Toxtyper 3.0

VITATOX 2020, 7. - 9. 9. 2020

Spolehlivý, rychlý a jednoduchý screening

RADANAL, RECIPE, BRUKER

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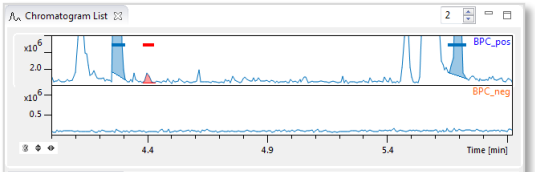
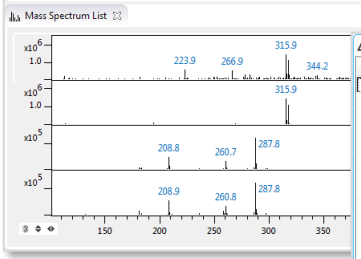
Country Sales Manager Bruker s.r.o.

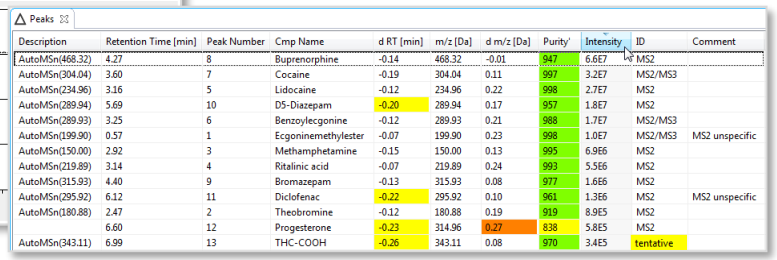
Toxtyper workflow for toxicology screening

Push-Button Sample Submission

UHPLC-MS^n analysis







Description	Retention Time [min]	Peak Number	Cmp Name	d RT [min]	m/z [Da]	d m/z [Da]	Purity'	Intensity	ID	Comment
AutoMSn(468.32)	4.27	8	Buprenorphine	-0.14	468.32	-0.01	947	6.6E7	MS2	
AutoMSn(304.04)	3.60	7	Cocaine	-0.19	304.04	0.11	997	3.2E7	MS2/MS3	
AutoMSn(234.96)	3.16	5	Lidocaine	-0.12	234.96	0.22	998	2.7E7	MS2	
AutoMSn(289.94)	5.69	10	D5-Diazepam	-0.20	289.94	0.17	957	1.8E7	MS2	
AutoMSn(289.93)	3.25	6	Benzoylecgonine	-0.12	289.93	0.21	988	1.7E7	MS2/MS3	
AutoMSn(199.90)	0.57	1	Ecgoninemethylester	-0.07	199.90	0.23	998	1.0E7	MS2/MS3	MS2 unspecific
AutoMSn(150.00)	2.92	3	Methamphetamine	-0.15	150.00	0.13	995	6.9E6	MS2	
AutoMSn(219.89)	3.14	4	Ritalinic acid	-0.07	219.89	0.24	993	5.5E6	MS2	
AutoMSn(315.93)	4.40	9	Bromazepam	-0.13	315.93	0.08	977	1.6E6	MS2	
AutoMSn(295.92)	6.12	11	Diclofenac	-0.22	295.92	0.10	961	1.3E6	MS2	MS2 unspecific
AutoMSn(180.88)	2.47	2	Theobromine	-0.12	180.88	0.19	919	8.9E5	MS2	
	6.60	12	Progesterone	-0.23	314.96	0.27	838	5.8E5	MS2	
AutoMSn(343.11)	6.99	13	THC-COOH	-0.26	343.11	0.08	970	3.4E5	tentative	

Result:
List of identified compounds

Fully automated data processing and reporting

- MS^n spectra extraction
- Spectral library matching
- Result report generation

Simple and fully automated workflow from sample injection to final result in 12 min

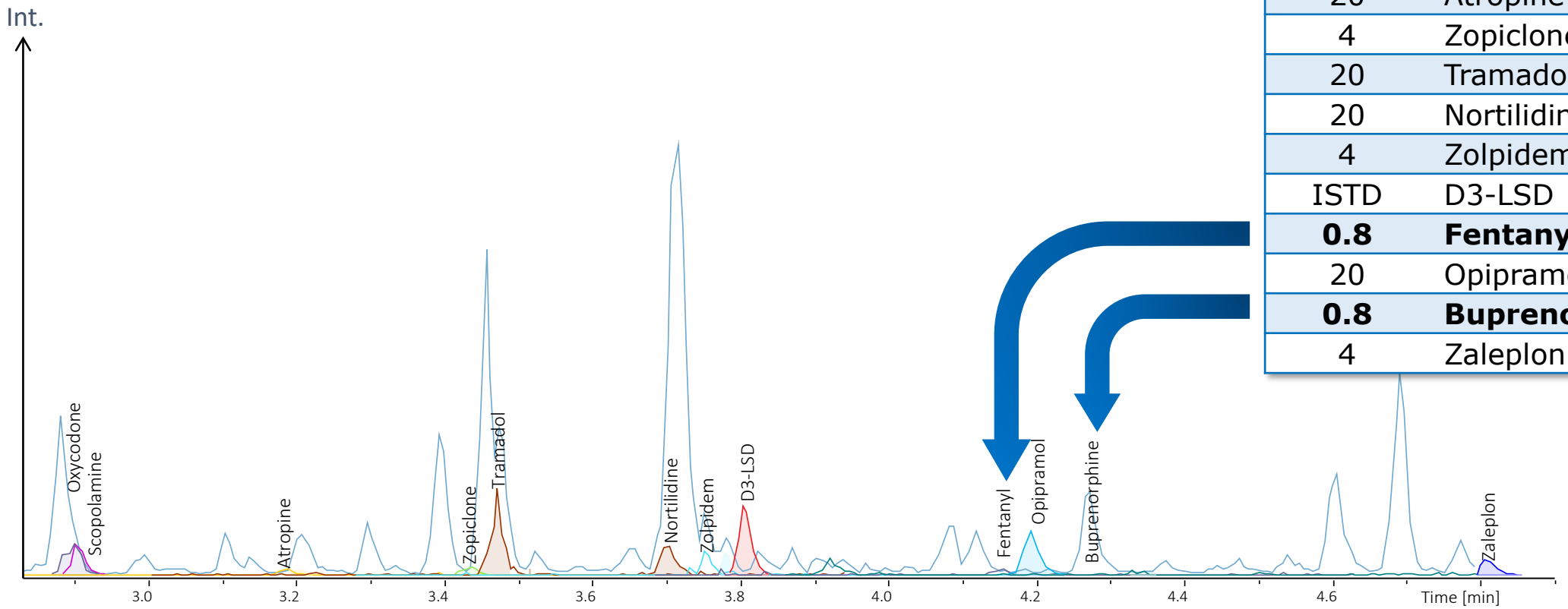
Choice of sample preparation

- The Toxtyper works with various proven sample preparations, depending on sample specimen and main application.
- A selection of SOPs is part of the Toxtyper Tutorial, e.g.:
  - **Protein precipitation (PP)** for fast and simple sample preparation and preservation of glucuronides
  - **Liquid-liquid extraction (LLE)** or
  - **Solid phase extraction (SPE)**

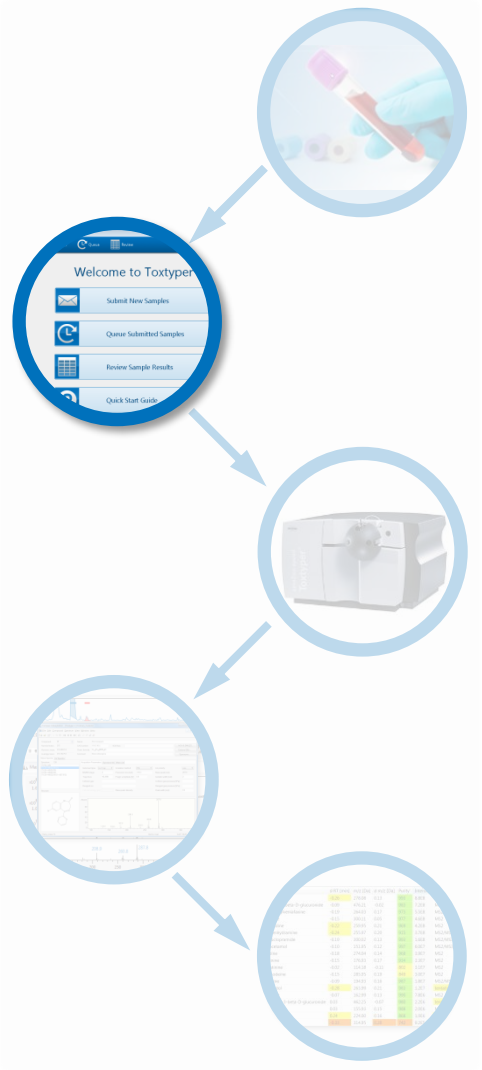
Drug identification in urine after LLE

Detection of Fentanyl and Buprenorphine at concentrations < 1 ng/mL after liquid-liquid extraction (LLE, 1 ml urine)

ng/mL	Compound
20	Oxycodone
20	Scopolamine
20	Atropine
4	Zopiclone
20	Tramadol
20	Nortilidine
4	Zolpidem
ISTD	D3-LSD
0.8	**Fentanyl**
20	Opipramol
0.8	**Buprenorphine**
4	Zaleplon

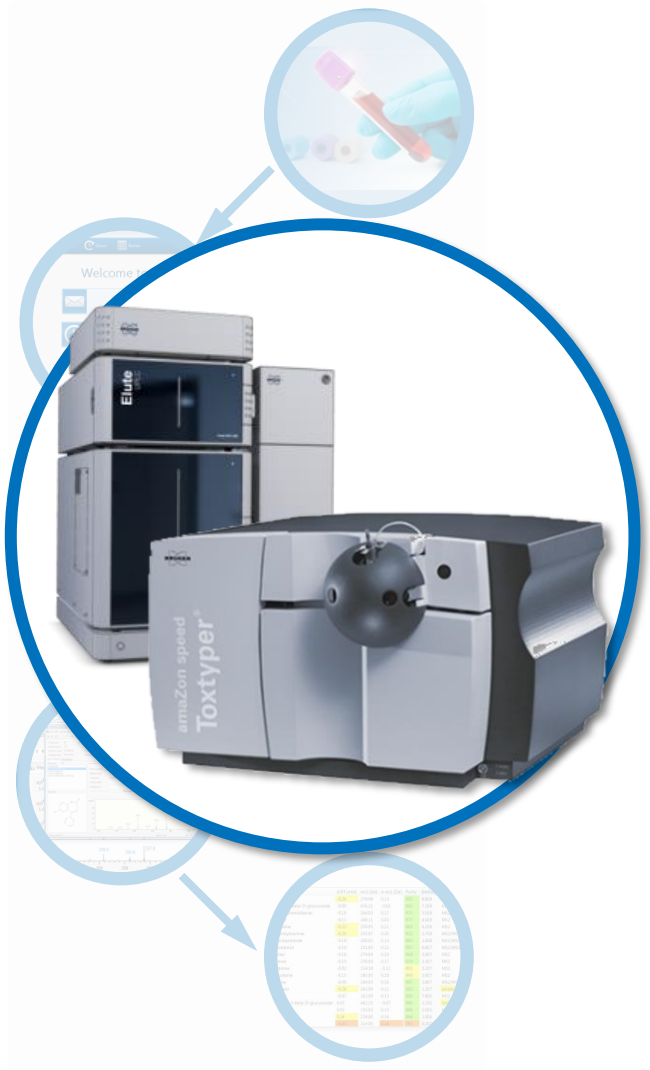


Easy to use software



- Toxtyper software designed for non-MS experts
- Intuitive user interface for easy sample management

