Advanced Tandem Mass Spectrometry Solutions for Targeted and Comprehensive Forensic Toxicology Screening

Joe Anacleto

Applied Markets, Bruker

Forensic Toxicology Challenges

Driving under the influence

Drug facilitated sex crimes

Post mortem analyses…

Drugs, poisons, metabolites…

New synthetic drugs and chemicals…

Blood, urine, saliva, hair, tissues, pills, tablets, powders, liquids, herbs…

Forensic Toxicology Challenges

Driving under the i

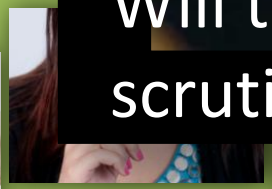
ortem analyses…

salts, cathinones

Drugs, poisons, me

als…

Will the data stand up to scrutiny in a court of law?



Blood, urine, saliva, hair, tissues, pills, tablets, powders, liquids, herbs…

Evolving Analytical Instrument Toolset

The variety of needs…

Immunoassays

Gas Chromatography

Liquid Chromatography

GC-Mass Spectrometry

Traditional Key Technologies

GC-MS/MS (Triple Quad)

LC-MS/MS (Triple Quad)

LC-MS^n (Ion Trap)

LC-High Resolution (QTOF)

Expanding Mass Spectrometry Technologies

…demands a variety of technologies.

Expanding Mass Spectrometry Toolset

Triple Quadrupole Systems (GC or LC)

- Multiple Reaction Monitoring (MRM) mode delivers the highest levels of selectivity and sensitivity
- The ideal technology for targeted quantitative analyses!
- Slower scanning and unit resolution limits versatility for screening and unknown identification application

Ion Trap Systems

- Higher resolution and faster scanning enables screening and identification workflows
- Rapid MS, MS^2 and MS^3 acquisition enhances identification confidence
- Ultra fast positive/negative switching enables acquisition of both polarities in one run

High Resolution QTOF Systems

- Ultra-high resolution MS and MS/MS for the highest confidence identification
- Can use resolution, accurate mass, isotopic patterns, fragmentation patterns and accurate mass fragments for unknown identification
- The fastest scanning for the most comprehensive data acquisition and ability to do retrospective analysis since “all” the information is collected

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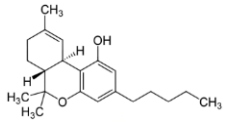
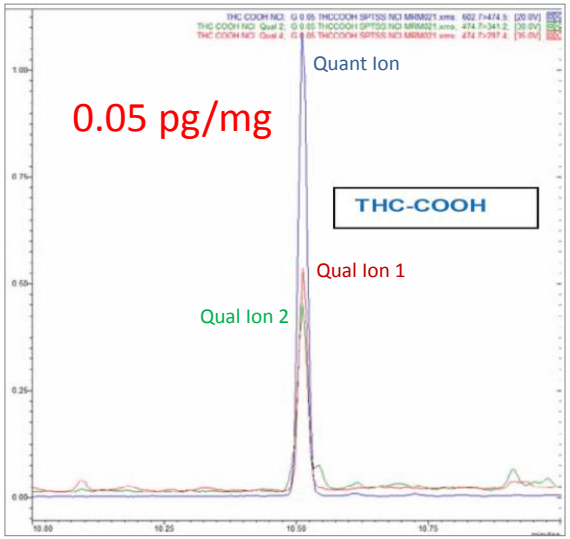
Bruker SCION™ TQ GC-MS/MS

Quantification of THC in hair samples

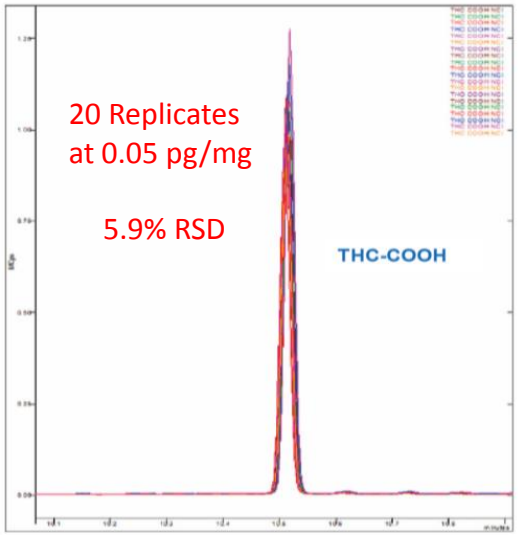
- The active substance in cannabis is 9-tetrahydrocannabinol (9-THC) and is metabolized in the body to an inactive metabolite 9-tetrahydrocannabinoic acid (THC-COOH)
  - In order to prove regular cannabis consumption, hair analysis is preferred over urine or blood analysis
  - The absorption of THC-COOH into hair is very low, making quantitation challenging due to the very high sensitivity required
- Society of Hair Testing for analysis in forensic cases, the limit of quantification for THC-COOH in hair should be less than 0.2 pg/mg while United States regulations recommend a detection limit of 0.05 pg/mg



MS Conditions	
Ionisation mode	Negative CI, 150 eV
Source temperature	265°C
Transfer line temperature	300°C
Resolution	2.0 Da
Detector	Electron Multiplier with Extended Dynamic Range (EDR)

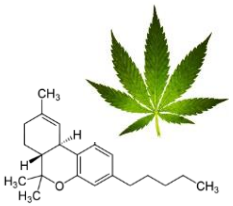


Spiked Control Hair Extract

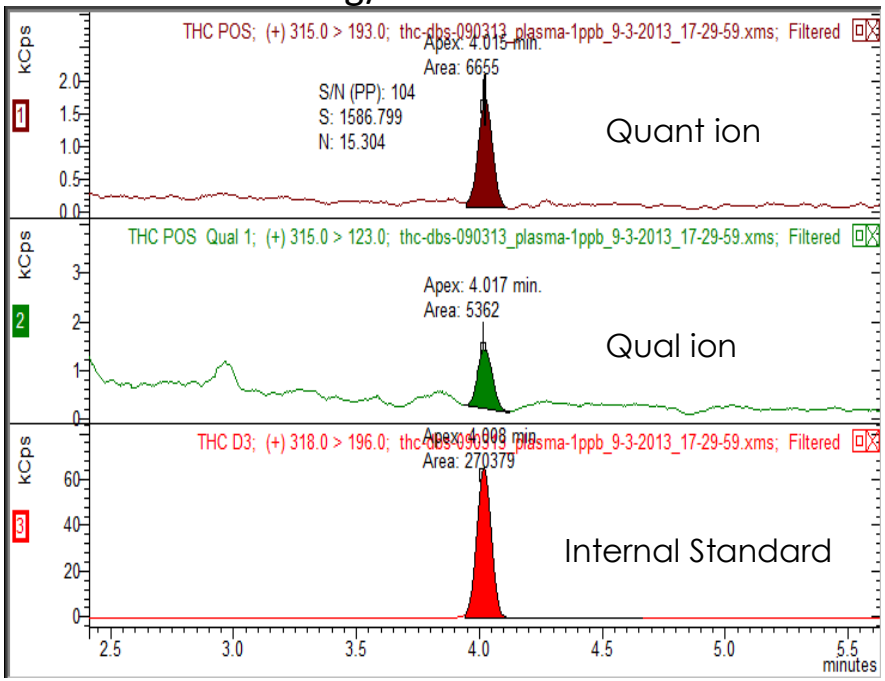


Bruker EVOQ™ LC-MS/MS

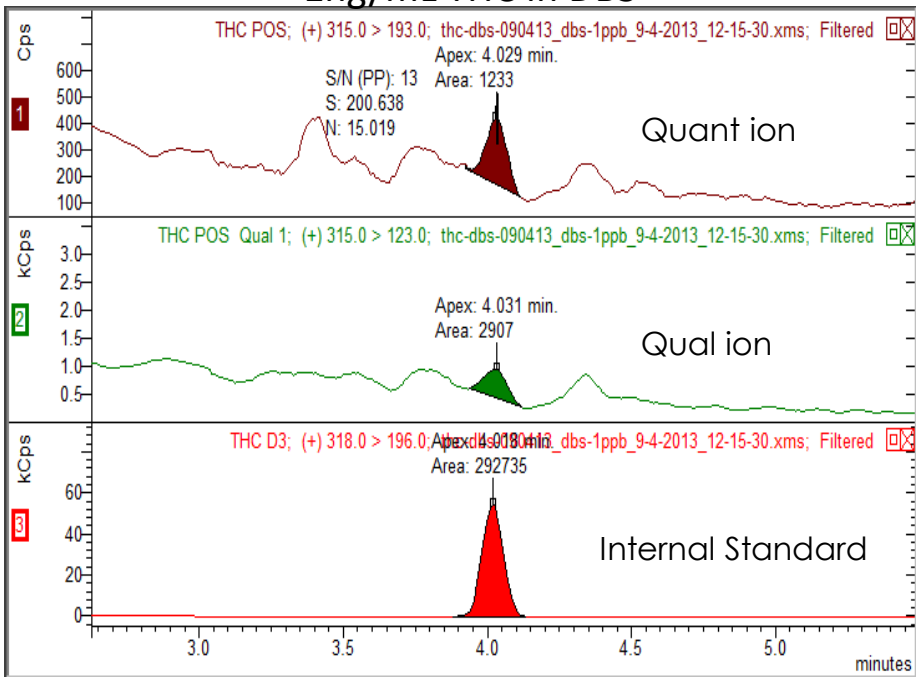
Quantification of THC in plasma and dried blood spots



