

Dried Blood Spots (DBS) and Dried Urine Spots (DUS) for Monitoring of Alcohol Markers and New Psychoactive Substances

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Urine contaminated with bacteria (e.coli)

Leads to alteration of EtG in urine samples

Helander A, Dahl H (2005) **Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption.** Clin Chem 51(9):1728–1730

Helander A, Olsson I, Dahl H (2007) **Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption.** Clin Chem 53(10):1855–1857

Urine contaminated with bacteria

Leads to Degradation of EtG in urine samples

Baranowski S, Serr A, Thierauf A, Weinmann W, Grosse Perdekamp M, Wurst FM, Halter CC (2008) **In vitro study of bacterial degradation of ethyl glucuronide and ethyl sulphate.** Int J Legal Med 122 (5):389–393

Thierauf A, Serr A, Halter CC, Al-Ahmad A, Rana S, Weinmann W (2008) **Influence of preservatives on the stability of ethyl glucuronide and ethyl sulphate in urine.** Forensic Sci Int 182 (1–3):41–45

Collection of urine on paper.... Stabilization of EtGstops degradation by bacteria

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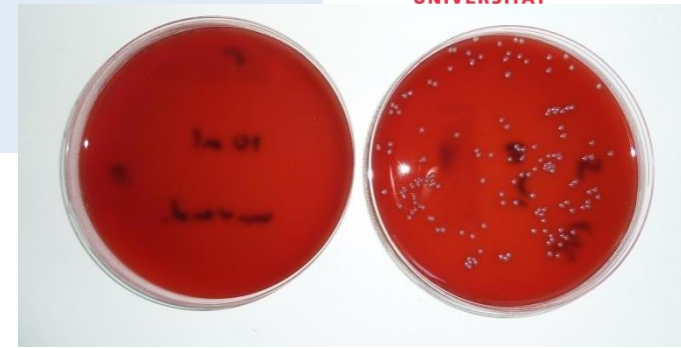
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UNIVERSITÄT



pH Paper



Whatmann
No. 903 filter
paper

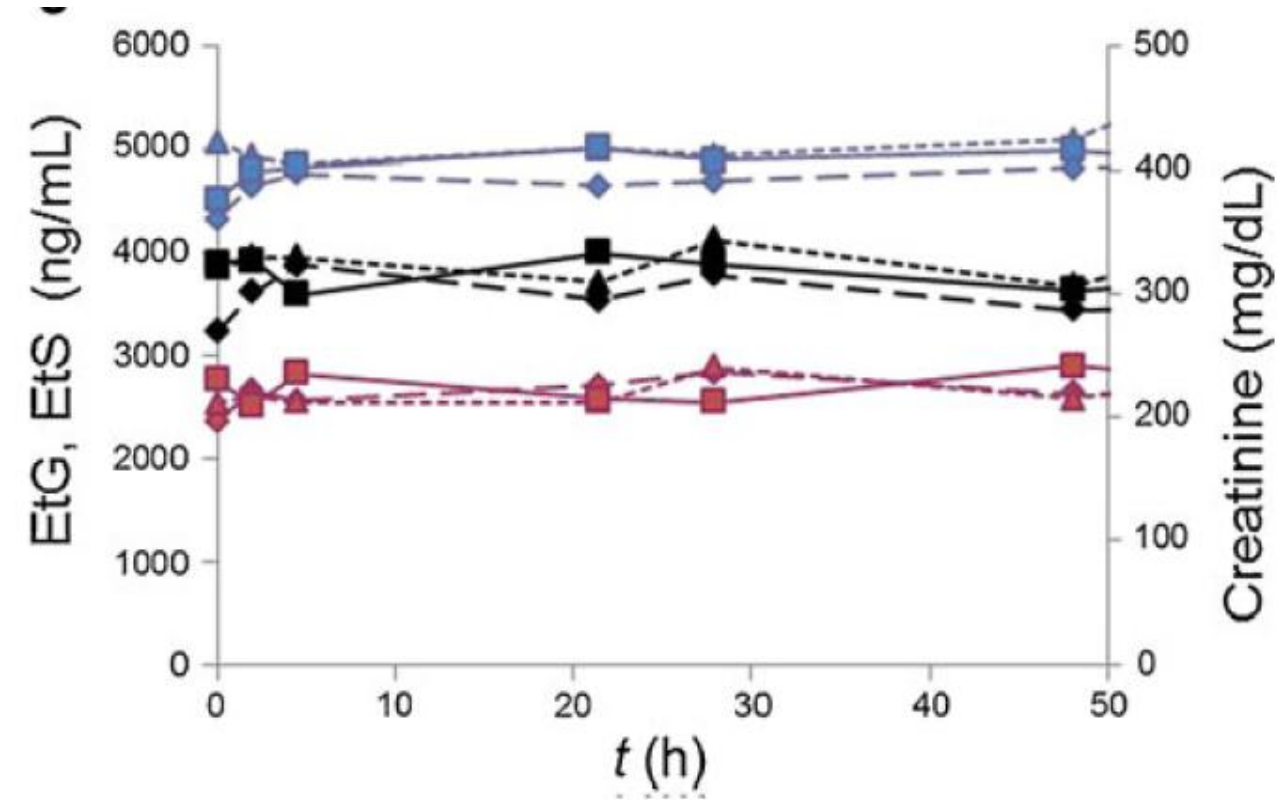


Columbia-blood-agar-plates
with escherichia coli (e. coli)

left: no colonies visible

right:
Colonies of escherichia coli
visible

Urine (sterile filtered) (storage: 48 hours, 37 °C)

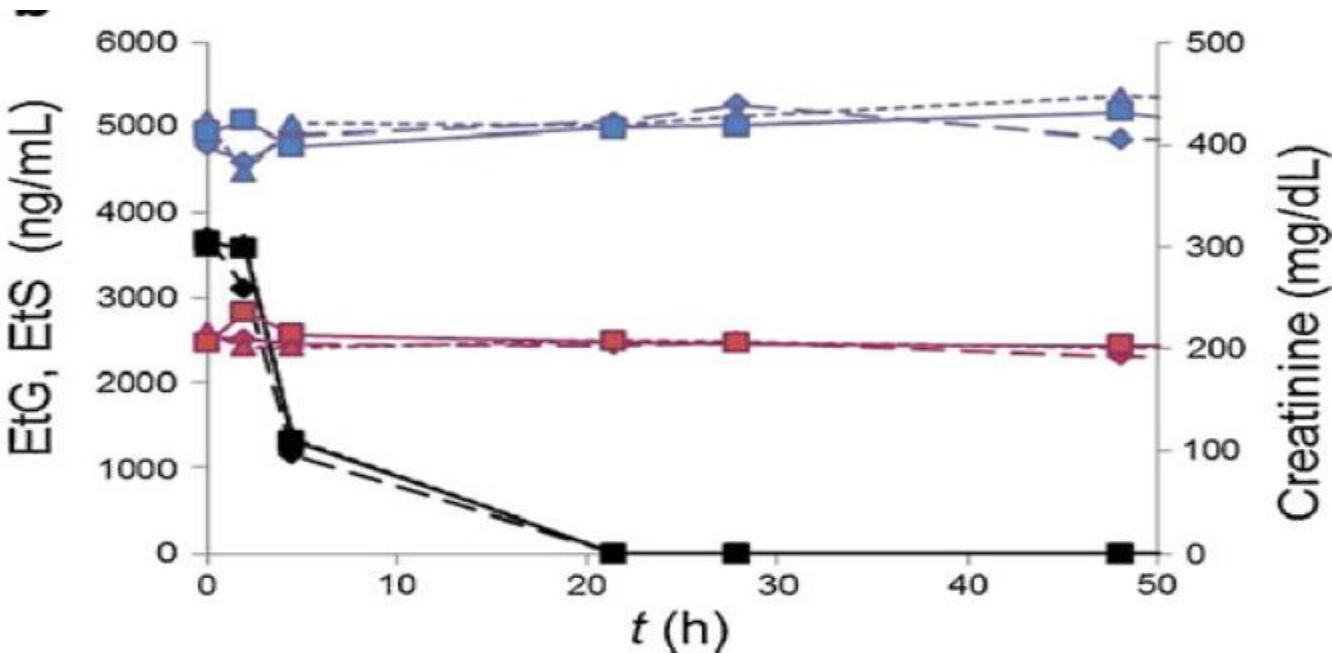


Blue: EtS

Black: ethyl
glucuronide EtG

Red: creatinine

Urine contaminated with e. coli (10^6 CFU) (incubation for 48 hours, 37 °C)



Blue: EtS

Red: creatinine

Black: ethyl glucuronide

Collection of urine incubated with e. coli on pH-paper strips or filter paper subsequent drying (no further degradation)

Anal Bioanal Chem

DOI 10.1007/s00216-011-5687-7

ORIGINAL PAPER

Inhibition of bacterial degradation of EtG by collection as dried urine spots (DUS)

Ana Hernández Redondo • Christiane Körber •
Stefan König • Andreas Längin • Ali Al-Ahmad •
Wolfgang Weinmann

Received: 22 September 2011 / Revised: 20 December 2011 / Accepted: 20 December 2011

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Kaufmann E, Alt A (2008) Detection of ethyl glucuronide in dried human blood using LC-MS/MS. *Int J Legal Med* 122(3):245–249

Winkler M, Kaufmann E, Thoma D, Thierauf A, Weinmann W, Skopp G, Alt A (2011) Detection of ethyl glucuronide in blood spotted on different surfaces. *Forensic Sci Int* 210(1–3):243–246

Hernández Redondo A, Schroeck A, Kneubuehl B, Weinmann W. Determination of ethyl glucuronide and ethyl sulfate from dried blood spots. *Int J Legal Med*. 2013 Jul;127(4):769-75. doi: 10.1007/s00414-012-0815-2. Epub 2013 Jan 3.

