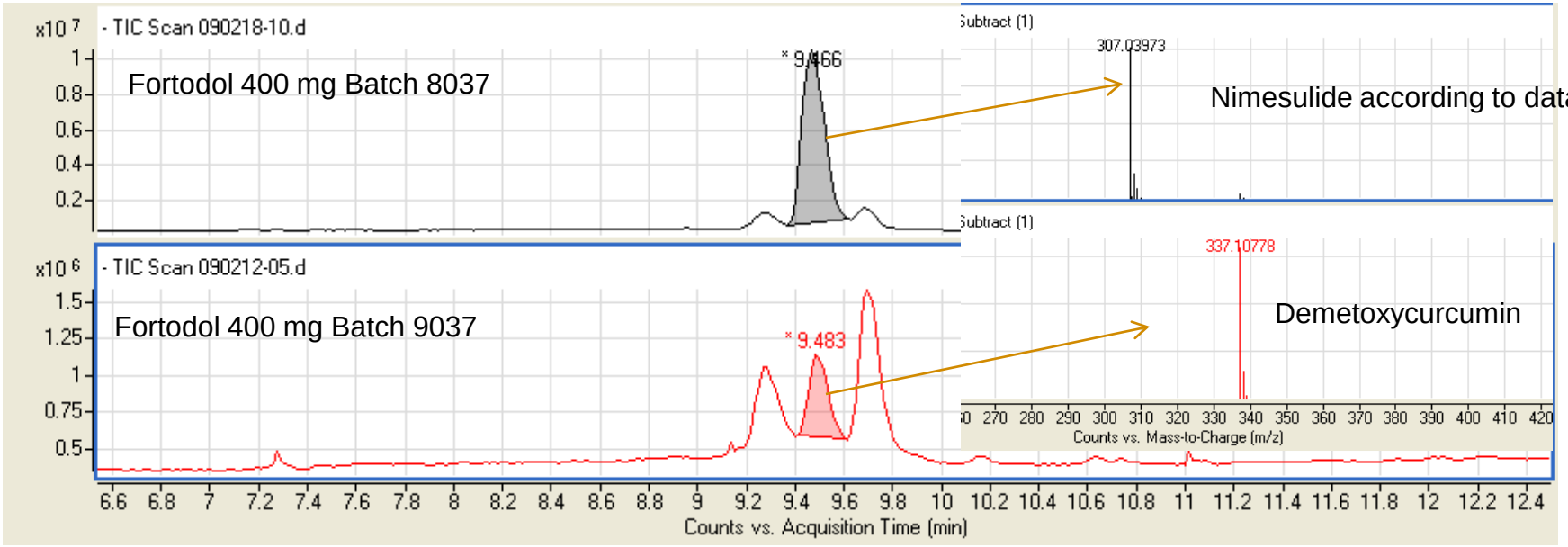
MPA laboratory performed first analyses based on the following hypotheses