1ng/mL THC in Plasma

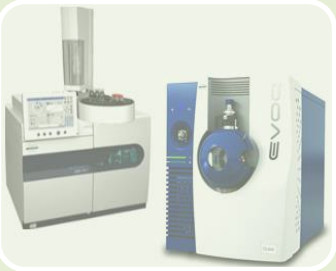


1ng/mL THC in DBS



- Excellent sensitivity for plasma samples at 1ng/mL THC (SN>10, 3µL of sample)
- R2=0.999 with response factor RSD<10%
- RSD for 1ng/mL plasma 3.6%, DBS 8.8%, respectively

Expanding Mass Spectrometry Toolset



Triple Quadrupole Systems (GC or LC)

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Bruker Toxtyper LC-MS^n System

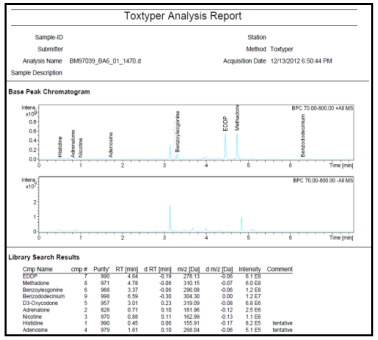
Complete solution for rapid forensic toxicology screening

Ready to use, pre-defined UHPLC-MS^n methods for rapid screening

- Built-in spectral library that screens for over 900 compounds in 12 minutes
- Covers both basic and acidic analytes (positive and negative) in one run

Complete software solution with an easy to use graphical interface

- Fully automated data analysis and report generation
- Optional detailed interrogation of raw data and retrospective analysis
- Sub-libraries and optional metabolite library (Prof. Hans Maurer) with > 3000 metabolites



Fully compatible with standard sample preparations methods (blood and urine)

- Protein precipitation (PPT), Alkaline Liquid/Liquid (LLE) extraction protocol, Solid Phase Extraction (SPE)

Bruker Toxtyper System

Typical workflow

Sample Preparation

PPT, LLE or SPE

Serum: **1 mL, LLE (pH 9)**

Urine: **100 µL, Precipitation with ACN**

Toxtyper OpenAccess™ software for sample management

LC-MS^n Data acquisition on amaZon speed ion trap

Automated data analysis and library search

Automated ID report generation

Typical Detection Limits:

- Serum, LLE: 1-50 ng/ml
- Urine, precipitation: 10-100 ng/ml
- Urine, LLE: 1-10 ng/ml
- Whole blood: 1-10 ng/ml

Bruker Toxtyper System

Typical workflow

Sample Preparation

PPT, LLE or SPE

↓

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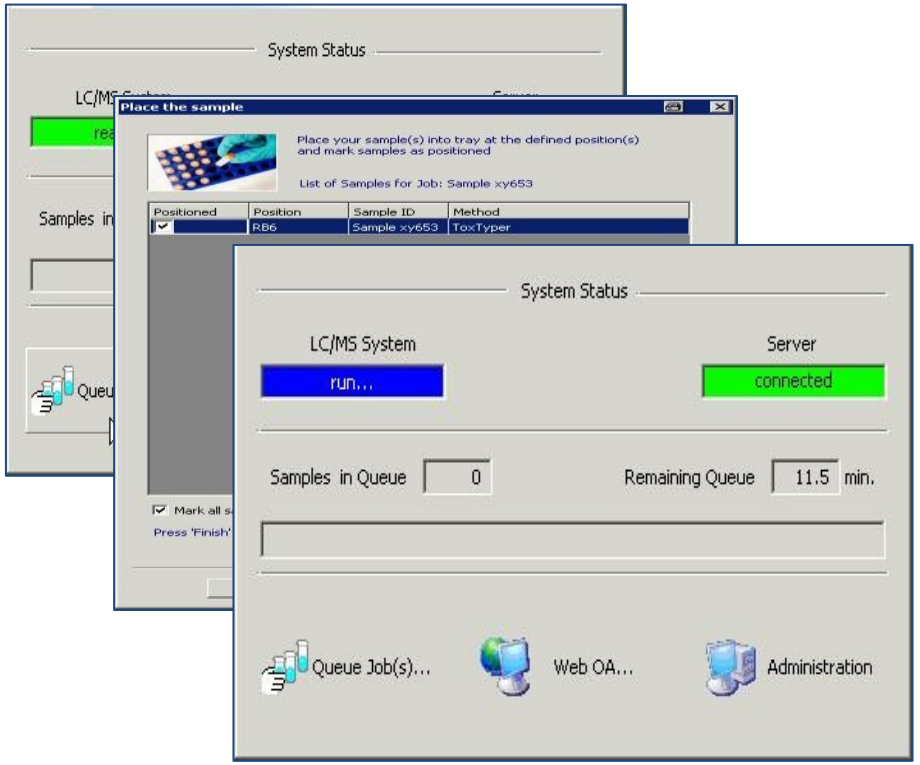

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Typical workflow

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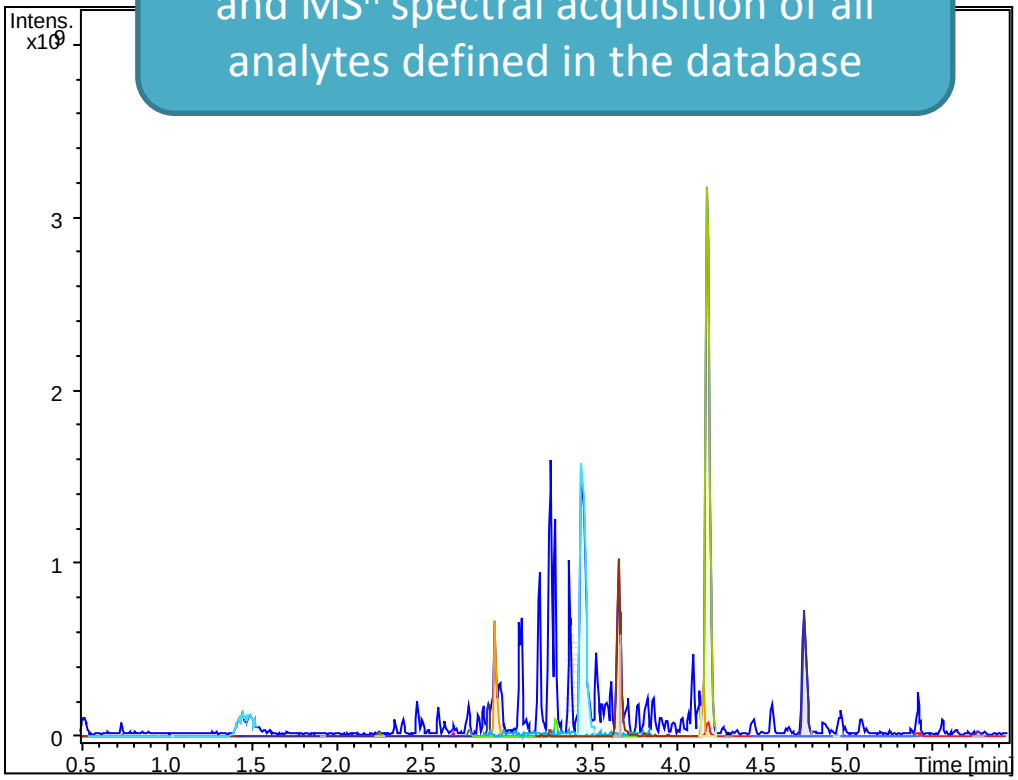
Predefined, retention time based MS and MS^n spectral acquisition of all analytes defined in the database



SmartICC™ – (Ion Charge Control) Method to control the number of ions in the trap to prevent space charge. Achieved by using previous scans to extrapolate ion flux for the ions of interest.

