RÄTTSMEDICINALVERKET
NATIONAL BOARD OF FORENSIC MEDICINE

Screening and confirmation strategies in postmortem toxicology

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VIRTUAL/ONLINE SYMPOSIUM:
CURRENT TRENDS IN FORENSIC TOXICOLOGY
MAY 22—24, 2018

Who am I?

- Graduated 1989 in analytical chemistry
- Began forensic toxicology 1990
- PhD in human toxicology 2001
- Docent in forensic toxicology 2007
- Professor in forensic toxicology 2017

These are the learning objectives

- recognize the pros and cons of different techniques and methodologies
- evaluate and select appropriate methodology for the analysis of drugs in post-mortem cases
- design strategies for successful screening and confirmation

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Screening strategies

Immuno assay platforms

- Homogenous assays
  - EMIT, CEDIA …
- Heterogenous assays
  - RIA, ELISA …

Chromatographic platforms

- GC-NPD
- LC-UV
- GC-MS
- LC-MS/MS
- LC-TOF
- LC-QTOF

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Confirmation strategies

Explaining the immuno assay

- Covers the analytes that cross-react with the immuno assay

Pharmacology based

- Compounds with similar effects
  - Opioids
  - Sedatives

Chemistry based

- Compounds with similar properties
  - Extraction
  - Chromatography
  - Detection

Prevalence based

- Compounds that occur together or that are prevalent
  - Amphetamine and carboxy-THC urine

Qual or Quant?

- Case
  - Homocide, Suicide, Accident, *Intox*
- Matrix
  - Brain, blood, urine, liver, hair
- Compound
  - Markers, medications, DoA, NPS

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Debate

Journal of Analytical Toxicology, 2016;40:318–320
doi: 10.1093/jat/bkw013
Advance Access Publication Date: 13 March 2016
Letter to the Editor

OXFORD

Letter to the Editor

To Measure or Not to Measure? That is the NPS Question

Dimitri Gerostamoulos[1,2,*], Simon Elliott[3], H. Chip Walls[4], Frank T. Peters[5], Matthew Lynch[1,2], and Olaf H. Drummer[1,2]

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Qualitative confirmation

Method characteristics

• Simple preparation	✓ LLE
• Short run time	✓ < 10 min
• High sensitivity	✓ Sub-ng LOQ
• High selectivity	✓ Q-TOF

Sample preparation

- 1.0 g blood + 500 µL 1M TRIS-buffer, pH 10.2
- 3 mL diethylether
- Extract and centrifuge
- Freeze (-80ºC) for 15 minutes, decant
- Evaporate (40ºC, N2)
- Reconstitute in 100 µL methanol
- Inject 5 µL

Chromatography

- Agilent 1290 Infinity + Agilent 6550 Q-TOF
- YMC-Triart C18, 2.0 x 50 mm, 1.9 µm (C18)
- Mobile phase A 0.05 % HFo in 10 mM ammonium formate
- Mobile phase B Methanol
- 0.75 mL/min, gradient chromatography (60°C) 8.5 min/spl

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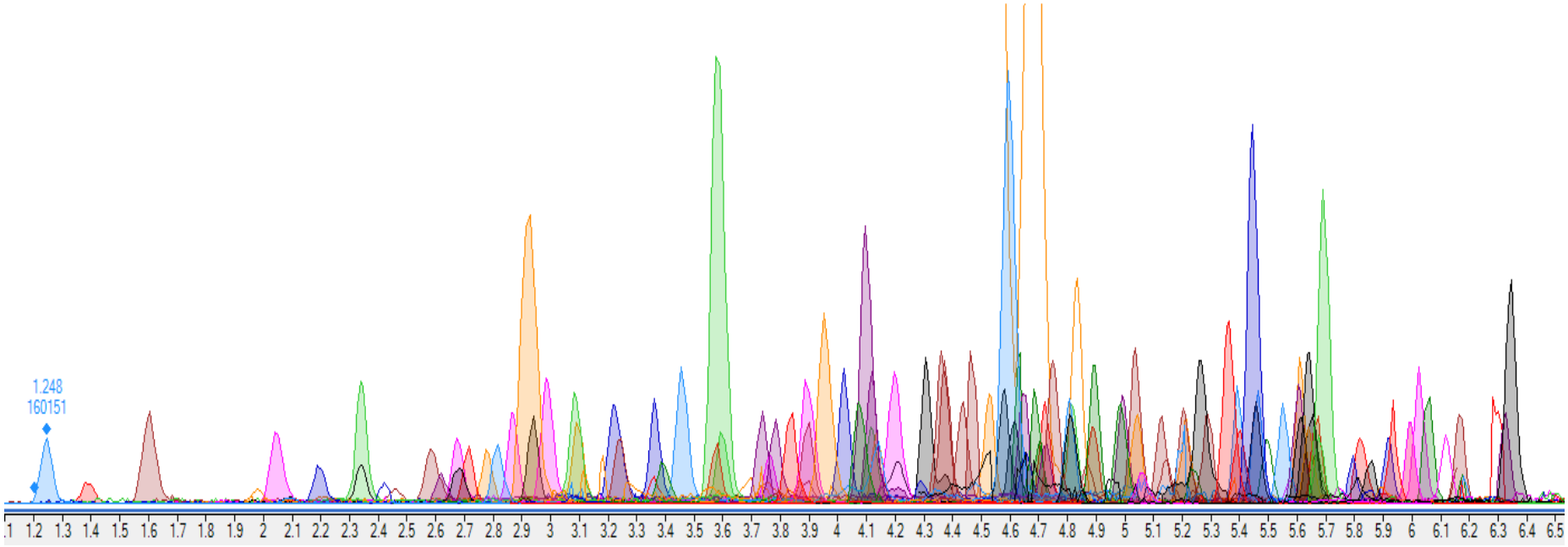
Chromatography