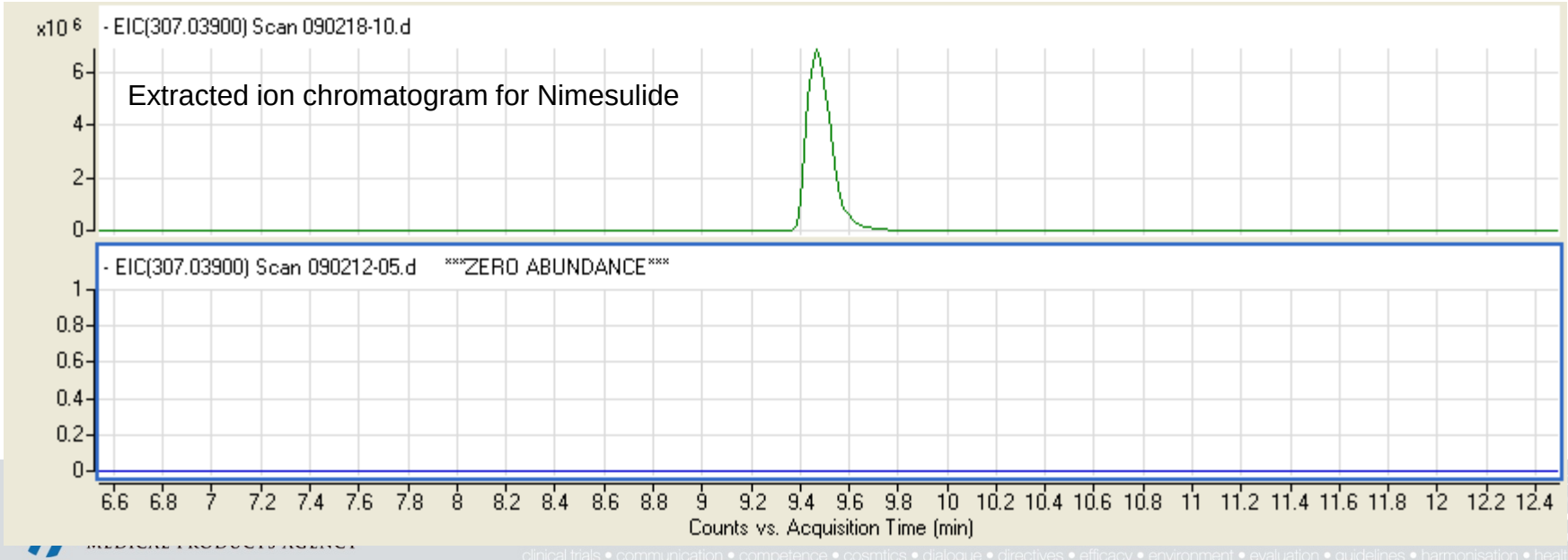
- Adulteration with antiinflammatory/analgesic substance?
- Contamination with aflatoxins, pesticides?
- Solvent giving toxic effects?
- High content of curcumin in concentrated extract of *Curcuma longa* (turmeric)?