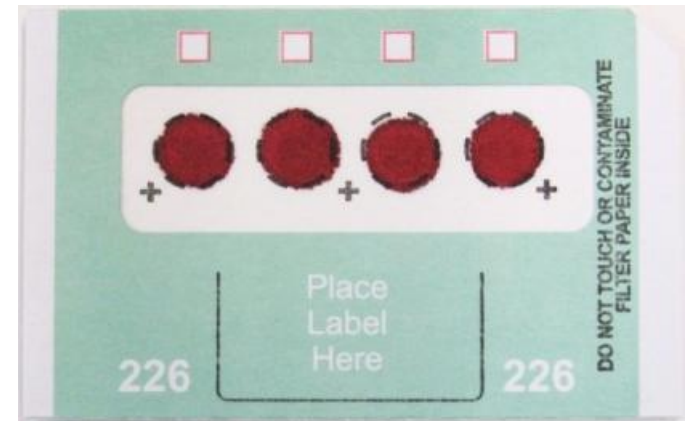
EtG and EtS on DBS

Int J Legal Med
DOI 10.1007/s00414-012-0815-2

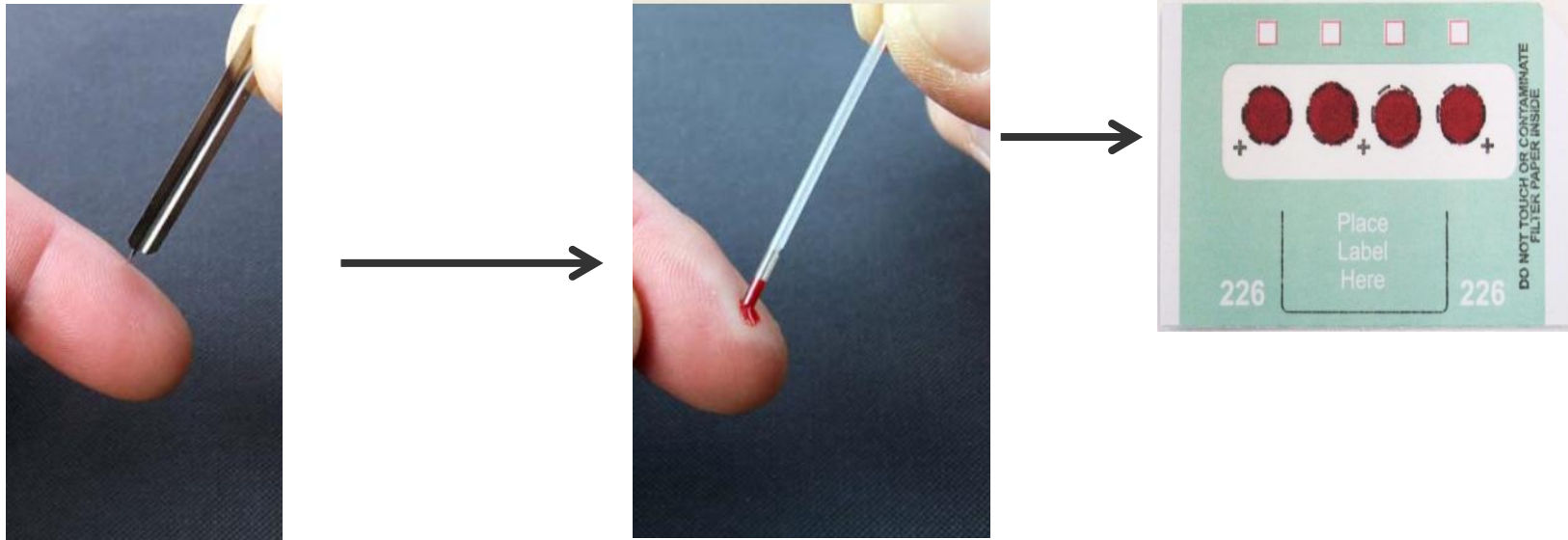
ORIGINAL ARTICLE

Determination of ethyl glucuronide and ethyl sulfate from dried blood spots

Ana Hernández Redondo • Alexandra Schroeck •
Beat Kneubuehl • Wolfgang Weinmann



Sample Collection: capillary blood



Blood collection with lancette and capillary (or pipette)

Sample Preparation



10 - 30 μL
blood
3 h drying

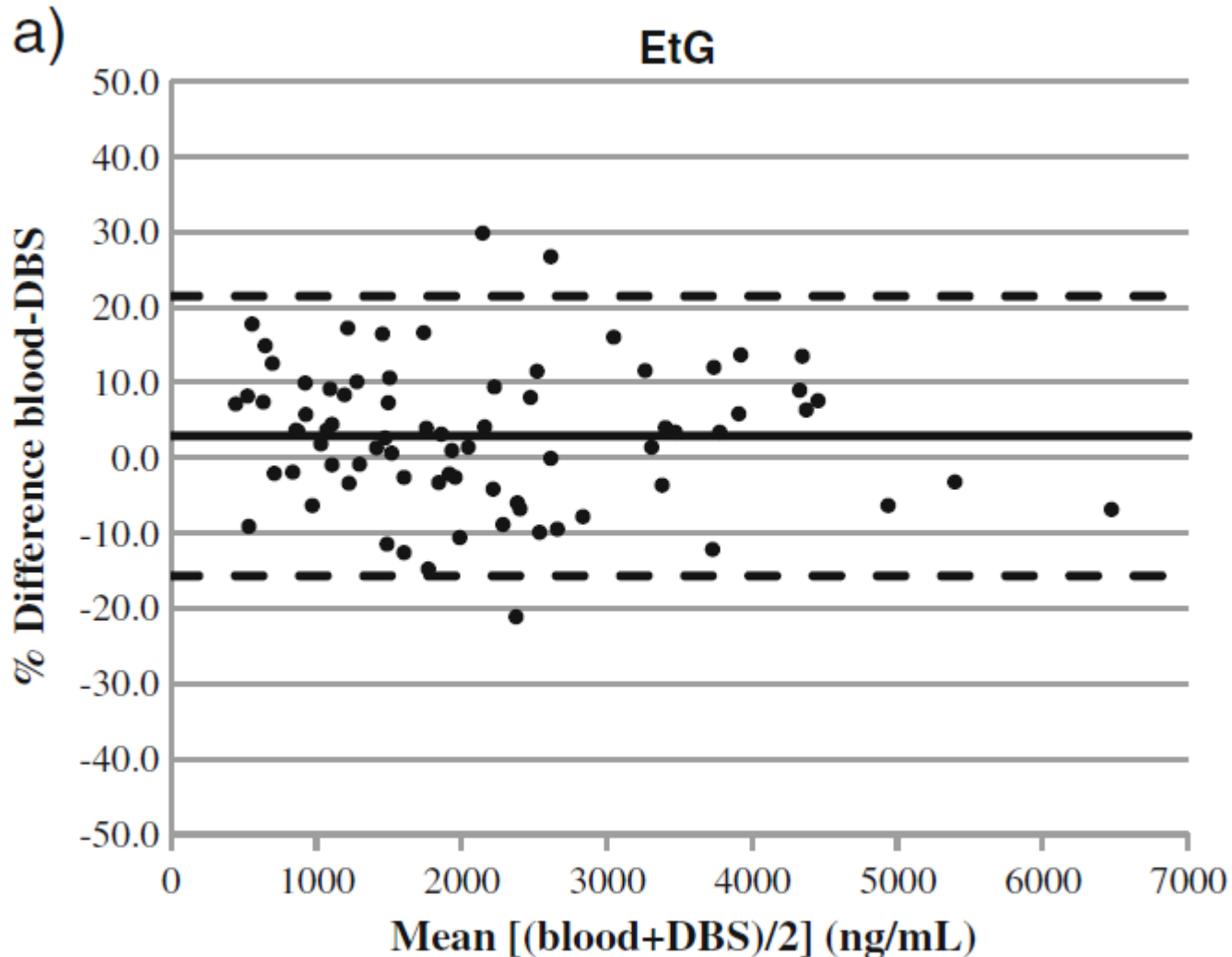


Punch out
DBS



Extraction
500 μL MeOH

EtG and EtS on DBS (Bland-Altman Difference plots)



Phosphatidylethanol (PEth)

Quantitation by LC-MS/MS in Whole Blood

H. Gnann¹, W. Weinmann¹, C. Engelmann¹, A. Thierauf¹, F. M. Wurst², G. Skopp³, M. Winkler⁴, V. Auwärter¹, S. Dresen¹, N. Ferreiros Bouzas¹

¹ Institute of Legal Medicine, University Medical Centre, Albertstrasse 9, 79104 Freiburg, Germany

² Department of Psychiatry and Psychotherapy II, Christian Doppler Clinic, Paracelsus Medical University, Salzburg, Austria

³ Institute of Legal Medicine and Traffic Medicine, University Hospital, Voss-Str. 2, 69115 Heidelberg, Germany

⁴ Institute of Legal Medicine, University of Ulm, Prittwitz Str.6, 89075 Ulm, Germany

Facts about PEth

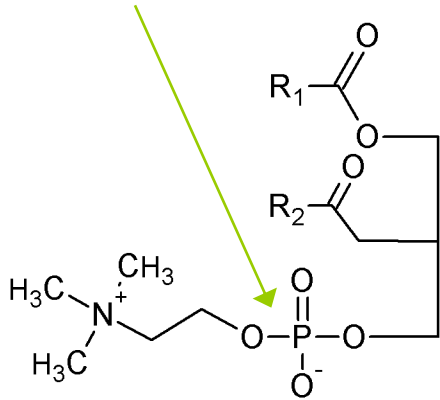
- abnormal phospholipid
- direct alcohol consumption marker
- formed in cell membranes of red blood cells
- stable 3 weeks in refrigerator or freezer -80°C

