Department of Exper. & Clinical Toxicology
Saarland University, Homburg, Germany

Gas Chromatography (-Mass Spectrometry) in Bioanalysis:

Univ.-Prof. Dr. Dr. h.c. Hans H. Maurer
(hans.maurer@uniklinikum-saarland.de)
(http://wwwalt.med-rz.uniklinik-saarland.de/med_fak/pharma-toxi/index.html)

Department of Exper. & Clinical Toxicology
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Teaching

Pharmacology and Toxicology for Students of Pharmacy
with the basics of anatomy, physiology, pathophysiology and drug therapy

Toxicology for Students of Medicine, Dentistry, Human Biology

Research

Drug Metabolism
Analytical Toxicology

Medical Healthcare

ClinTox Service (24/7)

Medical Healthcare: ClinTox Service (24/7)

- **Diagnosis and Prognosis of Poisonings**
- **Indication for (invasive) treatment**
- **Monitoring of the efficiency of detoxication**
- **Differential diagnostic exclusion of poisonings**
- **Drug determinations for brain death diagnosis**
- **Monitoring of polytoxicomania patients (abuse of alcohol, drugs and/or medicaments)**
- **Detection of adverse drug reactions or interactions**
- **Monitoring of Munchhausen Syndrome Patients**
- **Monitoring of non-compliant patients (TDM)**
- **Some forensic analyses**

Toxicological Analyses - How ?

Screening	**Which class of poison?**
Identification	**Which poison?**
Quantification	**Which concentration (therap/tox.)?**
Quality Control	**Were appropriate procedures correctly used?**
Interpretation	**Correlation of the analytical results with the clinical signs**

Toxicological Analyses – Which methods?

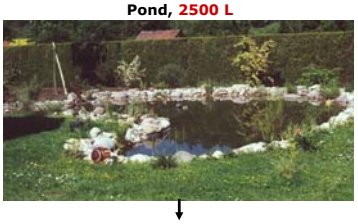
Requirements for an ideal method

- **Identification and quantification of as many poisons as possible with one single method in one single step**
- **Highest selectivity, validity**
- **Short turnaround time**
- **Easy handling**
- **Constant availability**
- **Reasonable cost**

Which Concentrations must be Analyzed ?

1 cube sugar  **2,5 g**
2500 mg

Pond, 2500 L



Sugar Concentration = 1 mg/L = 1000 µg/L = 1 µg/mL

Which Concentrations can be Analyzed ?

1 kg Sugar

1 kg
1 000 g
1 000 000 mg
1 000 000 000 µg

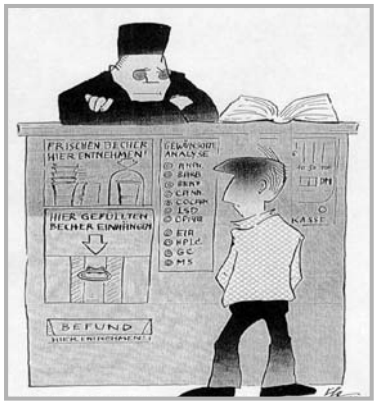
Bostal Lake, ca. 8 000 000 000 L

Sugar Concentration = 0,125 µg/L = **0,125 ng/mL**

Toxicological Analysis - Which Methods ?

- **Spot Tests**
- **IA** — **FPIA, EIA, ELISA, RIA, LIA**
- **(HP)TLC** — **Chemical detection, UV, (FT)IR**
- **GC** — **FID, NPD, ECD, MS**
- **GC-MS** — **EI, PICI, NICI**
- **HPLC** — **UV, DAD, FD, ECD, MS**
- **CE** — **UV, DAD, FD, ECD, MS**
- **LC-MS, CE-MS** — **ESI, APCI**
- **(AAS, ICP-MS)**

Toxicological Expertise: Dream of the Judge (Clinician)



On-site Drug Testing
(roadside, bedside, workplaceside ....)

IA vs GC-MS Study

12 cases with positive Triage®8 not confirmed by GC-MS (10.8%)

AMP (4)	BAR (1)	THC (1)
TCA (4)	MTD (1)	
BZO (2)	OPI (2)	

8 cases with negative Triage®8 despite GC-MS detection (7.2%)

BZO (3)	AMP (1)
TCA (3)	COC (1)

77 cases with GC-MS detection of drugs principally not detectable by Triage®8 (69.8%)

Non-OP Analgesics (43)	Antiepileptics (9)
Non-TCA AD (16)	Opioids (8)
Neuroleptics (15)	Cardiovascular drugs (6)
Hypnotics (12)	Others (4)

(von Mach/Weber/Meyer/Weilemann/Maurer/Peters, TDM, 2007)

Management of the CT Lab Services in Homburg/Saar

One toxicologist is on duty around the clock

- **Quantification of suspected compounds in blood by IA, GC-MS, LC-MS**
- **Quantification of solvents in blood and urine by HS-GC or GC-MS**

- **Comprehensive screening in urine by GC-MS (STA), IA: Can/Coc**
- **Limited screening in blood by GC-MS, LC-MS**
- **Quantification of relevant compounds by GC-MS, LC-MS, IA**

- **Screenings for rare compounds by LC-MS, Spot Tests**
- **Quantification of rare compounds by ELISA, UV, LC-MS, GC-MS**

- **Analytical, toxicological, clincal interpretation and consultation**

When GC-MS or LC-MS/MS ?

One toxicologist is on duty around the clock

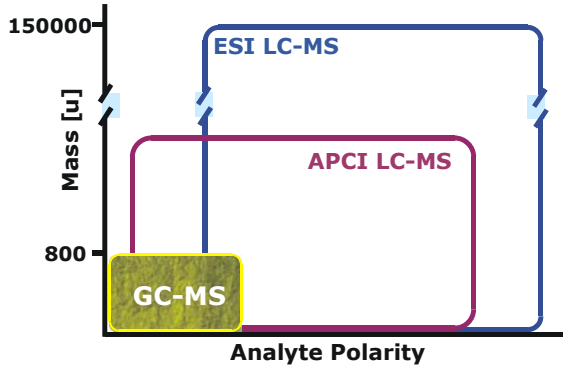
- Quantification of suspected compounds in blood by IA, GC-MS, LC-MS
- Quantification of solvents in blood and urine by HS-GC or GC-MS

- Comprehensive screening in urine by GC-MS (STA), IA: Can/Coc
- Limited screening in blood by GC-MS, LC-MS
- Quantification of relevant compounds by GC-MS, LC-MS, IA

- Screenings for rare compounds by LC-MS, Spot Tests
- Quantification of rare compounds by ELISA, UV, LC-MS, GC-MS

- Analytical, toxicological, clincal interpretation and consultation

MS Coupling Techniques



Toxicological Analysis - Which MS Type ?

GC-MS, GC-MS/MS ??

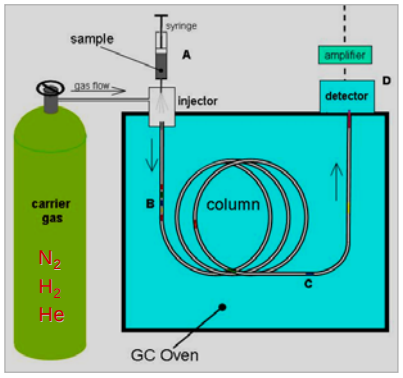
LC-MS, LC-MS/MS ??

TOF ??

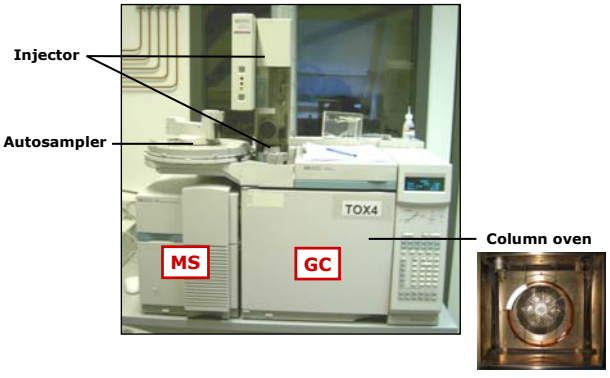
Quad ??

Trap ??