UV-chromatogram

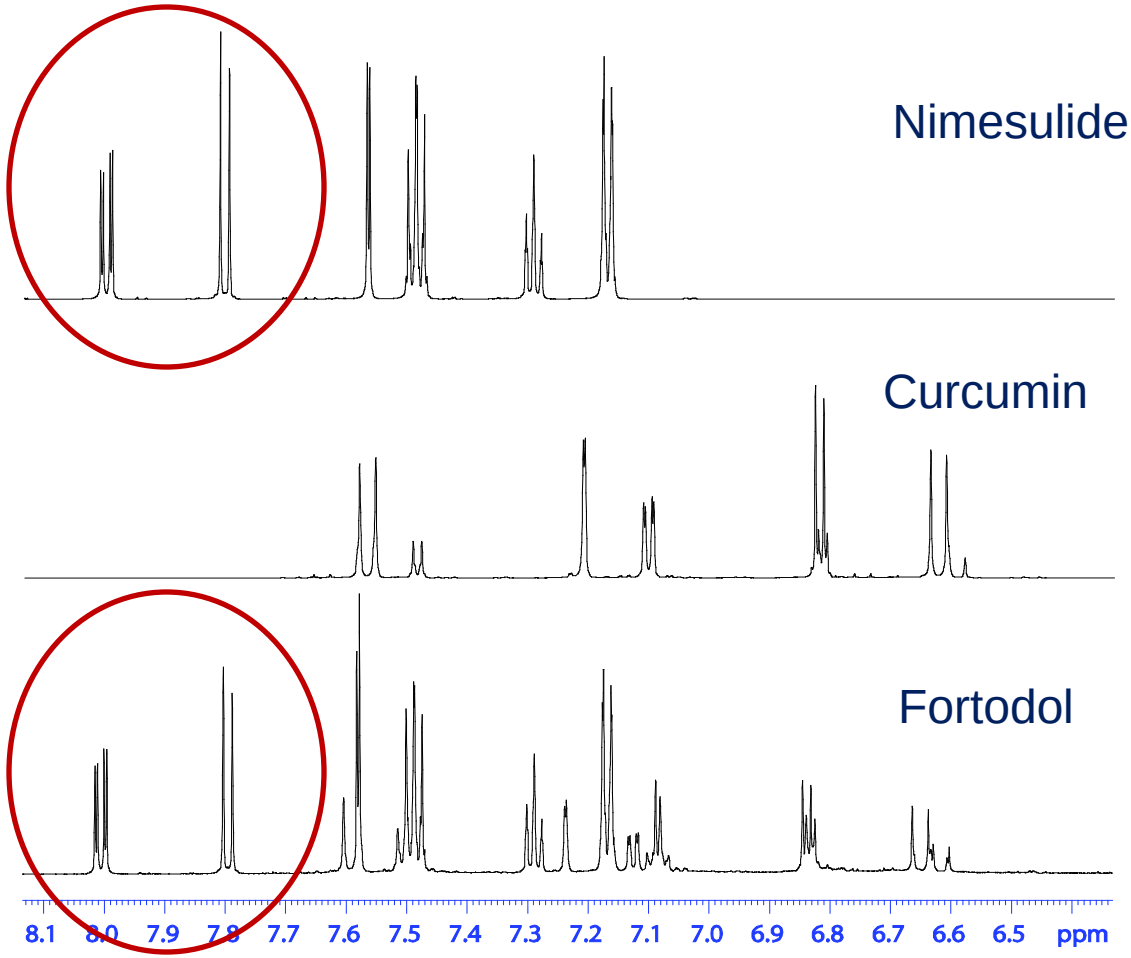


Electrospray, negative ionisation





Analysis with NMR confirm identity



Arlanda, Sweden, Pangea IV, 20-27 Sept. 2011

Pangea IV, Sweden

- **5307 gram powder**
- **42 432 tablets**
- **150 syringes and injection needles**
- **11 vials**
- **550 tubes with cream**
- **323 capsules**
- **400 wet potency tissues**