- $t_{1/2}$: 4 days
- detectable up to 29 days after sobriety

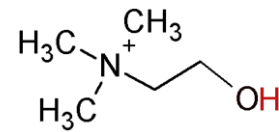
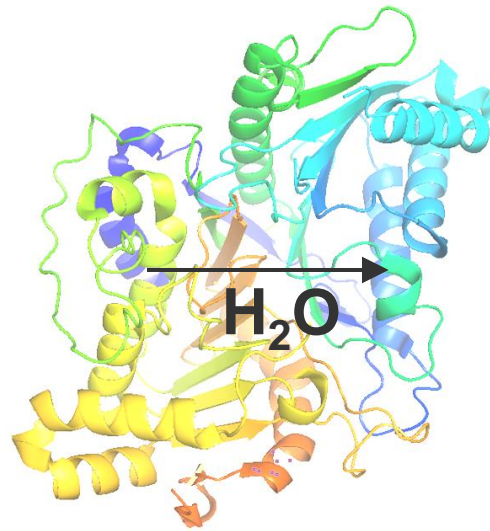
Introduction

synthesis of PEth

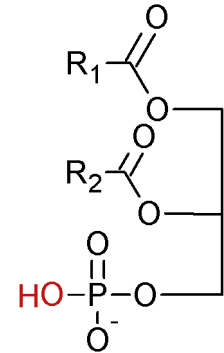
phospholipase D



phosphatidylcholine



choline

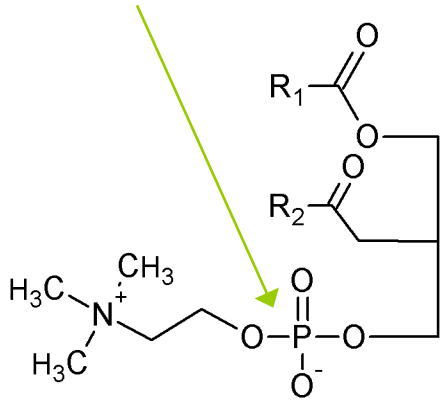


phosphatidic acid

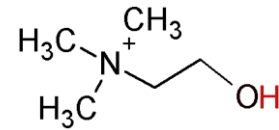
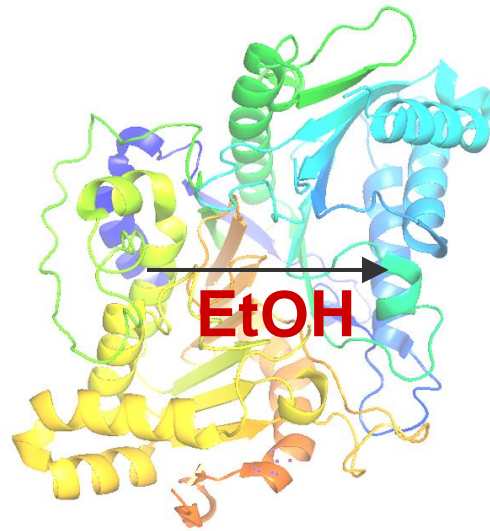
Introduction

synthesis of PEth

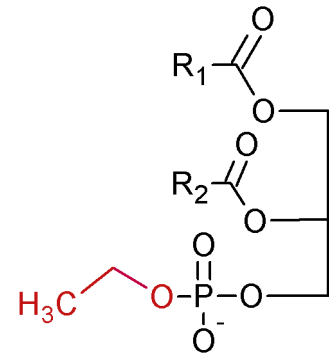
phospholipase D



phosphatidylcholine



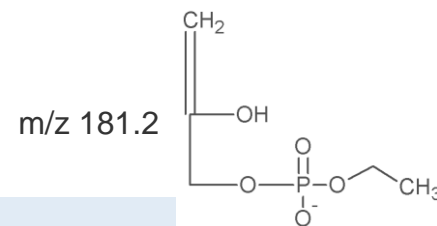
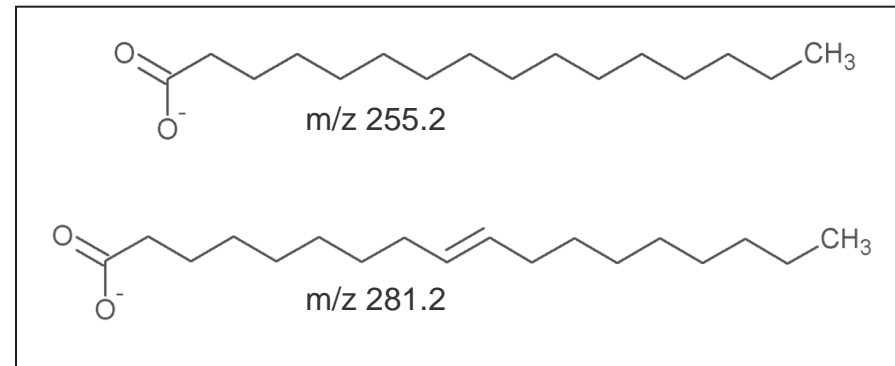
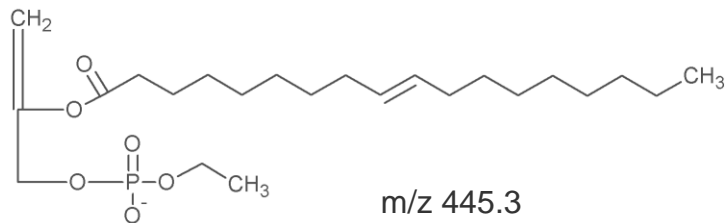
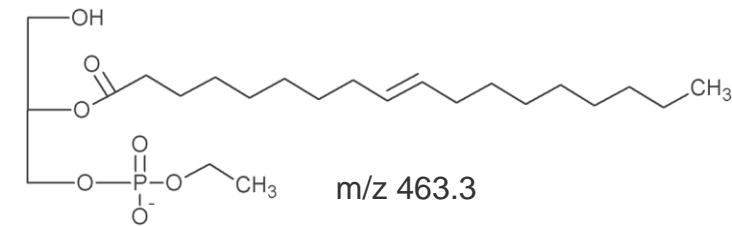
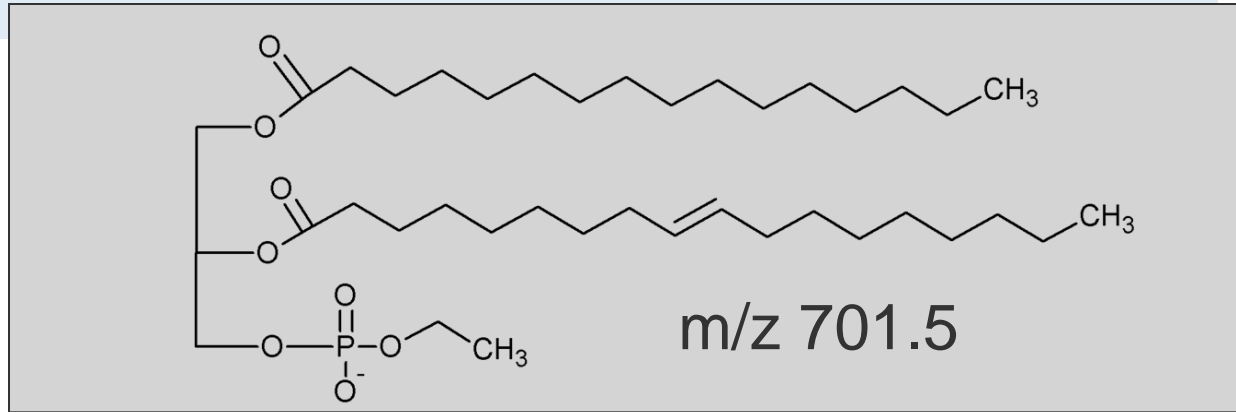
choline



phosphatidylethanol

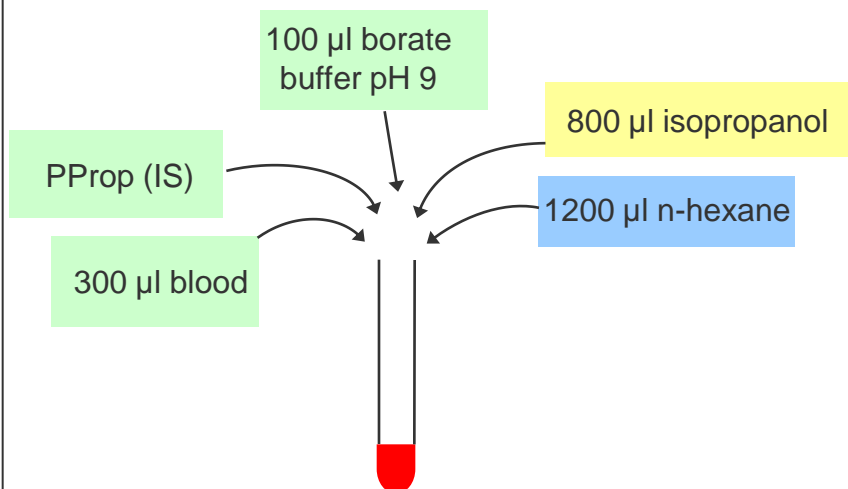
Introduction

PEth 16:0/18:1 and fragments



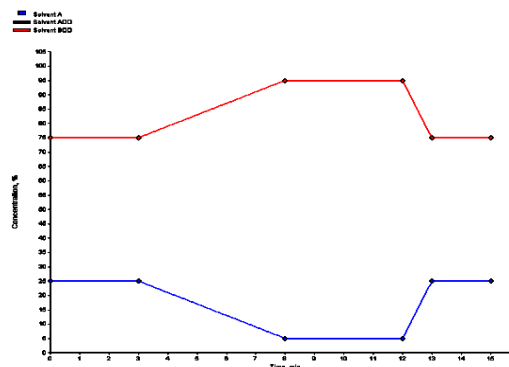
Method

Extraction



Shake 10 min, centrifuge, evaporate the supernatant and redissolve in 150 µl solvent

LC-parameter



Column:
Luna Phenyl Hexyl
50 x 2 mm, 3 µm,
Phenomenex

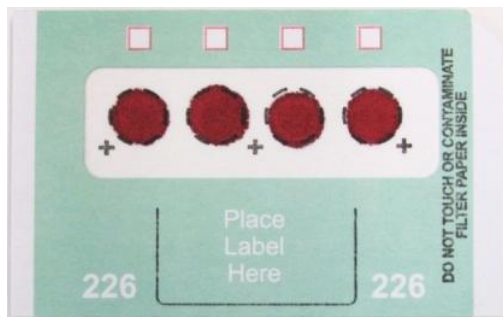
solvents: A= ammonium acetate 2 mMol/l,
B= methanol:acetone (95:5; v/v)

gradient: 15 min, flow rate: 400 µl/min

MS/MS

- electrospray ionisation
- negative mode
- precursor scans, enhanced product ion scans, multiple reaction monitoring (MRM)

Sample Preparation: DBS for PEth



30 μ L Venous Blood
(Li-Heparin)