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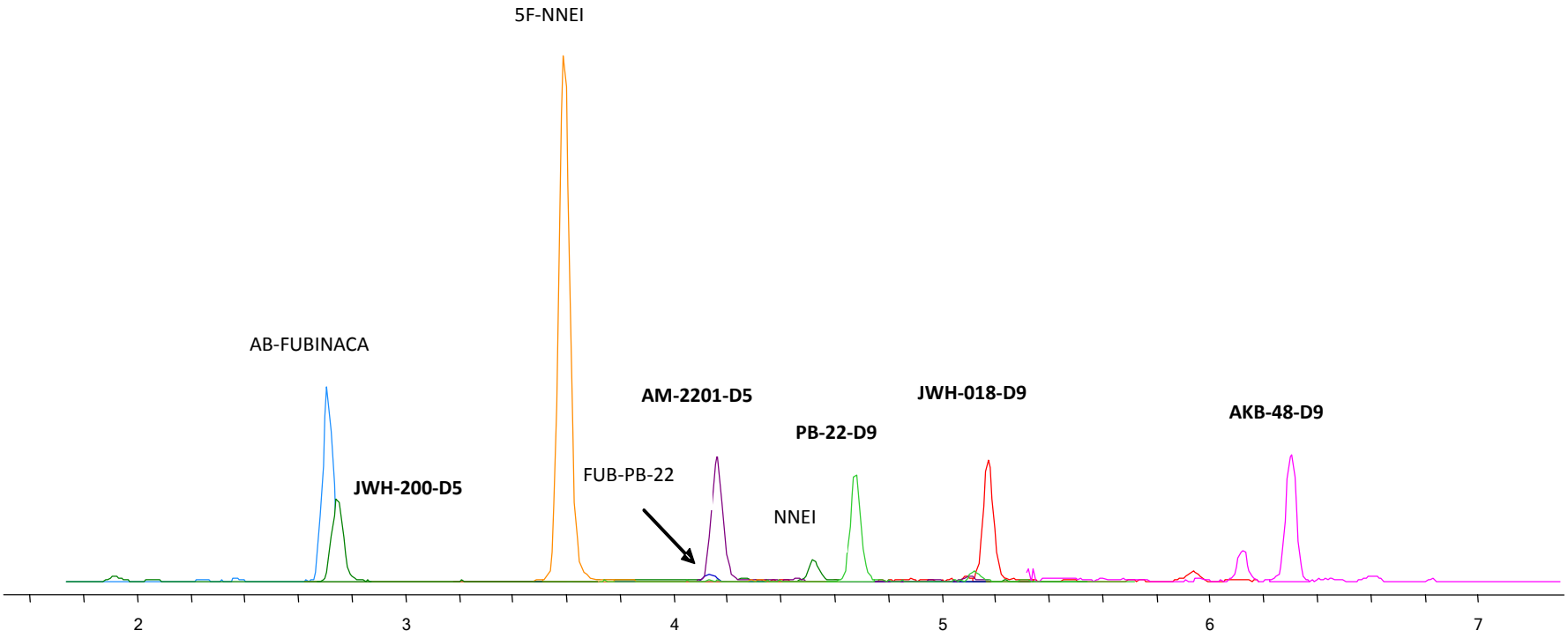
Case example



Acquisition strategy

- All MS data acquired
- MS/MS data from a list of m/z
  - Preferred list containing acq parameters

Single Search | Batch Search | Batch Summary | Edit Compounds | Spectral Search | Browse Spectra | Edit Spectra

Mass

Precursor ion:

Tolerance: 200 ppm mDa

Ion polarity: (Any)

Ionization mode: (Any)