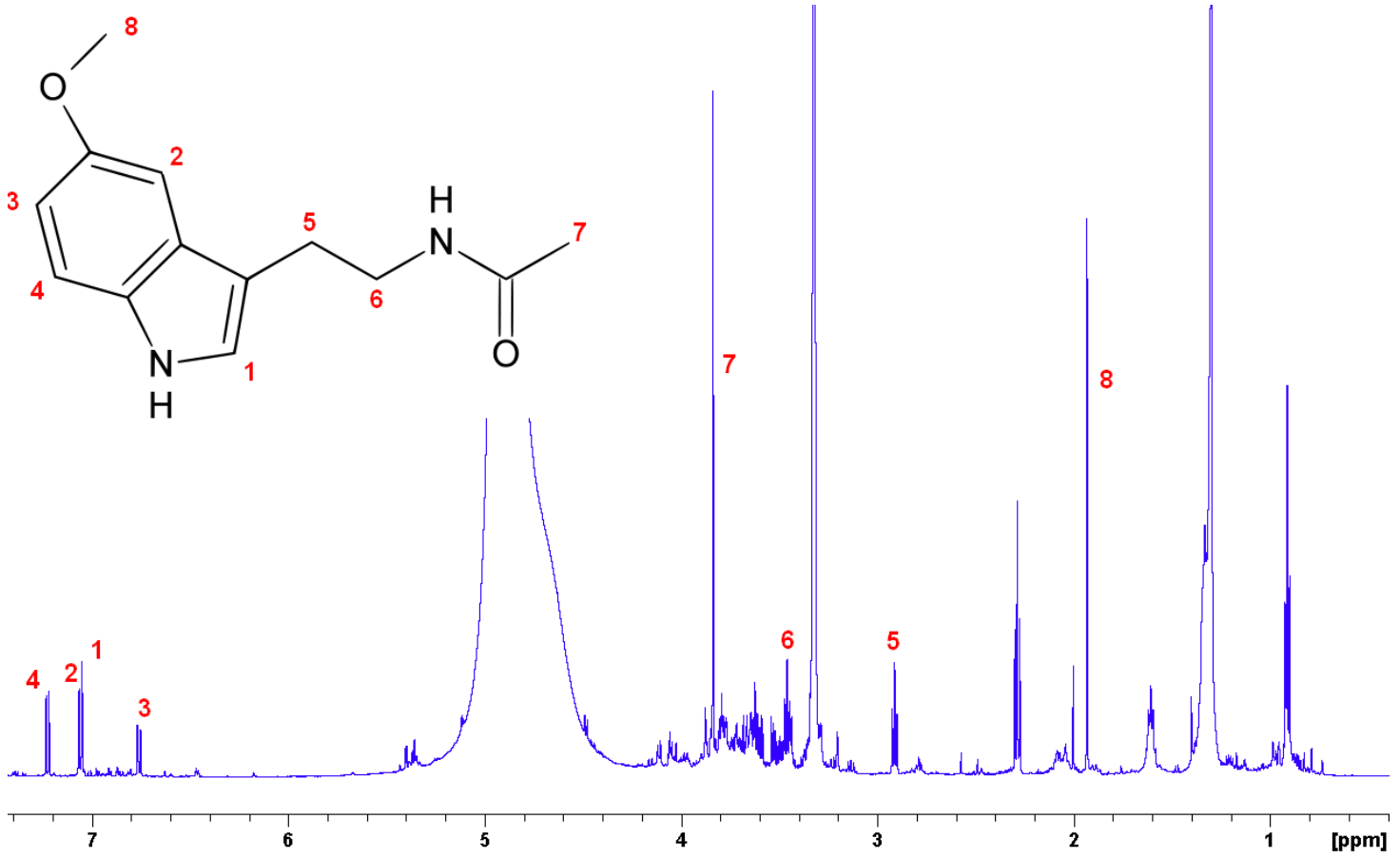
P57 Hoodia

Sibutramine, 8.7 mg in tablets from internet

“Breast enlargement"

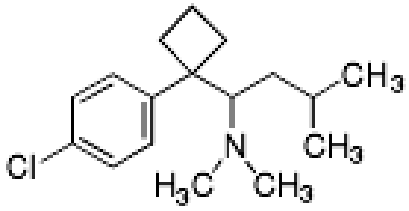
31 jars x 60 tablets

The tablets contained 1 mg melatonin



Melatonin found in "breast enlargement" tablets
Proton NMR spectrum (also confirmed with LC-QTOF-MS)

100% pure nature – with undeclared sibutramine



Sibutramine

Sibutramine found 16 mg in each capsule

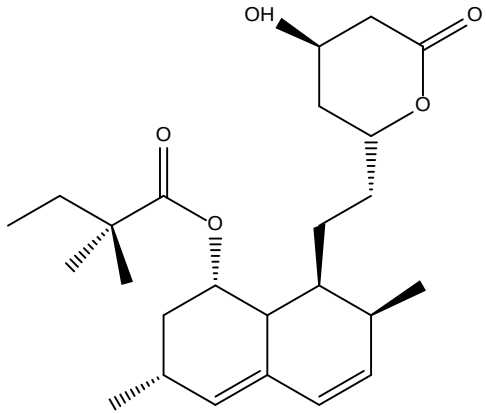
Pangea IV, global outcome

- **81 countries participated**
- **13 000 website closed down**
- **606 auction sites removed**
- **500 website under investigation**
- **Postal hubs**
  - 66 505 packages inspected
  - 8 695 packages seized
- **55 individuals arrested/under investigation**
- **2.5 million units seized to a value of 6.8 million USD**

