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

Wolfgang Weinmann

FTC Forensic Toxicology and Chemistry
University of Bern (CH)

Wolfgang.Weinmann@irm.unibe.ch

www.legal-highs.ch

www.irm.unibe.ch
Urine contaminated with bacteria (e.coli)

Leads to alteration of EtG in urine samples


Urine contaminated with bacteria leads to degradation of EtG in urine samples.


Collection of urine on paper.... Stabilization of EtG ..... stops degradation by bacteria

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

left: no colonies visible

right: Colonies of escherichia coli visible

Whatmann No. 903 filter paper

pH Paper
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)

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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

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EtG and EtS on DBS


EtG and EtS on DBS

Determinition 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

m/z 701.5

m/z 463.3

m/z 445.3

m/z 255.2

m/z 281.2

m/z 181.2
**Extraction**

- 100 µl borate buffer pH 9
- 800 µl isopropanol
- 1200 µl n-hexane
- 300 µl blood
- PProp (IS)

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 µL Venous Blood (Li-Heparin)

Punch out DBS

3 h drying

Extraction 500 µL MeOH
Separation of the four PEth homologues

PEth $16:0/16:0$ ➔ PEth $16:0/18:2$ ➔ PEth $16:0/18:1$ ➔ PEth $18:1/18:1$
PEPth versus CDT during withdrawal therapy of alcohol addicts

H. Gnann\textsuperscript{1}, C. Engelmann\textsuperscript{1}, A. Thierauf\textsuperscript{1}, G. Skopp\textsuperscript{2}, M. Winkler\textsuperscript{3}, F. Sporkert\textsuperscript{4}, W. Weinmann\textsuperscript{1}

\textsuperscript{1} Institute of Legal Medicine, University Medical Centre, Albertstrasse 9, 79104 Freiburg, Germany
\textsuperscript{2} Institute of Legal Medicine and Traffic Medicine, University Hospital, Voss-Str. 2, 69115 Heidelberg, Germany
\textsuperscript{3} Institute of Legal Medicine, University of Ulm, Prittwitz Str.6, 89075 Ulm, Germany
\textsuperscript{4} University Center of Legal Medicine, Lausanne-Geneva, Rue du Bugnon 21, CH-1011 Lausanne, Switzerland
- 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:**

- day 1 2 3 4 8 12 15 19 22 26 29 33

**For comparison:**

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

PEth 16:0/18:1
CDT %

pat 8

PEth 16:0 / 18:1 [ng/ml]

CDT [%]

day
Evaluation of phosphatidylethanol concentrations in drinking experiments

H. Gnann\textsuperscript{1}, A. Thierauf\textsuperscript{1}, W. Weinmann\textsuperscript{2}

\textsuperscript{1} Institute of Legal Medicine, University Medical Centre, Albertstrasse 9, 79104 Freiburg, Germany
\textsuperscript{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

- Drinking term: 1 %/h
- Days of blood sampling
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

- Drinking term: 1 %/h
- Days of 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
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  • 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
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

![Chemical structure of amphetamine derivatives]

- 2,5-DMA
- 3,4-DMA
- 3,4,5-TMA
- 4-MTA
- DOB
- DOET
- DOM
- Ethylamphetamine
- MDDMA
- PMMA
- 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
» Aminooindanes
Included Substance Classes

» Amphetamine derivatives
» 2C family
» Aminooindanes
» Cathinones

3-FMC  Mephedrone
4-MEC  Methcathinone
Butylone  Methedrone
Cathinone  Methylone
Ethcathinone  Naphyrone
Ethylone  Pencyclone
Flephedrone  Pyrovalerone
MDPPP
MDPV
Included Substance Classes

» Amphetamine derivatives
» 2C family
» Aminoisindanes
» Cathinones
» Piperazines

BZP
mCPP
MDBP
MeOPP
p-fluoro-BZP
TFMPP
Included Substance Classes

- Amphetamine derivatives
- 2C family
- Aminooxindanes
- Cathinones
- Piperazines
- Tryptamines

Chemical structures:
- 5-MeO-DALT
- 5-MeO-DMT
- AMT
- DiPT
- DMT
- DPT
- MiPT
Included Substance Classes

» Amphetamine derivatives
» 2C family
» Aminoindanes
» Cathinones
» Piperazinnes
» Tryptamines
» Other substances

- Desoxypipradol
- Ephedrine
- Ketamine
- Norephedrine
- PCP
Sample Preparation DBS

» 10 µL Blood, prepare DBS

» Extract (500 µL MeOH)

» Evaporate Solvent

» Inject, LC-MS/MS, MRM-method
QC Sample Chromatogram
(30 ng/mL)
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