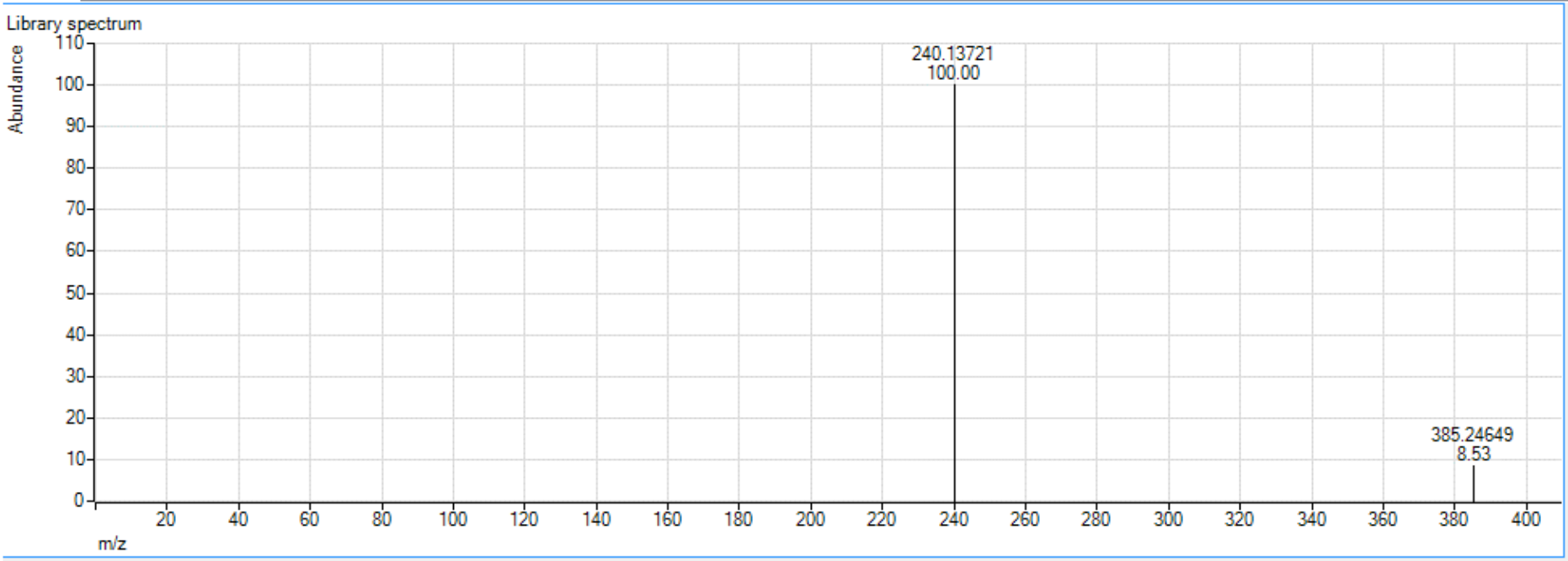
Collision energy

Tolerance: 2.0 eV

Spectra for compound: MMB-CHMINACA

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument
MMB-CHMINACA	(M+H)+	385.24860	10	Positive	ESI	QTOF
MMB-CHMINACA	(M+H)+	385.24860	20	Positive	ESI	QTOF
MMB-CHMINACA	(M+H)+	385.24860	40	Positive	ESI	QTOF

Graphic | Mass List



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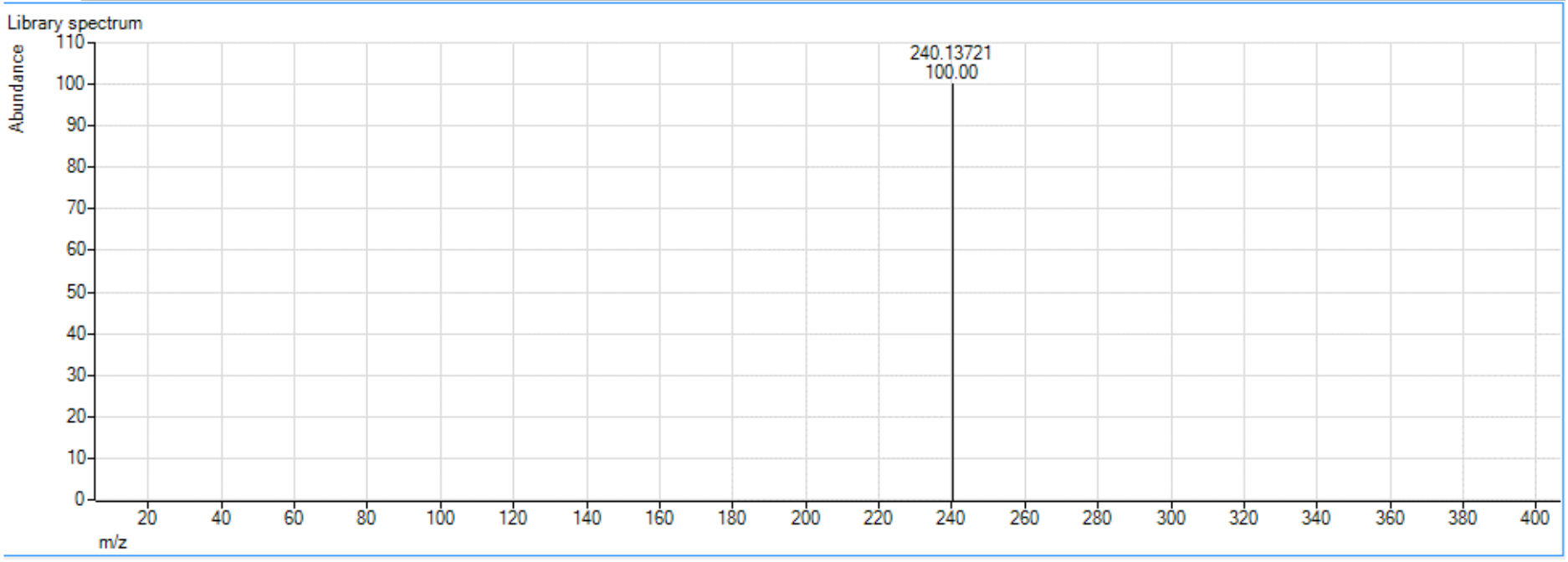