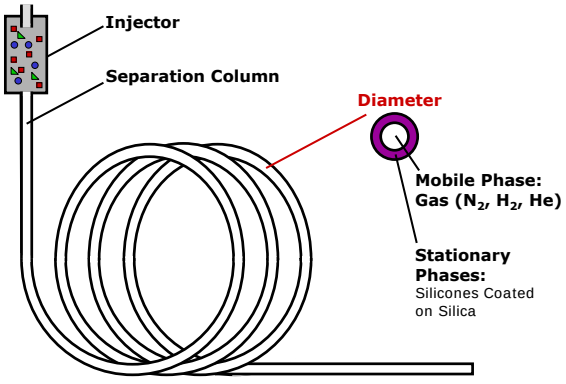
Gas Chromatograph



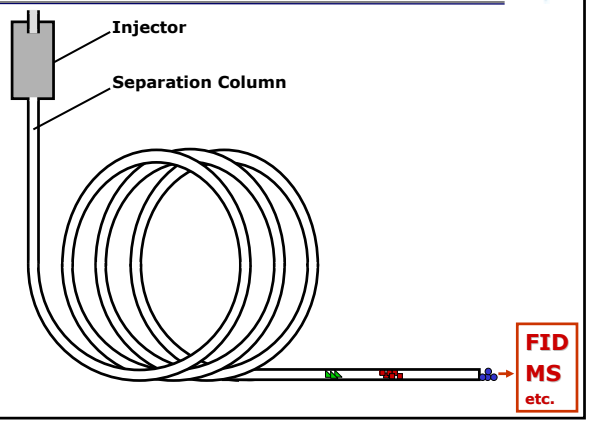
Gas Chromatograph



Separation of Compounds by GC

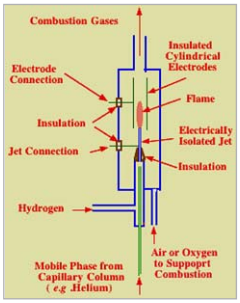


Separation of Compounds by GC



Flame Ionization Detector (FID)

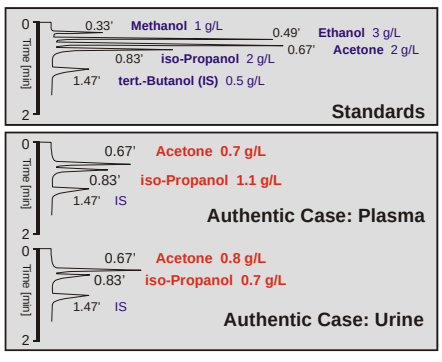
- FID burns compounds in column effluent in a hydrogen flame
- Organic compounds produce positive ions, collected at an electrode above the flame
- The generated current is measured
- Current changes over time sent to recorder => peaks on the chromatogram
- Peak area proportional to Carbon atoms
- Non-selective, rather sensitive and linear over many orders of magnitude



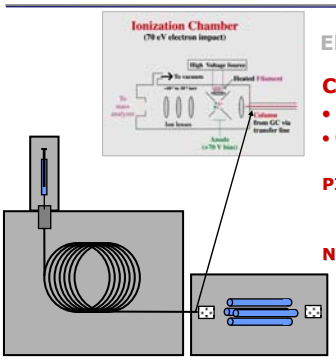
Determination of Alcohols/Solvents in Plasma and Urine by GC-FID

For alcohols:
HS, 80°C, 90°C

GC-FID, 0.1% SP-1000/ Carbopak C, 80°C



Gas Chromatography-Mass Spectrometry



Electron Ionization (EI)

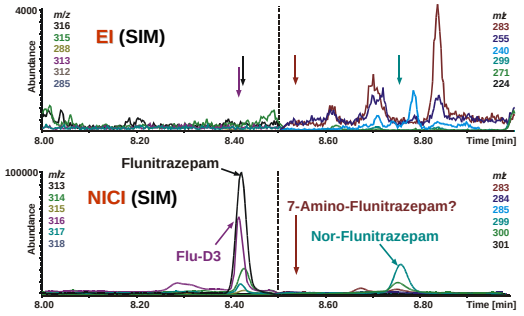
Chemical Ionization (CI):
- Reactant gas ionized (e.g. methane)
- Charge transfer to analyte

PICI: detection of positive ions
=> MH^+ (MW confirmation)

NICI: detection of negative ions
=> very sensitive
for analytes with electronegative groups

NICI Much More Sensitive Than EI
for Compounds with Electronegative Groups

Plasma spiked with 5 ng/mL each



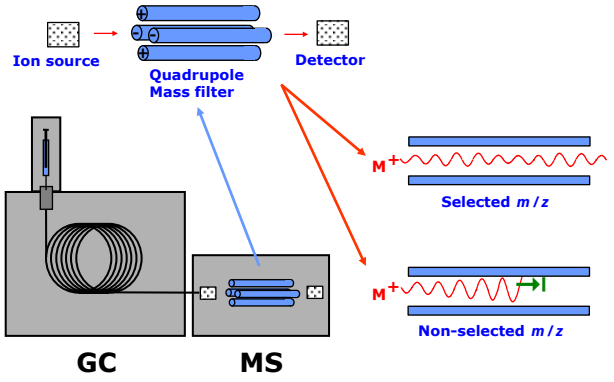
(Maurer/Kraemer/Kratzsch/Peters/Weber, Therap. Drug Monitor., 2002)

The Three Pillars of GC-MS Methods

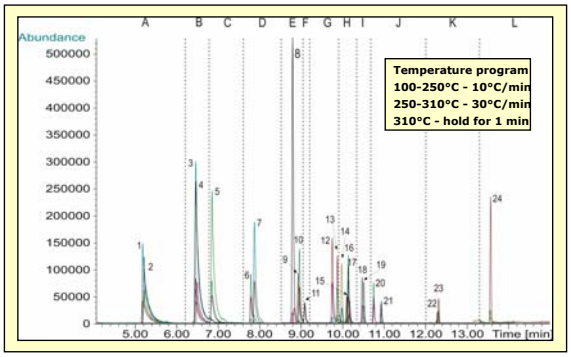
GC-MS Method

Sample Preparation	Separation	Detection
Pre-extraction steps • Homogenization • Conjugate cleavage	Chromatography • LC • GC • TLC	Photo Detection • UV/VIS, FD • DAD
Isolation/Extraction • Protein precipitation • LLE • SPE • SPME	Electrokin. Methods • CE • MECC	**Mass Spectrometry** • Ionization - EI, CI (PICI, NICI) - APCI, ESI • **Mass Analyzer** - **Quadrupole** - Ion Trap - TOF
Post-extraction steps • Reconcentration • Derivatization		Others • FID, NPD • ECD

Gas Chromatography-Mass Spectrometry

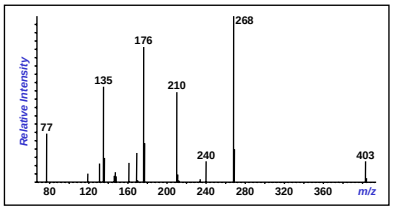


Reconstructed Mass Chromatograms:
High Separation Power, High Selectivity



(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Identification of Drugs/Poisons by Mass Spectrometry



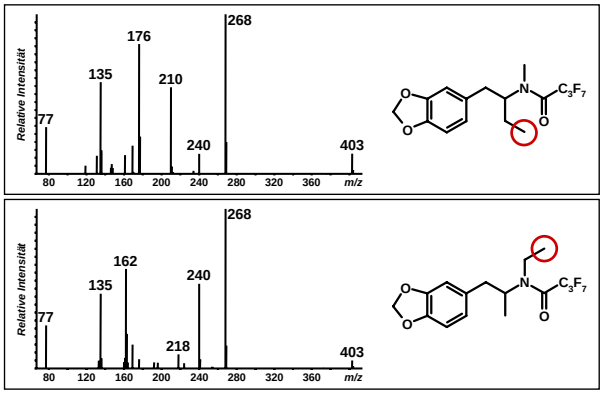
finger print

identification
of individuals

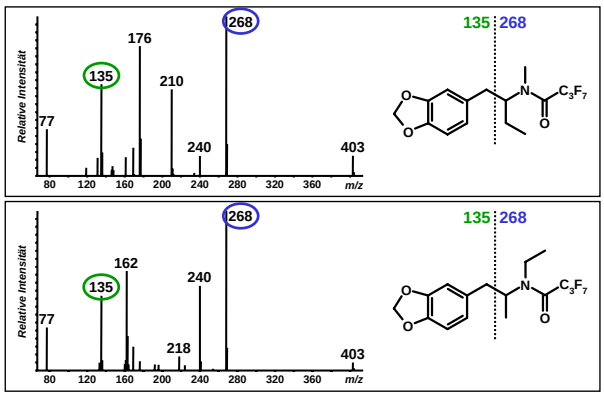
mass spectrum

identification
of molecules

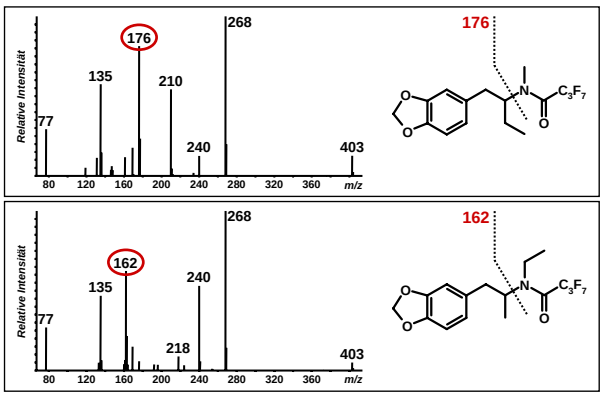
Mass Spectrometry – How Specific?