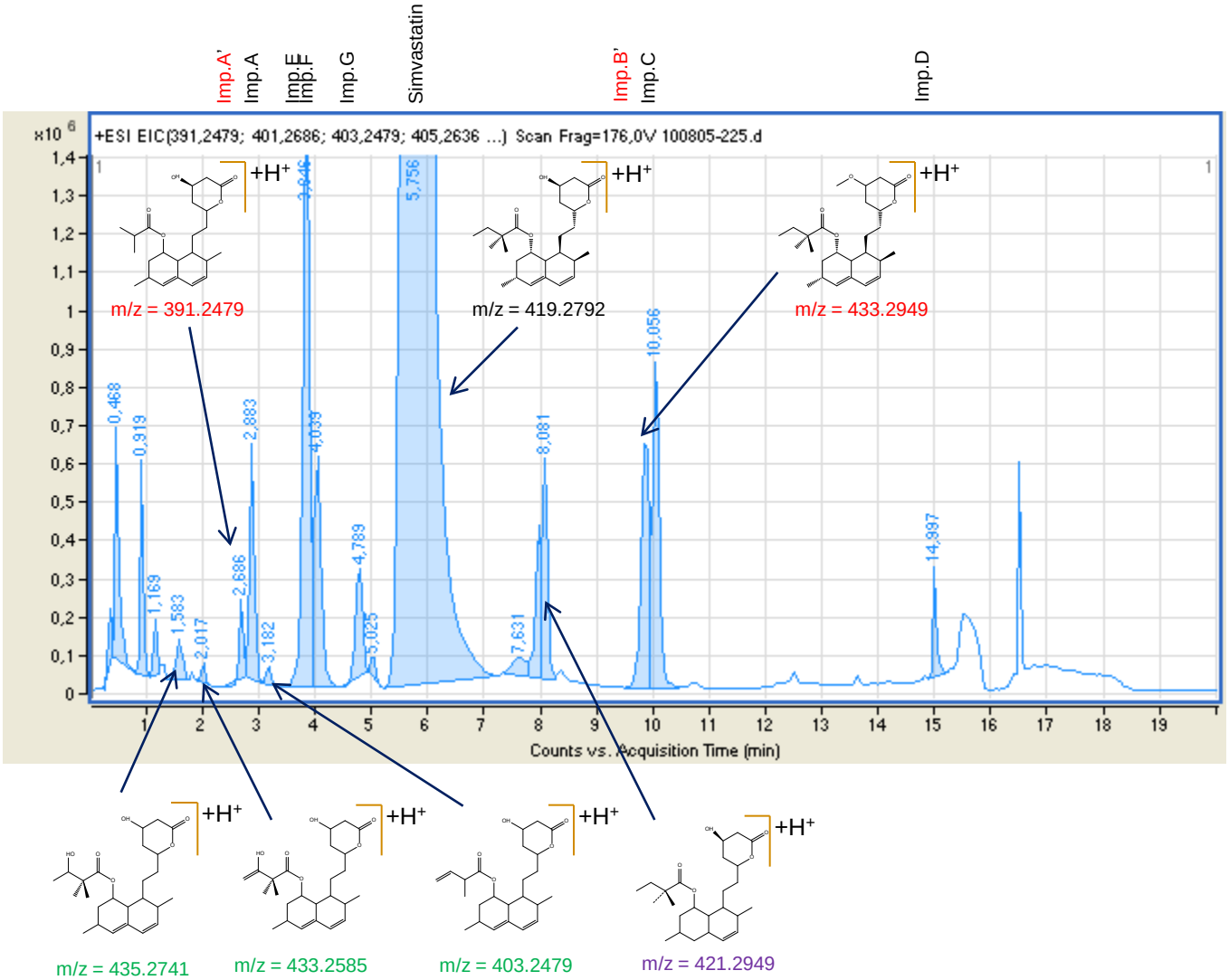
Is it possible to trace suspected illegal API?