HPLC-MS^n data acquisition



Bruker Column Kit

- Acclaim® RSLC 120 C18
  2.2 µm, 120Å 2.1 x 100 mm

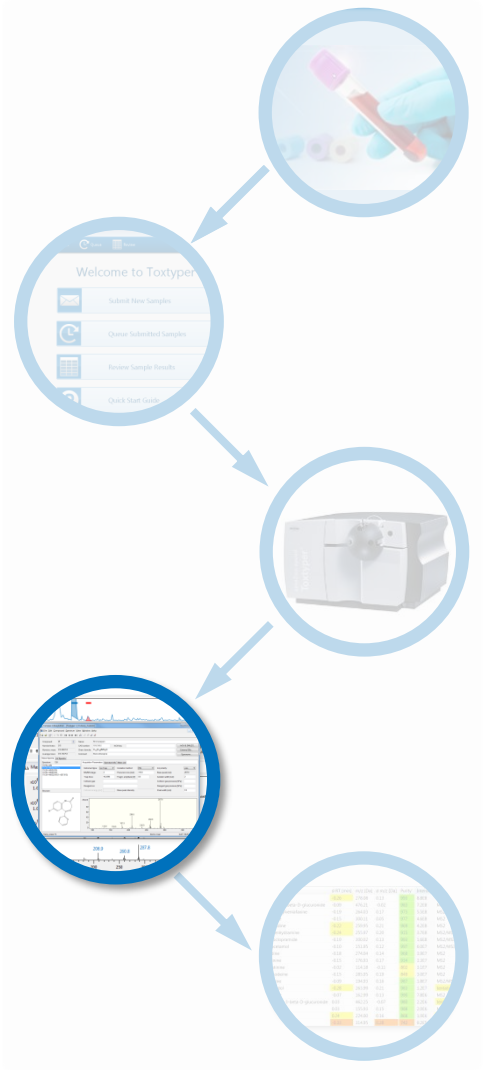
Gradient

- H_2O/ACN, 2 mM ammonium formate, 0.1% HCOOH
- 11 minutes total run time

AutoMS^2/MS^3 Data Acquisition

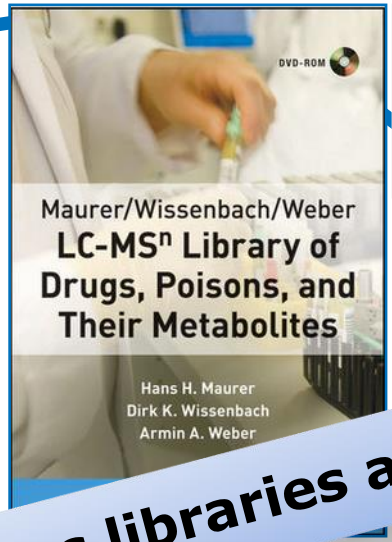
- Electrospray Ionization
- Zero delay alternating polarity (ZDA™)
- UltraScan 70-800 *m/z* @ at 32.000 *m/z* s^{-1}
- **Scheduled Precursor List (SPL)** containing precursor mass and retention time information of all library compounds to trigger data dependent acquisition of MS^n spectra

Compound identification using library search



Compound	CAS	Monoisotopic neutral mass	Formula	Polarity	RT [min]
2-Amino-5-chloropyridine	1072-98-6	128.01	C5H5ClN2	POSITIVE	2.05
2-Hydroxyethylflurazepam	20971-53-3	332.07	C17H14ClFN2O2	POSITIVE	10.11
3-Hydroxybromazepam	13132-73-5	331.00	C14H10BrN3O2	POSITIVE	6.07
6-O-Acetylmorphine	2784-73-8	327.15	C19H21NO4	POSITIVE	3.56
7-Aminoflunitrazepam	34084-50-9	283.11	C16H14FN3O	POSITIVE	5.17
Alpha-hydroxyalprazolam	37115-43-8	324.08	C17H13ClN4O	POSITIVE	9.48
Alprazolam	28981-97-7	308.08	C17H13ClN4	POSITIVE	10.18
			H18N2O3	NEGATIVE	8.88
			9H13N	POSITIVE	3.49
			7H21NO	POSITIVE	9.00
			H19NO4	POSITIVE	4.34
			1H16N2	POSITIVE	2.05
			10BrN3O	POSITIVE	7.40
			H41NO4	POSITIVE	8.18
Butalbital	77-26-9	224.12	C11H16N2O3	NEGATIVE	7.28
Clobazam	22316-47-8	300.07	C16H13ClN2O2	POSITIVE	10.78
Clonazepam	1622-61-3	315.04	C15H10ClN3O3	POSITIVE	9.98
Cocaethylene	529-38-4	317.16	C18H23NO4	POSITIVE	6.13
Cocaine	50-36-2	303.15	C17H21NO4	POSITIVE	5.16
Codeine	76-57-3	299.15	C18H21NO3	POSITIVE	3.32
Creatinine	60-27-5	113.06	C4H7N3O	POSITIVE	0.50
D3-Clomipramine	136765-29-2	317.17	C19H20ClN2D3	POSITIVE	10.42
D4-Haloperidol	136765-35-0	379.17	C21H19ClD4FNO2	POSITIVE	8.33
D5-Diazepam	65854-76-4	289.10	C16H8ClN2OD5	POSITIVE	11.43
Desalkylflurazepam	2886-65-9	288.05	C15H10ClFN2O	POSITIVE	10.48

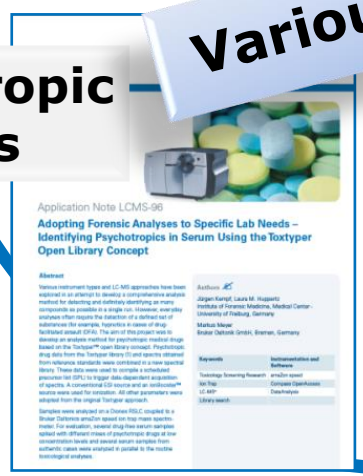
Toxtyper & DOA Drugs of Abuse

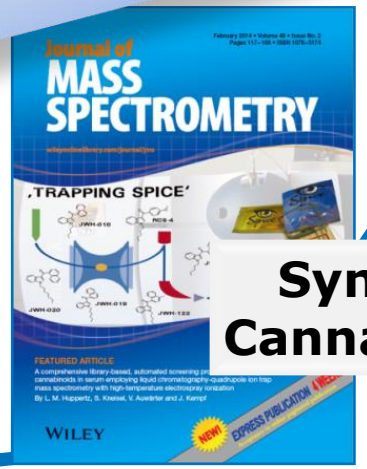


Maurer/ Wissenbach/ Weber

Various libraries available

Psychotropic Drugs





Synthetic Cannabinoids

Toxtyper 3.0 library: 1187 compounds from various classes

Antidepressants
Amitriptyline
Amitriptylinoxide
Clomipramine
Desipramine
Dibenzepin
Dosulepine
Doxepin
Duloxetine
Imipramine
Melitracen
Nortriptyline
Oxypertine
Protriptyline
Reboxetine

Analgesics
Acetylsalicylic acid
Amidopyrine
Fentanyl
Nifenazone
Salicylamide
Salicylic acid

Benzodiazepines
7-Aminoflunitrazepam
Alprazolam
Bromazepam
Brotizolam
Chlordiazepoxide
Clobazam
Clobenzepam
Clonazepam
Clotiazepam
Delorazepam
Desalkylflurazepam
Diazepam
Estazolam
Flunitrazepam
Lorazepam
Lormetazepam
Medazepam
Metaclazepam
Midazolam
Nitrazepam
Nordazepam
Oxazepam
Prazepam
Temazepam
Tetrazepam

Opiates
Codeine
Heroin
Hydrocodone
Morphin-3-beta-D-glucuronide
Morphine
Morphine-6-beta-D-glucuronide
Oxycodone
Oxymorphone
Papaverin

Antifungal, Antibacterial …
Griseofulvin
Isoconazole
Naftifine
Ornidazole
Phthalylsulfathiazole
Sulfadiazine
Sulfadoxine
Sulfaethidole
Sulfalene
Sulfamerazine
Sulfamethizole
Sulfapyridine
Terconazole
Tolnaftate

Metabolites
Desmethyl-chlordiazepoxide
Desmethyl-Citalopram
Desmethylclobazam
Desmethylclomipramine
Desmethylclozapine
Desmethyl-Mirtazapine
Desmethylvenlafaxine
Nordoxepine
THC-COOH
THC-OH

Amphetamines
Amphetamine
MBDB
MCPP
MDA
MDDMA
MDEA
MDMA
Methamphetamine
PMMA
Pseudoephedrine
Sibutramin

Pesticides
Alachlor
Flurochloridone
Isoproturon
Metamitron
Methabenzthiazuron
Methoprotryne
Metsulfuron-methyl
Monolinuron
Napropamide
Sebuthylazine
Terbuthylazine
Terbutryn
Triasulfuron

Antihistaminics
Bamipine
Cyclizine
Isothipendyl
Mequitazine
Methaphenilene
Oxomemazine
Pyribenzamine
Pyrilamine
Tritoqualine

Synthetic Cannabinoids
AM-1220
AM-2233
JWH-007
JWH-015
JWH-018
JWH-019
JWH-020
JWH-072
JWH-073
JWH-081
JWH-122
JWH-122-5-fluoropentyl-derivate
JWH-200
JWH-210
JWH-250
JWH-307
JWH-387
JWH-398
JWH-412
Methanandamide
RCS-4 ortho-isomer