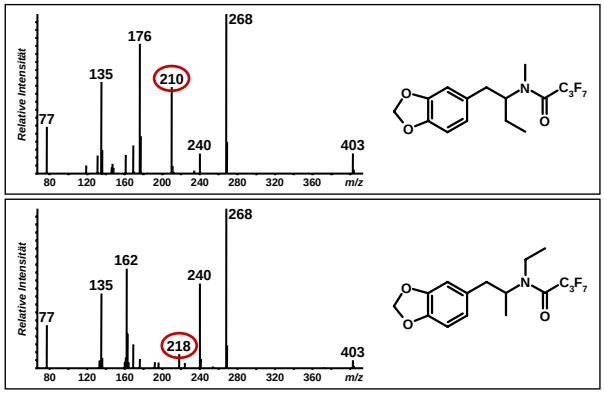
Mass Spectrometry – How Specific?



Mass Spectrometry – How Specific?



Mass Spectrometry – How Specific?



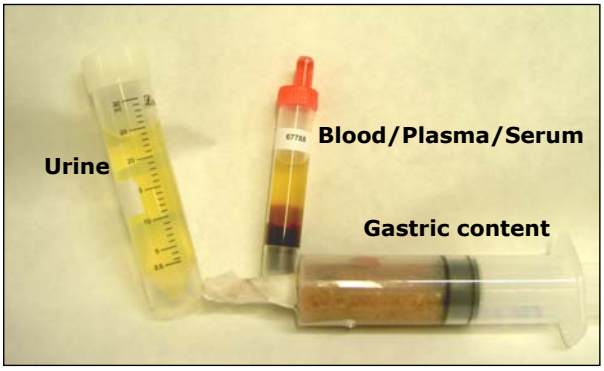
Prerequesites for GC and GC-MS Methods

(Bio)analytical Method

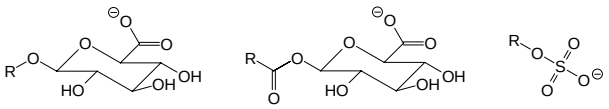
Sample Preparation	Separation	Detection
Pre-extraction steps • Homogenization • Conjugate cleavage **Isolation/Extraction** • Protein precipitation • LLE • SPE • SPME **Post-extraction steps** • Reconcentration • Derivatization	Chromatography • LC • GC • TLC Electrokin. Methods • CE • MECC	Photo Detection • UV/VIS, FD • DAD Mass Spectrometry • Ionization - EI, PICI, NICI - APCI, ESI • Mass Analyzer - Quadrupole - Ion Trap - TOF Others • FID, NPD • ECD

Biosamples in Forensic Toxicology

Biosamples in Clinical Toxicology



Cleavage (Hydrolysis) of Conjugates



ether glucuronide (acetal) | ester glucuronide (acylal) | sulfate ester

Acid hydrolysis	reflux with concentrated mineral acid
Enzymatic hydrolysis	ß-Glucuronidase (GRD) Arylsulfatase (ARS)

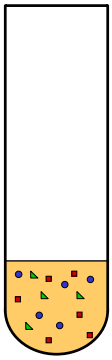
Sample Work-up Procedures for MAPs

- **Liquid-Liquid Extraction (LLE)**
- **Solid-phase Extraction (SPE)**
- **Solid-phase Microextraction (SPME)**

Für LC-MS, nicht für GC-MS:

- **On-line Extraction (e.g. Turbulent-Flow)**
- **Deproteinization (plasma)**
- **Direct Injection (urine)**

Extraktion der Wirk-/Giftstoffe



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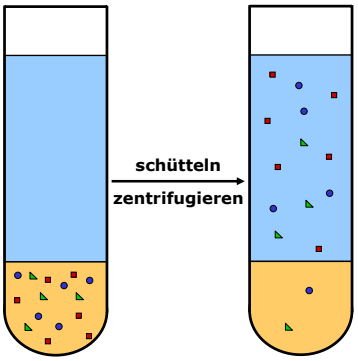
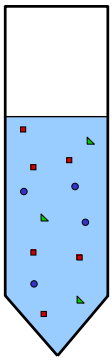
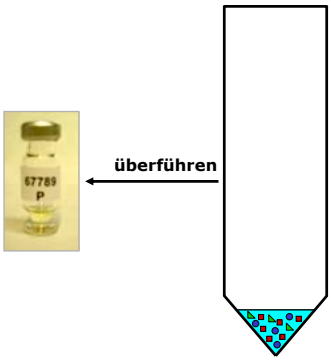


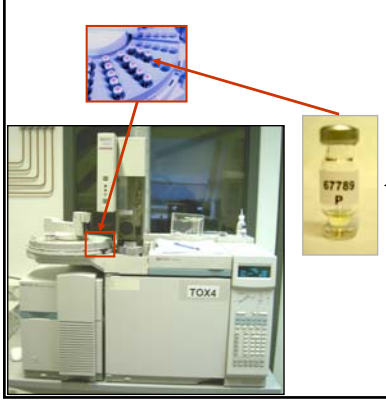
Eindampfen des Extraktes



Aufnahme des Rückstands



GC-Injektion über Autosampler



Plasma Sample Preparation I (LLE)

Plasma (1 mL + trimipramine-d_3)

LLE (diethyl ether/ethyl acetate, pH 7/12)

GC-MS (HP1)

EI, scan/SIM

NICI, scan/SIM

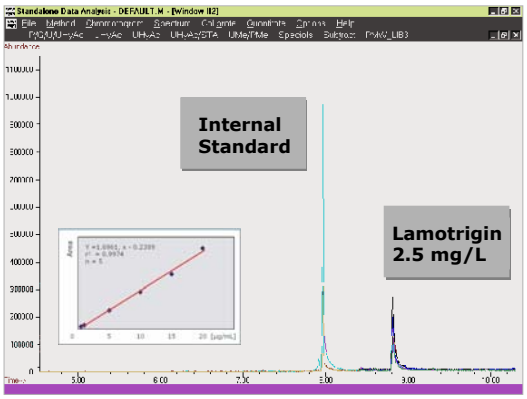
LC-MSD (RP 8, 5 µm; ACN/AF)

APCI, scan/SIM, (CID, 2 voltages)

not suitable for all drugs

(Maurer, MPW, part 1, 2007)
(Maurer/Kratzsch/Weber/Peters/Kraemer, J. Mass Spectrom., 2002)

Universal GC-MS Quantification Procedure



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EI, scan/SIM

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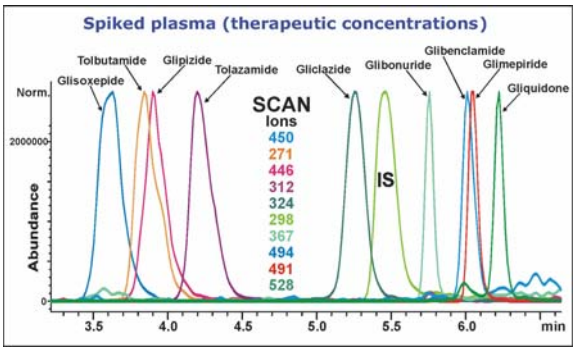
LC-MSD (RP 8, 5 µm; ACN/AF)

APCI, scan/SIM, (CID, 2 voltages)

not suitable for all drugs

(Maurer, MPW, part 1, 2007)
(Maurer/Kratzsch/Weber/Peters/Kraemer, J. Mass Spectrom., 2002)

MAP for 9 OADs in Plasma by LLE and LC-APCI-MS (SIM)



(Maurer/Kratzsch/Kraemer/Peters/Weber, J. Chromatogr.B, 2002)

Sample Work-up Procedures for MAPs

- Liquid-Liquid Extraction (LLE)
- **Solid-phase Extraction (SPE)**
- Solid-phase Microextraction (SPME)

Für LC-MS, nicht für GC-MS:

- On-line Extraction (e.g. Turbulent-Flow)
- Deproteinization (plasma)
- Direct Injection (urine)

Apparatus for Solid-Phase Extraction

Plasma Sample Preparation II (SPE)

0.5-1 mL Plasma + IS + 2 mL H_2O

SPE (Confirm HCX 130 mg, 3 mL)

Elution (MeOH/NH_3 98:2)