Study outline:

- Fingerprinting of API using LC-MS/MS impurity profile
- Separation and detection of Simvastain related impurities using modern LC technology (UPLC-like conditions)
- Highly selective and sensitive MS-QTOF detection
- Chemometric models to interpret data



LC-MS optimization



Selectivity – sensitivity - validation

- **LOQ simvastatin 8 ppm**
- **About 40 times lower as compared to UV detection**
- **Intraday precision at typical impurity level was about 4-6%**
- **Several new impurities (<< 0.1%) were identified**

Dataset study

39 APIs originated from 9 different providers

21 Finished Products coming from 14 different manufacturers

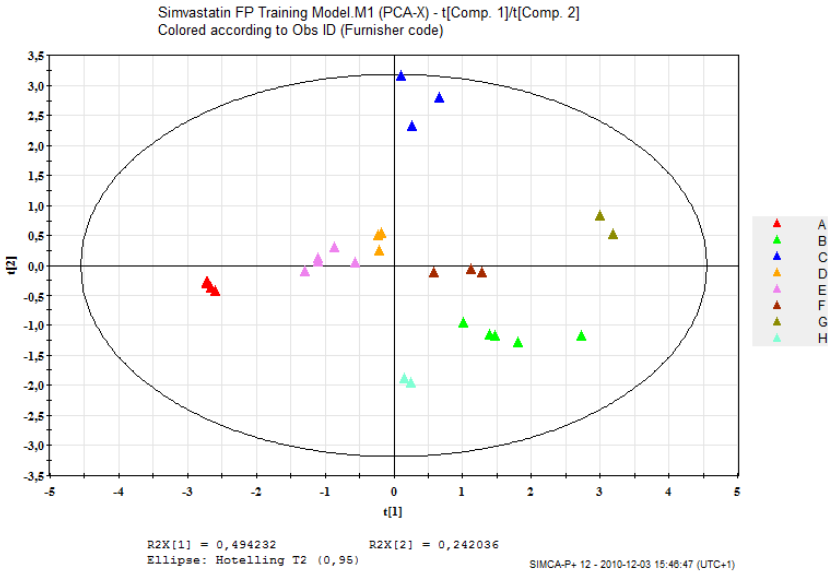
LC/MS impurity fingerprinting of 15 impurities related to Simvastatin.

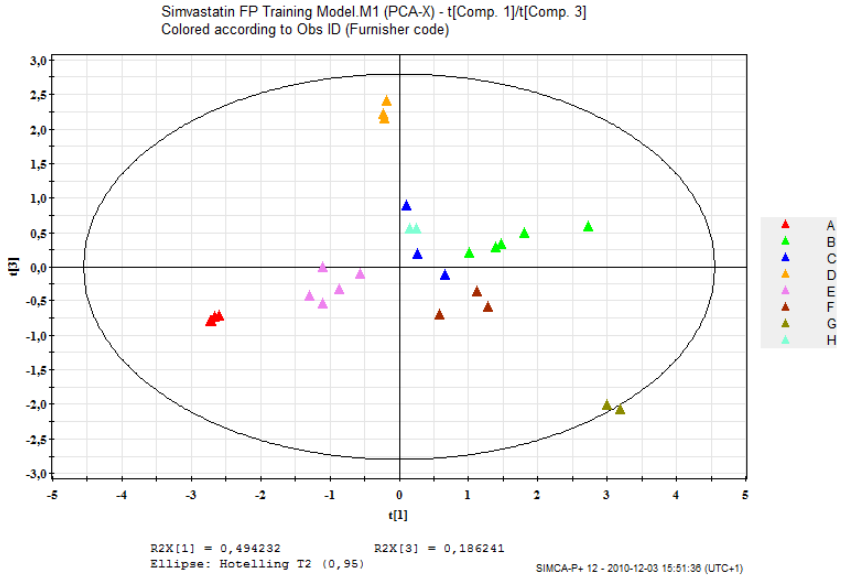
Data mining

6-variables PCA training model

Built on relative areas of 6 impurities:

- m/z = 403.2479
- Impurity E
- Impurity F
- Impurity G
- Impurity B'
- Impurity B