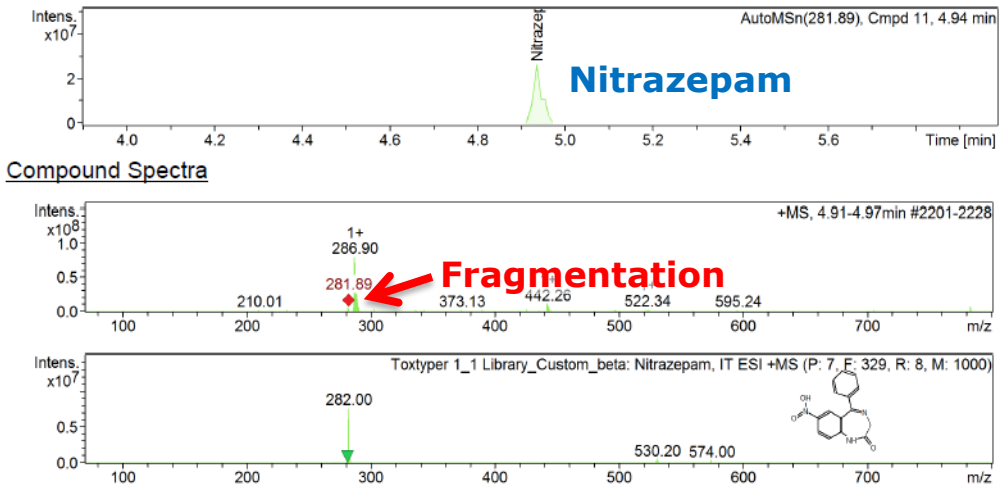
And many more …

Identification by MS^2 and MS^3: Nitrazepam

Extracted ion chromatogram

MS Spectra

experimental vs. library



Identification by MS^2 and MS^3: Nitrazepam

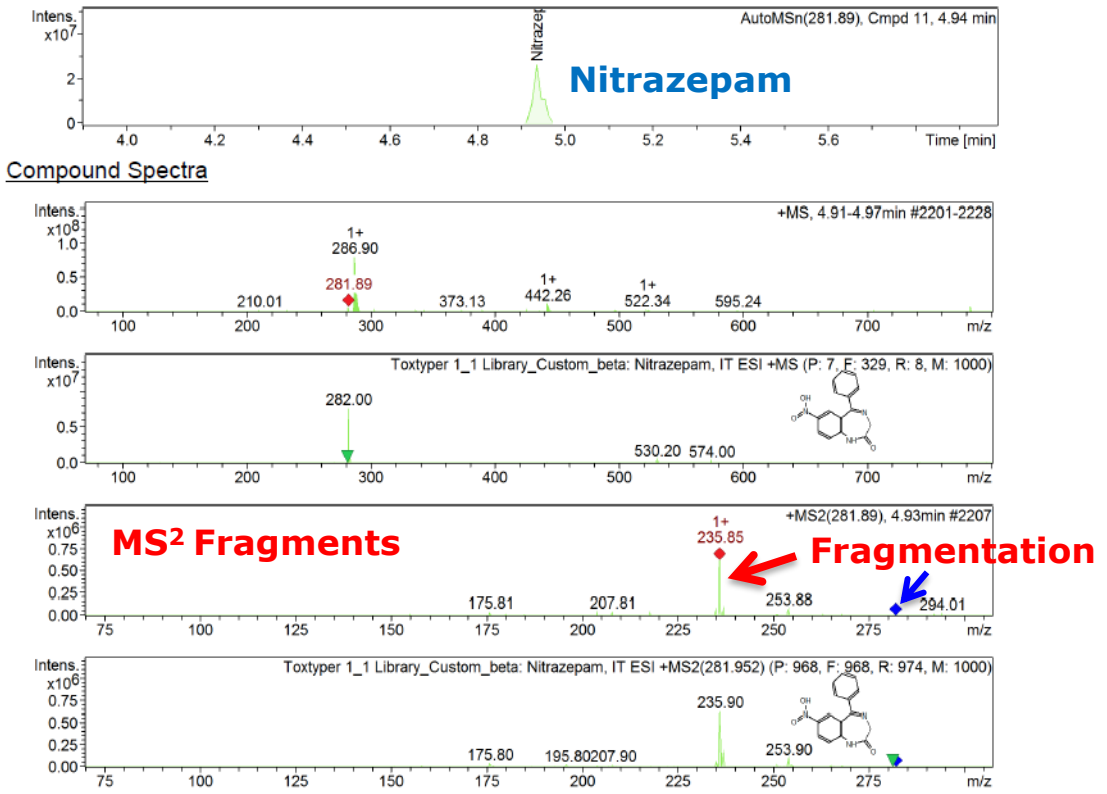
Extracted ion chromatogram

MS Spectra

experimental vs. library

MS^2 Spectra

experimental vs. library



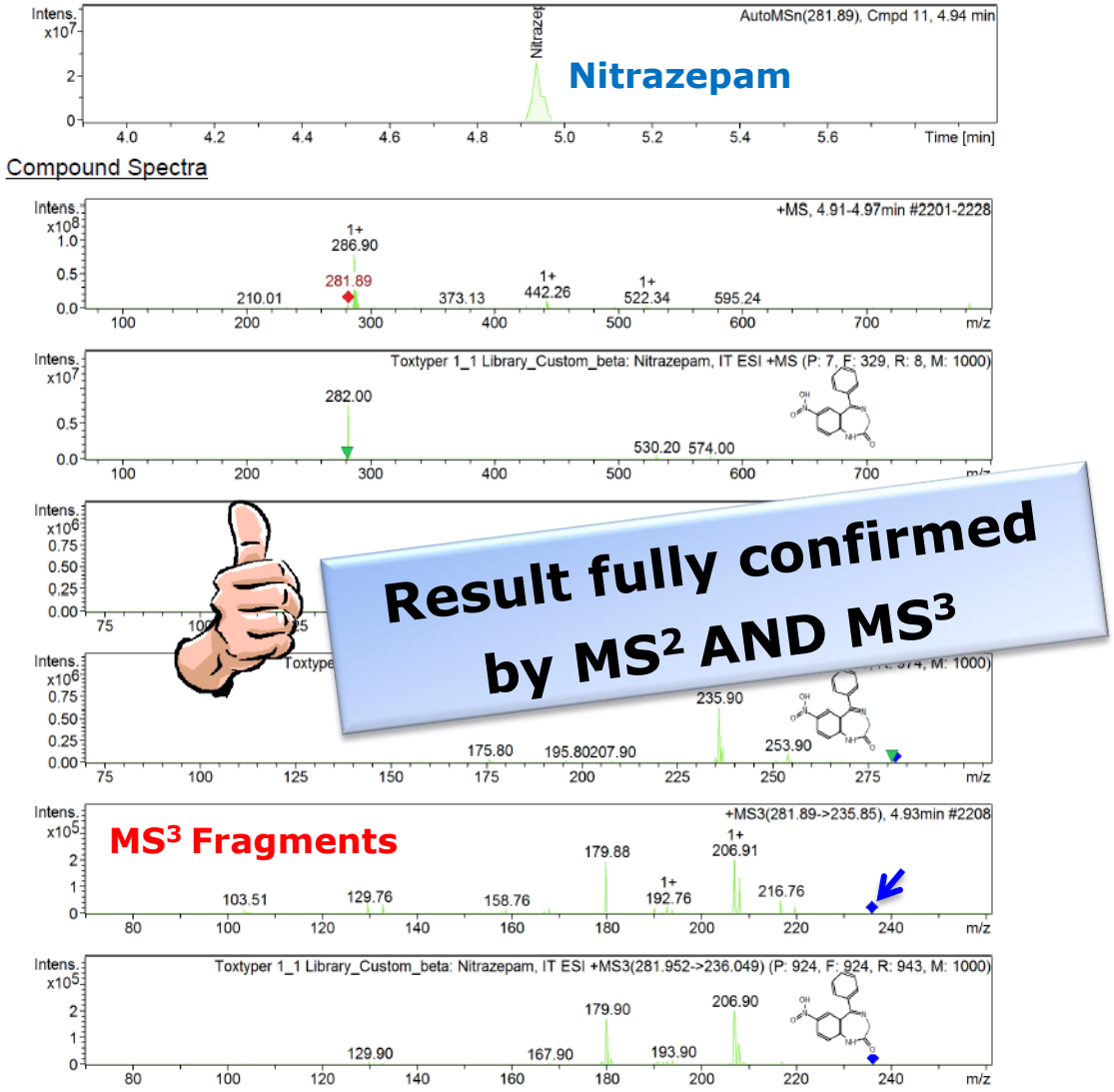
Identification by MS^2 and MS^3: Nitrazepam

Extracted ion chromatogram

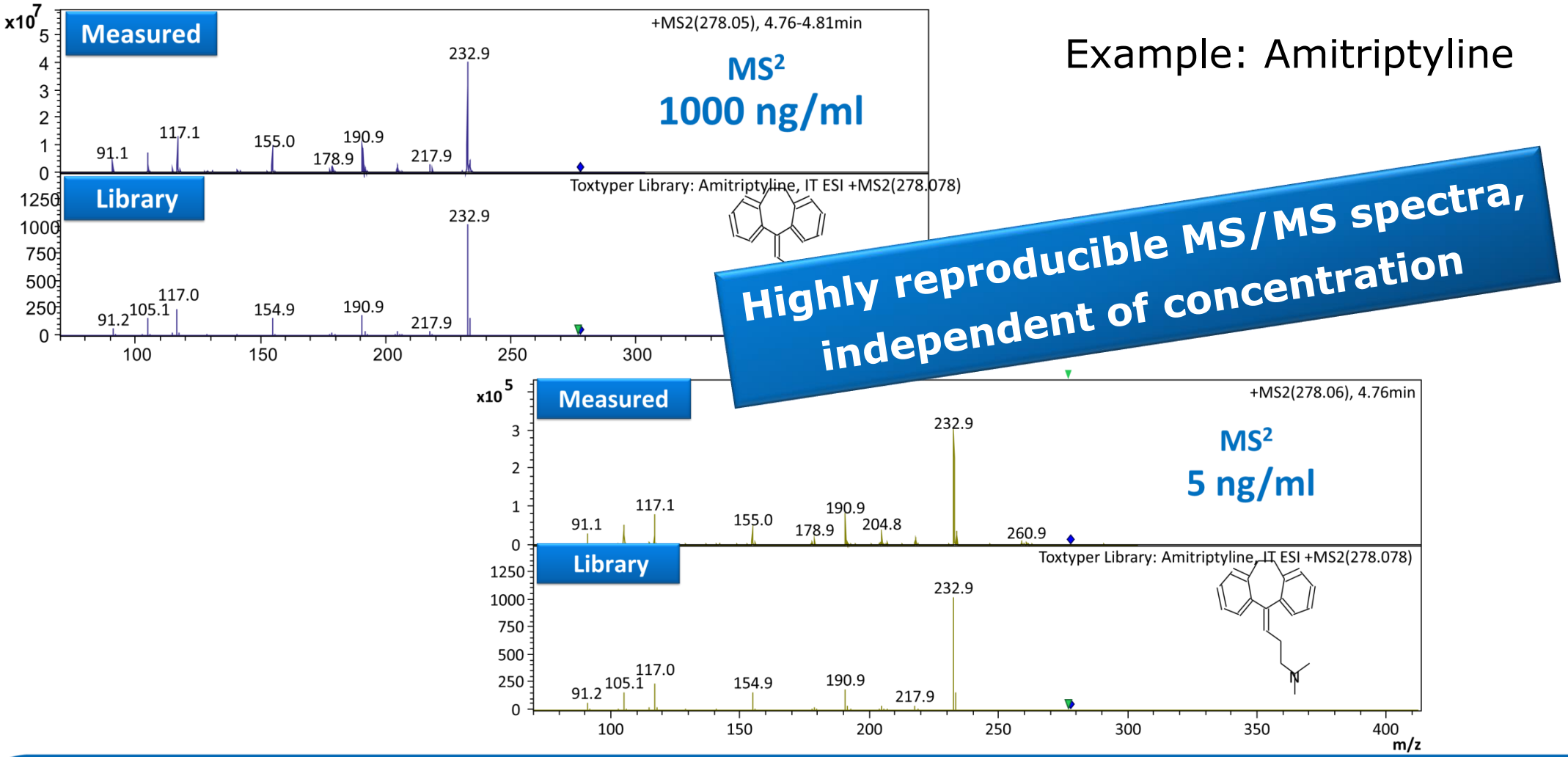
MS Spectra
experimental vs. library

MS^2 Spectra
experimental vs. library

MS^3 Spectra
experimental vs. library



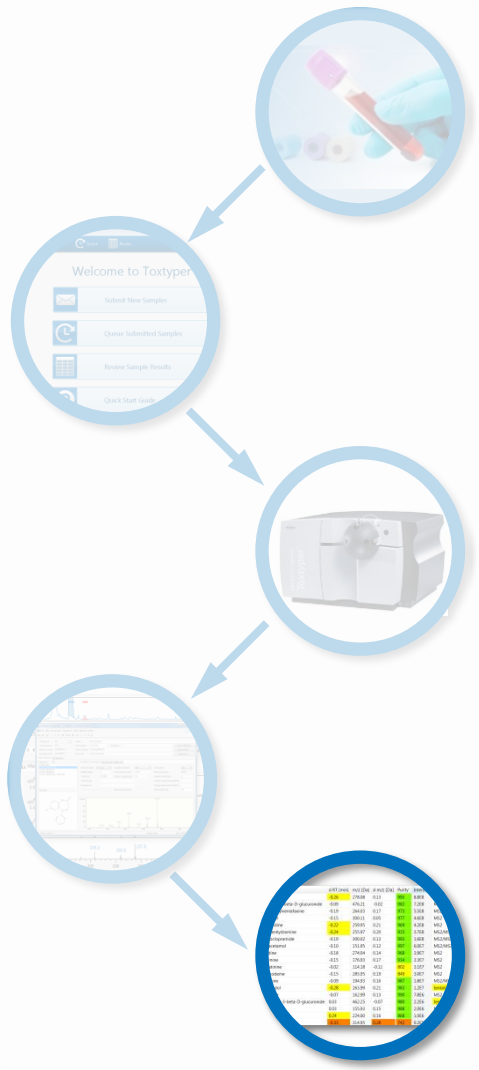
MS^n reproducibility independent of concentration