Bruker Toxtyper System

Typical workflow

Sample Preparation

PPT, LLE or SPE

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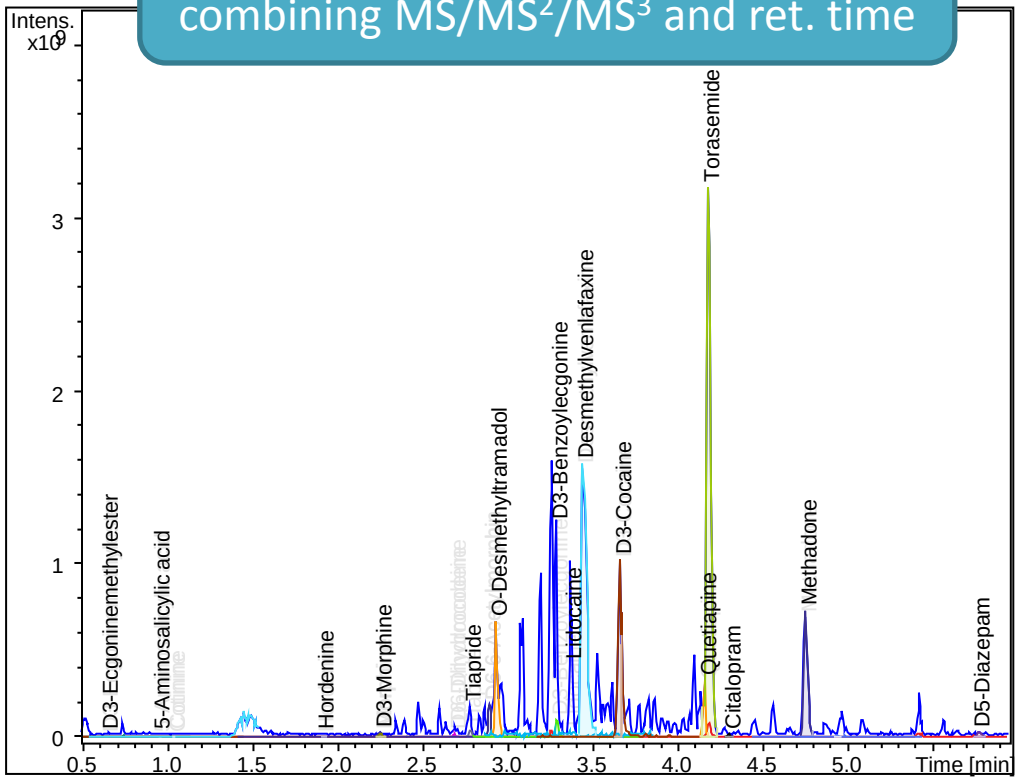
Purity score based matching combining MS/MS^2/MS^3 and ret. time



SmartFRAG™ – Ensures consistent and reproducible fragmentation for different compound classes on all Toxtyper systems

Bruker Toxtyper System

Typical workflow

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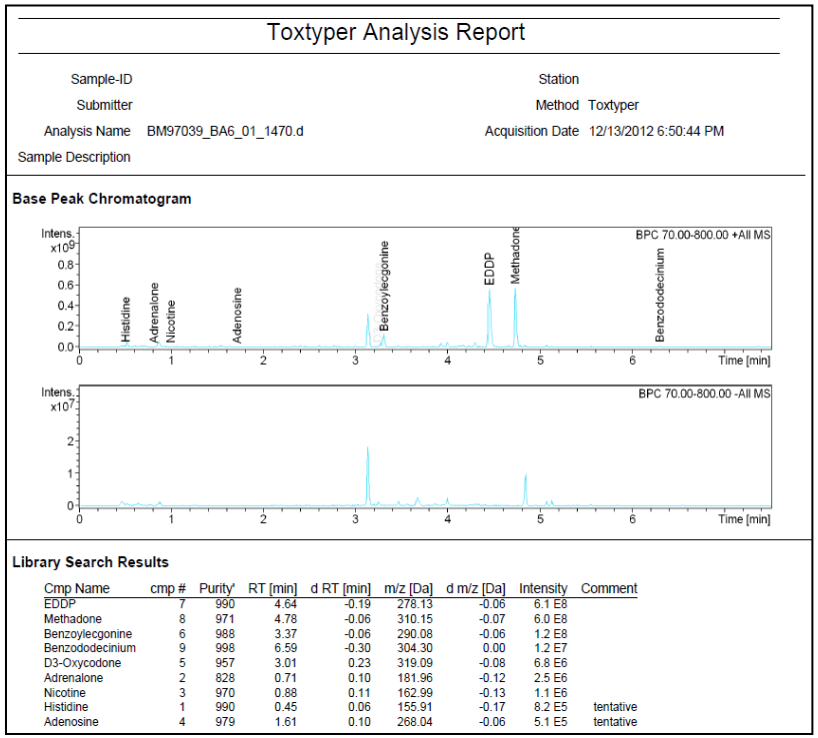
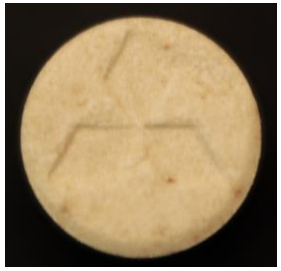


Bruker Toxtyper System

Seized Goods: Suspected 'XTC' tablet



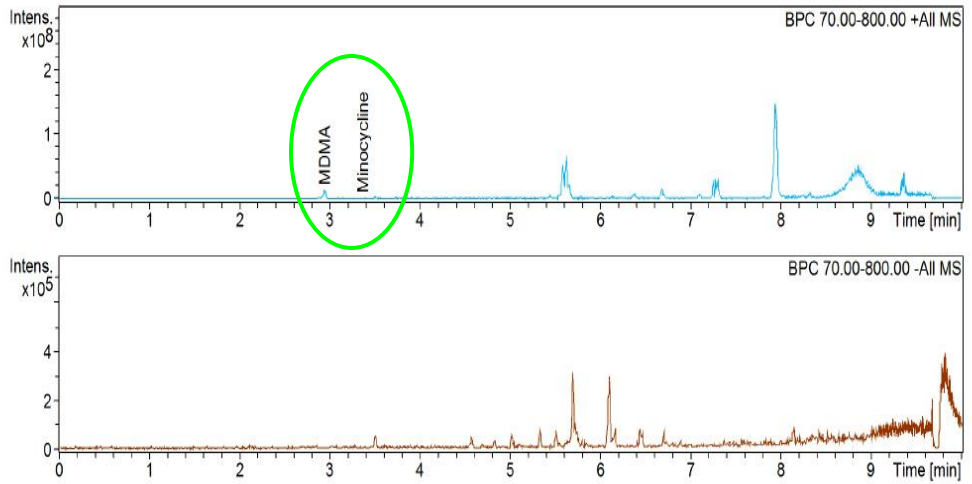
Sample Preparation:

- Pulverise tablet
- Dissolve approximately 100 mg in 2 ml methanol
- Filter
- Dilute 1:10,000 with methanol
- Inject onto Toxtyper system

Toxtyper Analysis Report

Sample-ID	140409_Mitsu	Station	amaZon speed
Submitter	lh	Method	Toxtyper
Analysis Name	140409_Mitsu_002_BD2_01_985.d	Acquisition Date	4/9/2014 8:24:23 AM
Sample Description			