Evaporation (N_2, 56°C)

Derivatization, GC-MS (HP1/5)

EI, scan/SIM

NICI, scan/SIM

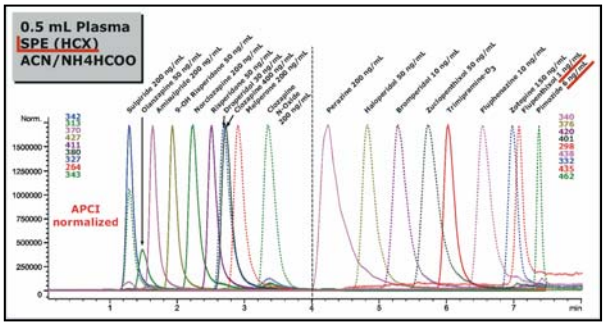
LC-MSD (RP 8, 5µm; ACN/AF)

APCI, scan/SIM (CID, 2voltages)

suitable for DOA and very low-dosed drugs

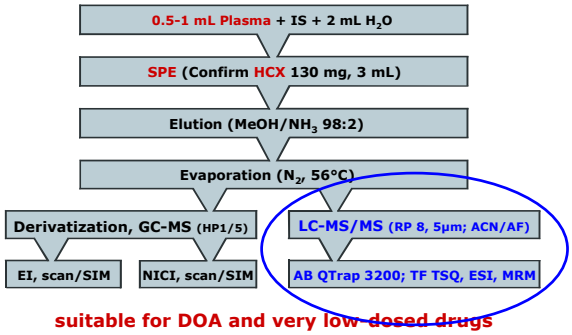
(Peters/Kraemer/Maurer, Clin. Chem. 2002)
(Kratzsch/Weber/Peters/Kraemer/Maurer, J. Mass Spectrom., 2003)

MAP for Neuroleptics in Plasma by LC-APCI-MS after SPE



(Kratzsch/Weber/Peters/Kraemer/ Maurer, J. Mass Spectrom., 2003)

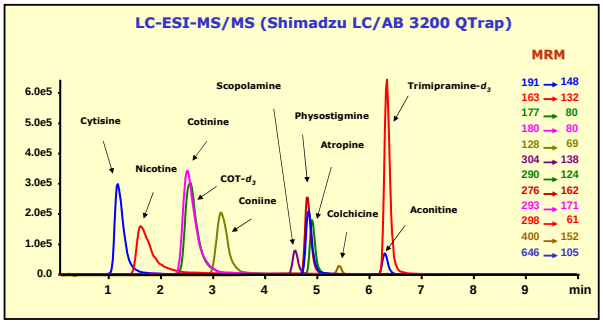
Plasma Sample Preparation II (SPE)



suitable for DOA and very low-dosed drugs

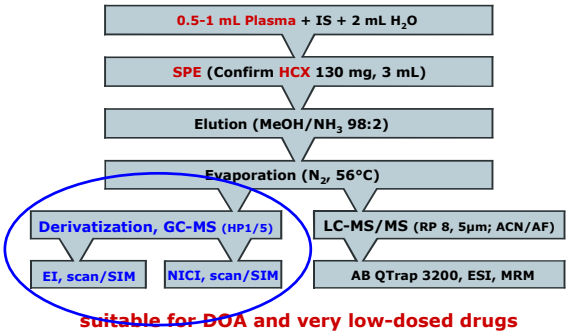
(Peters/Kraemer/Maurer, Clin. Chem. 2002)
(Kratzsch/Weber/Peters/Kraemer/Maurer, J. Mass Spectrom., 2003)

MAP for Toxic Alkaloids in Plasma by LC-MS(/MS) after SPE



(Beyer/Peters/Kraemer/Maurer, J Mass Spectrom, 2007)

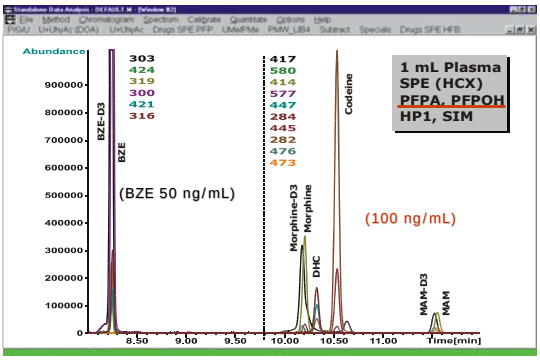
Plasma Sample Preparation II (SPE)



suitable for DOA and very low-dosed drugs

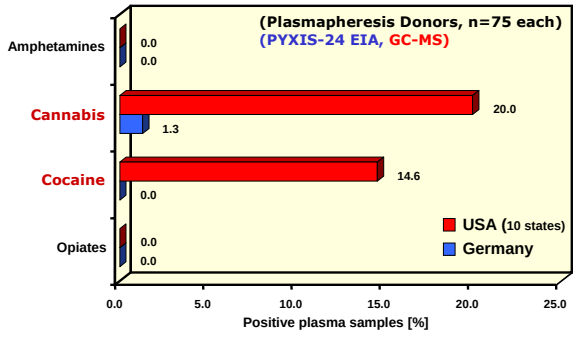
(Peters/Kraemer/Maurer, Clin. Chem. 2002)
(Kratzsch/Weber/Peters/Kraemer/Maurer, J. Mass Spectrom., 2003)

Confirmation of DOAs in Plasma by GC-MS



(Peters/Maurer/Hellstern, Vox Sang, 2003)

Prevalence of Illicit Drugs in Donor Blood in GER vs. USA

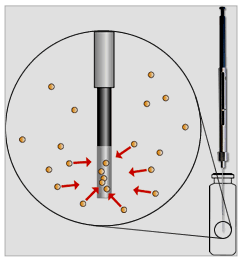


(Peters/Maurer/Hellstern, Vox Sang, 2003)

Solid-phase Microextraction (SPME)

Work-up by SPME:

- Deproteinization
- Centrifugation
- SPME of Supernatant



(Pragst, Anal Bioanal Chem, 2007)

Derivatization

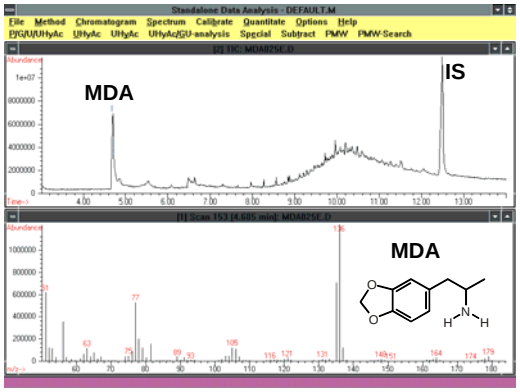
Improvement of

- Gas chromatographic characteristics
- Volatility of polar compounds
- Sensitivity by halogenation for ECD or NICI
- Separation of enantiomers via diastereomers

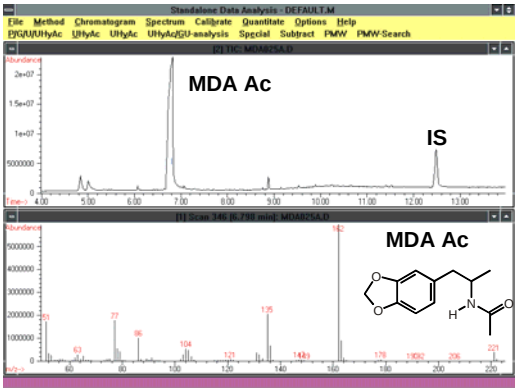
Common derivatization reactions

- (Perfluoro)acylation for alcohols, phenols and amines
- Silylation (TMS, TBDMS) for alcohols, phenols, carboxylic acids, and amines
- Methylation for carboxylic acids, phenols, (and alcohols)