Example: Amitriptyline

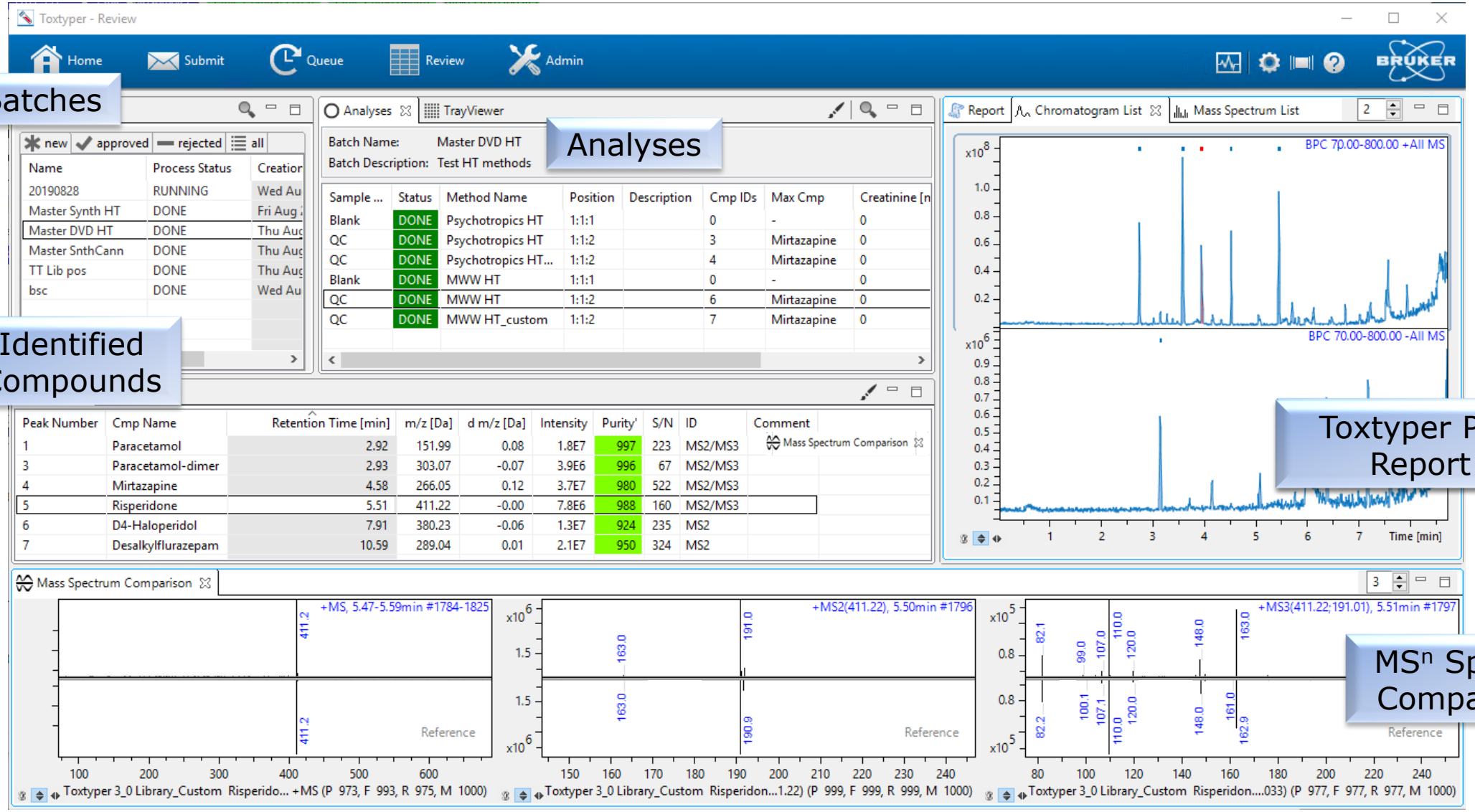
Highly reproducible MS/MS spectra, independent of concentration

Automated result reporting



- Toxtyper provides a **simple list with identified compounds** and few tentative hits which require manual validation.
- **Color coding** can be applied in order to facilitate the review process.
- The Toxtyper software allows for fast **interactive result evaluation** using chromatogram and MS^n spectra views.
- All results are available as a **PDF report** that can be directly printed or sent via e-mail.

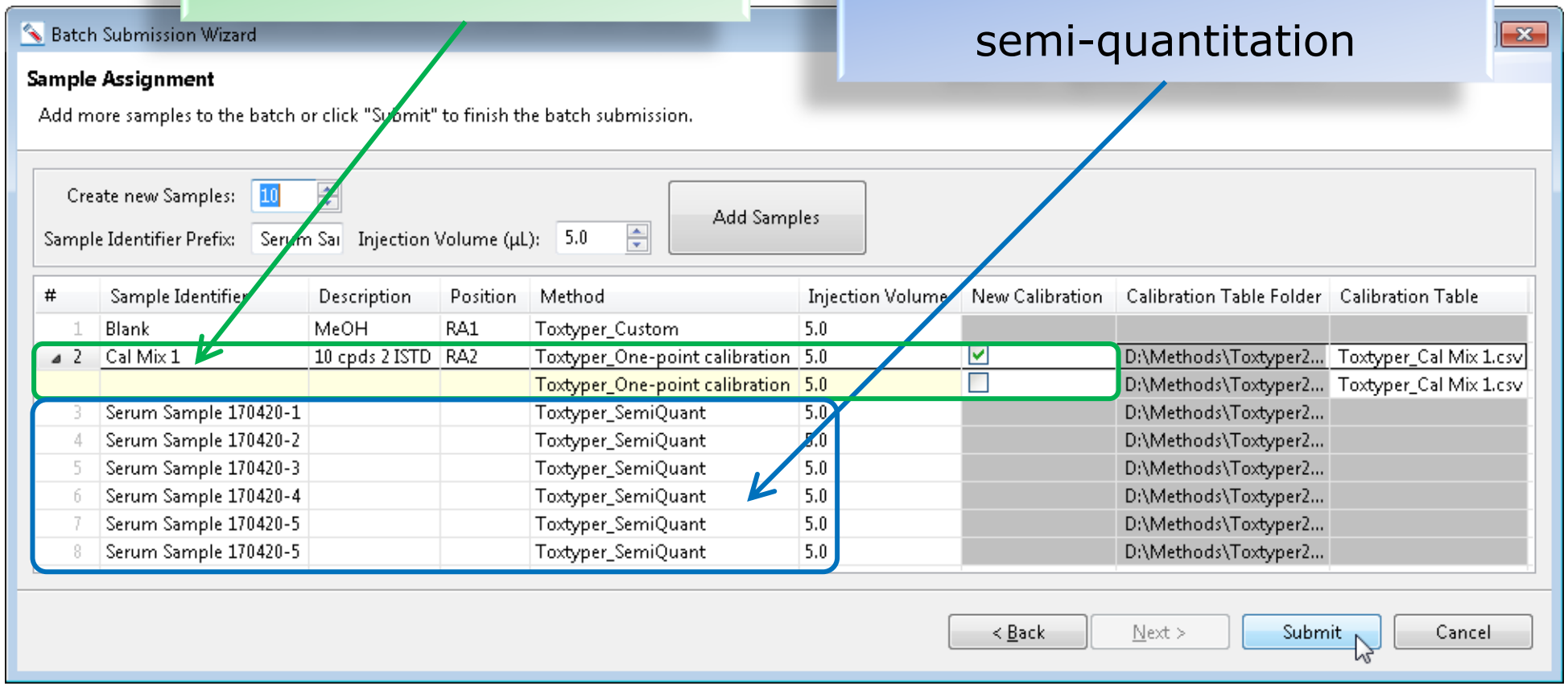
Toxtyper review perspective: All at a glance



Batch submission for semi-quantification

1. Injection of calibration standards

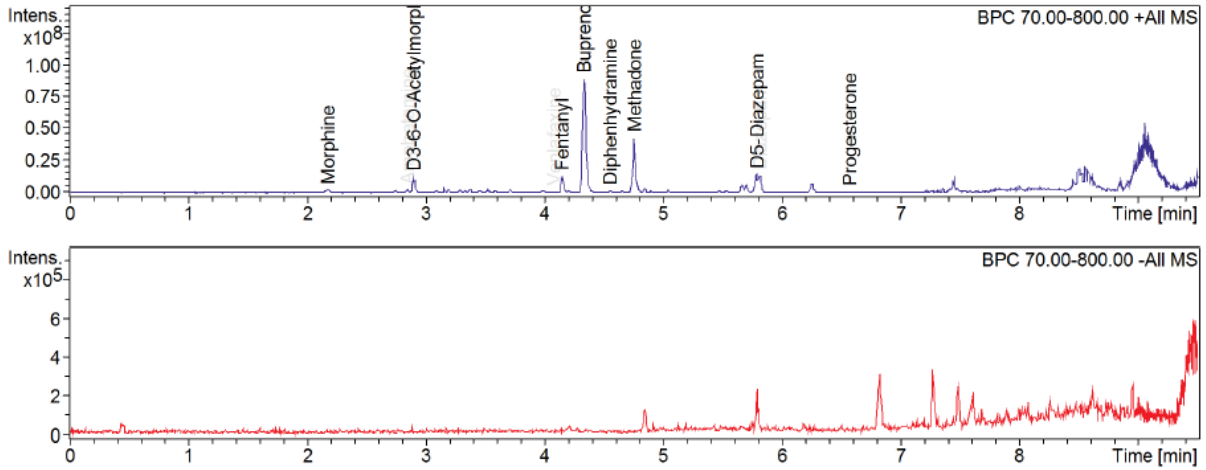
2. Sample analysis including semi-quantitation



Semi-quantification results

Sample-ID	SQM 50	Station	Toxtyper2
Submitter	Tony Technician	Method	Toxtyper_SemiQuant (1.1.1)
Analysis Name	SQM 50_RB2_01_507.d	Acquisition Date	11/9/2016 3:10:50 PM
Sample Description			