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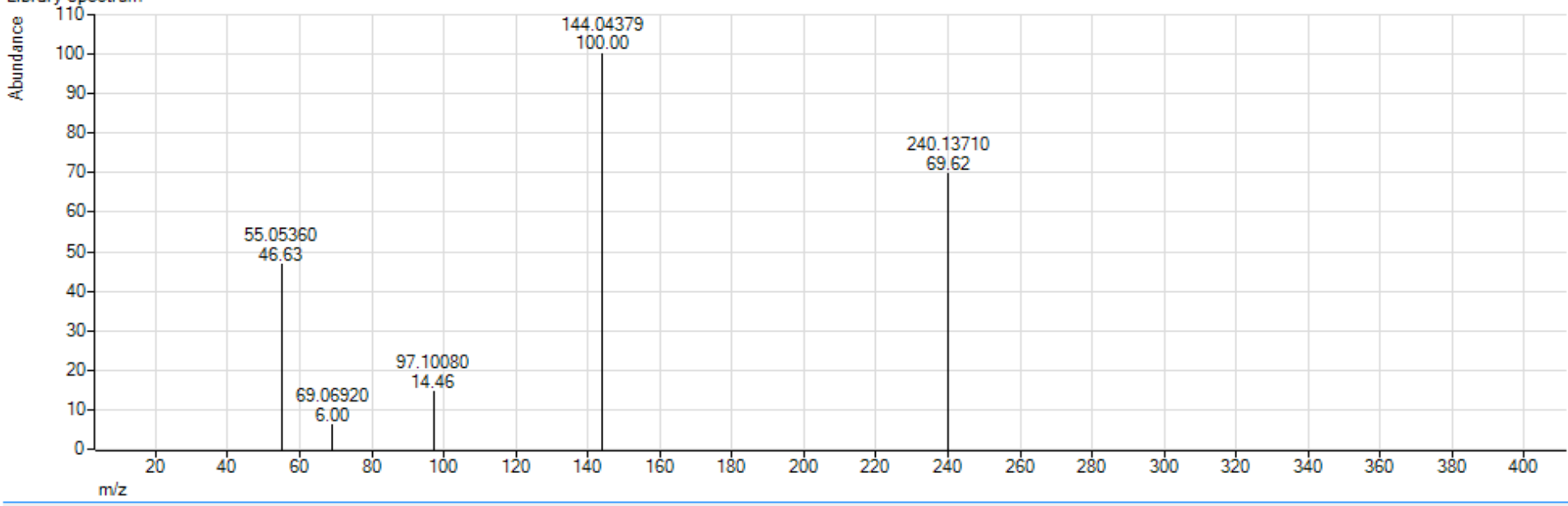
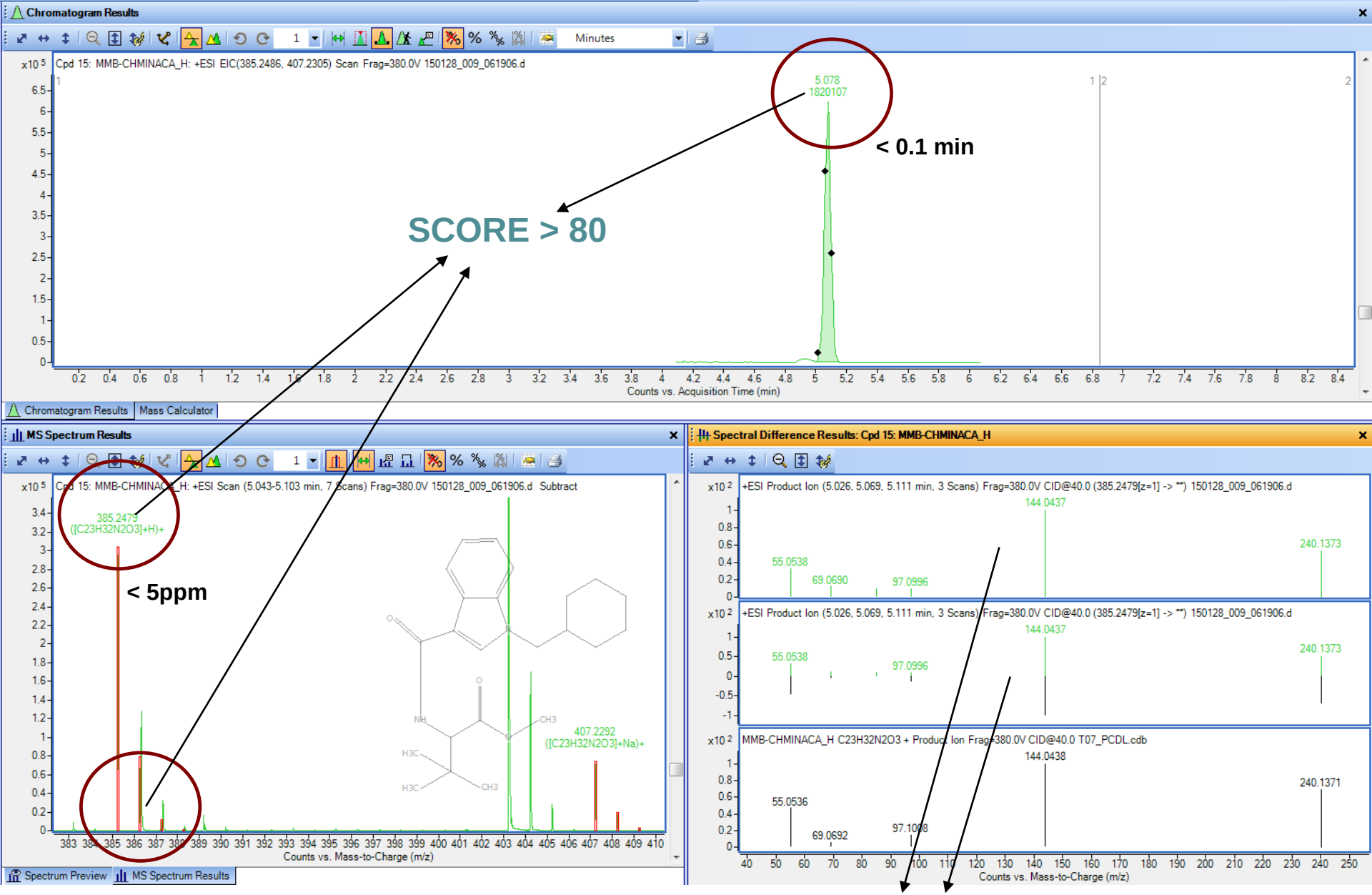
Graphic | Mass List