Base Peak Chromatogram



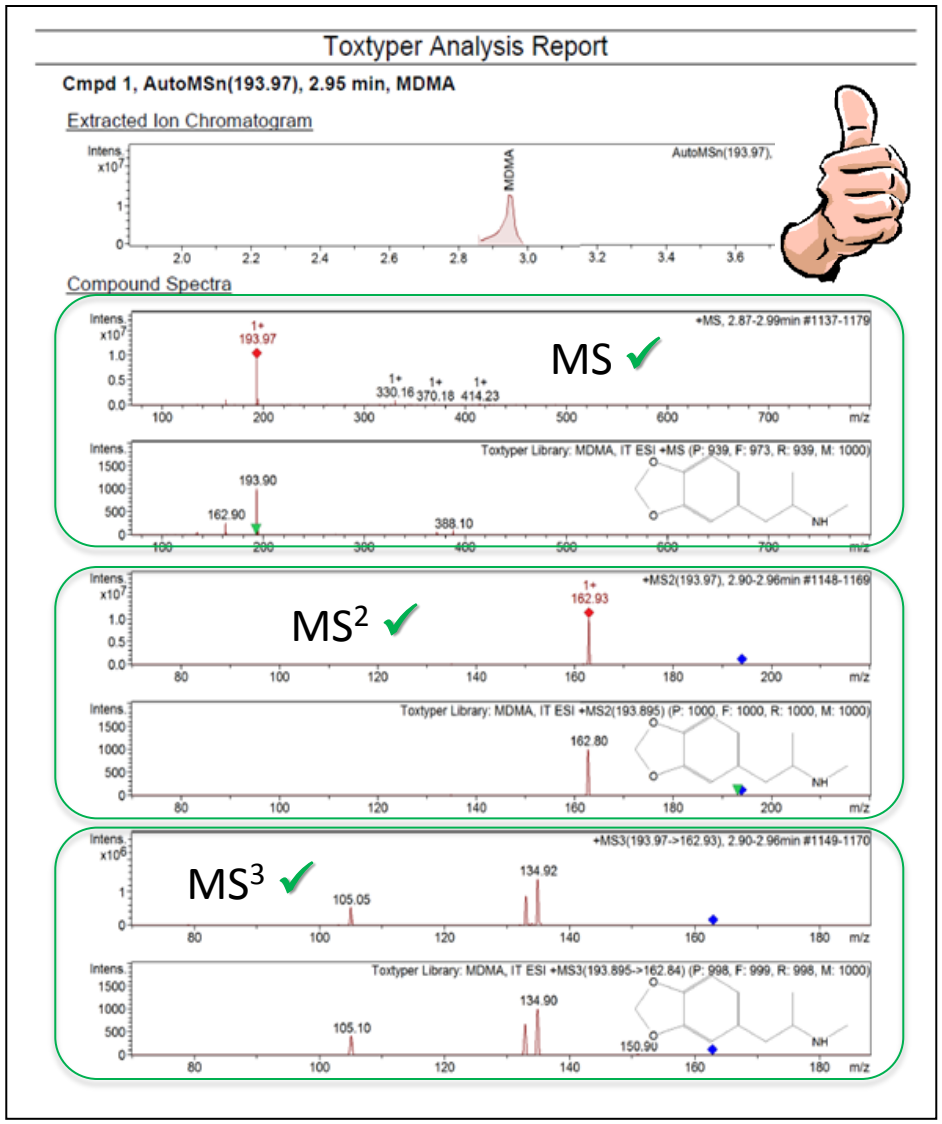
Library Search Results

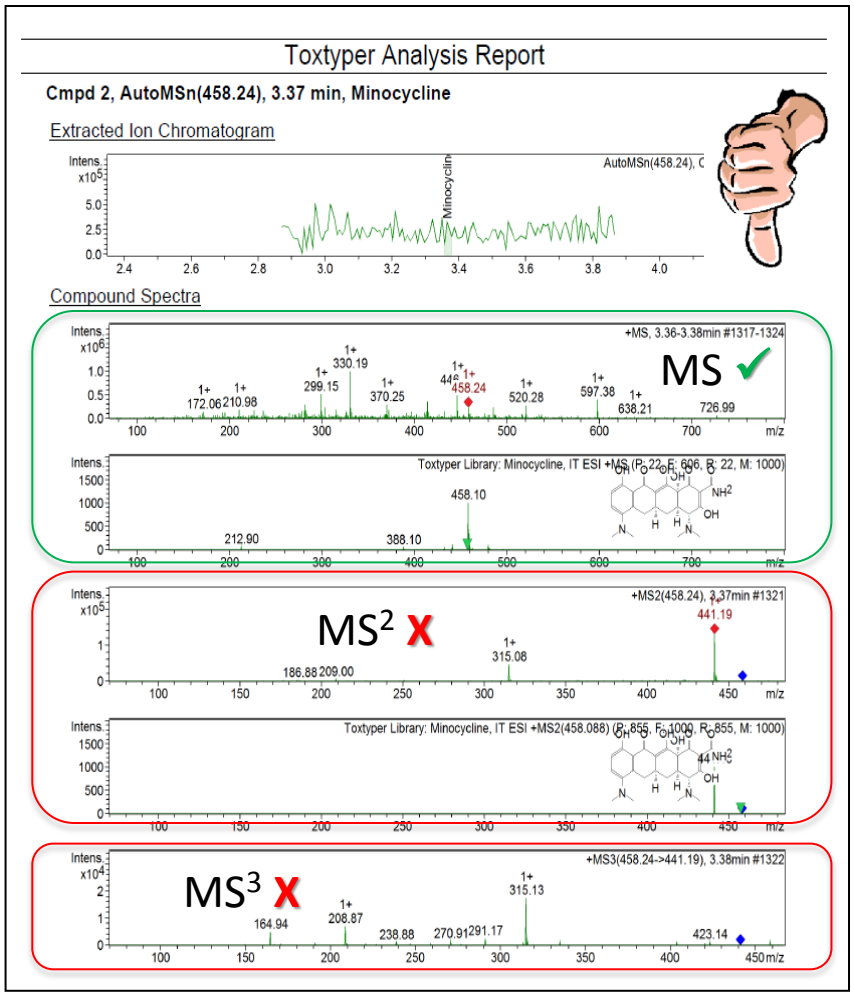
Cmp Name	cmp #	Purity'	RT [min]	d RT [min]	m/z [Da]	d m/z [Da]	Intensity	ID	Comment
MDMA	1	999	3.14	-0.19	193.97	-0.15	1.3 E7	MS2/MS3	
Minocycline	2	855	3.19	0.18	458.24	0.05	3.2 E5	MS2	tentative

**Data, courtesy of Institute of Forensic Medicine, Forensic Toxicology, University Medical Center Freiburg, Germany*

Bruker Toxtyper System

Suspected 'XTC' tablet data evaluation





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Bruker Toxtyper System

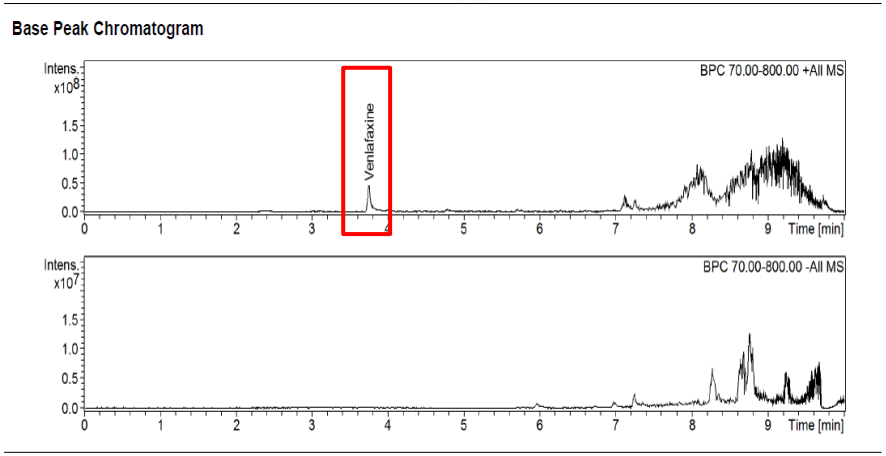
Post Mortem Analysis – Stomach Contents and Blood

Analysis of tablet from stomach:

- Wash
- Pulverise
- Extract into MeOH
- Dilute 1:1,000
- Inject 2 µl

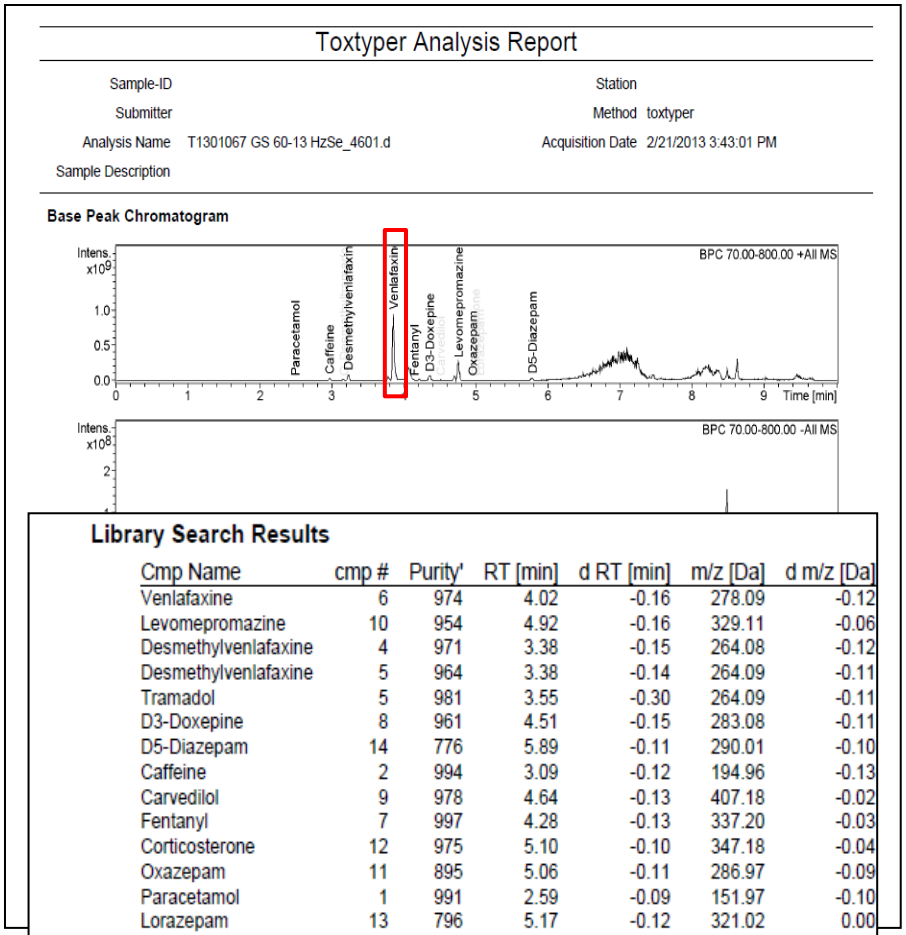
Analysis of serum sample (cardiac blood)

Venaflaxine found to be 1000 ng/ml



Library Search Results

Cmp Name	cmp #	Purity'	RT [min]	d RT [min]	m/z [Da]	d m/z [Da
Venlafaxine	1	983	4.02	-0.27	278.15	-0.0

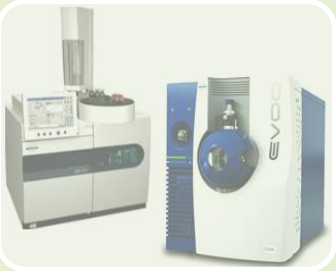


Library Search Results

Cmp Name	cmp #	Purity'	RT [min]	d RT [min]	m/z [Da]	d m/z [Da]
Venlafaxine	6	974	4.02	-0.16	278.09	-0.12
Levomepromazine	10	954	4.92	-0.16	329.11	-0.06
Desmethylvenlafaxine	4	971	3.38	-0.15	264.08	-0.12
Desmethylvenlafaxine	5	964	3.38	-0.14	264.09	-0.11
Tramadol	5	981	3.55	-0.30	264.09	-0.11
D3-Doxepine	8	961	4.51	-0.15	283.08	-0.11
D5-Diazepam	14	776	5.89	-0.11	290.01	-0.10
Caffeine	2	994	3.09	-0.12	194.96	-0.13
Carvedilol	9	978	4.64	-0.13	407.18	-0.02
Fentanyl	7	997	4.28	-0.13	337.20	-0.03
Corticosterone	12	975	5.10	-0.10	347.18	-0.04
Oxazepam	11	895	5.06	-0.11	286.97	-0.09
Paracetamol	1	991	2.59	-0.09	151.97	-0.10
Lorazepam	13	796	5.17	-0.12	321.02	0.00
O-Desmethyltramadol	3	987	3.01	0.16	250.02	-0.16

**Data courtesy of Institute of Forensic Medicine, Forensic Toxicology, University Medica*

Expanding Mass Spectrometry Toolset



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Bruker ToxScreener Solution

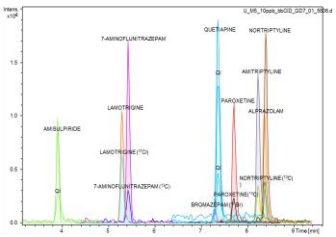
Comprehensive and sensitive forensic toxicology screening

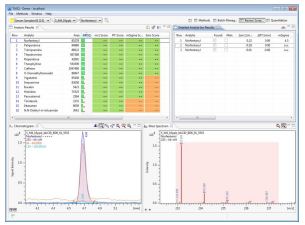
A complete solution for comprehensive Forensic Toxicology screening of body fluids and human tissues

- Includes a complete systems with high performance QTOF and UHPLC system, column, accurate mass toxicology database, complete analysis protocol, system test kit, processing software and technical Support

Innovative technologies that deliver the highest sensitivity and confidence in results

- Market leading QqTOF platforms that deliver the highest performance:
  - Dual Ion funnel and High Definition Collision cell for sustained sensitivity delivering low false positive findings
  - 10 bit digitizer allows accurate screening over the widest concentration range





Powerful software tools that enable the entire workflow and rapid data analysis

- Application specific software enabling colour coded “sample at glance” rapid data review and interrogation of screening results
- Additional advanced software tools for unknown identification capabilities

Bruker ToxScreener Solution

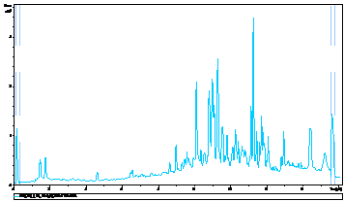
Typical workflow

Sample Preparation

PPT, LLE or SPE

UHPLC-QTOF Acquisition

Continuous acquisition of TOF MS **and bbCID MS/MS**



Find "All" Components

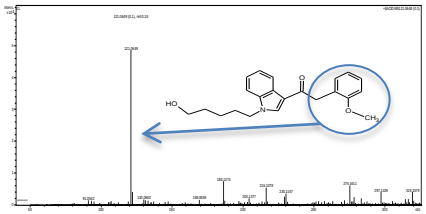
High resolution XIC of every compound desired, detect peaks and integrate

Screen Found Components

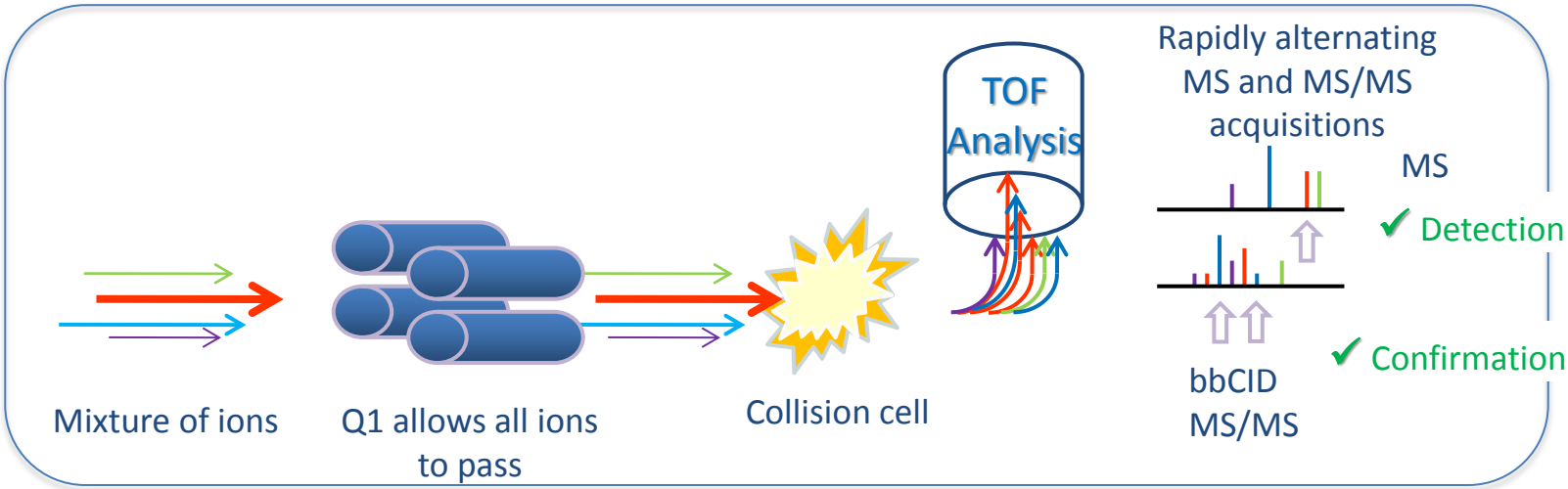
Check compounds against retention time and accurate mass database

Confirm Found Components

Match and score retention time, accurate mass, isotopes & diagnostic ions.