Base Peak Chromatogram



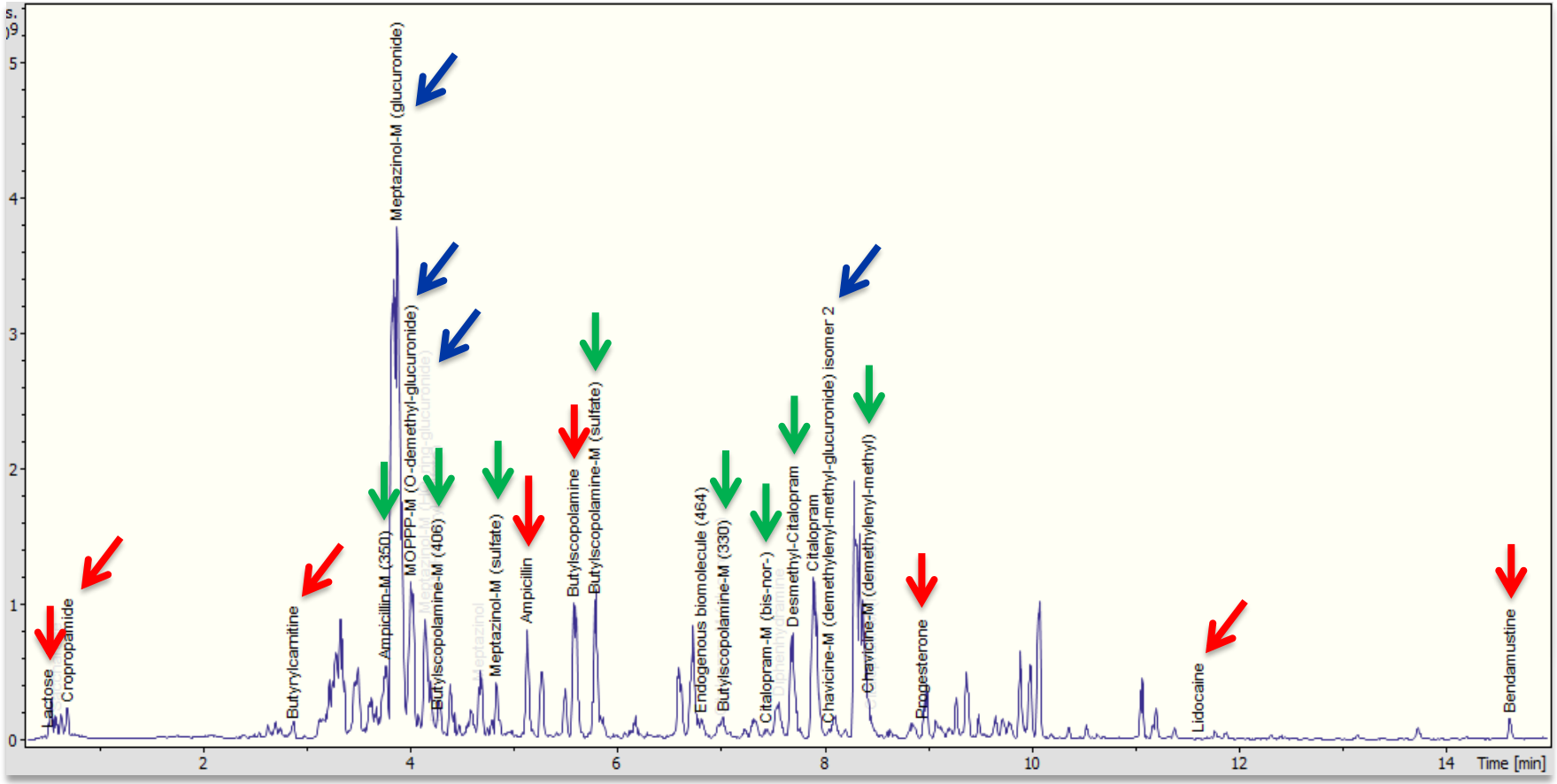
Library Search Results

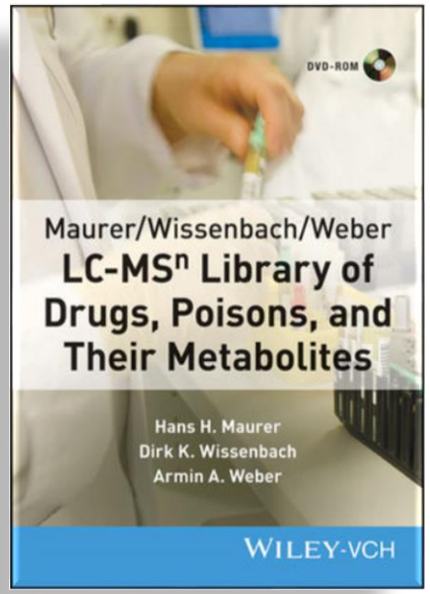
Cmp Name	Cmp #	Purity'	RT [min]	d RT	m/z [Da]	d m/z	Intensity	Semi-quant Conc.	ID	Comment
Buprenorphine	6	917	4.33	-0.08	468.31	0.00	1.0 E8	90 ng/mL	MS2	
Methadone	8	982	4.76	-0.02	310.11	0.11	4.3 E7	54 ng/mL	MS2/MS3	
Diazepam	10	972	5.81	-0.11	284.89	0.19	1.5 E7	164 ng/mL	MS2	
D5-Diazepam	9	986	5.79	-0.10	289.95	0.16	1.5 E7	109 ng/mL	MS2	
Fentanyl	5	994	4.15	-0.13	337.21	0.02	1.5 E7	12 ng/mL	MS2	
D3-6-O-Acetylmorphine	3	970	2.90	-0.08	331.17	0.00	1.3 E7	44 ng/mL	MS2	
Amphetamine	2	993	2.85	0.07	136.07	0.03	2.6 E6	262 ng/mL	MS2/MS3	
Morphine	1	887	2.18	-0.02	285.99	0.15	2.6 E6	80 ng/mL	MS2	
Diphenhydramine	7	866	4.55	0.16	256.02	0.15	4.9 E5		MS2/MS3	
Progesterone	11	822	6.57	-0.26	314.99	0.24	4.8 E5		MS2	
Venlafaxine	4	835	4.08	0.06	278.01	0.20	3.4 E5		tentative	MS2 unspecific

Concentration values are listed for all calibrated target compounds

Maurer/Wissenbach/Weber library for urine drug screening

Identification of parent drugs and metabolites including glucuronides





Reporting of results MWW library in alphabetical order

Extract of Toxtyper MWW report. Grouping of drugs and their metabolites.

Butylscopolamine and metabolites

Citalopram and metabolites

Meptazinol and metabolites

Butylscopolamine	38	983	5.59	360.28	-0.18	1.1 E9	MS2/MS3
Butylscopolamine artifact (-H2O)	53	934	8.10	342.22	-0.12	1.7 E8	MS2/MS3
Butylscopolamine-M (330)	44	956	7.03	330.20	-0.10	1.7 E8	MS2/MS3
Butylscopolamine-M (406)	28	931	4.26	406.25	-0.15	1.3 E8	MS2/MS3
Butylscopolamine-M (HO-aryl)		831	4.26	406.25	-0.15	1.3 E8	MS2/MS3
Butylscopolamine-M (HO-aryl-sulfate)	27	873	4.25	456.23	-0.13	8.0 E7	MS2/MS3
Butylscopolamine-M (sulfate)	39	958	5.78	440.19	-0.09	1.1 E9	MS2/MS3
Butyrylcarnitine	13	845	2.87	232.06	0.04	6.1 E6	MS2/MS3
Chavicine-M (demethylenyl-)	62	721	10.03	274.09	0.01	1.3 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl)	56	929	8.43	288.15	-0.05	3.0 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl-glucuronide)		875	8.43	288.15	n/a	3.0 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl-glucuronide) isomer 1	42	897	6.82	464.28	-0.18	9.0 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl-glucuronide) isomer 2		880	6.82	464.28	-0.18	9.0 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl-glucuronide) isomer 2	52	995	8.05	464.25	-0.15	8.2 E7	MS2/MS3
Chavicine-M (demethylenyl-methyl-glucuronide) isomer 1		993	8.05	464.25	-0.15	8.2 E7	MS2/MS3
Citalopram	51	981	7.90	325.18	-0.07	1.2 E9	MS2/MS3
Citalopram-M (N-oxide)	57	912	8.45	341.07	0.03	1.5 E7	MS2/MS3
Citalopram-M (bis-nor-)	48	857	7.44	297.16	-0.06	7.1 E7	MS2/MS3
Citalopram-M (glucuronide)	43	953	7.01	501.20	-0.10	4.1 E7	MS2/MS3
Clobazam-M/artifact (HO-glucuronide +NH4)	64	810	11.54	510.35	-0.25	5.2 E6	MS2
Corticosterone	47	792	7.40	347.26	-0.21	8.9 E6	MS2/MS3
Creatinine-M/artifact (227)	4	997	0.53	227.01	0.09	2.9 E8	MS2/MS3
Cropropamide	7	782	0.78	241.04	0.06	3.1 E7	MS2/MS3
Desmethyl-Citalopram	50	991	7.70	311.25	-0.19	8.0 E8	MS2/MS3
Diisooctylphthalate	72	978	16.70	391.27	-0.17	3.0 E7	MS2/MS3
Diphenhydramine	49	910	7.56	256.11	-0.28	2.8 E8	MS2/MS3
Diphenhydramine-M/artifact (N-oxide dimer)	55	946	8.29	543.34	-0.34	1.9 E9	MS2/MS3
Isovalerylcarnitine	17	796	3.56	245.91	0.19	4.9 E7	MS2/MS3
Lactose	2	841	0.50	365.12	-0.02	2.0 E7	MS2/MS3
Meptazinol	30	959	4.67	234.12	-0.13	3.8 E8	MS2/MS3
Meptazinol-M (HO-ring-glucuronide)	25	890	4.15	426.26	-0.16	8.9 E8	MS2/MS3
Meptazinol-M (glucuronide)	21	979	3.88	410.26	-0.16	3.8 E9	MS2/MS3
Meptazinol-M (nor-glucuronide)	22	974	4.00	396.27	-0.17	1.2 E9	MS2/MS3
Meptazinol-M (sulfate)	32	972	4.83	314.16	-0.06	4.1 E8	MS2/MS3

Toxtyper versus Toxtyper MWW method Summary

	Toxtyper	MWW (2018)
Total no. of library compounds	1187	> 4500
Metabolites	Some	3000
Polarity	Positive and negative ion mode MS and MS^n	Positive ion mode MS^n spectra only
Retention time information	Yes, for all compounds	No
Use of SPL* for MS^n acquisition	Yes	No, completely untargeted, data dependent acquisition
Identification based on	Precursor *m/z* MS^n spectral information Retention time	Precursor *m/z* MS^n spectral information Related Metabolites

*Scheduled Precursor List

- **Toxtyper users**
  and their main applications

Selected Toxtyper References

Forensics/Clinical – Forensics – Clinical – Police

- **Dr. Michael Böttcher**, MVZ Labor Dessau GmbH, Germany
- **Dr. Eric Franssen,** Onze Lieve Vrouwe Gasthuis OLVG Amsterdam, Netherlands
- **Dr. George W. Hime**, Miami Dade County Medical Examiner, USA
- **Prof. Dr. Thomas Krämer**, Forensic & Pharmacology, Zürich, Switzerland
- **Dr. Natalia Krupina**, Moscow Regional Bureau of Forensic Medicine, Russia
- **Prof. Dr. Volker Auwärter**, Jürgen Kempf, Laura Huppertz; Institute of Forensic Medicine Freiburg; Germany
- **Dr. Ivana Černá, Ústřední vojenská nemocnice, Praha, Česká republika**
- **Prof. Markus R. Meyer, (Prof. Dr. Dr. Hans Maurer)**, Experimental & Clinical Toxicology, Homburg, Germany
- **Prof. Dr. Wieland**, Katharinenhospital, Stuttgart, Germany → SYNLAB Leinfelden
- **Prof. Dr. Denis Hochstrasser, Dr. Pierre Lescuyer**; Hôpitaux Universitaires de Genève, Switzerland

Selected Toxtyper References

Forensics/Clinical – Forensics – Clinical – Police

- **Dr. Christian Vidal**, State Police (LKA) Lower Saxony, Germany
- **Dr. Michael Pütz, Dr. Lars Müller,** Federal Police (BKA) Wiesbaden, Germany
- **Colonel Dr. Theerin Sinchai,** Royal Thai Police, Thailand
- **Dr. Kristian Wittke**, State Police (LKA) Berlin, Germany

Dr. Michael Böttcher, MVZ Labor Dessau

User Profile

- Biggest private drug testing lab in Germany (Limbach group)
- Therapeutic drug monitoring, drugs of abuse testing, workplace drug testing, intoxication cases, clinical drugs testing, especially for addiction medicine.
- Use of mainly GC-MS for the General Unknown Screening before Toxtyper

Toxtyper value for the general unknown screening (GUS)

- Rapid sample preparation for urine samples: no hydrolysis, no derivatization
- Comprehensive MWW library including metabolites (glucuronides!!!)
- Ease of training and simple data evaluation
  - saves time and money by increasing through-put
  - facilitates overnight and weekend service, e.g. in case of intoxications

Since 2016 a second Toxtyper system is in use for saliva screening.