Library spectrum




Chromatogram Results
Cpd 15: MMB-CHMINACA_H: +ESI EIC(385.2486, 407.2305) Scan Frag=380.0V 150128_009_061906.d
5.078
1820107
< 0.1 min
SCORE > 80
Counts vs. Acquisition Time (min)
Chromatogram Results
Mass Calculator
MS Spectrum Results
Cpd 15: MMB-CHMINACA_H: +ESI Scan (5.043-5.103 min, 7 Scans) Frag=380.0V 150128_009_061906.d Subtract
385.2479
([C23H32N2O3]+H)+
< 5ppm
407.2292
([C23H32N2O3]+Na)+
Counts vs. Mass-to-Charge (m/z)
Spectrum Preview
MS Spectrum Results
Spectral Difference Results: Cpd 15: MMB-CHMINACA_H
+ESI Product Ion (5.026, 5.069, 5.111 min, 3 Scans) Frag=380.0V CID@40.0 (385.2479[z=1] -> **) 150128_009_061906.d
144.0437
240.1373
55.0538
69.0690
97.0996
MMB-CHMINACA_H C23H32N2O3 + Product Ion Frag=380.0V CID@40.0 T07_PCDL.cdb
144.0438
240.1371
55.0536
69.0692
97.1008
Counts vs. Mass-to-Charge (m/z)
SCORE > 80

Establishing thresholds

- Negative donor blood (N=5)
  - 200 pg/g
  - 100 pg/g
  - 50 pg/g
- Parameters
  - Retention time, Mass accuracy, Area, MS-score, MS/MS-score

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Cases first 9 months (%-pos)

• Petty drug offences	• 773	(19%)
• DUID	• 924	(12%)
• Post mortem	• 211	(13%)
• Violent crimes	• 44	(5%)
• Criminal justice	• 32	(66%)
• Other	• 18	(6%)

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Findings first 9 months

AB-FUBINACA	146
MMB-CHMINACA	93
THJ-018	47
AB-CHMINACA	30
FUB-AKB-48	20
5F-PB-22	20
AB-PINACA	16
5F-AKB-48	16
FUBIMINA	15

And 22 other cannabinoids

Summary

- Sensitive
  - Thresholds below the *10-percentiles*
- Flexible
  - Succesfully updated

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Quantitative confirmation

Interpretation help

- Several matrices
  - Blood, urine, hair
- Several analytes
  - Parent compounds, metabolites, degradation products
    - Ratios in blood
    - Ratios in urine

Toxic or lethal concentrations?

Table 2
Comparison between poisonings and other causes of death: median (range) drug concentrations in blood in the age group of 14–44 years in Finland 2000–2008.

	Fatal poisonings	Other causes of death
Codeine, mg/l	1.4 ↑↑ (0.02–34)	0.09 (0.02–6.9)
Morphine, mg/l	0.07 (0.02–2.5)	0.07 (0.02–1.6)
Codeine/morphine	22.5 ↑↑ (0.5–850)	5.9 (0.3–275)
Tramadol, mg/l	5.3 ↑↑ (0.3–140)	0.6 (0.1–25)
O-desmethyltramadol, mg/l	0.8 ↑↑ (0–4.9)	0.2 (0–3.9)
Tramadol/*O*-desmethyltramadol	7.7 (2.0–300)	6.4 (1.1–47)
Methadone, mg/l	0.35 (0.07–2.0)	0.30 (0.06–1.9)
Buprenorphine, μg/l[a]	1.4 ↑ (0.2–100)	1.2 (0.002–74)
Norbuprenorphine, μg/l[a]	0.8 ↓ (0.2–89)	1.3 (0.1–200)
Buprenorphine/norbuprenorphine[a]	1.8 ↑↑ (0.1–18)	0.86 (0.01–33)

↑ Significantly higher concentration, $p < 0.05$.
↑↑ Significantly higher concentration, $p < 0.001$.