Broad-Band Collision Induced Dissociation (bbCID)



- **Channel 1 TOF MS (low CE):** **No isolation and no fragmentation**, full scan MS spectra are acquired across every LC peak
- **Channel 2 broadband CID (high CE):** **No isolation but all ions are fragmented** in the collision cell, MS/MS spectra are acquired for every LC peak
- Rapidly alternate between both channels (approximately 1s cycle time), no threshold
- Continuous generation of MS & MS/MS spectra across each peak: no risk of missing anything
  - False positive reduction
  - Enhanced confirmation

Bruker ToxScreener Database

1	m/z	RT	sum formula	name	CAS	QI 1	QI 2	QI 3
404	301.0738	7.89	C16H13N2O2Cl1	Clobazam	(22316-47-8)	259.0633	224.0944	
405	318.1003	7.89	C16H13N2O2Cl1NH4^1+	Clobazam (NH4)	(22316-47-8)	259.0633	224.0944	
406	316.1211	6.32	C17H18ClN3O	Clobenzepam	(1159-93-9)	271.0633	229.0289	256.0398
407	479.1995	11.33	C26H32ClFO5	Clobetasone butyrate	(25122-57-0)	71.0491	343.1459	371.1408
408	256.1462	6.31	C14H22N1O1Cl1	Clobutinol	(14860-49-2)	58.0651		
409	162.0138	6.69	C6H8N1Cl1S1	Clomethiazole	(533-45-9)	113.0294	126.0372	
410	315.1622	9.03	C19H23N2Cl1	Clomipramine	(303-49-1)	86.0964	58.0651	242.0731
411	316.0483	7.56	C15H10N3O3Cl1	Clonazepam	(1622-61-3)	270.0554	302.0453	214.0418
412	230.0246	3.83	C9H9N3Cl2	Clonidine	(4205-90-7)	212.9981	186.9824	44.0495
413	346.0986	6.01	C14H20N3O3Cl1S1	Clopamide	(636-54-4)	250.0048	112.1121	169.0163
414	401.1448	9.97	C22H25ClN2OS	Clopenthixol Peak 1	(982-24-1)	271.0343	231.0030	100.0757
415	401.1448	10.12	C22H25ClN2OS	Clopenthixol Peak 2	(982-24-1)	271.0343	231.0030	100.0757
416	322.0663	11.78	C16H16ClNO2S	Clopidogrel	(113665-84-2)	184.0524	155.0258	212.0473
417	344.0982	8.96	C18H18N3Cl1S1	Clothiapine	(2058-52-8)	287.0404	255.0684	313.0561
418	319.0666	10.17	C16H15ClN2OS	Clotiazepam	(33671-46-4)	291.0717	154.0685	218.0731
419	327.1371	7.19	C18H19N4Cl1	Clozapine	(5786-21-0)	270.0793	84.0808	296.0949
420	318.1699	5.46	C18H23N1O4	Cocaethylene	(529-38-4)	196.1332	82.0651	150.0913
421	304.1543	4.89	C17H21N1O4	Cocaine	(50-36-2)	182.1176	82.0651	105.0335
422	300.1594	3.39	C18H21N1O3	Codeine	(76-57-3)	215.1067	243.1016	225.0910
423	322.1413	3.39	C18H21N1O3Na^1+	Codeine (Na)	(76-57-3)	215.1067	243.1016	322.1414
424	400.1754	6.28	C22H25N1O6	Colchicine	(64-86-8)	358.1649	310.1200	341.1384
425	347.2216	8.74	C21H30O4	Corticosterone	(50-22-6)	121.0648	329.2111	311.2006
426	361.2009	7.37	C21H28O5	Cortisone	(53-06-5)	163.1117	343.1904	121.0648
427	177.1022	3.73	C10H12N2O1	Cotinine	(486-56-6)	80.0495	98.0600	70.0651
428	319.2631	13.08	C21H34O2	CP 47-497	(70434-82-1)	233.1900		
429	336.2897	13.08	C21H34O2NH4^1+	CP 47-497 (NH4)	(70434-82-1)	233.1900		
430	301.2525	13.08	C21H33O^1+	CP 47-497 Fragm 301	(70434-82-1)	233.1900		
431	333.2788	13.51	C22H36O2	CP 47-497-C8	(70434-92-3)	247.2056		
432	350.3053	13.51	C22H36O2NH4^1+	CP 47-497-C8 (NH4)	(70434-92-3)	247.2056		
433	315.2682	13.51	C22H35O^1+	CP 47-497-C8 Fragm 315	(70434-92-3)	247.2056		
434	377.3050	11.98	C24H40O3	CP 55-940	(83002-04-4)	121.1012	215.1430	233.1900
435	415.2609	11.98	C24H40O3K^1+	CP 55-940 (K)	(83002-04-4)	121.1012	215.1430	233.1900
436	399.2869	11.98	C24H40O3Na^1+	CP 55-940 (Na)	(83002-04-4)	121.1012	215.1430	233.1900
437	394.3315	11.98	C24H40O3NH4^1+	CP 55-940 (NH4)	(83002-04-4)	121.1012	215.1430	233.1900
438	311.0945	10.74	C18H15ClN2O	Croconazole	(77175-51-0)	125.0153	208.0883	69.0447
439	324.1529	7.52	C19H21N3S	Cyamemazine	(3546-03-0)	237.0481	58.0651	100.1121
440	267.1855	6.94	C18H22N2	Cyclizine	(82-92-8)	167.0855	152.0621	
441	167.0855	6.94	C13H11^1+	Cyclizine Fragm 167	(82-92-8)	167.0855	152.0621	
442	276.1746	7.95	C20H21N	Cyclobenzaprine	(303-53-7)	216.0934	231.1168	84.0808
443	292.1907	6.16	C17H25NO3	Cyclopentolate	(512-15-2)	72.0808	129.0699	157.1012

UNIVERSITY OF HELSINKI
FACULTY OF MEDICINE

UNIVERSITÄTS KLINIKUM FREIBURG
Institute of Forensic Medicine, Forensic Toxicology

- Chemical formula / name for ~2000 compounds:

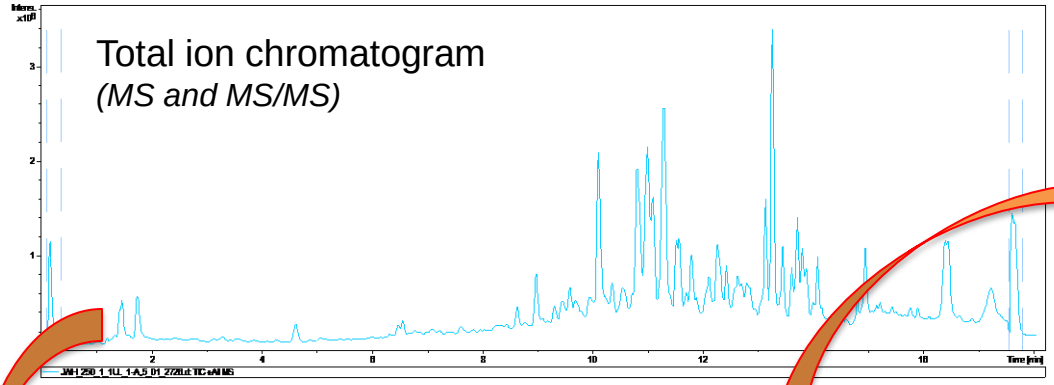
  ~1250 compounds of forensic relevance, contribution Helsinki/Freiburg;