Dr. Michael Böttcher, MVZ Labor Dessau

Bruker "Customer Insight"

LC-MS for Toxicology: Pushing the Limits o Speed and Sensitivity in Drug Screening

Ground-breaking LC-MS solutions enable general unknown analysis of drugs in biological samples, at the specialized laboratory of Dr. Michael Böttcher, MVZ Labor-Dessau GmbH

Dr. Michael Böttcher's specialist medical laboratory uses cutting-edge toxic instrumentation to offer screening services for clinics and organizations Europe.

"We rely on Bruker's robust LC-MS/MS solution, the Toxtyper™, to rapidly biological samples for drugs and drugs of abuse, with unprecedented accuracy.

"We were extremely impressed with the sensitivity, ease of use and rapid sample preparation."

"Post-run time is also greatly reduced on the Toxtyper™ compared to GC-MS. Data-mining is more efficient, and you don't need as much experience."

Dr. Michael Böttcher, MVZ Labor Dessau

Streamlining sample preparation: GC-MS vs. Toxtyper

Timetable for GC/MS (1 urine sample)

- Enzymatic hydrolysis:	**2.25 h**
- Extraction:	0.60 h
- Derivatization:	0.30 h
- Chromatographic run + cooling:	0.30 h (17 min +2 min)
	3.45 h = 3 h 25 min

-- All sample preparation steps with different recovery

-- Different „on board“ stability for the different substances

-- Urine samples to be analysed batchwise

-- Acidic substances need separate extraction and derivatization

Timetable for Toxtyper (1 sample)

- Enzymatic hydrolysis:	-
- Extraction:	0.50 h
- Derivatization:	-
- Chromatographic run:	0.50 h (TT-
	1.00 h

Allows continuous analysis for all kind of samples

-- No extraction losses

-- No additional stability problems

-- Glucuronides detectable (MWW 2014 library)

“It became clear that the hydrolyzing process for urine analysis is really limiting in GC-MS. If you can avoid it, it’s a big step forward. Oral fluid is such a clean matrix; the Toxtyper™ can achieve extreme sensitivity, which was unexpected at the beginning.”

- Rapid sample preparation enables random access for urgent samples.
- For GC-MS this is uneconomical as sample prep is cumbersome and thus done batch-wise.

Prof. Dr. Wieland / Dr. Shipkova

User Profile

- Central Institute for Laboratory Medicine and Clinical Chemistry, Klinikum Stuttgart
- Toxtyper was purchased in 2016 by Prof. Wieland and Dr. Shipkova

Toxtyper value

- Use of Toxtyper for screening of drugs and toxins in blood, saliva and urine.
- Replacement of GC-MS and a couple of targeted LC-MS/MS methods, e.g. for benzodiazepines, pregabalin and as well as synthetic opioids
- *"The Toxtyper is user-friendly, provides a reasonable solution for a 24 h/365 day emergency setting, and will not overburden the technical skills of laboratory technicians with minimal LC-MS experience"*[1)]

In 2018 Prof. Wieland and Dr. Shipkova changed to SYNLAB (Leinfelden) and purchased a Toxtyper to offer the same analytics at the new site.

[1)] *Clinical Mass Spectrometry 4–5 (2017) 11–18*

Identification and detection limits in urine

Analyte	LLOI/LLOD Toxtyper "MWW" [µg/L]	LLOI/LLOD Toxtyper "DOAL" [µg/L]	Cut Off Immunoassay [µg/L]	LLOD of GC- or LC with MS Detection [µg/L]
Acetylcodeine	11/40	10/20	300	10
Amphetamine	n.d.	100/200	500	150
Benzoylecgonine	22/50	9/10	300	75[1]
Methadone	3/5	2/5	300	40
Nordiazepam	80/200	50/100	200	125
Sufentanil	2/10	1/1	n.a.	1
Pregabalin	430/2000	430/500	n.a.	200

Urinary creatinine concentrations were between 50 and 350 mg/dl. n.d. = not determined; n.a. = not applicable. LLOI = lower limit of identification; LLOD = lower limit of detection.

[1] confirmation by GC-MS was based on cocaine detection.

* LLOI was calculated, LLOD was experimentally established.

Simplified automated sample preparation for urine with Tecan robot: Hydrolysis, precipitation (incl. addition IS), 10fold dilution

- MWW:
  Maurer/Wissenbach/Weber method and library
- DOAL
  Toxtyper drugs of abuse method and library (83 compounds)

Publications on urine (2017) and oral fluid screening (2018)

Contents lists available at ScienceDirect

Clinical Mass Spectrometry

journal homepage: www.elsevier.com/locate/clinms

Detection of drugs of abuse in urine using the Bruker Toxtyper™: Experiences in a routine clinical laboratory setting

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ARTICLE INFO

Keywords:
Drugs of abuse screening
Assay performance
Ion trap mass spectrometry
Routine laboratory

ABSTRACT

Urine screening can be used to detect misuse of illicit drugs and validate opioid replacement therapy comp
It is common that immunochemical assays are combined with GC-MS for these applications. Bruker has r
released an ion trap mass spectrometer, called Toxtyper™, with the potential to replace current screen
gorithms to detect drug misuse.

Here, we compare our current strategy of urine screening for misuse of cannabis, amphetamines, c
opiates, benzodiazepine, methadone, sufentanil, and pregabalin to the Toxtyper protocols provided
manufacturer.

The analytical performance of the instrument was determined on a selected drug panel and with 18
samples being compared to establish concordance between our currently established approach and the To

The lower limits of detection and identification for acetylcodeine, amphetamine, benzoylecgonine,
done, and nordiazepam were below the common cut-offs for immunological screening assays and compar
GC-MS. Imprecision and accuracy, both within- and between-series, were consistently < 25%. To
screening for pregabalin and sufentail was less sensitive than a targeted LC-MS/MS assay. Concordance
predefined criterion of > 90% for all drugs, except for pregabalin. Cannabis misuse could not be detected
the limited sensitivity of the Toxtyper assay protocols used and the inherent imprecision of the assay.

Our study has revealed that a considerable portion of our current time-consuming protocol for scr
drugs of abuse in urine, based on the combination of multiple analytical methods, could be consolidated
Toxtyper for a majority of the most-relevant substances in our patient population.

Ther Drug Monit. 2018 Jun 6. doi: 10.1097/FTD.0000000000000544. [Epub ahead of print]

Evaluation of an Ion Trap LC-IT/MS Instrument (Toxtyper) for Drug of Abuse Screening in Oral Fluid.

Plecko T[1], Berbalk K, Wieland E, Shipkova M.

Author information

1 Zentralinstitut für Klinische Chemie und Laboratoriumsmedizin, Klinikum Stuttgart, Stuttgart, Germany.

Abstract

BACKGROUND: Oral fluid (OF) is increasingly used as an alternative sample matrix in drug of abuse (DOA) screening. Screening is commonly performed by immunoassays and results confirmed using laborious GC-MS based methods. Therefore, an easy to operate ion trap mass spectrometric (IT-MS) commercial screening method (Toxtyper, Bruker Daltronik, Bremen, Germany) combined with a laboratory-developed sample preparation procedure have been evaluated for their application to OF.

METHODS: Oral fluid samples were subjected to protein precipitation followed by HybridSPE-phospholipid extraction. Chromatographic separation was achieved by ultra-high performance liquid chromatography (UHPLC); MS2/MS3 spectra were recorded by IT-MS and analyzed using a library provided by the manufacturer (Bruker Daltronik). The lower limit of detection (LLOD), linearity, imprecision, inaccuracy, and specificity (interferences, matrix effects) were investigated for methadone, buprenorphine, pregabalin, fentanyl, amphetamine, MDMA (3,4-Methylendioxy-N-methylamphetamin), cocaine, acetylcodeine and nordiazepam, after

Prof. Maurer, Experimental and Clinical Toxicology, Saarland University

Prof. Hans Maurer

RESEARCH ARTICLE

WILEY

Blood plasma level determination using an automated LC–MS^n screening system and electronically stored calibrations exemplified for 22 drugs and two active metabolites often requested in emergency toxicology

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Abstract

Fast and comprehensive qualitative and quantitative methods preferably by gas chromatography–mass spectrometry (GC–MS) and/or liquid chromatography–mass spectrometry (LC–MS) are needed to support the (differential) diagnosis of acute poisonings in emergency toxicology. One option is a commercially available qualitative screening solution based on LC–MS^n (Bruker Daltonik Toxtyper™, TT). Identified and toxicologically relevant compounds should be quantified to assess severity of poisonings. The aim of the present study was to test the TT system for quantification simultaneous with the screening process in blood plasma exemplified for 22 relevant drugs and two active metabolites. A standard liquid–liquid extraction was used for sample work-up followed by 1:5 dilution of the final extracts. They were analyzed using the TT system consisting of a Bruker amaZon speed ion trap and a Thermo Fisher Dionex Ultimate 3000 LC system. Plasma levels were assessed using full-scan data and an electronically stored five-point calibration. The calibration model was linear for the studied ranges and could be used for at least two months. The method was validated according to international guidelines. The acceptance criteria recommended for emergency toxicology for accuracy and precision were fulfilled for all tested compounds, but bromazepam, lorazepam, oxycodone, and prothipendyl could reliably be determined only above the therapeutic range. In conclusion, the presented procedure allowed the combination of a comprehensive LC–MS^n screening with fast automated assessment of plasma levels for emergency toxicology of tested compounds.

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DOI: 10.1002/dta.2466

Calibrator and QC concentrations of 24 target compounds

TABLE 1 Final plasma concentrations in µg/L of the analytes in calibrators (Cal 1–5) and quality control samples (QC) LOW, therapeutic (TH), HIGH, and dilution (DILU) as well as the used weightings of linear calibration model and the therapeutic ranges according to Schulz et al.[32]

Analyte	Weighting	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	QC LOW	QC TH	QC DILU	QC HIGH	Therapeutic Range
9-Hydroxyrisperidone	$1/x^2$	100	500	1,000	1,500	2,000	120	100	800	1,600	10–100
Amitriptyline	$1/x^2$	100	500	1,000	1,500	2,000	120	200	800	1,600	50–200
Bromazepam	equal	1,000	1,500	2,000	2,500	3,000	1,200	200	1,200	2,400	50–200
Carbamazepine	$1/x^2$	2,500	5,000	10,000	15,000	20,000	3,000	12,000	-	16,000	2,000–12,000
Citalopram	$1/x^2$	100	500	1,000	1,500	2,000	120	200	800	1,600	20–200
Clozapine	$1/x^2$	100	500	1,000	1,500	2,000	120	600	800	1,600	100–600
Codeine	$1/x^2$	250	500	1,000	1,500	2,000	300	250	800	1,600	30–250
Diazepam	equal	500	1,000	1,500	2,000	2,500	600	2,000	1,000	2,000	200–2,000
Diphenhydramine	equal	500	1,000	1,500	2,000	2,500	600	1,000	1,000	2,000	100–1,000
Doxepin	$1/x^2$	100	500	1,000	1,500	2,000	120	150	800	1,600	20–150
Fluoxetine	$1/x$	250	500	1,000	1,500	2,000	300	500	800	1,600	120–500
Lorazepam	equal	500	1,000	1,500	2,000	2,500	600	250	1,000	2,000	20–250
Mirtazapine	$1/x^2$	150	500	1,000	1,500	2,000	180	300	800	1,600	50–300
Nordiazepam	equal	750	1,000	1,500	2,000	2,500	900	800	1,000	2,000	200–800
O-Desmethyltramadol	$1/x^2$	100	500	1,000	1,500	2,000	120	300	800	1,600	100–300
Oxazepam	equal	1,000	1,500	2,000	2,500	3,000	1,200	2,000	1,200	2,400	500–2,000
Oxycodone	$1/x^2$	250	500	1,000	1,500	2,000	300	100	800	1,600	5–100
Paracetamol (acetaminophen)	$1/x^2$	2,500	30,000	60,000	90,000	120,000	3,000	20,000	-	96,000	10,000–20,000
Prothipendyl	$1/x^2$	50	125	250	375	500	60	10	200	400	5–10
Quetiapine	$1/x^2$	100	500	1,000	1,500	2,000	120	500	800	1,600	100–500
Risperidone	$1/x$	50	125	250	375	500	60	60	200	400	20–60
Tramadol	$1/x$	250	500	1,000	1,500	2,000	300	300	800	1,600	100–300
Venlafaxine	$1/x^2$	100	500	1,000	1,500	2,000	120	400	800	1,600	100–400
Verapamil	$1/x^2$	100	500	1,000	1,500	2,000	120	350	800	1,600	50–350