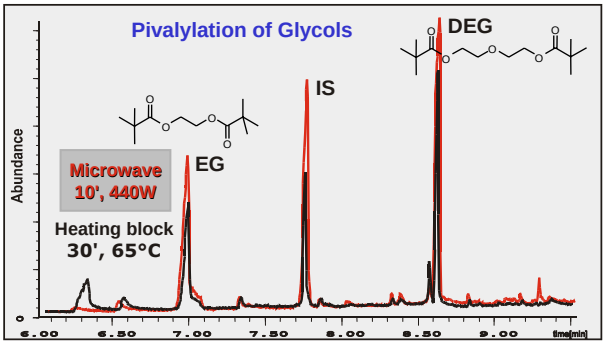
Poor GC Properties of Polar Analytes



Improvement of GC Properties by Derivatization

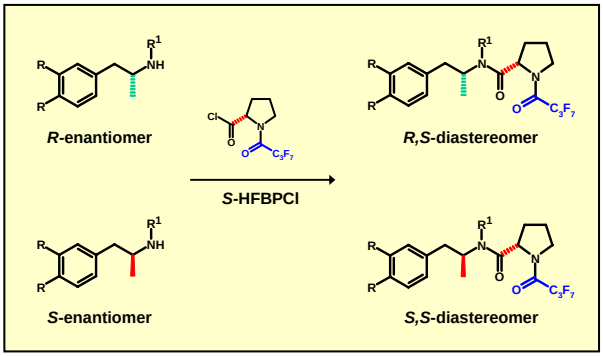


Heating Block vs Microwave Assisted Derivatization



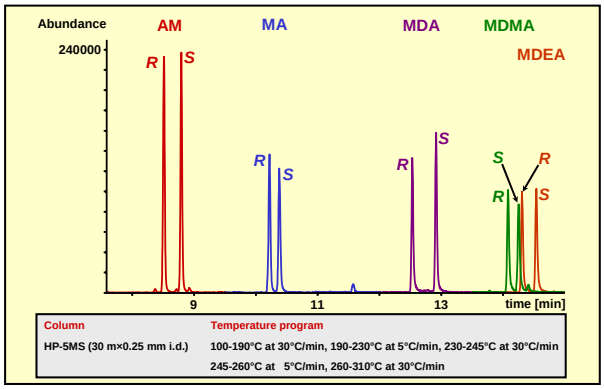
(Maurer/Peters/Paul/Kraemer, J. Chromatogr.B, 2001)

Chiral Derivatization Enantiomers to Diastereomers



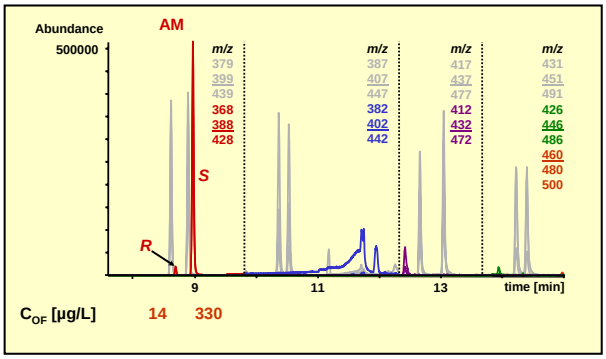
(Peters/Kraemer/Maurer, Clin. Chem., 2002)
(Peters/Samyn/Lamers/Riedel/Kraemer/de Boeck/Maurer, Clin. Chem., 2005, 2007)

Separation of HFBP-Derivatives of all Analytes in Oral Fluid Extract



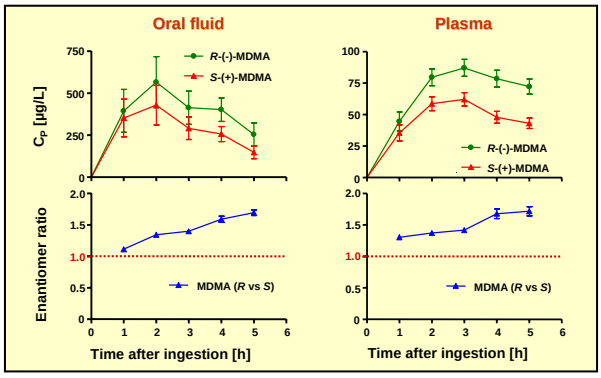
Column	Temperature program
HP-5MS (30 m×0.25 mm i.d.)	100-190°C at 30°C/min, 190-230°C at 5°C/min, 230-245°C at 30°C/min 245-260°C at 5°C/min, 260-310°C at 30°C/min

Chiral GC-MS Analysis of DOA in Oral Fluid Therapeutic Ingestion of Dexedrine (*S-(+)*-AM) ?



(Peters/Samyn/Kraemer/Riedel/Maurer, Clin. Chem., 2007)

Oral Fluid and Plasma Data from a Driving Performance Study (75 mg rac. MDMA)



(Peters/Samyn/Kraemer/Riedel/Maurer, Clin. Chem., 2007)

Steps of Toxicological Analysis

Screening	**Which class of poison?**
Identification	**Which poison?**
Quantification	Which concentration (therap/tox.)?
Quality Control	Were appropriate procedures correctly used?
Interpretation	Correlation of the analytical results with the clinical signs, autopsy, criminal investigation

What is our Concept for Urine Screening ?

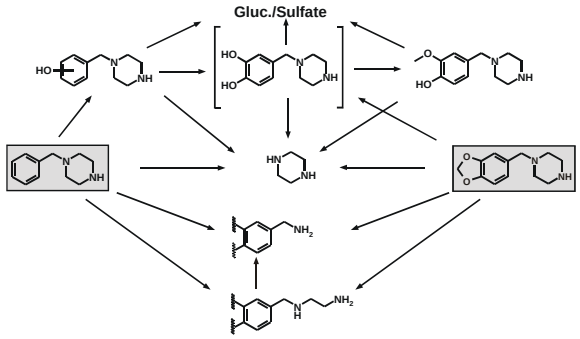
- **Comprehensive (non-target) screening for >2000 drugs**
- **Simultaneous confirmation of >2000 drugs by GC-MS**

=> more than 20 drug classes, hundreds of metabolites

high-throughput: >2000 drugs in one sample

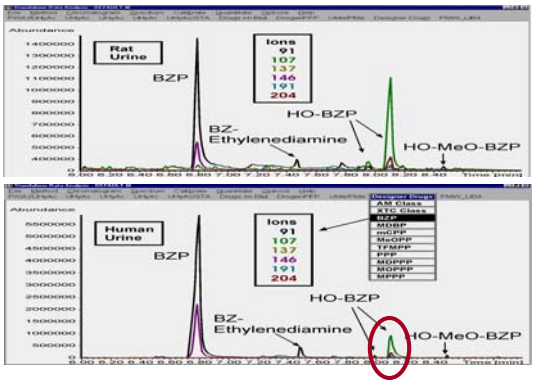
(Maurer, Clin Chem Lab Med, 2004; Clin Biochem, 2005; J Mass Spectrom, 2006)

Metabolic Pathways of Benzylpiperazines



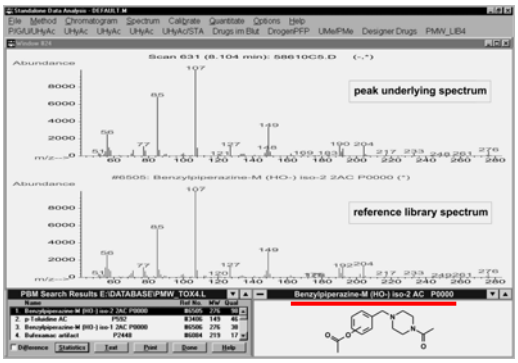
(Staack/Fritschi/Maurer, J. Chromatogr.B, 2002)

Detection of BZP and its Metabolites in Rat and Human Urine by GC-MS (STA)



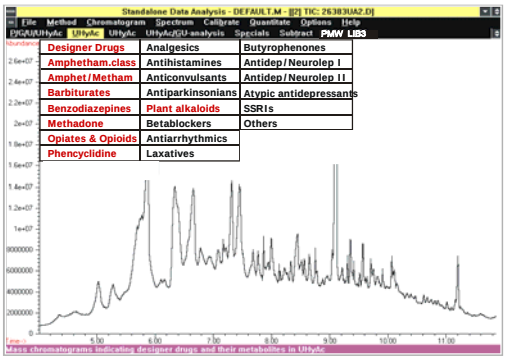
(Staack/Fritschi/Maurer, J. Chromatogr.B, 2002)

Detection of BZP Metabolites in Urine by GC-MS using MPW_2007



(Staack/Fritschi/Maurer, J. Chromatogr.B, 2002)

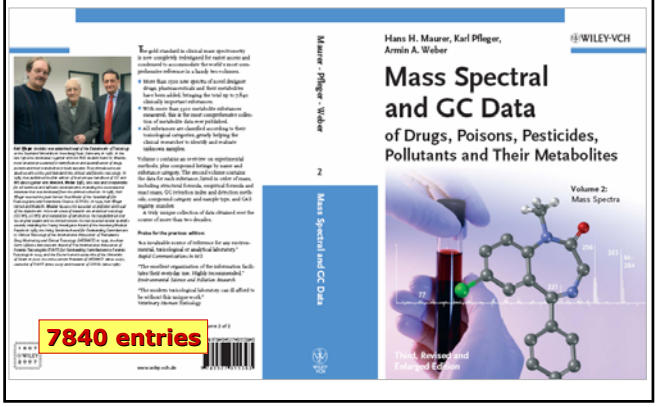
General Screening Procedure (STA) for Confirmation and Differentiation in Urine



Designer Drugs	Analgesics	Butyrophenones
Amphetham.class	Antihistamines	Antidep/Neurolep I
Amphet/Metham	Anticonvulsants	Antidep/Neurolep II
Barbiturates	Antiparkinsonians	Atypic antidepressants
Benzodiazepines	Plant alkaloids	SSRIs
Methadone	Betablockers	Others
Opiates & Opioids	Antiarrhythmics	
Phencyclidine	Laxatives	

(Maurer, Clin Chem Lab Med, 2004)

Maurer/Pfleger/Weber, 3rd Edition, 2007



Steps of Toxicological Analysis

Screening	Which class of poison?
Identification	Which poison?
Quantification	**Which concentration (therap/tox.)?**
Quality Control	**Were appropriate procedures correctly used?**
Interpretation	Correlation of the analytical results with the clinical signs, autopsy, criminal investigation

What is our Concept for Plasma Analysis ?