3 h drying

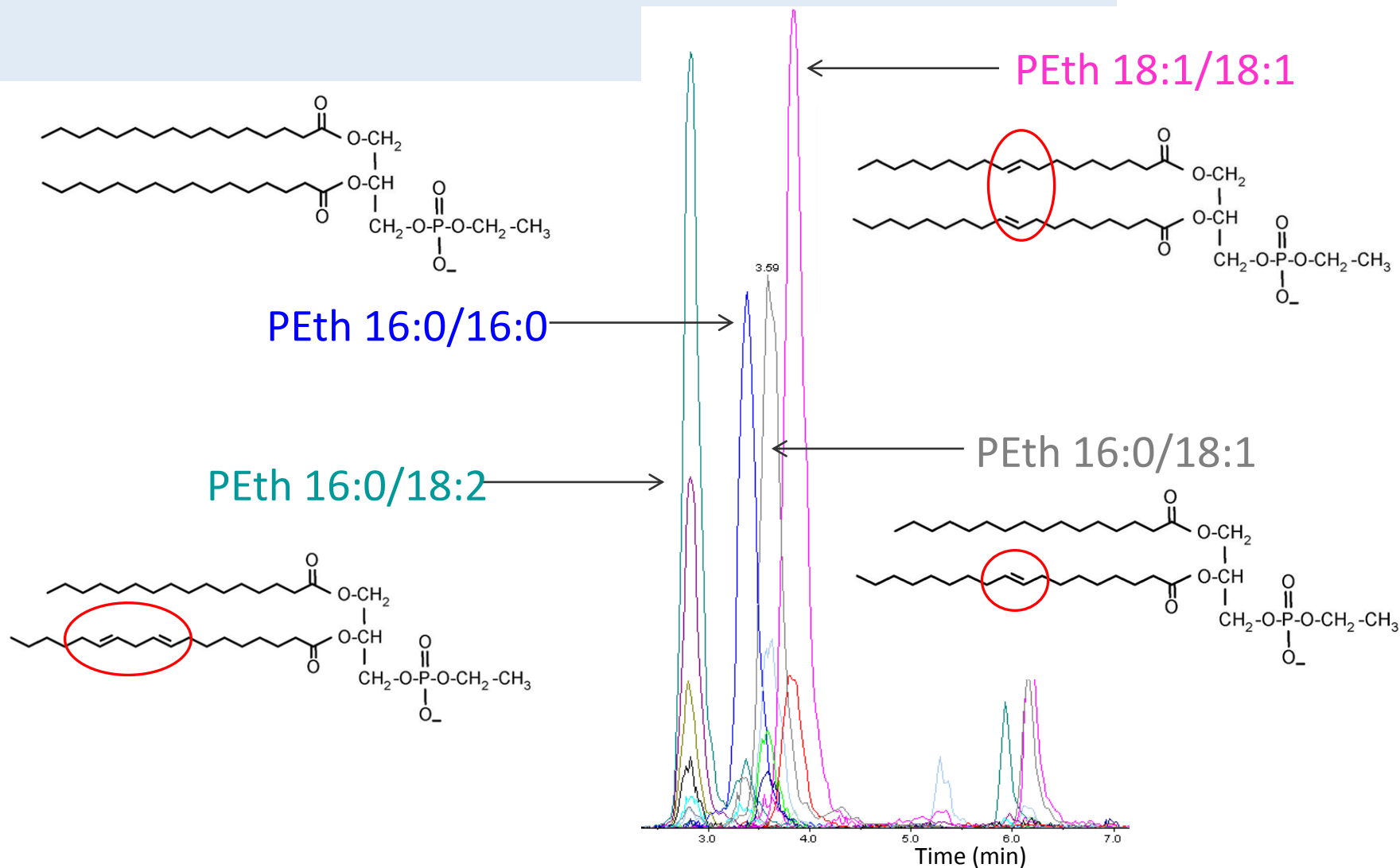


Punch out
DBS



Extraction
500 μ L MeOH

Separation of the four PEth homologues



PEth versus CDT during withdrawal therapy of alcohol addicts

H. Gnann¹, C. Engelmann¹, A. Thierauf¹, G. Skopp², M. Winkler³,
F.Sporkert⁴, W. Weinmann¹

¹ Institute of Legal Medicine, University Medical Centre, Albertstrasse 9, 79104 Freiburg, Germany

² Institute of Legal Medicine and Traffic Medicine, University Hospital, Voss-Str. 2, 69115 Heidelberg, Germany

³ Institute of Legal Medicine, University of Ulm, Prittwitz Str.6, 89075 Ulm, Germany

⁴ University Center of Legal Medicine, Lausanne-Geneva, Rue du Bugnon 21, CH-1011 Lausanne, Switzerland

experimental design

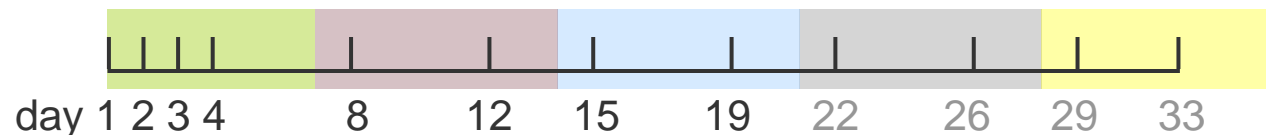
- 12 inpatient alcoholics were obtained during withdrawal therapy
- blood and urine samples were collected during the first 19 days (one patient up to 33 days)

Monitored parameters:

PEth (blood)

CDT (serum)

days of blood collection:



for comparison:

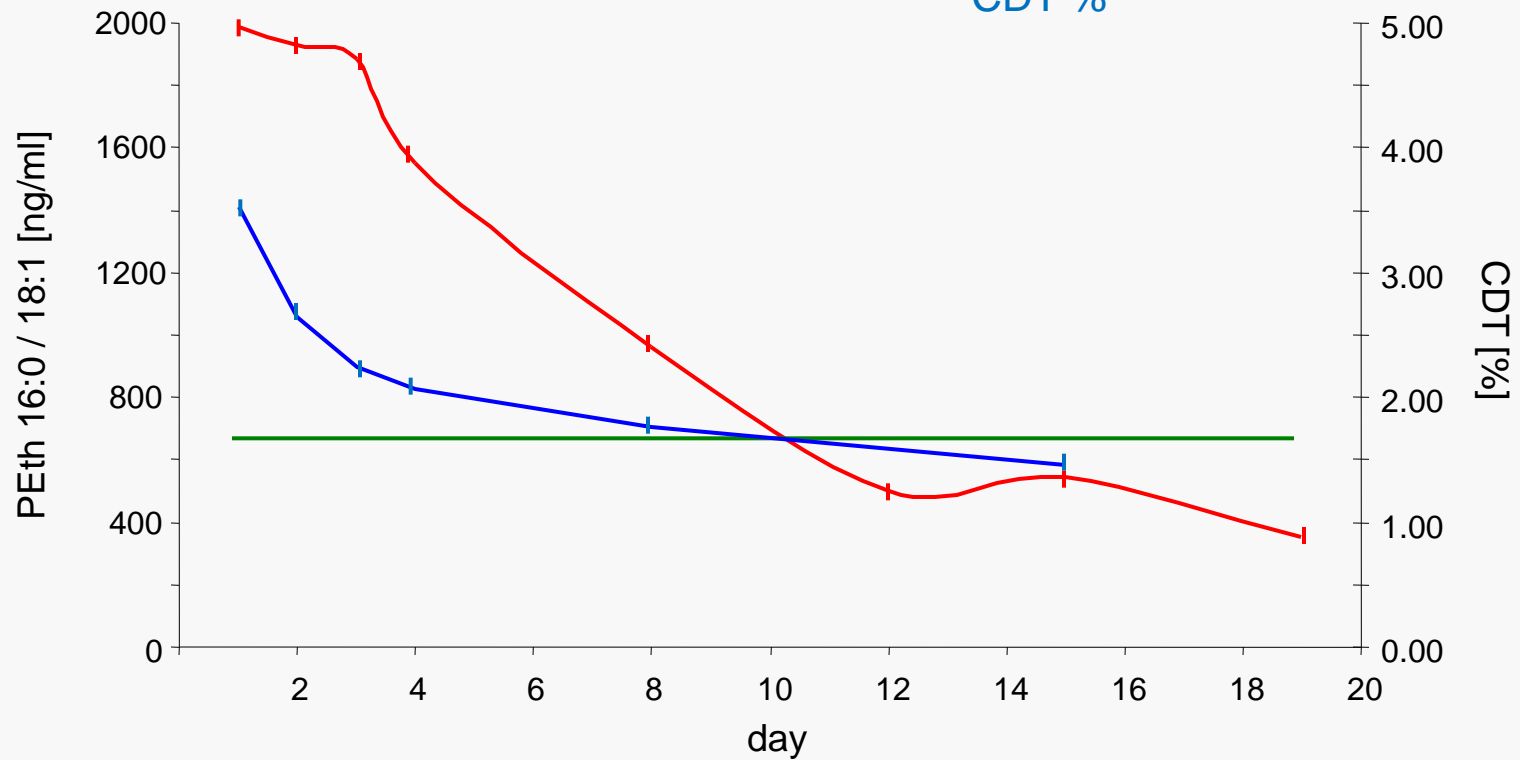
- PEth values of 78 social drinkers
- a questionnaire for the alcohol consume was filled out by the volunteers

Representative Results

pat 8

PEth 16:0/18:1

CDT %



Evaluation of phosphatidylethanol concentrations in drinking experiments

H. Gnann¹, A. Thierauf¹, W. Weinmann²

¹ Institute of Legal Medicine, University Medical Centre, Albertstrasse 9, 79104 Freiburg, Germany