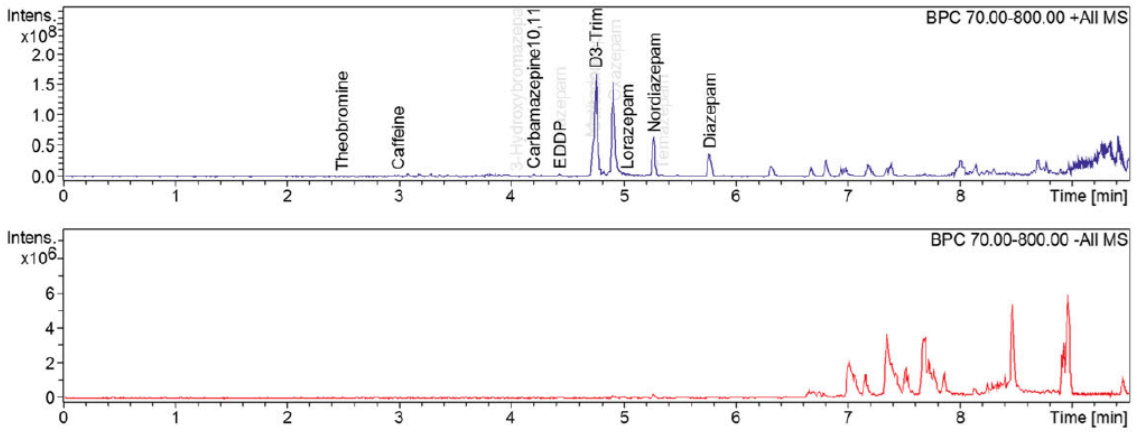
Calibration covers the therapeutic to toxic range

Toxtyper Results and Conclusions

Toxtyper Analysis Report

Sample-ID	114773_P_LLE_TTQ	Station	
Submitter		Method	toxtyper_semiquant (2.0)
Analysis Name	114773_P_LLE_TTQ_BC3_01_5294.d	Acquisition Date	5/17/2016 5:54:52 AM
Sample Description			

Base Peak Chromatogram



Library Search Results

Cmp Name	Cmp #	Purity'	RT [min]	d RT	m/z [Da]	d m/z	Intensity	Semi-quant Conc.	ID	Comment
3-Hydroxybromazepam	6	985	4.05	-0.04	331.94	0.06	6.9 E5		MS2/MS3	MS2 unspecific
Bromazepam	9	976	4.44	-0.09	315.91	0.10	3.6 E6	<1000 ng/mL	MS2	
Caffeine	2	971	3.00	-0.09	194.86	0.23	4.2 E6		MS2/MS3	
Carbamazepine	14	990	4.73	-0.15	236.89	0.21	3.3 E7	3089 ng/mL	MS2	
Carbamazepine10,11-epoxide	7	918	4.21	-0.08	252.94	0.16	5.0 E6		MS2	
D3-Trimipramine	15	922	4.75	-0.22	298.14	0.10	1.8 E8		MS2	
Diazepam	20	936	5.76	-0.16	285.00	0.08	4.1 E7	2299 ng/mL	MS2	
EDDP	8	970	4.44	-0.20	278.10	0.09	5.9 E6		MS2	
Lorazepam	17	899	5.02	-0.15	320.97	0.05	6.4 E5	<500 ng/mL	MS2/MS3	MS2 unspecific
Methadone	13	956	4.72	-0.06	310.14	0.08	5.8 E7		MS2/MS3	
Nordiazepam	18	977	5.27	-0.10	270.92	0.14	6.6 E7	>2500 ng/mL	MS2	
Oxazepam	16	977	4.92	-0.15	286.88	0.18	4.0 E6	<1000 ng/mL	MS2/MS3	MS2 unspecific
Temazepam	19	977	5.34	-0.17	300.96	0.11	4.3 E6		MS2/MS3	MS2 unspecific
Theobromine	1	900	2.49	-0.10	180.84	0.23	1.5 E6		MS2	

4 | CONCLUSIONS

The current method allowed a reliable and fast blood plasma screening and level assessment for 24 analytes in 24/7 emergency toxicology. The method was successfully integrated into the Toxtyper™ screening solution and validated according to international guidelines and recommendations. The blood levels of the analytes could be assessed automatically and quickly, based on a stored five-point calibration model and were given together with the blood screening results in a pdf report. However, the method was limited to blood level assessment in emergency toxicology to identify acute overdosing or poisoning.

George W. Hime, Elisa Shoff
Miami Dade Medical Examiner

George W. Hime, M.S., Assistant Laboratory Director and Elisa Shoff, Toxicologist II, Miami-Dade Medical Examiner Dept. Toxicology Laboratory, Miami, USA

"The Toxtyper has been our go-to instrument for postmortem casework containing novel substances, substances that are otherwise undetectable via GCMS, and unknown substances we have not yet seen in the lab. This instrument has been a blessing to our laboratory over the last several years of dealing with the flood of new illegal drugs in Miami. From synthetic cathinones to synthetic fentanyls we have detected and identified them all reliably and with high confidence. "

Benefits of using Toxtyper:
"Reliable, rugged, sensitivity beyond expectation, and ease of maintenance and operation are all features of this instrument. Full spectral MS3 data at pg/mL sensitivities in postmortem whole blood or tissues cannot be beat in our work by any other instrument."

Toxtyper in the "opioid crisis": Use of smaller sub-libraries and SPE sample preparation for urine, blood and tissue samples to enhance sensitivity for the detection of highly potent NPS such as designer opioids.

George W. Hime, Elisa Shoff
Miami Dade Medical Examiner

Qualitative Identification of Fentanyl Analogs and Other Opioids in Postmortem Cases by UHPLC-Ion Trap-MS^n

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Article

Abstract

Since 2013, the Miami-Dade County Medica
the number of opioid-related deaths. The ma
nyl into the local heroin supply. From 2014
increase in fentanyl-related deaths, followe
novel fentanyl analogs were identified in r
acetyl fentanyl. In 2016, four additional fen
butyryl fentanyl, furanyl fentanyl and carfer
to address this epidemic, a method was de
analgesic compounds in postmortem sampl
ion trap mass spectrometry with MS^n capab
from 0.1 to 5 ng/mL, with a majority having
specimens from ~500 postmortem cases we
initial screening results. Of those cases, 375 were positive for illicit fentanyl and/or one or more fentanyl analogs. Due to the potency of these compounds, they were almost always included in the cause of death. Worth emphasizing and extremely alarming is the detection of carfentanil in 134 cases, 104 of which were initially missed by gas chromatography mass spectrometry. By incorpo-

Table III. Comparison of number of positive cases for synthetic opioids on LC-Ion Trap MSn and GC–MS with respective limits of detection

Analog	No. of cases detected by LC-Ion Trap MS^n	No. of cases detected by GC–MS	Limits of detection on LC-Ion Trap MS^n (ng/mL)	Limits of detection on GC–MS (ng/mL)
Acetyl fentanyl	13	4	0.2	5
Beta-hydroxythiofentanyl	9	0	0.1	a
Butyryl fentanyl	3	1	0.2	0.5
Carfentanil	134	30	0.1	1
Furanyl fentanyl	37	9	0.5	2.5
4-Fluoroisobutyryl fentanyl	22	16	0.5	1
U-47700	4	1	0.5	–

[a]Beta-hydroxythiofentanyl cannot be detected via GC–MS.

Prof. Volker Auwärter, Dr. Jürgen Kempf
Inst. of Forensic Medicine, Freiburg

User Profile

- Leading national institute in forensic toxicology
- World-renowned expertise in the analysis of New Psychoactive Substances (NPS)
- Drug testing (forensic and clinical), intoxication and death case analysis, full post-mortem toxicology, drug market monitoring

Toxtyper value

- Comprehensive and cost-effective pre-screening
- Quick and easy update of Toxtyper libraries to keep pace with emerging NPS
- Use for all kind of biological samples as well as for drug preparations

"It is often the case that, if they could, customers would always like to have the whole picture of xenobiotics present in the sample. But money is a restriction for them: if you want to conduct a full toxicological analysis, the price will be very high. This is why it's valuable to us to be able to offer broad screening with the Toxtyper™, covering almost all relevant analytes, at a lower price. It's a benefit to us and to them." explains Prof. Dr. Auwärter.

From: Bruker Customer Insights

Example: Semi-quantification of designer benzodiazepines using Toxtyper

Dr. Jürgen Kempf

- Use of a benzodiazepine sub-library
- LODs in serum between 1-25 ng/mL
- Two exceptions: 3-OH-Bromazepam and Demoxepam (50 ng/mL)

Name	LOD [ng/mL]
2-OH-Ethylflurazepam	25
3-OH-Bromazepam	50
3-OH-Flubromazepam	25
3-OH-Phenazepam	25
7-Aminoclonazepam	10
7-Aminoflunitrazepam	1
7-Aminonitrazepam	1
Adinazolam	5
α-OH-Alprazolam	5
α-OH-Midazolam	5
α-OH-Triazolam	5
Alprazolam	1
Bromazepam	10
Chlordiazepoxide	5
Clobazam	1
Clonazepam	5
Clonazolam	5
Cloniprazepam	1
Clotiazepam	1
Delorazepam	5

Name	LOD [ng/mL]
Demoxepam	50
Desalkylflurazepam	10
Deschloroetizolam	1
Diazepam	5
Diclazepam	1
Etizolam	1
Flubromazepam	5
Flubromazolam	5
Fludiazepam	10
Flunitrazepam	1
Flunitrazolam	5
Flurazepam	1
Fonazepam	5
Loprazolam	1
Lorazepam	5
Lormetazepam	5
Meclonazepam	5
Medazepam	5
Metizolam	1
Midazolam	1

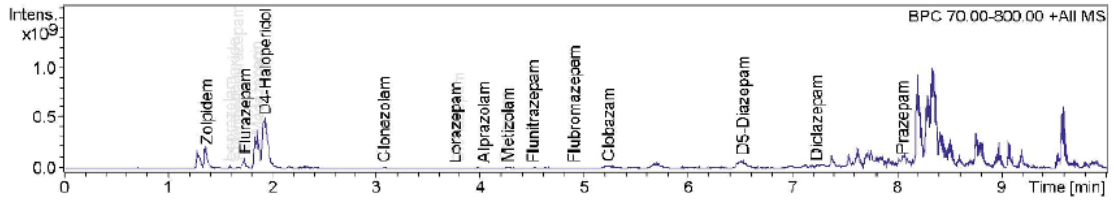
Name	LOD [ng/mL]
Norflunitrazepam	5
Nifoxipam	-
Nimetazepam	10
Nitrazepam	25
Nitrazolam	5
Norclobazam	10
Nordazepam	5
Oxazepam	25
Phenazepam	5
Prazepam	1
Pyrazolam	5
RO-5-4864	10
Temazepam	1
Tetrazepam	5
Triazolam	5
Zaleplone	5
Zolpidem	1
Zopiclone	5