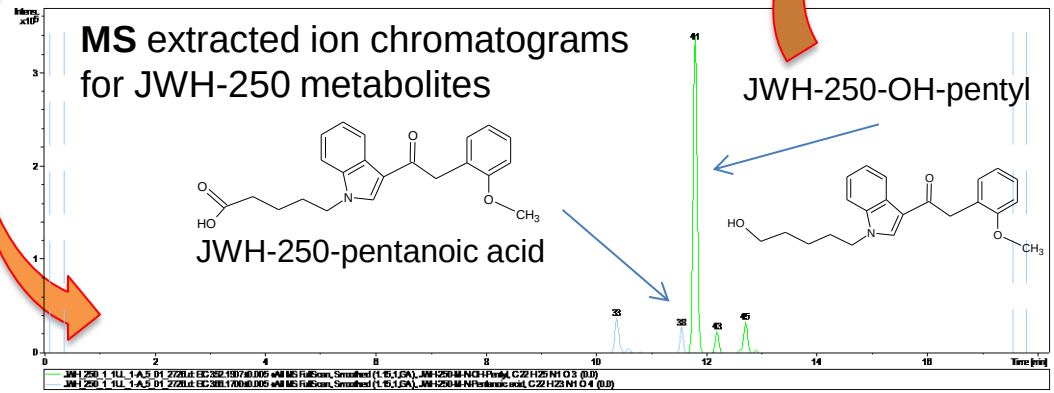
  plus ~750 pesticides

- Detailed information for all:
  Retention time
  Adducts
  Fragments (fullscan)
  Isomers
  Qualifier ions
  (for ~1500 compounds)

Bruker ToxScreener Data Processing

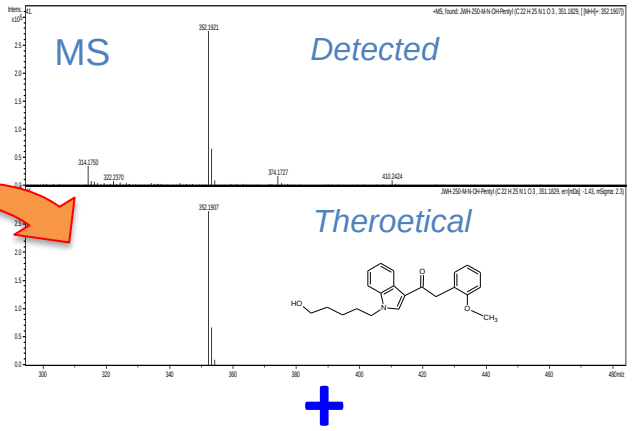
Urine sample from suspected JWH-250 user

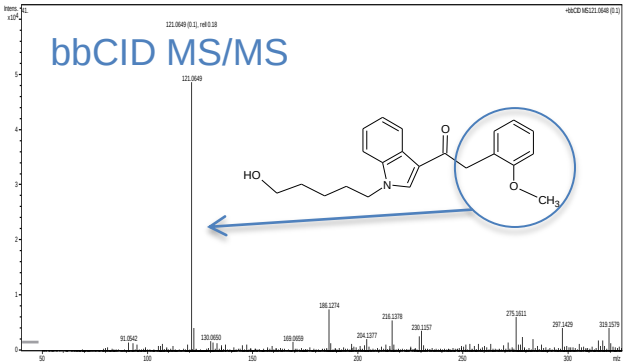




1) Retention Time Match

2) Accurate Mass & Isotope Pattern Match





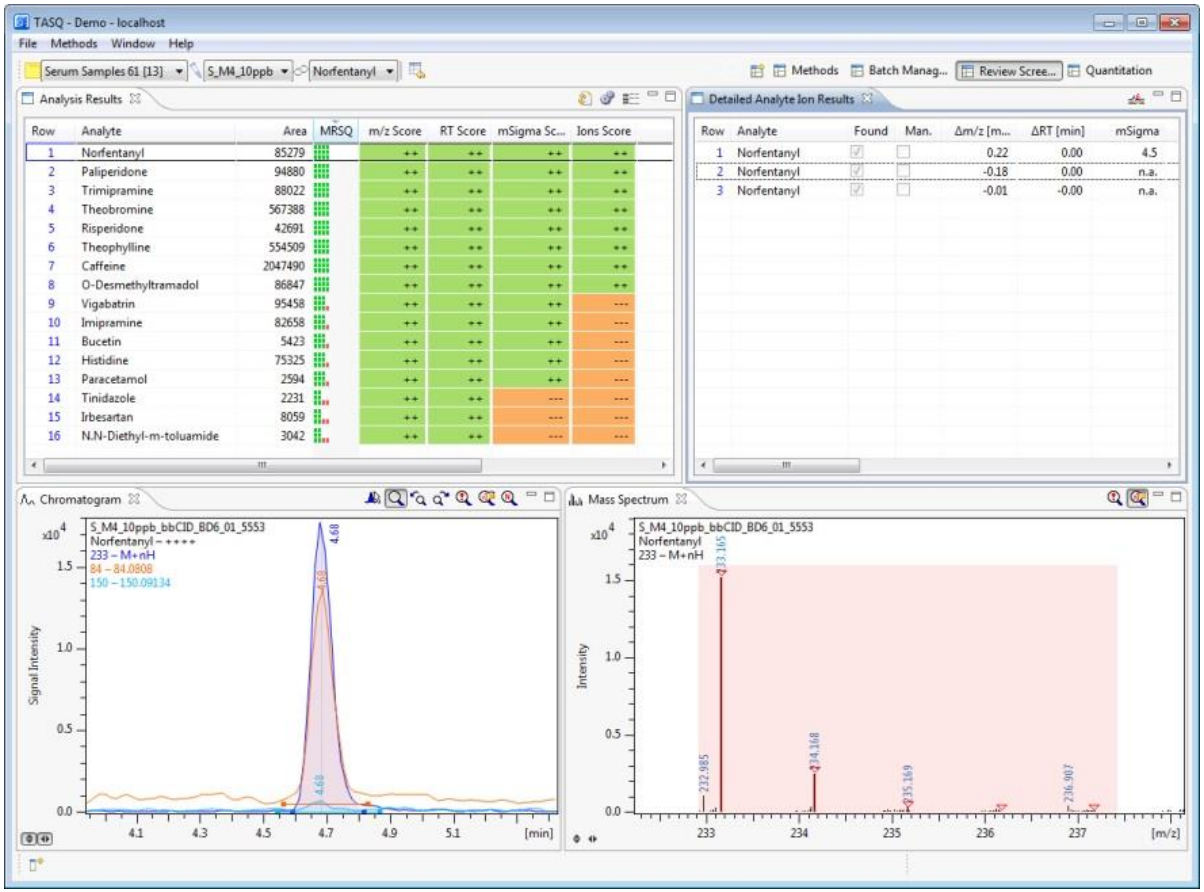
3) Accurate Mass Fragments Match → Results Table

Data Courtesy of Hjelt Institute, Department of Forensic Medicine, University of Helsinki, Finland