Häkkinen et al. Forensic Science International 222 (2012) 327–331

Norbup/Bup ratio patients

Table IV. Urine Concentrations of Buprenorphine and Norbuprenorphine, Daily Dose, and Creatinine Concentrations from 16 Patients Under Ongoing Subutex Treatment for Heroin Dependence

Patient	BUP (µg/L)	NorBUP (µg/L)	Daily Dose (mg)	Creatinine (g/L)	BUP/ Creatinine (µg/g)	NorBUP/ Creatinine (µg/g)
1	36	48	1	0.6	60	80
2	40	142	6	0.5	80	284
3	174	690	8	1.7	102	406
4	543	1510	8	2.8	194	539
5	87	473	10	0.7	124	676
6	52	274	12	0.5	104	548
7	61	60	12	0.8	76	75
8	538	2050	12	2.4	224	854
9	72	559	12	0.9	80	621
10	222	611	14	1.6	139	382
11	537	1020	14	2.1	256	486
12	45	265	16	0.6	75	442
13	35	204	16	0.6	58	340
14	31	116	16	0.2	155	580
15	433	646	24	0.5	866	1290
16	1080	1700	32	1.5	720	1130

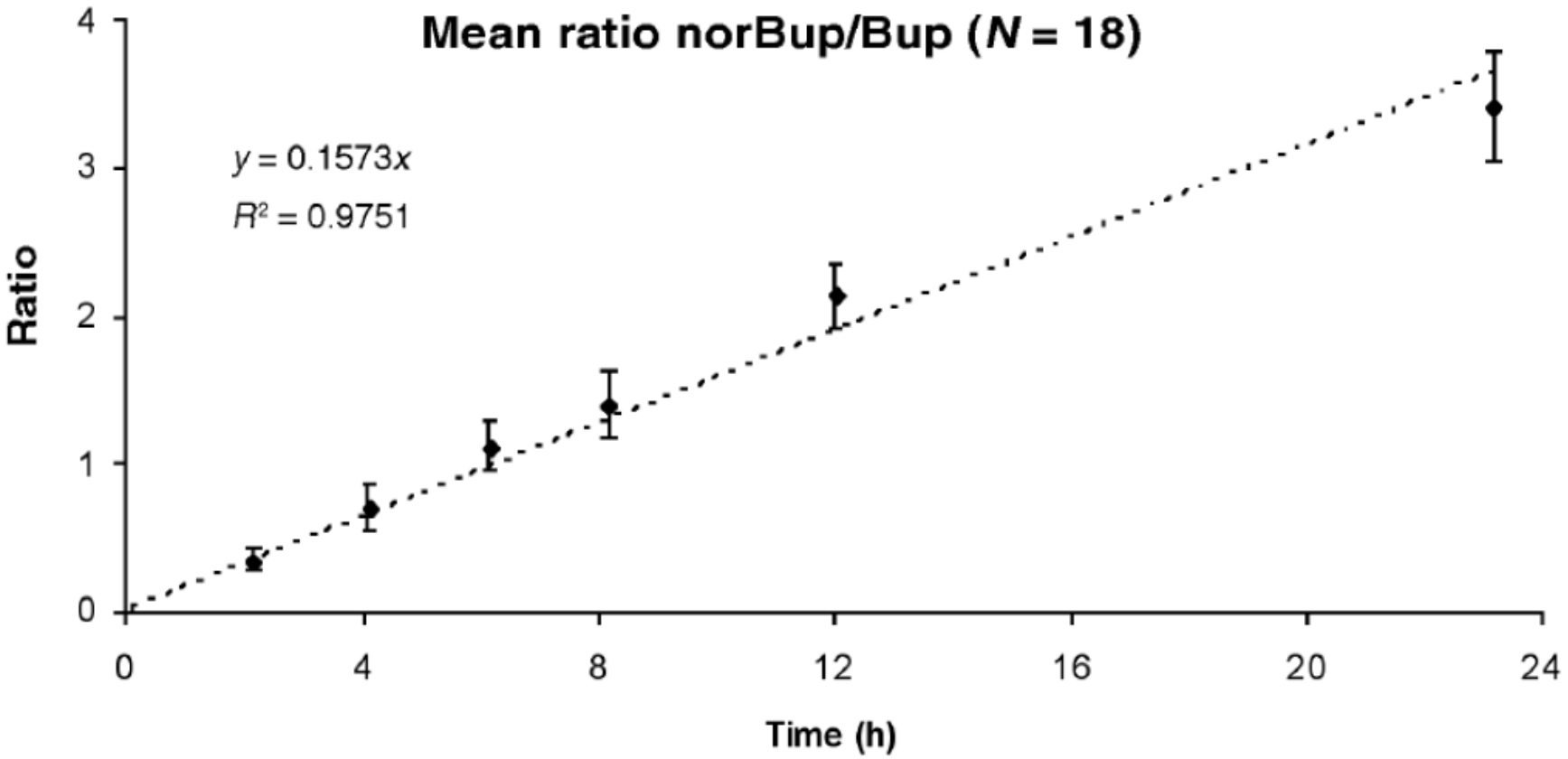
Analysis of Buprenorphine, Norbuprenorphine, and Their Glucuronides in Urine by Liquid Chromatography–Mass Spectrometry