Application Note LCMS 130: Detection and semi-quantitative determination of designer benzodiazepines in serum using LC-MSn

Example: Semi-quantification of designer benzodiazepines using Toxtyper

Sample - ID		Station	Trapinski
Submitter	JK	Method	benzotyper b06a semiquant
Analysis Name	BenzoMix I QC 5ngml_13890.d	Acquisition Date	8/9/2016 6:11:48 PM
Sample Description	Serum		

Base Peak Chromatogramm



Library Search Results

Cmp Name	Cmp #	Purity	RT [min]	d RT	m/z [Da]	d m/z	Intensity	Semi-quant Conc.	ID	Comment
D4-Haloperidol	9	938	1.92	0.08	380.06	0.14	4.7 E8	48 ng/ml	MS2/MS3	
D3-Doxepin	7	951	1.85	0.07	282.96	0.14	3.7 E8	49 ng/ml	MS2/MS3	
Zolpidem	1	973	1.37	0.04	308.02	0.08	10.0 E7	7 ng/mL	MS2/MS3	
Flurazepam	5	992	1.74	0.08	387.93	0.27	8.9 E7	6 ng/mL	MS2/MS3	
Prazepam	21	996	8.04	0.07	324.94	0.16	8.4 E7	5 ng/mL	MS2/MS3	
D5-Diazepam	19	991	6.52	-0.16	289.88	0.12	7.5 E7		MS2/MS3	
Medazepam	8	912	1.86	0.12	270.84	0.16	2.9 E7	6 ng/mL	MS2/MS3	
Midazolam	6	990	1.74	0.08	325.89	0.21	2.5 E7	4 ng/mL	MS2/MS3	
Loprazolam	2	947	1.59	0.07	465.00	0.10	2.2 E7	6 ng/mL	MS2/MS3	
7-Aminoflunitrazepam	4	982	1.66	0.09	283.89	0.11	1.9 E7	5 ng/mL	MS2/MS3	
Metizolam	15	946	4.26	-0.05	328.90	0.10	1.7 E7	6 ng/mL	MS2/MS3	
Diclazepam	20	940	7.22	-0.05	319.03	-0.03	1.4 E7	5 ng/mL	MS2/MS3	
Clonazolam	10	981	3.07	0.23	353.92	0.18	1.2 E7	7 ng/mL	MS2/MS3	
Flunitrazepam	16	990	4.51	0.28	313.90	0.20	8.0 E6	5 ng/mL	MS2/MS3	
Alprazolam	13	964	4.03	-0.05	308.88	0.22	6.8 E6	5 ng/mL	MS2/MS3	
Clobazam	18	999	5.23	-0.08	300.89	0.11	5.5 E6	6 ng/mL	MS2/MS3	
Triazolam	14	991	4.25	-0.08	342.91	0.09	5.3 E6	6 ng/mL	MS2/MS3	
Flubromazepam	17	842	4.90	-0.07	332.84	0.16	2.8 E6	19 ng/mL	MS2/MS3	
Clonazepam	12	959	3.79	0.27	315.80	0.20	1.3 E6	< 5 ng/ml	MS2/MS3	
Chlordiazepoxide	3	822	1.62	0.11	299.89	0.11	7.4 E5	< 5 ng/ml	MS2/MS3	MS2 unspecific
Lorazepam	11	706	3.76	0.33	321.03	-0.03	6.0 E5	< 5 ng/ml	tentative	tentative

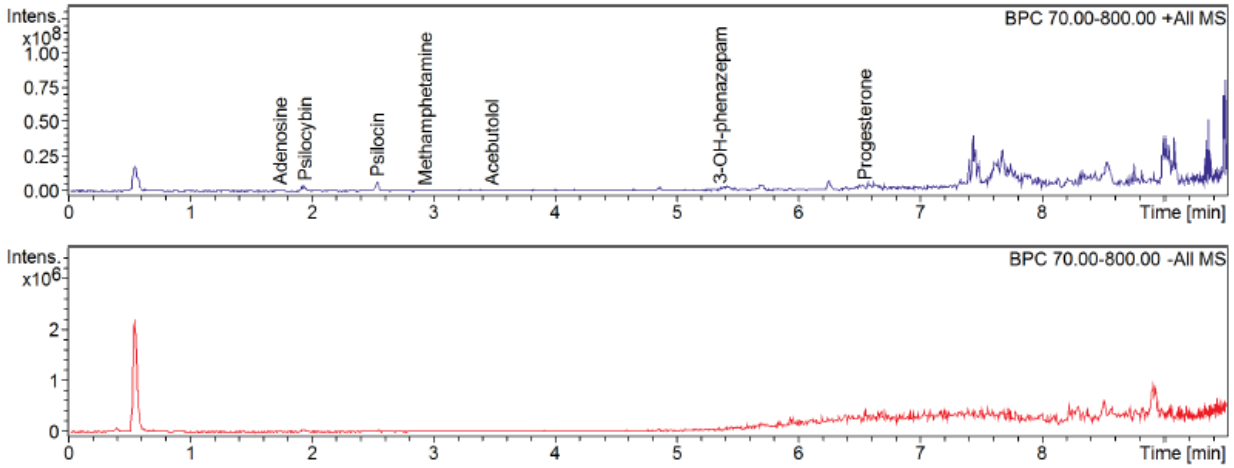
After a one-point calibration, qualitative and semi-quantitative results can be obtained from the same Toxtyper analysis

Application Note LCMS 130: Detection and semi-quantitative determination of designer benzodiazepines in serum using LC-MSn

State Police Lab (LKA) Brandenburg "Magic Mushrooms"

Sample-ID	[redacted]	Station	Toxtyper_2.0
Submitter	[redacted]	Method	Toxtyper_Custom (2.0)
Analysis Name	Psilos Realprobe_RA5_01_46.d	Acquisition Date	3/22/2017 3:17:06 PM
Sample Description			

Base Peak Chromatogram



Library Search Results

Cmp Name	Cmp #	Purity'	RT [min]	d RT	m/z [Da]	d m/z	Intensity	ID	Comment
Psilocin	3	872	2.54	-0.06	204.99	0.14	6.8 E6	MS2/MS3	
Psilocybin	2	977	1.94	-0.07	285.04	0.06	3.8 E6	MS2	
Adenosine	1	998	1.74	0.13	268.02	0.08	8.1 E5	tentative	
Progesterone	7	877	6.54	-0.28	315.22	0.01	6.7 E5	MS2	
Methamphetamine	4	986	2.94	-0.14	150.04	0.09	6.1 E5	MS2	
3-OH-phenazepam	6	861	5.36	0.22	367.20	-0.20	5.7 E5	MS2	
Acebutolol	5	870	3.48	0.03	337.21	0.00	3.0 E5	tentative	MS2 unspecific

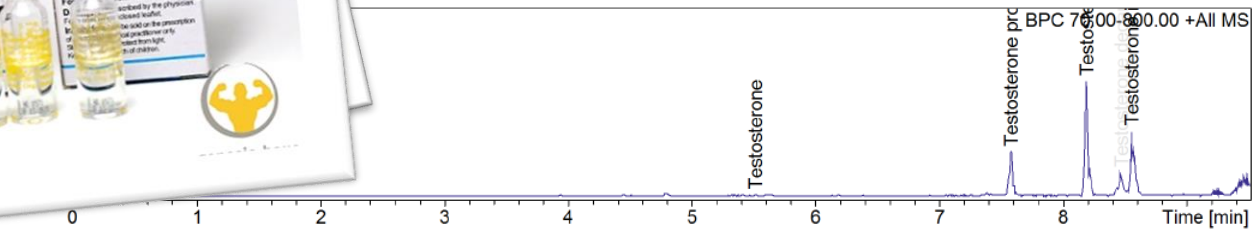
Identification of **Psilocin and Psilocybin**. Methamphetamine is due to contamination of the packaging material.

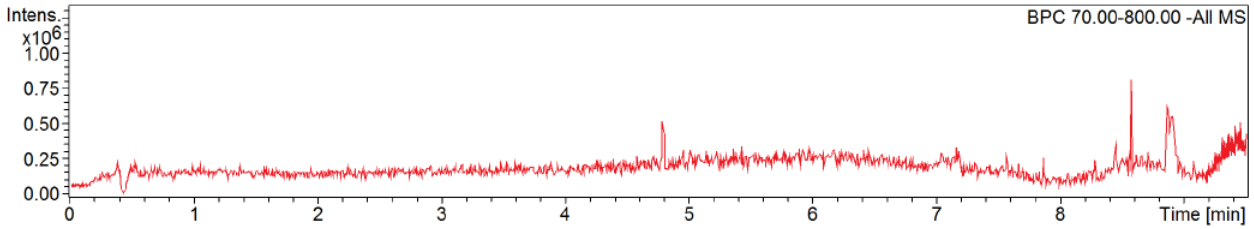
Data courtesy of Landeskriminalamt Brandenburg (State Police Lab), Eberswalde , Germany

State Police Lab (LKA) Brandenburg "yellow oil sample" → Sustanon®

Toxtyper Analysis Report

Station Toxtyper_2.0
Method Toxtyper_Custom (2.0)
Acquisition Date 3/20/2017 3:13:47 PM



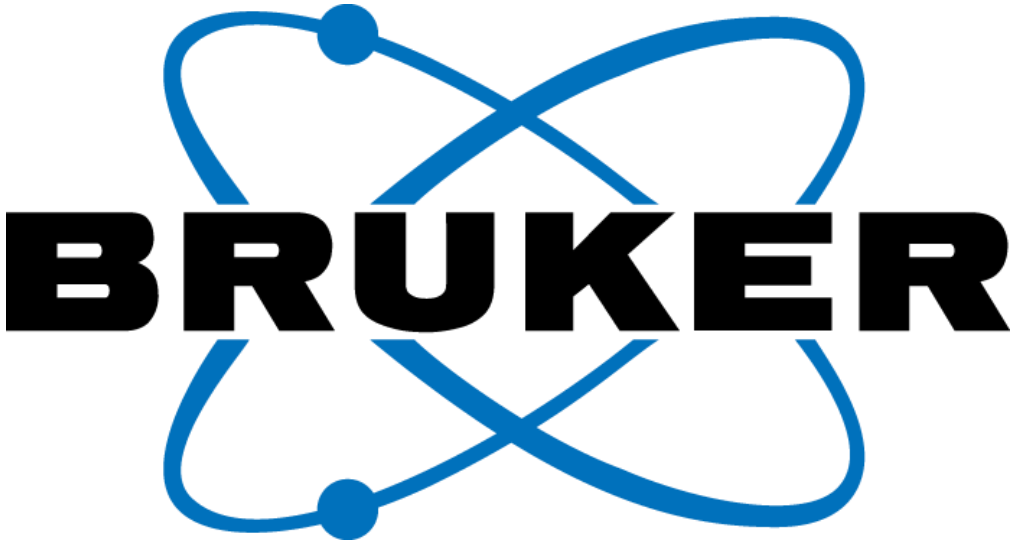


Library Search Results

Cmp Name	Cmp #	Purity'	RT [min]	d RT	m/z [Da]	d m/z	Intensity	ID
Testosteron phenylpropionate	3	961	8.19	-0.10	421.29	-0.02	1.2 E9	MS2/MS3
Testosterone isocaproate	5	939	8.55	-0.13	387.32	-0.04	6.6 E8	MS2/MS3
Testosterone propionate	2	966	7.58	-0.13	345.23	-0.01	4.5 E8	MS2/MS3
Testosterone decanoate	4	829	8.47	-0.30	443.40	-0.04	2.3 E8	MS2/MS3
Testosterone	1	910	5.51	-0.11	289.18	-0.08	5.7 E6	MS2/MS3

Identification of **Testosteron and its esters**

Data courtesy of Landeskriminalamt Brandenburg (State Police Lab), Eberswalde , Germany



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