² Institute of Forensic Medicine, Medical Faculty, University of Bern, Buehlstrasse 20, CH-3012 Bern, Switzerland

experimental design

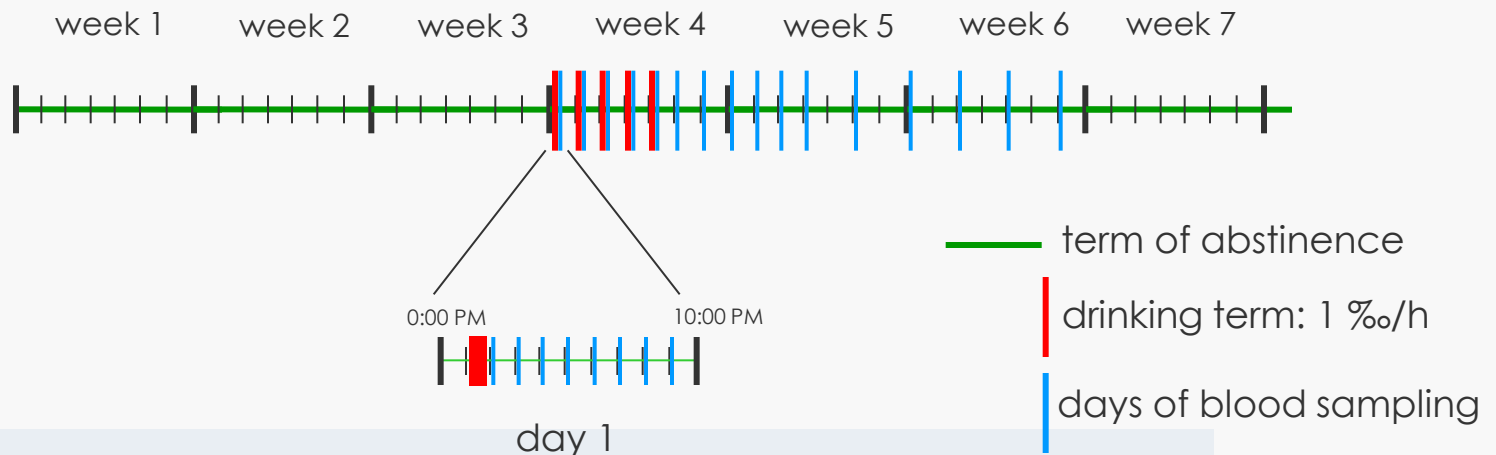
drinking experiment: 5 days

- 11 volunteers (21-76 years old)
- drinking amounts from 155 to 340 mL Vodka (40%), dependent on weight and height

Monitored parameters:

- PEth 16:0/18:1
- Ethanol
- γ -GT
- CDT

Drinking and blood sampling

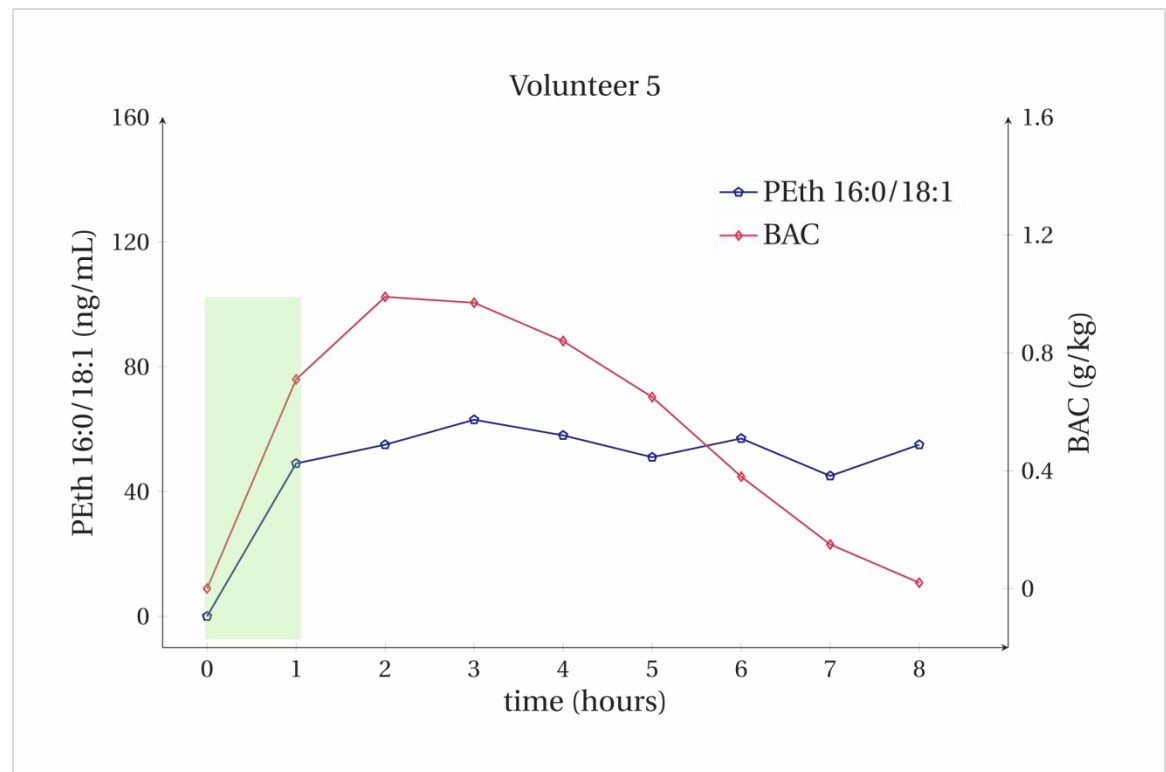


PEth and BAC formation during the first 8 hours

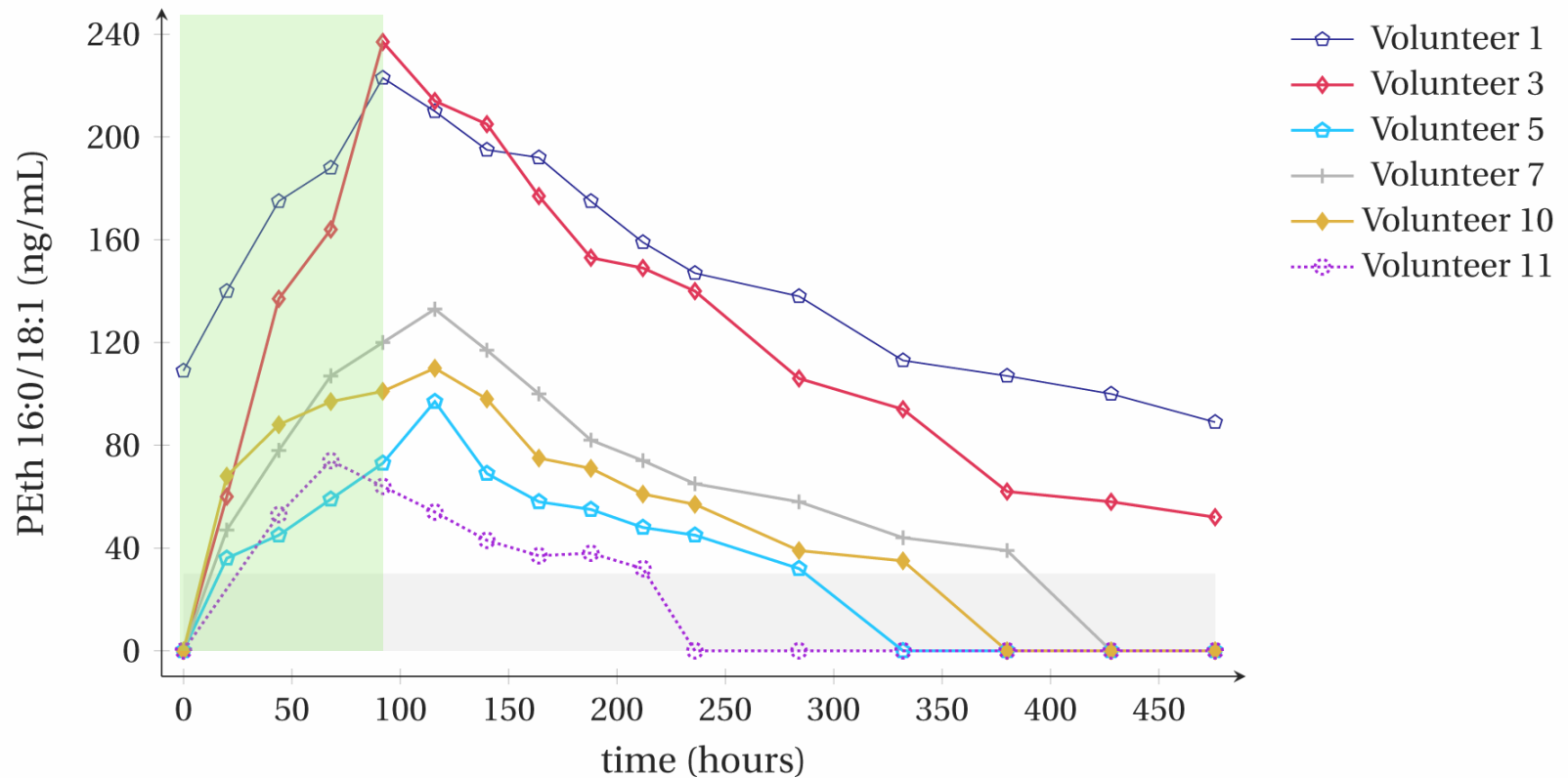
Formation of PEth
during the first two
hours in all volunteers

maximum BACs:
0.09% - 0.18%

mean PEth formation
in the first two hours:
up to 75 ng/mL



PEth formation and elimination during 21 days



experimental design

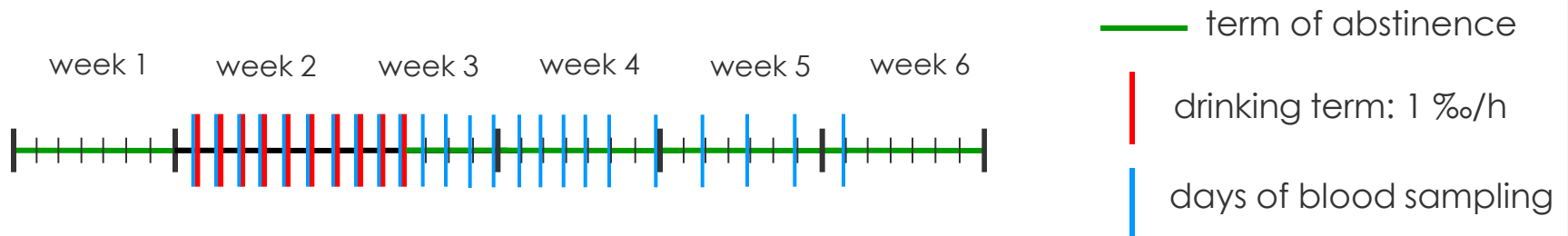
drinking experiment: 10 days

- 4 volunteers (27-31 years old)
- Drinking amounts 220 to 300 mL Vodka (40%), dependent on weight and height