Journal of Analytical Toxicology, Vol. 27, October 2003

Robert Kronstrand*, Tor G. Seldén, and **Martin Josefsson**
National Board of Forensic Medicine, Department of Forensic Chemistry, University Hospital, SE-581 85 Linköping, Sweden

Mean ratio = 3,6

Ratios after single intake



Kronstrand R, Nyström I, Andersson M, Gunnarsson L, Hägg S, Josefsson M, Ahlner J. Urinary detection times and metabolite/parent compound ratios after a single dose of buprenorphine.
J Anal Toxicol. 2008 Oct;32(8):586-93.

Example urine buprenorphine

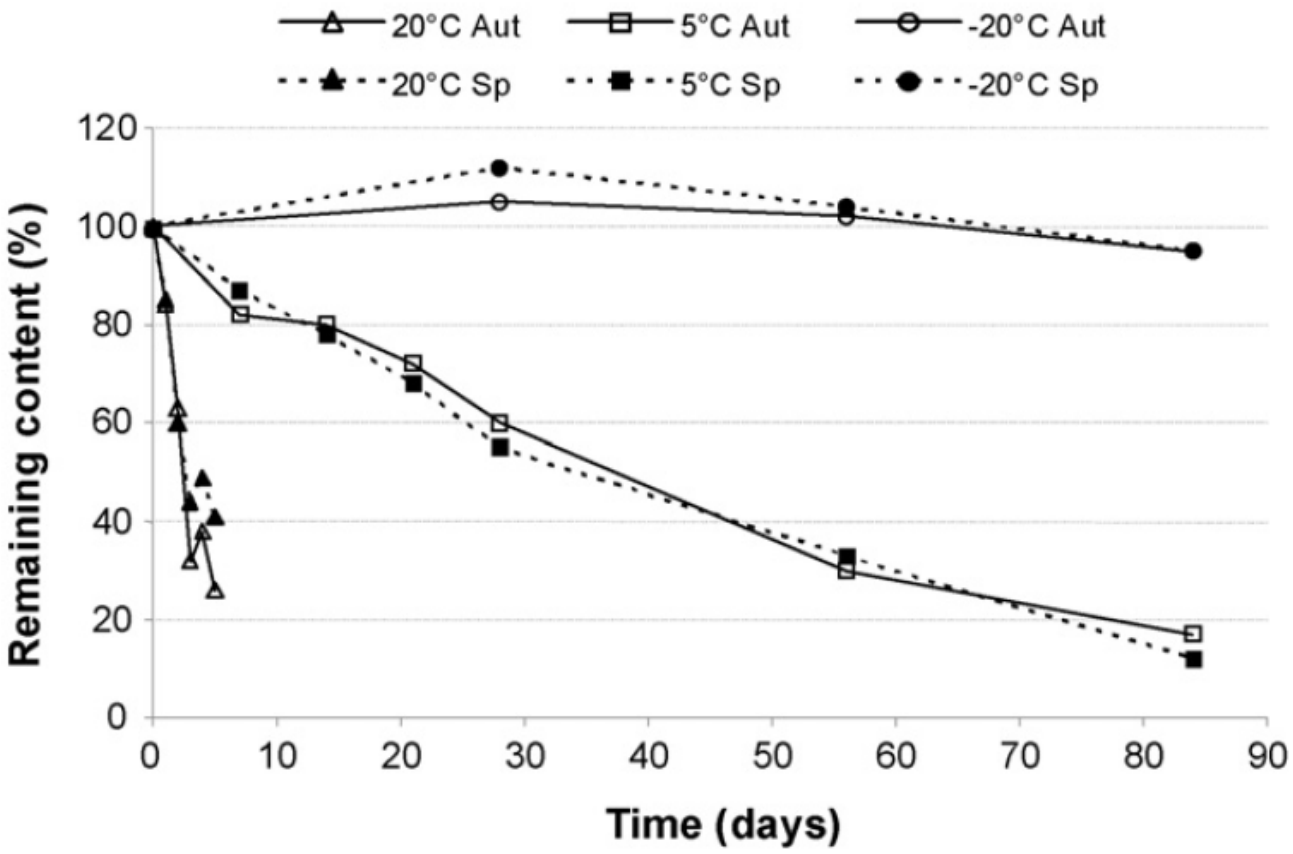
	Femoral blood	Urine	Vitreous
Buprenorphine	1.8 ng/g	0.18 µg/mL	
Norbuprenorphine	1.1 ng/g	0.01 µg/mL	
Amphetamine	0.15 µg/g		
Glucose			0.2 mM
Ethanol	0.85 ‰	1.50 ‰	