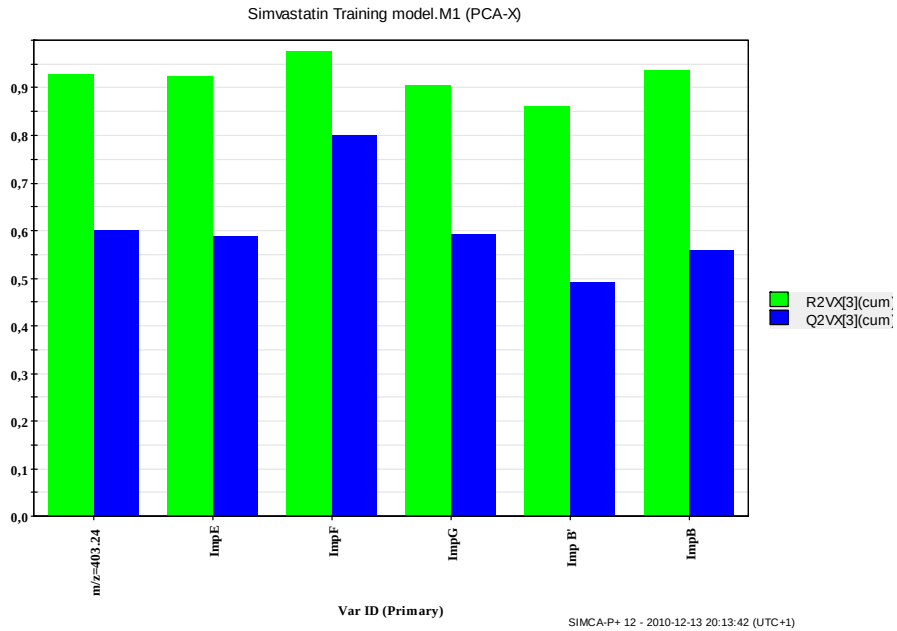




Explained cumulative Variation: 92.2%

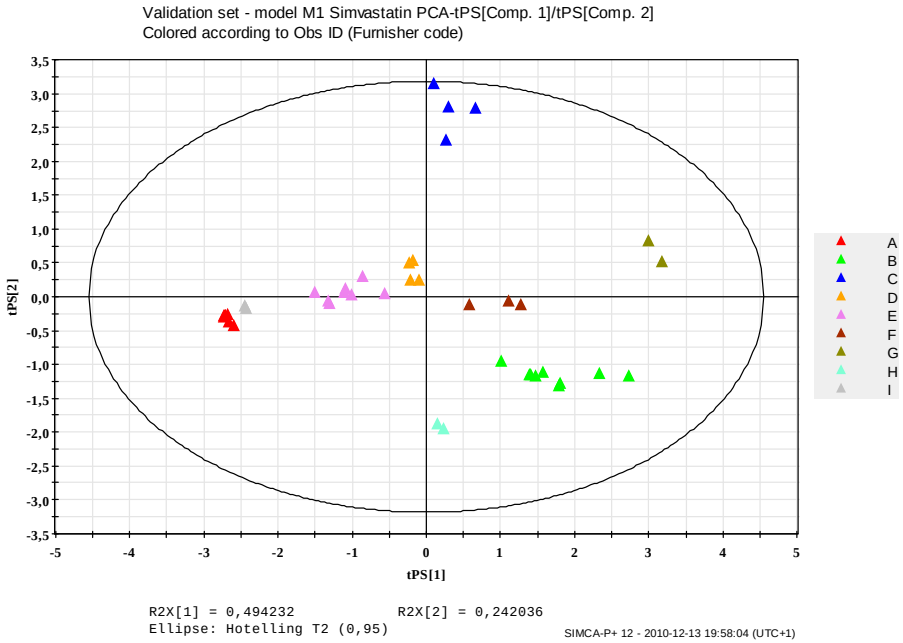
Validation

Cross validation



Very good explained variation R2 e 0.9
Good predicted variation Q2 e R2 – 0.3

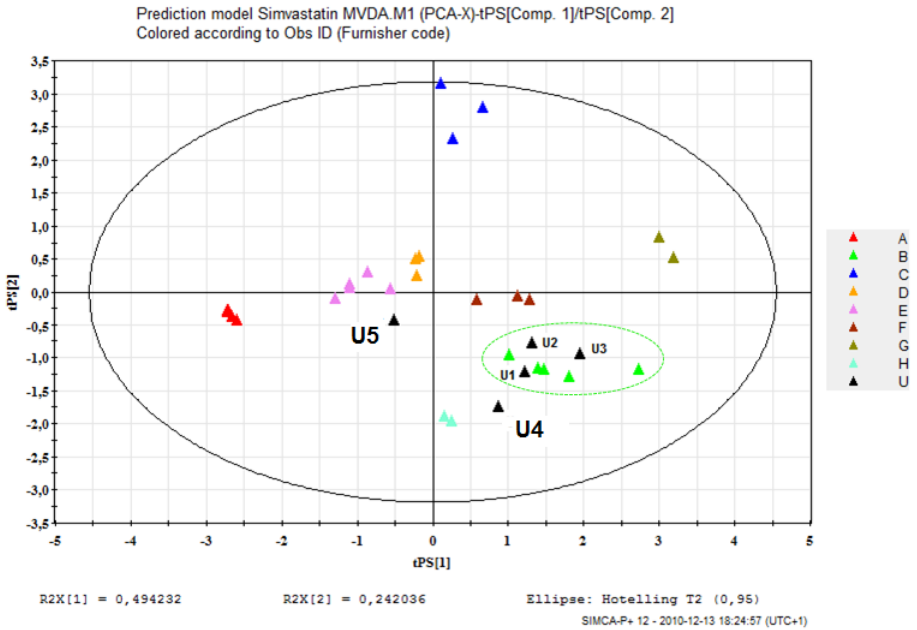
External Validation Test

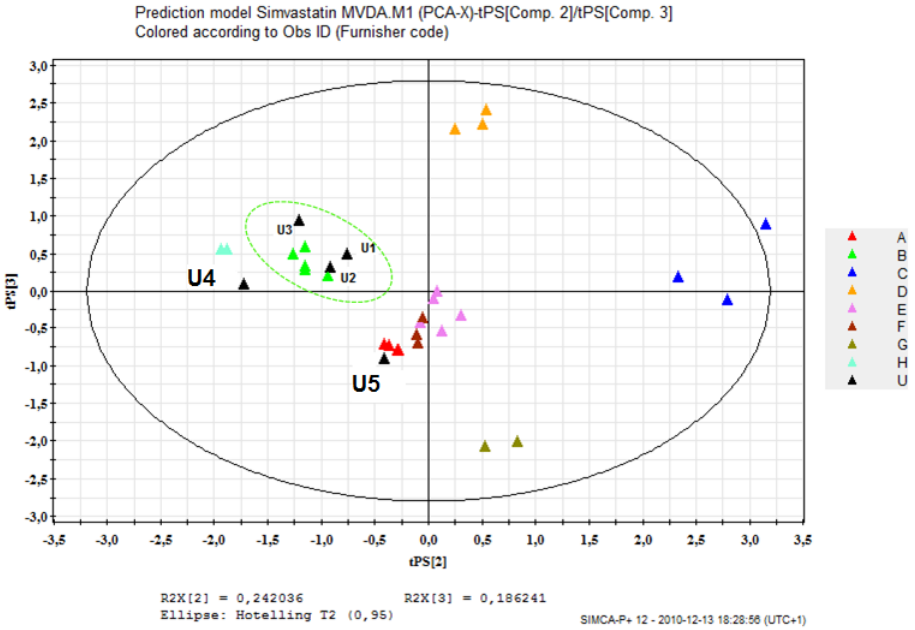


All validation samples (16) fit to their respective groups

Application of the PCA model

Application to 5 unknown test samples





Samples U1, U2 and U3: Furnisher B
Samples U4 and U5: unknown Furnishers

The Swedish OMCL have developed an analytical strategy for testing of suspected counterfeit medicines

- Screening with LC-MS for identification

  - retention time, UV-spectra, exact mass and MS-MS data

- Analysis with NMR

  -verify identity and structure elucidation possible

- Assay by quantitative NMR, (or by LC-MS or by HPLC)
- Preliminary studies also show that it may be possible to trace illegal API both as substances and in finished products