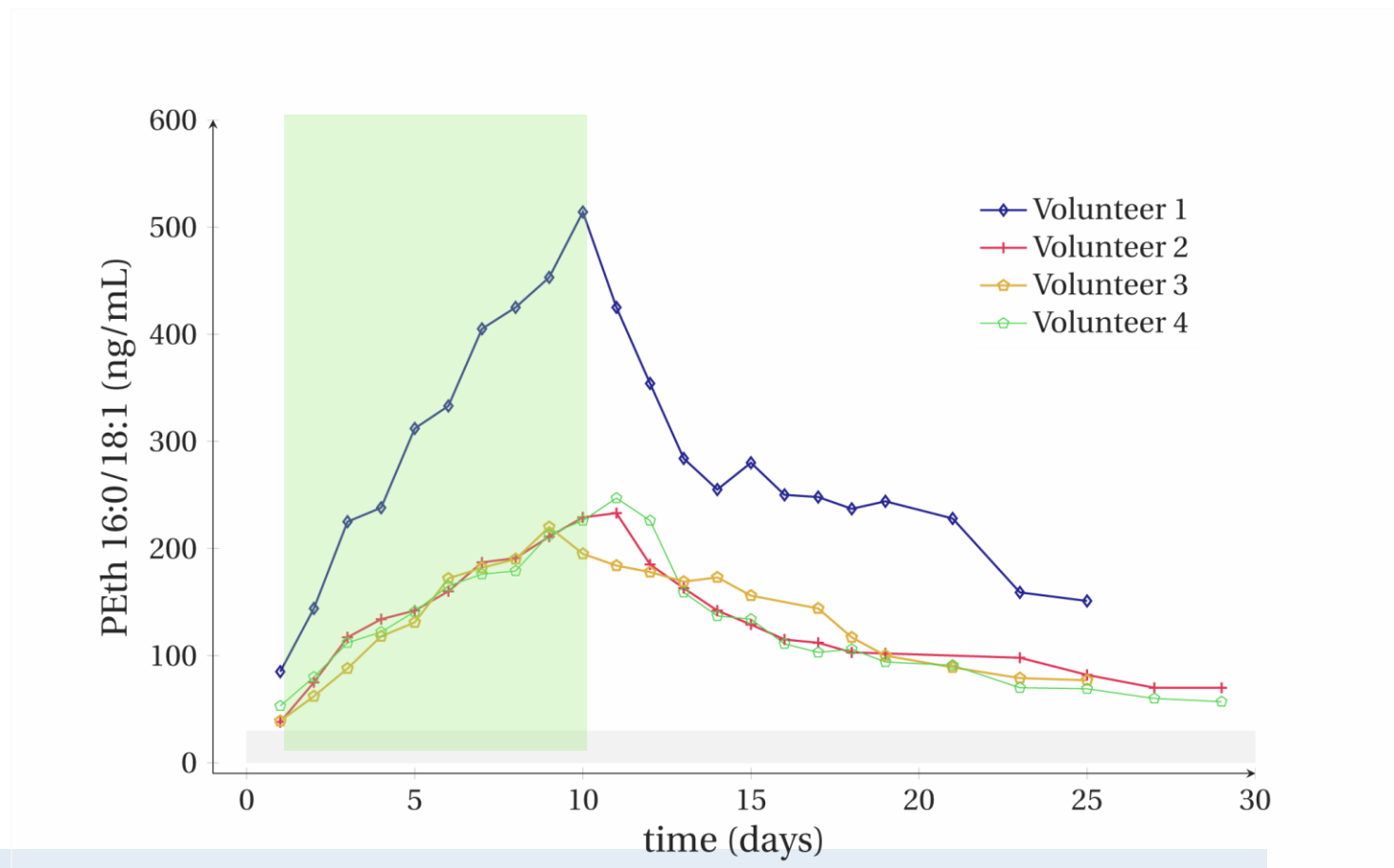
Monitored parameters:

- Peth 16:0/18:1

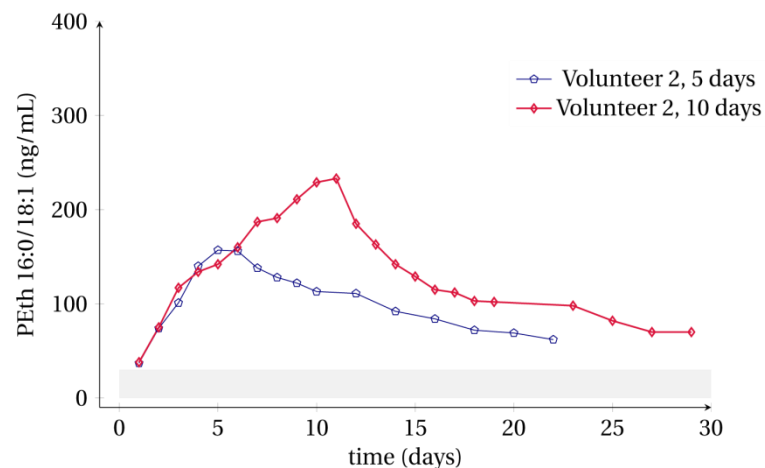
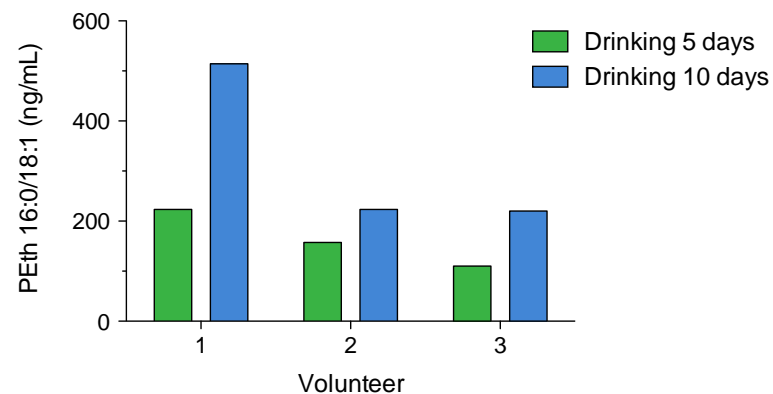
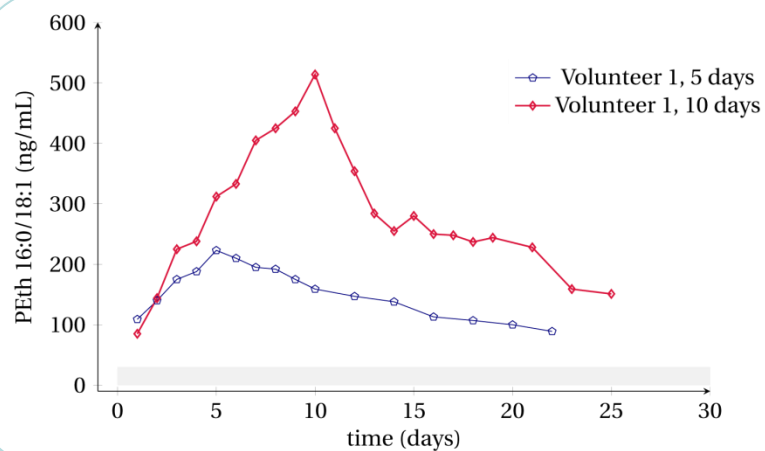
Drinking and blood sampling



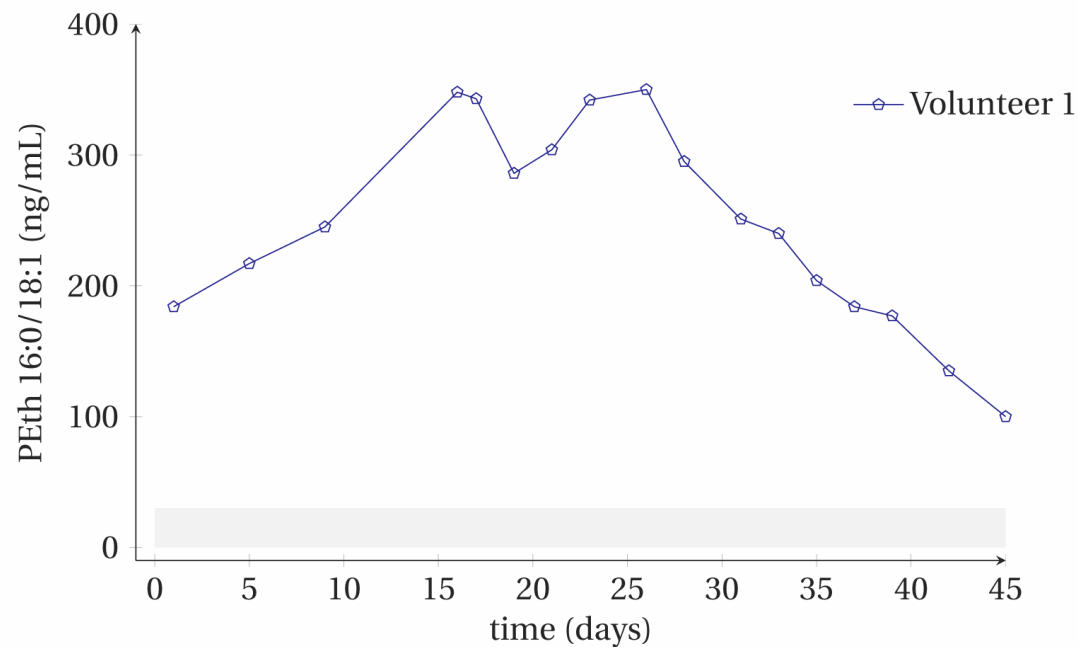
PEth formation and elimination during 29 days



Comparison 5 days – 10 days by two volunteers



Drinking over 4 weeks



- no abstinence term before starting the experiment
- only one volunteer
- 270 mL Vodka, 3 times a week over 4 weeks

Conclusions from drinking experiments

- > Peth is formed immediately when ethanol is present
- > values to 250 ng/mL by 5 days drinking and 550 ng/mL by 10 days drinking could be reached

Conclusions from drinking experiments

- > long term drinking (3 times per week, 4 weeks):
volunteer reached Peth values about 400 ng/mL
- > Cut off value for alcohol misuse:
suggestion of **800 ng/mL for Peth 16:0/18:1**
- > **PEth closes the gap between EtG and CDT and for some questions („week end drinking“) may be more sensitive than CDT**