Multi-Analyte Procedures for Screening for and Validated Quantification of Various Drugs by GC-MS, LC-MS or LC-MS/MS

high-throughput: hundreds of drugs in one sample

(Maurer, Clin Chem Lab Med, 2004; Clin Biochem, 2005; J Mass Spectrom, 2006)

Department of Exper. & Clinical Toxicology
Saarland University, Homburg, Germany

GC-MS Assay for

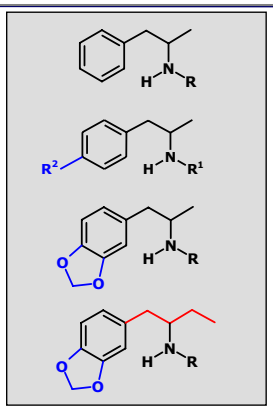
Validated Quantification of

Amphetamine and Designer Drugs

in Blood Plasma

(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Amphetamines and Amphetamine-derived Designer Drugs



Amphetamines

AM	R = H
MA	R = CH_3
EA	R = CH_2CH_3

Other Amphetamines

PMA	R^1 = H	R^2 = OCH_3
PMMA	R^1 = CH_3	R^2 = OCH_3
MTA	R^1 = H	R^2 = SCH_3
HO-AM	R^1 = H	R^2 = OH
PHOL	R^1 = CH_3	R^2 = OH

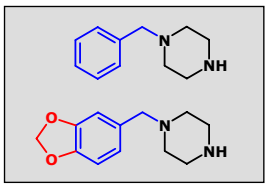
Methylenedioxy Amphetamines

MDA	R = H
MDMA	R = CH_3
MDEA	R = CH_2CH_3

Methylenedioxy Butylamines

BDB	R = H
MBDB	R = CH_3

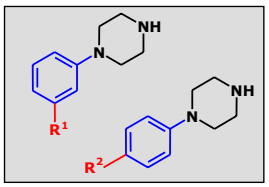
Piperazine-derived Designer Drugs



Benzyl Piperazines

BZP

MDBP



Phenyl Piperazines

TFMPP: R^1 = CF_3
mCPP: R^1 = Cl

MeOPP: R^2 = OCH_3

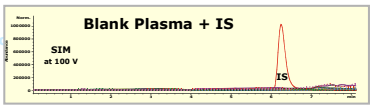
Method Development

- Initial Considerations
- Development of Parameters: Selection of IS
- Development of Separation System
- Development of Sample Preparation
- Optimization
- Validation

Prerequisites for Internal Standards

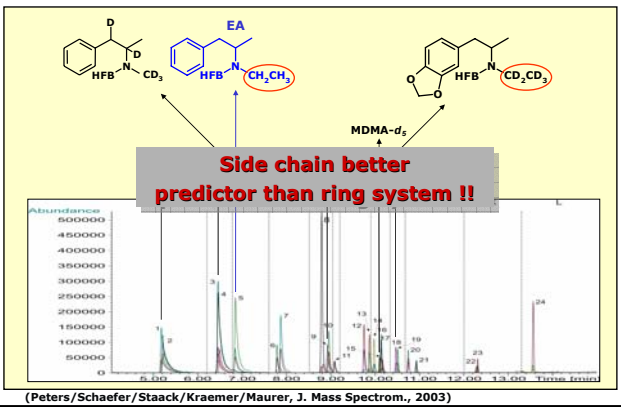
- IS should have similar physicochemical properties
- IS should compensate all variability (workup and measurement)
- No cross-contribution between analyte and IS
- IS must not cause relevant ion suppression/enhancement in LC-MS
- The use of non-labeled therapeutic drugs must be avoided !!

- Absence of interference should be checked using zero samples (blank + IS)
- In case of interferences, IS but not too much



(Maurer, Anal Bioanal Chem, 2007)

Choice of Internal Standard



(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Procedure

1 mL Plasma, 2 mL H_2O + ISs

SPE (Confirm HCX, 130 mg, 3 mL)

Elution ($MeOH:NH_3$, 98:2)

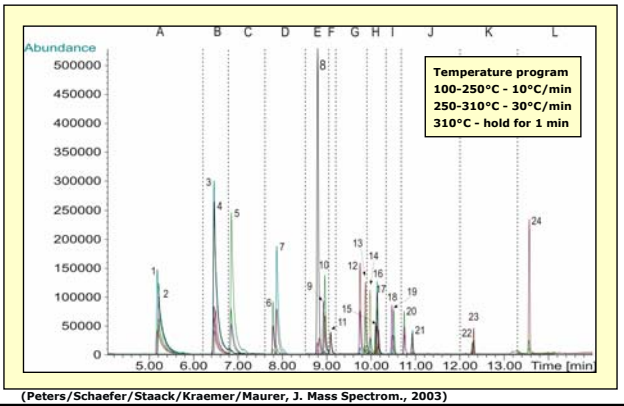
HFB (HFBA, 5´MW), 100 µL hexane

Washing with 200 µL 0.5 M Na_3PO_4

GC-MS (HP5, EI, SIM)

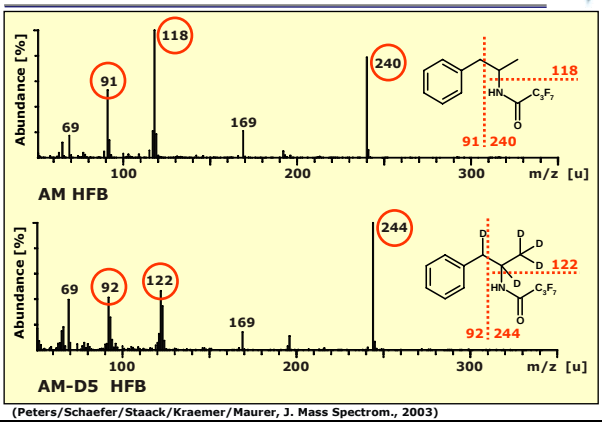
(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

GC Separation



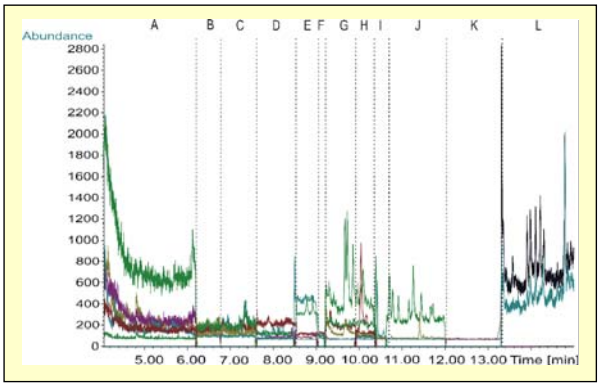
(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

MS Detection



(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Selectivity



(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Linearity

Range: 5 - 1000 µg/L

7 levels, 6 replicates for model

Weighted linear regression model (1/concentration²)

Single measurements for procedure

Analyte	Slope (n=8) mean ± SD	y-Intercept (n=8) mean ± SD	R^2 (n=8) range
AM	0.009 ± 0.0001	0.017 ± 0.0099	0.994 to 1.000
MA	0.009 ± 0.0001	-0.002 ± 0.0027	0.997 to 1.000
EA	0.055 ± 0.0047	-0.016 ± 0.0181	0.995 to 1.000
HO-AM	0.003 ± 0.0004	0.001 ± 0.0012	0.976 to 0.997
PMA	0.026 ± 0.0016	-0.009 ± 0.0088	0.993 to 1.000
PHOL	0.012 ± 0.0016	0.006 ± 0.0074	0.983 to 0.998
MDA	0.016 ± 0.0002	0.005 ± 0.0031	0.998 to 1.000
PMMA	0.004 ± 0.0002	-0.002 ± 0.0009	0.995 to 0.999
BDB	0.015 ± 0.0008	0.000 ± 0.0023	0.997 to 1.000
MTA	0.011 ± 0.0010	-0.004 ± 0.0053	0.991 to 0.997
BZP	0.006 ± 0.0008	-0.011 ± 0.0029	0.981 to 0.999
TFMPP	0.012 ± 0.0010	-0.001 ± 0.0014	0.996 to 1.000
MDMA	0.009 ± 0.0002	-0.002 ± 0.0020	0.998 to 1.000
MDEA	0.010 ± 0.0002	-0.003 ± 0.0020	0.998 to 1.000
MBDB	0.006 ± 0.0001	-0.001 ± 0.0016	0.996 to 1.000
mCPP	0.004 ± 0.0006	-0.002 ± 0.0009	0.998 to 1.000
MeOPP	0.009 ± 0.0011	-0.006 ± 0.0035	0.993 to 0.999
MDBP	0.053 ± 0.0060	-0.044 ± 0.0389	0.983 to 0.998