Example hair methadone

	Fem blood	Urine	Hair S1	Hair S2	Hair S3
Methadone	0.40		-	0.10	0.28
Buprenorphine	0.5	0.02	-	-	-
Norbup	0.6	0.08	-	-	-
7-aminoclo	0.02		0.034	0.011	-
Alprazolam	0.03		0.029	0.027	0.087
Zopiclone	-		0.18	0.051	0.034
Fentanyl	-		-	0.030	0.022

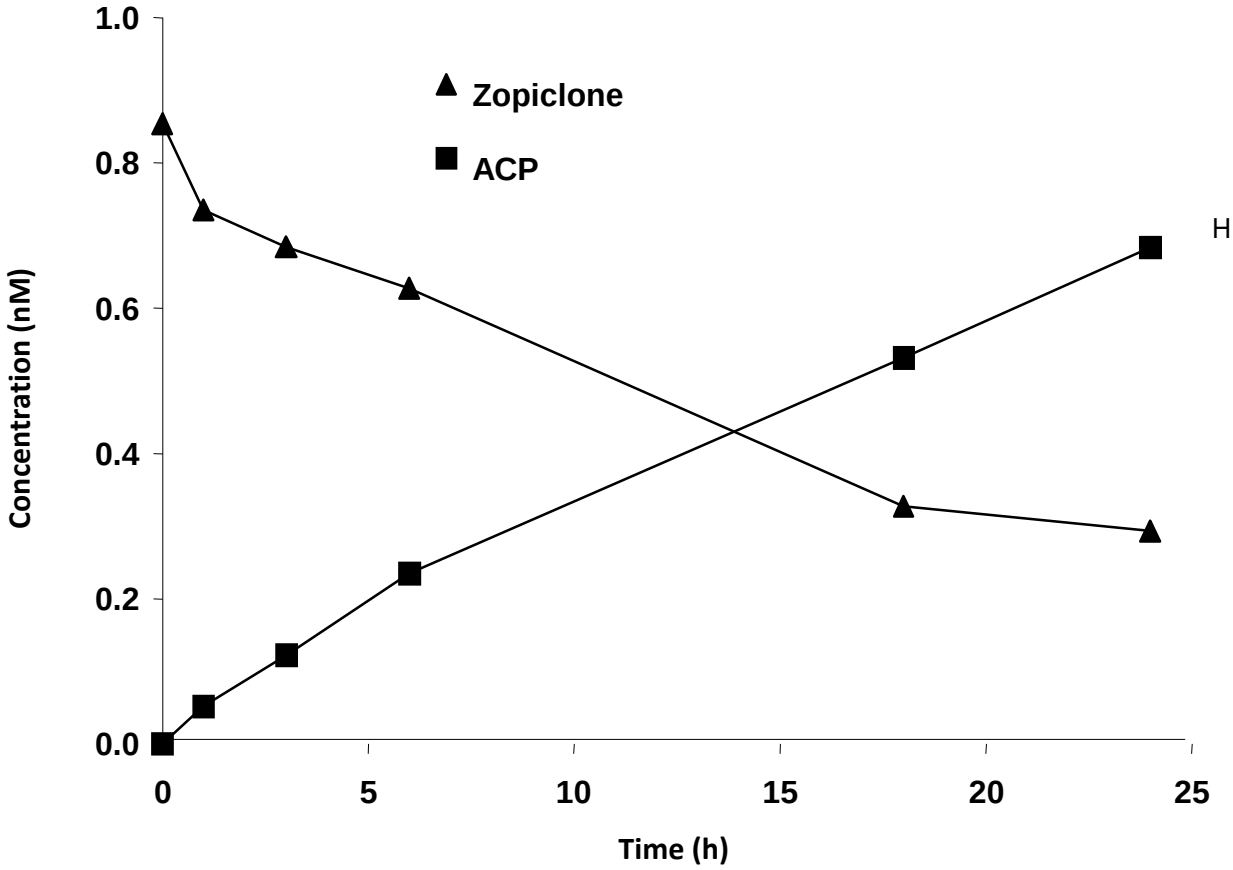
Example degradation product

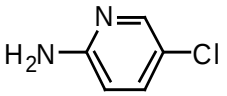
Zopiclone (in)stability

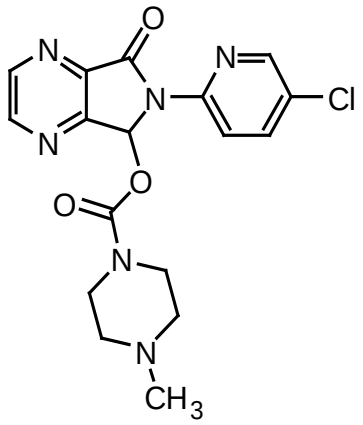


Stability studies of zopiclone Nilsson, Kugelberg, Kronstrand, Ahlner, Forensic Science International 2010

Formation of ACP







Interpretation

- Presence of ACP
  - intake of zopiclone
- Qunatitative value
  - Estimate the original concentratione of zopiclone

Summary

- Screening and confirmation strategies can vary depending on
  - Laboratory equipment and size
  - Laboratory workflow
  - Customer needs
  - Case load