Acknowledgements

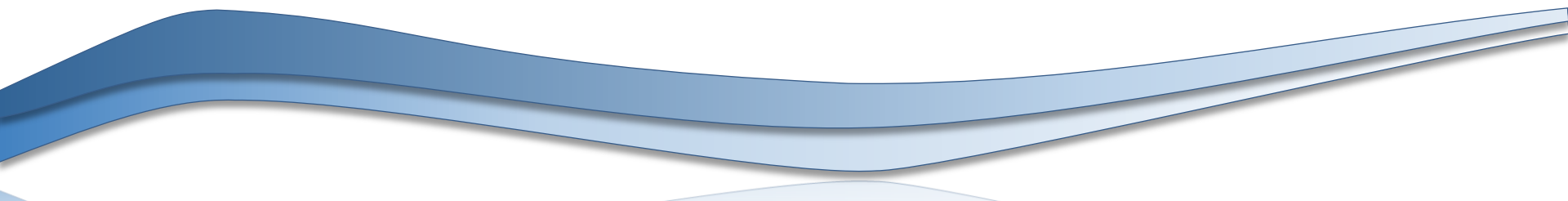
- Prof. Olof Beck (Karolinska Institute, Stockholm, Sweden)
- Project partners:
 - Dr. A. Faller, Prof. Dr. G. Skopp (Institute of Legal and Traffic Medicine, Heidelberg, Germany)
 - Dr. H. Gnann (Institute of Legal Medicine, Freiburg, Germany)



NPS New Psychoactive Substances

DBS: Dried Blood Spots

Lars Ambach, Wolfgang Weinmann
University of Bern, Switzerland



Research article

Received: 5 February 2013

Revised: 31 May 2013

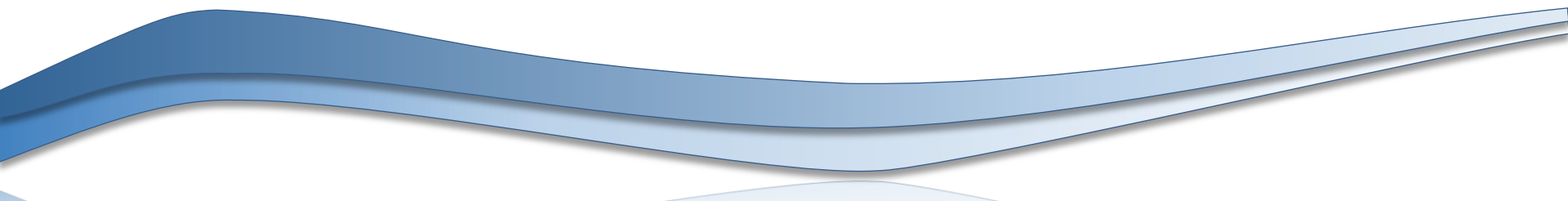
Accepted: 1 June 2013

Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1505

Rapid and simple LC-MS/MS screening of 64 novel psychoactive substances using dried blood spots

Lars Ambach,[†] Ana Hernández Redondo,[†] Stefan König and Wolfgang Weinmann*



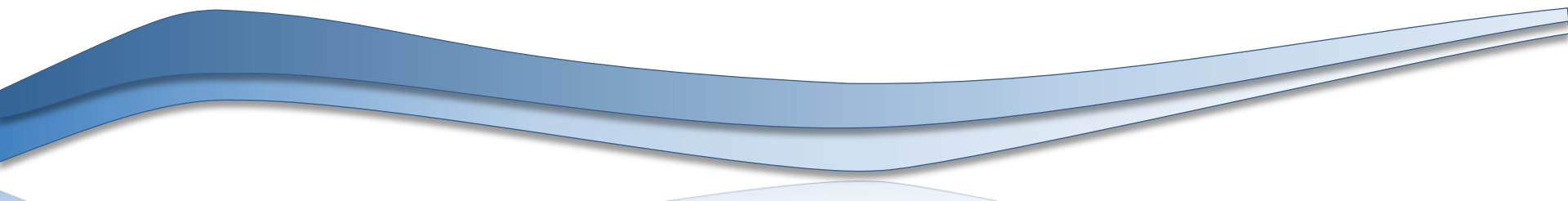
Legislation in Switzerland

Swiss Narcotics Law, Attachment List “e”

“New substances which are potentially psychoactive...”

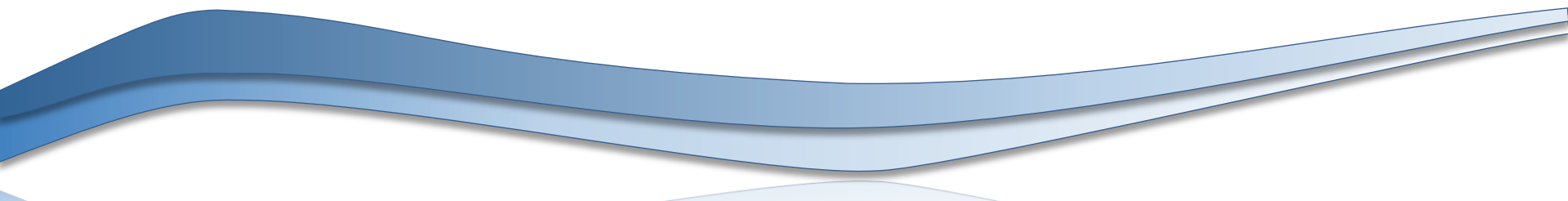
107 compounds (NPS), 7 classes (derivatives)

27 more to be listed 2013 (2nd half) (not published, yet)



DBS-analysis needs highly-sensitive LC-MS/MS for detection (Qtrap 5500)

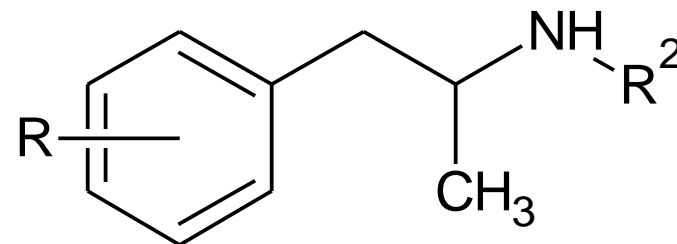
**due to low sample amount
(10 µL blood) – from venous blood or
capillary blood**



Included Substance Classes

» Amphetamine derivatives

2,5-DMA
3,4-DMA
3,4,5-TMA
4-MTA
DOB
DOET
DOM
Ethylamphetamine
MDDMA
PMA
PMMA
TMA-6



Included Substance Classes

» Amphetamine derivatives

» 2C family

2C-B

2C-D

2C-E

2C-H

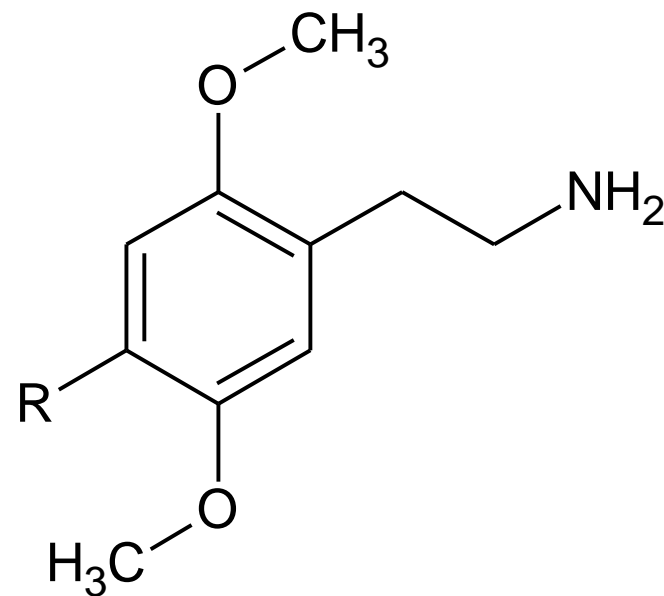
2C-I

2C-P

2C-T-2

2C-T-4

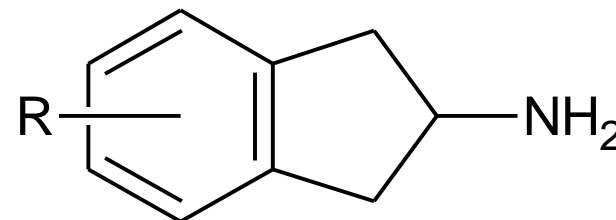
2C-T-7



Included Substance Classes

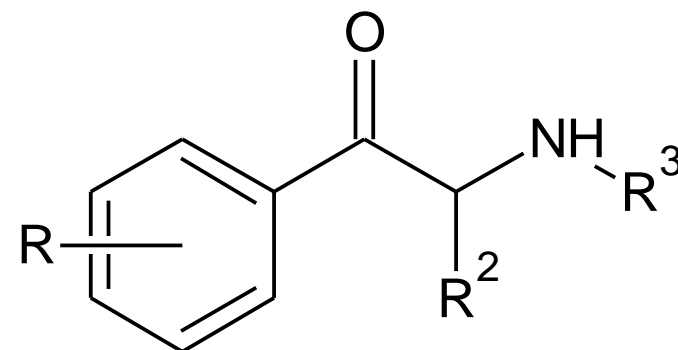
- » Amphetamine derivatives
- » 2C family
- » Aminoindanes

5-IAI
MDAI



Included Substance Classes

- » Amphetamine derivatives
- » 2C family
- » Aminoindanes
- » Cathinones



3-FMC

4-MEC

Butylone

Cathinone

Ethcathinone

Ethylone

Flephedrone

MDPPP

MDPV

Mephedrone

Methcathinone

Methedrone

Methylone

Naphyrone

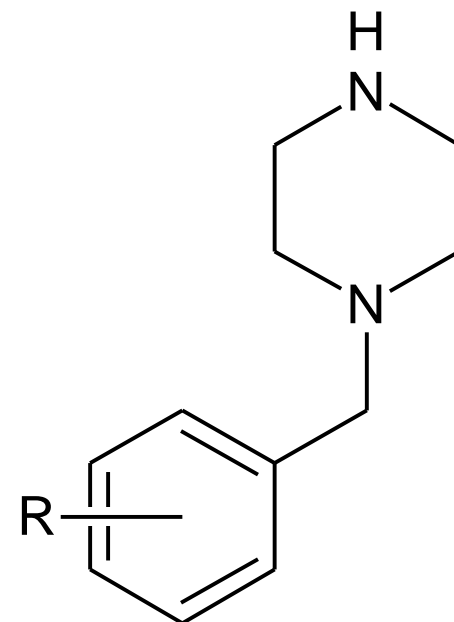
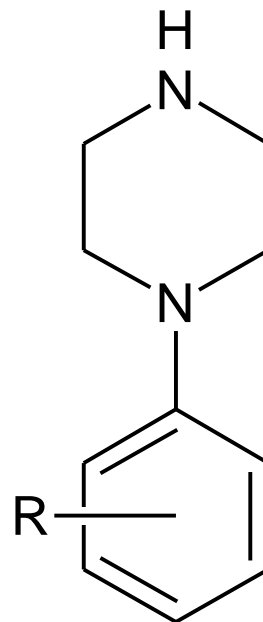
Pentylone