(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Validation

Accuracy: Within ±15% of the nominal value (±20% near LOQ)

Precision: ≤15% (≤20%) RSD

Extraction efficiency: 30 - (69 -) 110%

Processed sample stability: No indication of instability

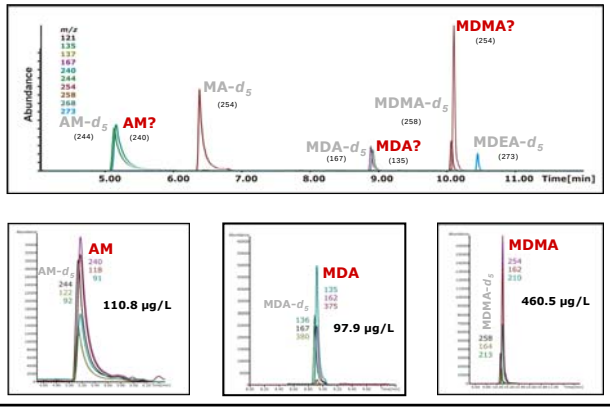
Freeze/thaw stability: Ratio of means within 90-110%
90% CI within 80-120% of control mean

Limits: Lowest point - LOQ with S/N ratios > 10
LOD - not systematically evaluated

Analyte	n=16 (8 days ▮ 2 replicates)												Extraction efficiency [%] mean ± SD	
	Repeatability RSD [%]				Intermediate precision RSD [%]				Accuracy Bias [%]					
	LQS	LOW	MED	HIGH	LQS	LOW	MED	HIGH	LQS	LOW	MED	HIGH	LOW (n = 5)	HIGH (n = 5)
AM	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.7
MA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.3
EA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.6
HO-A	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.7
PMA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.7
PHOL	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 0.6
MDA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.7
PMM	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.6
BDB	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.4
MTA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.7
BZP	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 1.3
TFMI	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 2.3
MDMA	18.7	9.5	0.8	1.5	18.7	9.5	1.9	2.2	5.8	-4.3	-6.0	-8.4	100 ± 4.9	89 ± 0.4
MDEA	1.9	4.4	2.0	1.5	3.7	4.4	2.0	1.9	-0.6	-5.6	-5.6	-7.9	103 ± 6.8	93 ± 1.1
MBDB	4.6	3.7	2.6	2.0	5.6	3.7	3.3	2.6	2.6	-3.2	-7.9	-9.2	100 ± 2.7	95 ± 3.8
mCPP	6.8	9.3	3.3	4.1	8.1	9.3	12.9	15.0	1.9	-6.0	2.8	-3.4	69 ± 0.9	63 ± 1.8
MeOPP	9.2	7.8	2.5	4.4	9.2	7.8	8.6	9.3	-6.3	-9.6	-0.6	-4.3	106 ± 7.6	96 ± 3.1
MDBP	15.3	8.8	6.3	6.4	15.3	9.0	14.9	17.7	12.9	-1.9	3.3	5.3	107 ± 7.8	82 ± 12.4

(Peters/Schaefer/Staack/Kraemer/Maurer, J. Mass Spectrom., 2003)

Authentic Case with AM, MDA and MDMA



Method Development

- Initial Considerations
- Development of Parameters
- Development of Separation System
- Development of Sample Preparation
- Optimization
- **Validation**

Validation Types

Full Validation

- For first implementation of a bioanalytical method
- For addition of new analytes to an existing assay

Partial Validation

- For method transfers between laboratories
- After changes of instrumentation
- After changes of matrix (e.g. plasma to urine)
- After change of sample processing procedure(s)

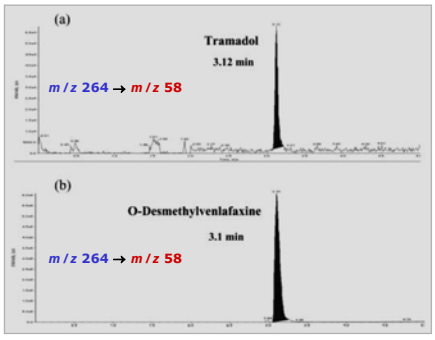
(Peters/Maurer, Accred Qual Assur, 2002)

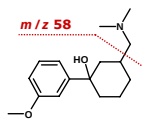
Validation Parameters

- Selectivity/Specificity
- Calibration Curve/Linearity
- Accuracy
  - Precision
  - Bias/Trueness
- Sensitivity/Limits
- Stability
- Recovery
- Matrix Effects for all LC-MS-based methods

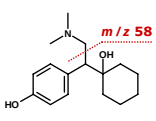
(Peters/Maurer, Accred Qual Assur, 2002)

Selectivity
An Important Issue in CT/FT (even in LC-MS-MS)





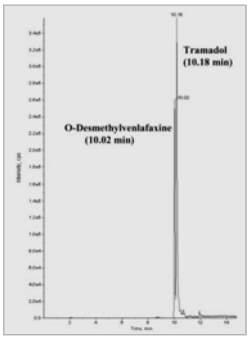
$[M+H]^+$ m/z 264



$[M+H]^+$ m/z 264

(Allen, Clin Toxicol, 2006)

Selectivity
An Important Issue in CT/FT (even in LC-MS-MS)



Improved selectivity

- better separation
- but: increased analysis time

Possible alternatives

- monitoring more selective transitions
- monitoring additional transitions

(Allen, Clin Toxicol, 2006)

Stability
An Important Issue in Forensic Toxicology

Processed sample stability

- Pool processed samples at high and low concentrations levels
- Inject aliquots at fixed time intervals

In-process, freeze/thaw and long-term stability

- Use QC samples at high and low concentration levels
- Analyze replicate control samples
  - from the same pool as stability samples, but analyze prior to freezing or thawing
- Analyze replicate stability samples after freezing or thawing

(Peters, Anal Bioanal Chem, 2007)

Experimental Design for Validation of GC-MS Procedures

Run	Linearity	Selectivity	Processed Sample Stability
0	42 calibration samples (7 concentration levels, 6 replicates each)	16 different matrix blanks 2x2 zero samples x spiked samples	18 injections of pooled extracts (every 3.8 h, at two concentrations)
Total	80 (80+x) injections		

Run	Calibration samples (7 levels)	Validation samples: Low P&A	Low Stability	Low Rec	Med P&A	High P&A	High Stability	High Rec	optional LLOQ P&A	optional Dil P&A	Total
1	(7)	2	6	-	2	2	6	-	2	2	25 (29)
2	(7)	2	-	2x5	2	2	-	-	2	2	23 (27)
3	(7)	2	-	-	2	2	-	2x5	2	2	23 (27)
4	(7)	2	6	-	2	2	6	-	2	2	25 (29)
5	(7)	2	-	-	2	2	-	-	2	2	13 (17)
6	(7)	2	6	-	2	2	6	-	2	2	25 (29)
7	(7)	2	-	-	2	2	-	-	2	2	13 (17)
8	(7)	2	-	-	2	2	-	-	2	2	13 (17)
Total	160 (192) injections										

(Habrdova/Peters/Theobald/Maurer, J. Mass Spectrom., 2005)

Do we Always Need Full Calibration ?

Full Calibration or One-Point Calibration?

A Retrospective Analysis

of Six Validated Assays

Frank T. Peters and Hans H. Maurer, Anal Chem, 2007

Calibration in Single Sample Analysis ?

Historic (stored) calibration curves

- No 'extra' calibration required saving time and resources
- Often long times between calibration and analysis
- Questionable because changes of important parameters likely

Full calibration at time of sample analysis

- Optimum situation with respect to result
- Comparatively high workload (usually ≥ 5 calibrators)
- Time-consuming (big disadvantage in emergency toxicology!)

One point calibration at time of sample analysis

- Compromise between necessary calibration, workload, and time
- Often used but reliability rarely systematically checked

Results obtained with one-point cablibration reliable?