Bruker ToxScreener Solution

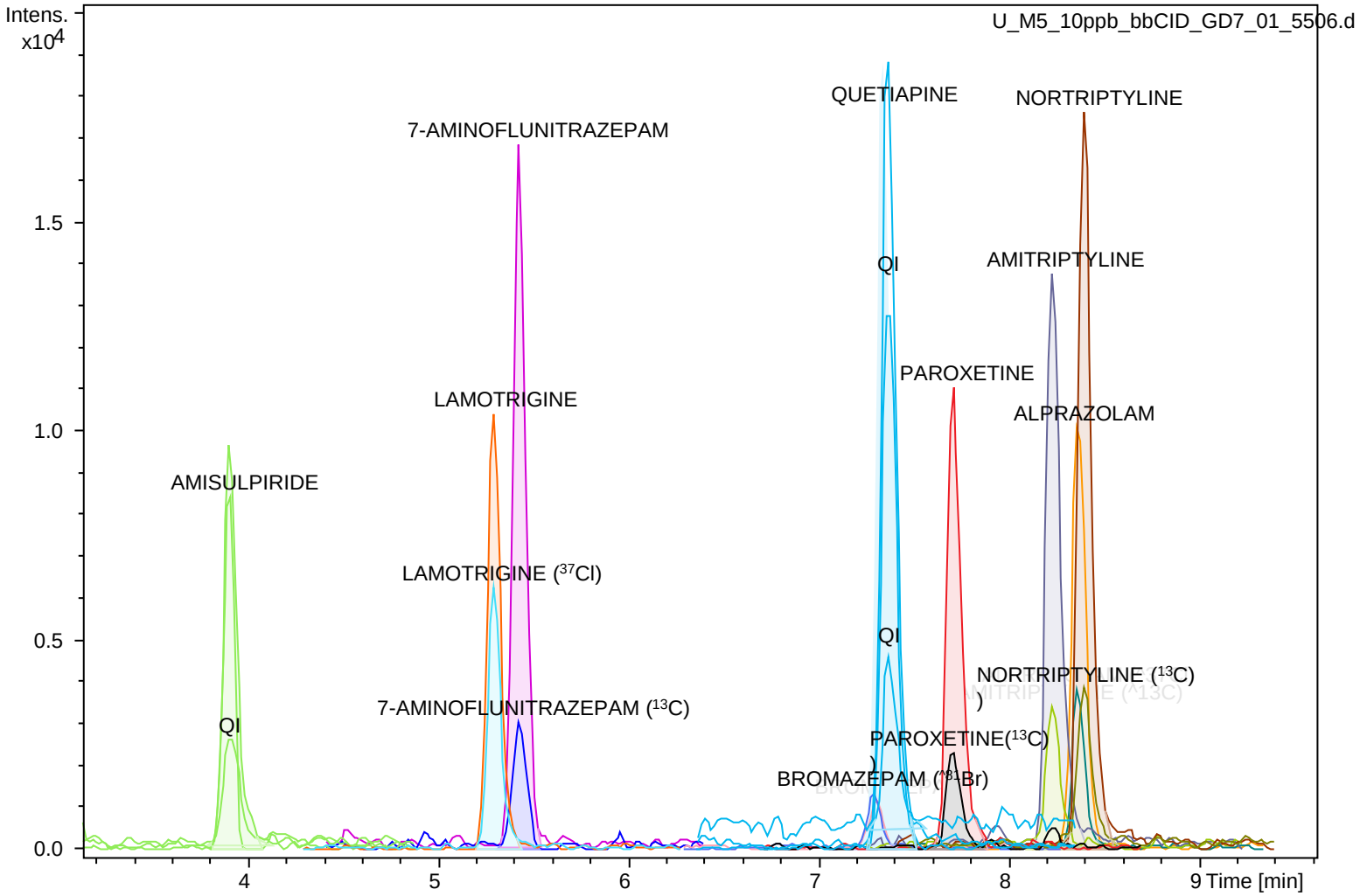
Automated Data Processing

“At-a-glance”review:
Fast and reliable identification of screened drugs scored against the database information.



Retention times, accurate mass, isotopic pattern and bbCID QI’s ions (with their intensity ratios) provide low false positive rates for enhanced confirmation in complex matrices

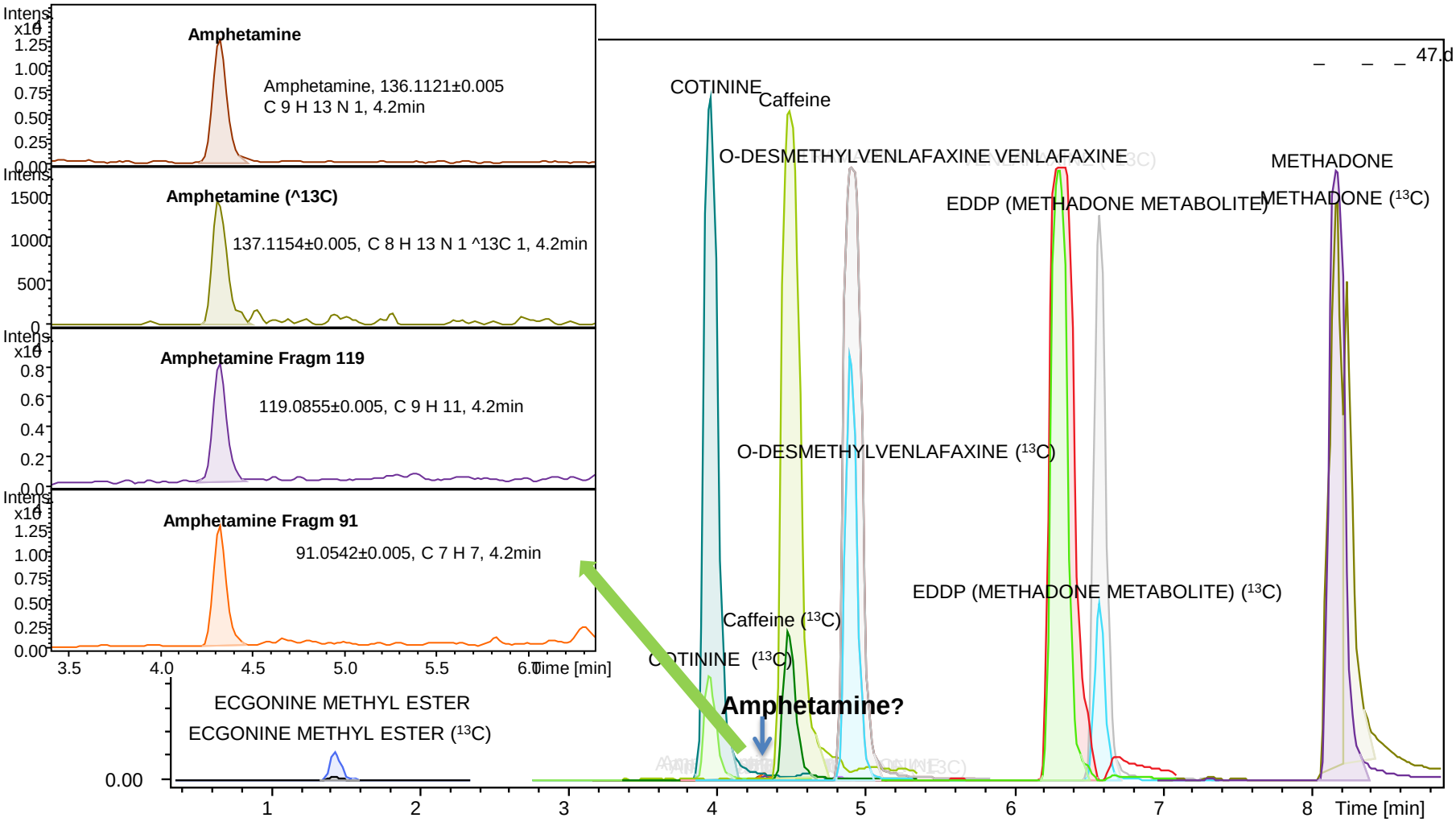
Bruker ToxScreener Sensitivity



Nine compounds spiked in urine at 10 ng/ml, all detected and confirmed, no false positives.

Bruker ToxScreener Solution

Example of positive Amphetamine finding, Post motem serum (LLE)



Summary

Bruker Forensic Toxicology Solutions

- The expanding portfolio of high performance MS technologies are playing an increasingly important role in addressing the growing challenges in forensic toxicology
  - From targeted quantitation to rapid or comprehensive screening workflows

- Bruker is focused on developing innovative solutions that deliver high performance, ruggedness and are easy to deploy in the forensic lab
  - Scion TQ GC-MS/MS and EVOQ TQ LC-MS/MS systems for targeted quant
  - The Toxtyper for rapid and very easy to use forensic screening
  - The ToxScreener for the highest performance in comprehensive screening

The variety of needs… *…demands a variety of technologies.*

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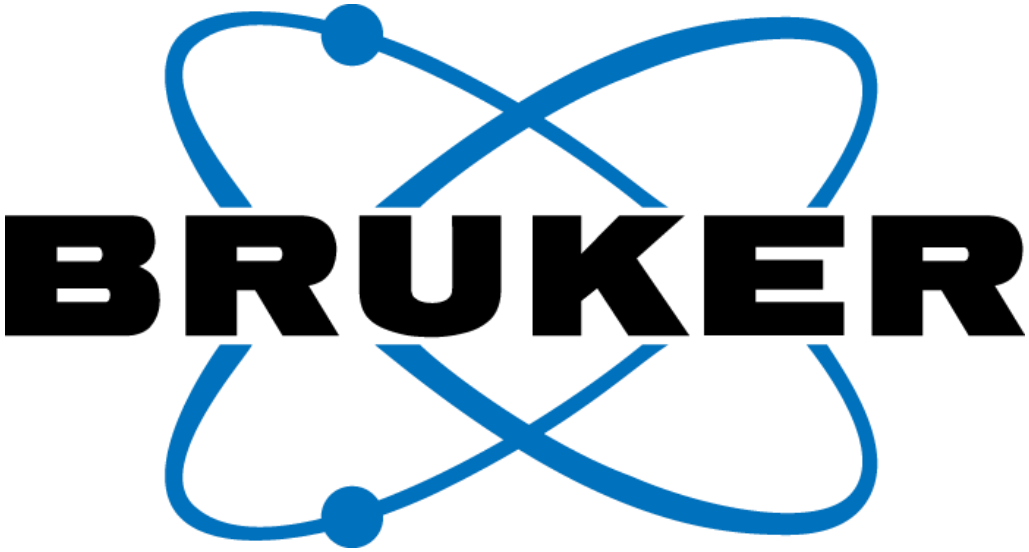

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