Pyrovalerone

Included Substance Classes

- » Amphetamine derivatives
- » 2C family
- » Aminoindanes
- » Cathinones
- » Piperazines

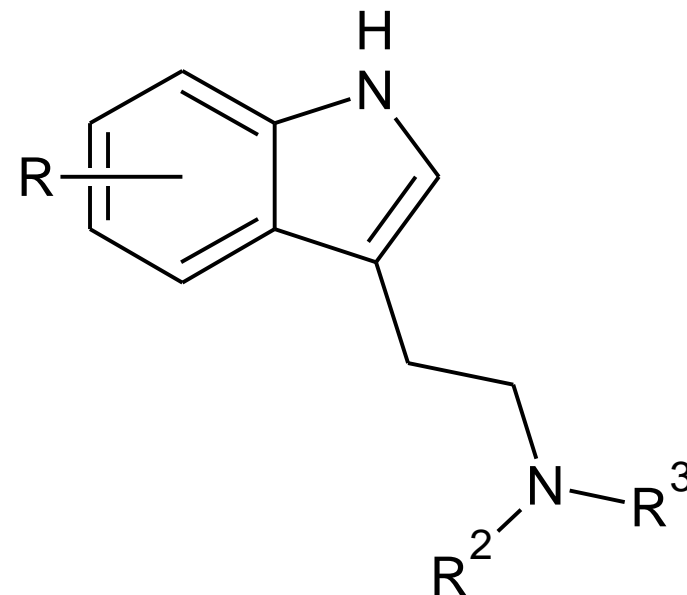
BZP
mCPP
MDBP
MeOPP
p-fluoro-BZP
TFMPP



Included Substance Classes

- » Amphetamine derivatives
- » 2C family
- » Aminoindanes
- » Cathinones
- » Piperazines
- » Tryptamines

5-MeO-DALT
5-MeO-DMT
AMT
DiPT
DMT
DPT
MiPT



Included Substance Classes

» Amphetamine derivatives

» 2C family

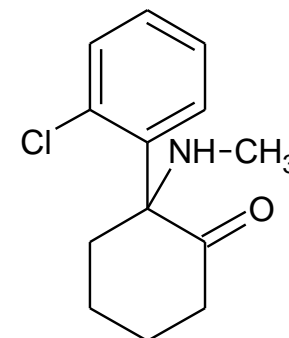
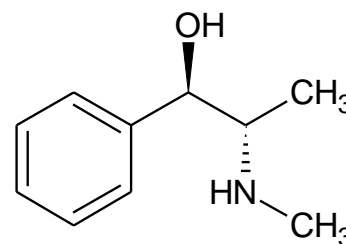
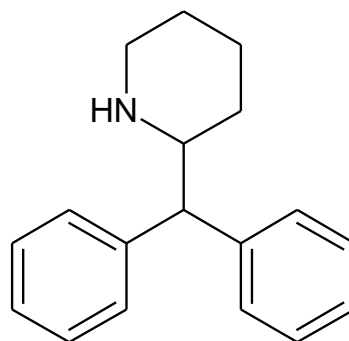
» Aminoindanes

» Cathinones

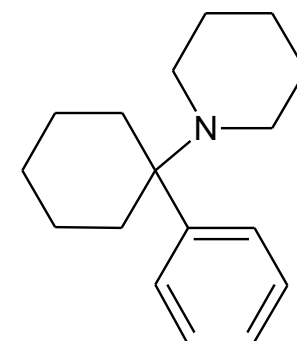
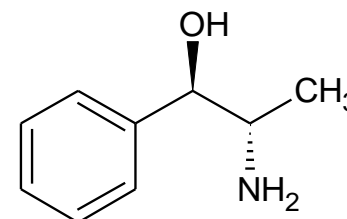
» Piperazines

» Tryptamines

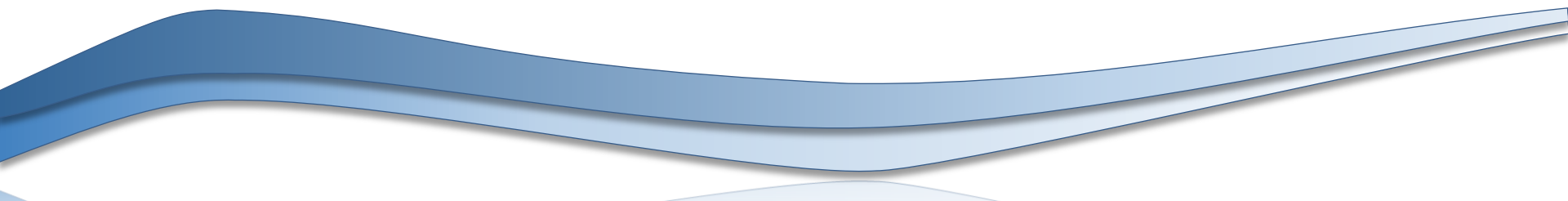
» Other substances



Desoxypipradol
Ephedrine
Ketamine
Norephedrine
PCP



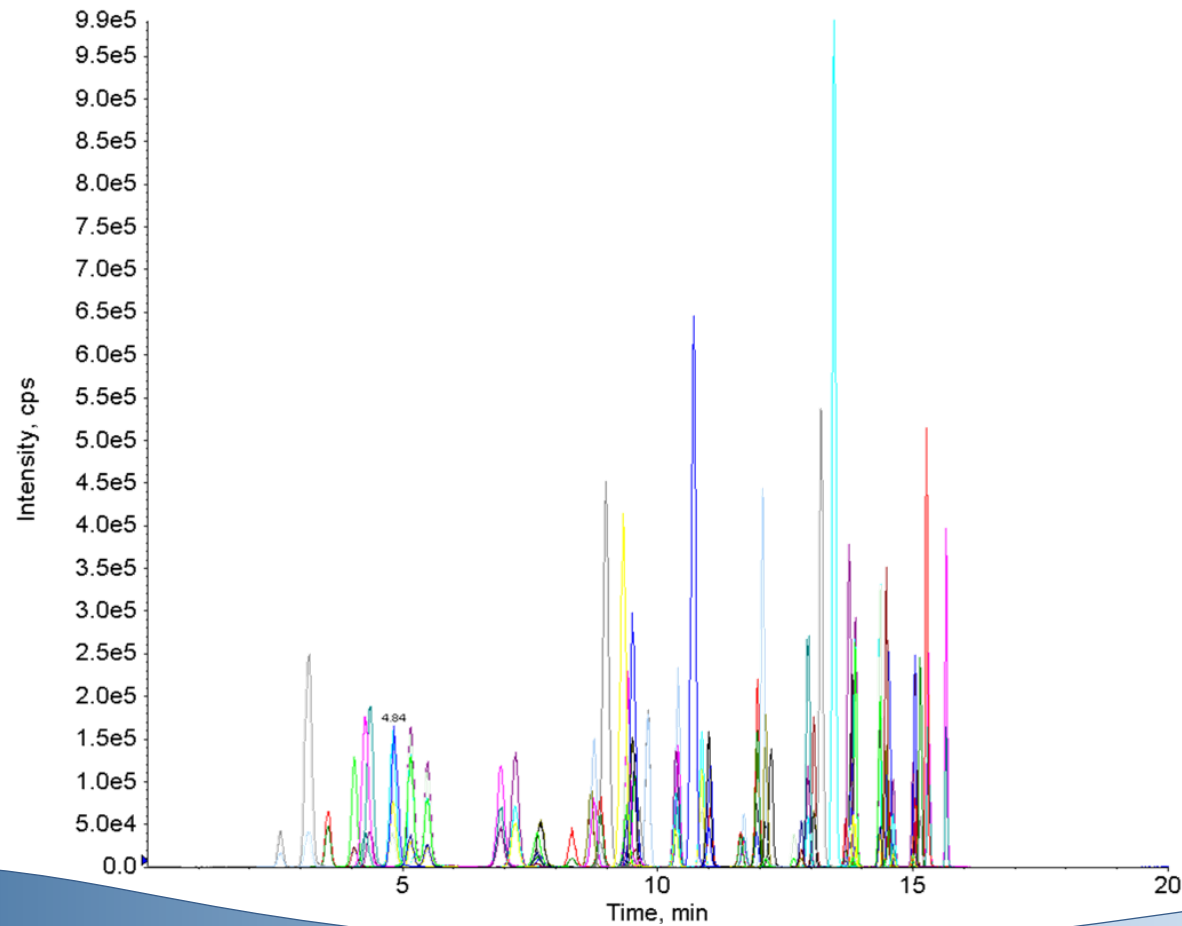
Sample Preparation DBS

- » 10 μ L Blood, prepare DBS
 - » Extract (500 μ L MeOH)
 - » Evaporate Solvent
 - » Inject, LC-MS/MS, MRM-method
- 

QC Sample Chromatogram (30 ng/mL)

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UNIVERSITÄT
BERN



Acknowledgements

Designerdrugs/Cannabinoids and PEth:

Volker Auwärter, Stefan Kneissel, Melanie Hutter (IRM Freiburg, Germany)

EU-Project (SPICE und SPICE II), Lead: V. Auwärter

BAG (Bundesamt für Gesundheit) - sponsoring L. Ambach (DD-project)

Team at FTC IRM Bern: Lars Ambach (NPS), Alexandra Schröck (Alkoholmarkers), Dr. Stefan König, Dr. Ana Hernandez*, Dr. Werner Bernhard, Thomas Wüthrich, Anita Iannone, Anja Kaiser, Sidonia Guggisberg, Patricia Coro*, Franziska Penitschka, Alain Broillet, Severine Krönert, Dipl.-Biol. Andreas Längin, Dr. Susanne Nussbaumer, Dipl.-Chem. Stephan Lanz, Petra Kindler, Sanne Kiekens*, Christiane Körber*, Yvonne Hari*. (*former group members).