Retrospective Analysis of Data from Six Validated Assays

Three GC-MS Assays for Plasma Quantification of

- MDA, MDMA, and MDEA enantiomers (I); Peters et al., Clin Chem, 2007
- 8 Drugs relevant to Diagnosis of Braindeath (II); Peters et al., TDM, 2005
- 18 AM- and piperazine-derived DD (III); Peters et al., JMS, 2003

Three LC-MS Assays for Plasma Quantification of

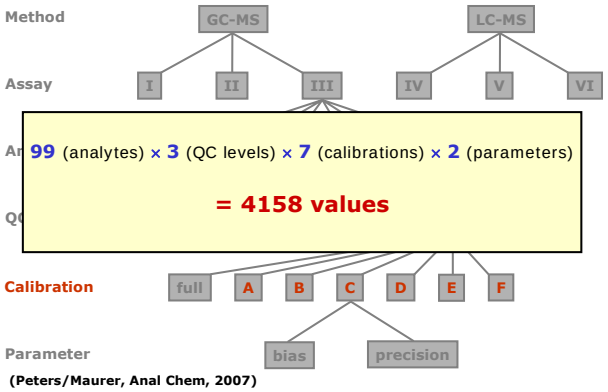
- 22 Beta-blockers (IV); Maurer et al., J Chromatogr A, 2004
- 23 BDZ, 3 BZ_1-agonists, and flumazenil (V); Kratzsch et al., JMS, 2003
- 15 Neuroleptics and 3 of their metabolites (VI); Kratzsch et al., JMS, 2003

Sample preparation

- Liquid-liquid extraction (II, V) or solid-phase extraction (I, III, IV, VI)
- Deuterated analogues used as IS for most (I, II), several (III, V), or no analytes (IV, VI)

(Peters/Maurer, Anal Chem, 2007)

Retrospective Data Analysis



(Peters/Maurer, Anal Chem, 2007)

Summary and Conclusions

Summary

- Retrospective calculation of one-point calibration data from existing validation data acquired with described experimental design
- Calibrators D obviously most suitable for one-point calibration
  - Acceptance criteria for bias (±15%, ±20% near LOQ) and precision (CV ≤15%, ≤20% near LOQ) fulfilled for most analytes
  - BUT: criteria not fulfilled for several BZD at low concentrations!

Conclusions

- One-point calibration can yield reliable results
- Reduction of analysis time and expense of resources possible
- Exceptions call for assessment of reliability for each single analyte

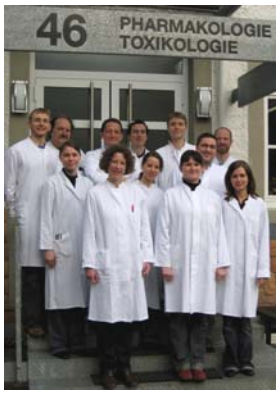
Full validation provides the data basis for assessment of reliability of one-point calibration!!

(Peters/Maurer, Anal Chem, 2007)

Department of Exper. & Clinical Toxicology Saarland University, Homburg, Germany

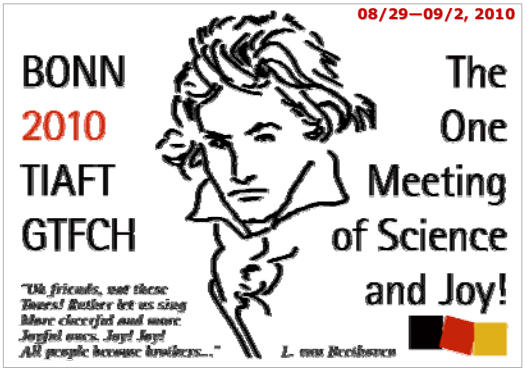
I would like to thank my scientific coworkers for their excellent work...

Dr. Frank T. Peters
Andreas Ewald
Markus Meyer
Melanie Mueller (JHH)
Anika-Anina Philipp
Daniela Remane
Christoph Sauer
Andrea Schwaninger
Dirk Wissenbach
Anne Kauffels
Nina Glaser
Stephanie Schaan
Matthias Sorge
Armin Weber



Greetings from Homburg/Saar

TIAFT Meeting in Germany



(http://www.gtfch.org/cms/)

Abkürzungsverzeichnis

AAS	Atomabsorptionsspektrometrie (für Metallbestimmungen)
AC	Acetylierung, acetyliert
ACE	Angiotensin Converting Enzyme
ACN	Acetonitril
ADH	Alkoholdehydrogenase
AF	Ammoniumformiat
AM	Amphetamin
APCI	Atmospheric Pressure Chemical Ionization
AT_1 Rezeptorblocker	Angiotensin Rezeptorblocker (Blutdrucksenker)
BDA	Benzodiazepin (Tranquilizer u.ä.)
BZE	Benzoylecgonin (Cocain-Metabolit)
CE	Capillarelektrophorese
CID	Collision-induced dissociation
COHb	Kohlenmonoxidhämoglobin
Cp	Plasmakonzentration
CT	Clinical Toxicology
DAD	Diodenarraydetektor (UV-Detektor)
DEG	Diethylenglycol
DOA	Drugs of Abuse
ECD	Electron-capture Detecto (Elektroneneinfang-Detektor)r
EG	Ethylenglycol
EI	Electron Ionization
EIA	Enzym-Immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray Ionization
FD	Fluoreszenzdetektor
FID	Flammenionisationsdetektor
FD	Fluoreszenzlicht-Detektor
FPIA	Fluoreszenzpolarisationsimmunoassay
FT	Forensic Toxicology
(FT)IR	(Fourier-Tansformations-)Infrarotlicht-Detektor
GC	Gaschromatographie
GC-MS	Gaschromatographie-Massenspektrometrie-Kopplung
GRD/ARS	Glucuronidase/Arylsulfatase
GSH	Gluthation
HFB	Heptafluorobutyrilierung, heptafluorobutyriliert
HPLC	Hochleistungsflüssigchromatographie
HS-GC	Headspace (Dampfraum) Gaschromatographie
HY	Hydrolyse, hydrolysiert
HPC	Heptafluorobutyrylprolylchlorid
IA	Immunoassay
ICP-MS	Inductively-coupled-plasma mass-spectrometry (für Metallbestimmungen)
IS	Interner (Analysen)-Standard
LC-MS	Liquidchromatographie-Massenspektrometrie-Kopplung
LIA	Lumineszenzimmunoassay
LLE	Liquid-liquid extraction
LOD	Limit of Detection
LOQ	Limit of Quantification

LSD	Lysergsäurediethylamid
MA	Methamphetamin
MAM	Monoactetyl-Morphin (Heroin-Metabolit)
mCPP	Chlorophenylpiperazin (Designer Droge)
MDA	Methylendioxyamphetamin (Designer-Droge)
MDBP	Methylendioxybenzylpiperazin (Designer Droge)
MDE(A)	Methylendioxyethylamphetamin (Designer-Droge)
MDMA	Methylendioxymethamphetamin (Ecstasy, Designer-Droge)
ME	Methylierung, methyliert
MeOPP	Methoxyphenylpiperazin (Designer Droge)
MS	Massenspektrometer, Massenspektrometrie
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoracetamid (Silylierungsreagenz)
NICI	Negative-ion chemical ionization
NPD	Nitrogen-Phosphorous-selective Detector
NSAID	Non-steroidal Anti-inflammatory Drug (Rheuma/Schmerzmittel)
OAD	Orales Antidiabeticum
PCP	Phencyclidin (Rauschdroge)
PG	Propylenglycol
p.i.	post ingestionem (nach der Einnahme)
PICI	Positive-ion chemical ionization
RIA	Radioimmunoassay
RP	Reversed-phase (Umkehrphasenchromatographie)
Scan mode	Zyklische Aufnahme vollständiger Massenspektren
SIM mode	Selected-ion monitoring mode (Zyklische Aufnahme ausgewählter Massenspuren)
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SSRI	Selektiver Serotonin-Reuptake Inhibitor (Antidepressivum)
STA	Systematische toxikologische Analyse
TDM	Therapeutic Drug Monitoring (Kontrolle der Plasmakonzentration zur Medikamenteneinstellung)
TFMPP	Trifluoromethylphenylpiperazin (Designer Droge)
THC	Tetrahydrocannabinol (Wirkstoff des Cannabis)
THC-COOH	THC-Carbonsäure (THC-Metabolit)
TLC	Thin-layer chromatography (Dünnschichtchromatographie)
TMS	Trimethylsilylierung, trimethylsilyliert
UV	Ultraviolettlicht-Detektor
XTC	Ecstasy (MDMA, Designer Droge)