Quantitation of "Liquid Ecstasy" (Gamma-Hydroxybutyric Acid) by Solid-Phase Dynamic Extraction/GC-MS in Urine

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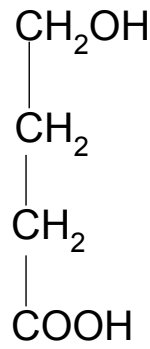
Introduction

Solid-phase dynamic extraction (SPDE) has been introduced by Chromtech (Idstein/Germany) and has been successfully applied for the forensic analysis of amphetamines in hair samples using a CTC/PAL headspace sampler coupled to a GC/MS quadrupole instrument [1].
Gamma-hydroxybutyric acid (GHB) has been abused as party drug ("Liquid Ecstasy") and as a rape drug in cases of sexual assault. GHB can be detected in urine and serum either by precipitation or by liquid/liquid extraction (LLE) and subsequent silylation [2] - or by conversion to gamma-butyrolactone (GBL), LLE and headspace-GC analysis [3]. GHB and GBL are volatile and evaporation can occur during solvent evaporation steps. SPDE offers the possibility to extract GBL directly from urine after acidic lactonisation of GHB.

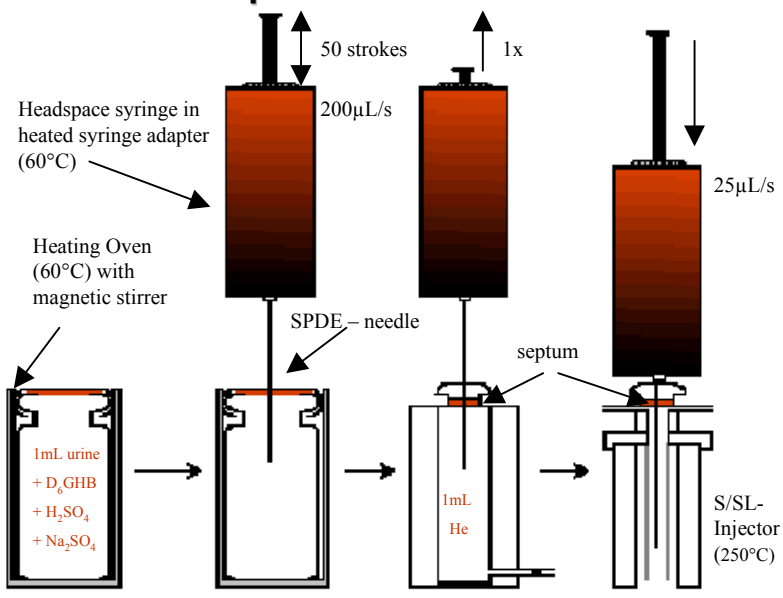
[1] F. Musshoff, D. W. Lachenmaier, L. Kroener, B. Madea.
Automated headspace solid-phase dynamic extraction for the determination of amphetamines and synthetic designer drugs in hair samples.
J. Chrom. A 958, 2002, 231-238
[2] A. G. Verstraete, E. Van de Velde, P. De Paepe, M. T. Rosseel.
Proc. 37th TIAFT Triennial Meeting, Krakow; T. Lech (Ed.), Inst. of Forensic Res. Publishers, Krakow, 2000, 195-201.
[3] M. A. LeBeau, M. A. Montgomery, M.L Miller, S. G. Burmeister.
Analysis of Biofluids for Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) by Headspace GC-FID and GC-MS.
J. Anal. Toxicol. 24, 2000, 421-428.

Experimental

1 mL urine was spiked with GHB and hexadeuterated D_6-GHB and treated with 0.15 mL sulfuric acid (98 %) in a 20 mL headspace vial for 5 min at 20 °C. 2 g Na_2SO_4 were added and the vial was placed into the autosampler tray.
The sample extraction by SPDE and subsequent injection was fully automated by the CTC/PAL-autosampler. A SPDE-syringe with internally coated canula (polydimethylsiloxane/activated charcoal, Chromtech/Idstein, Germany) was used for dynamic headspace extraction using 50 pumping-cycles at a vial temperature of 60 °C. Desorption was performed splitless with helium at 250 °C, SIM-mode was used for the detection of GBL and D_6-GBL (m/z 42/44, 56/60, 86/92 amu).



SPDE Technique



Development of the Method

Pre-Incubation-Time*	[m:ss]	5:00
Syringe-Temp.*	[°C]	60
Incubation-Temp.*	[°C]	60
Agitator-Speed	[rpm]	500
Agitator-On-Time	[m:ss]	0:30
Agitator-Off-Time	[m:ss]	0:05
Sample-Penetration	[mm]	28
Extraction-Strokes*		50
Extraction-Fill-Speed*	[µl/s]	200
Extraction-Eject-Speed*	[µl/s]	200
Desorption-Gas-Volume	[µl]	1000
Injector-Penetration	[mm]	45
Pre-Desorption-Time	[m:ss]	0:10
Desorption-Flow-Speed*	[µl/s]	25

The table above shows the optimized parameters.

A HP 6890 GC with a HP 5973 MSD equipped with a CTC Combi PAL autosampler was used to perform the analysis.

Starting with a PAL-Autosampler method written by Chromtech (Idstein, Germany) the parameters signed with * were modified step by step to optimize the extraction of GBL.

GC-Parameters:

Initial column temp.: 35 °C
Initial hold time: 3 min
Temperature ramp 1: 15 °C/min to 80 °C
Temperature ramp 2: 40 °C/min to 250 °C
Final hold time: 1 min

Column:

Optima d-3, 0.25 µm 30 m * 0.25 mm (Macherey-Nagel, Düren Germany)

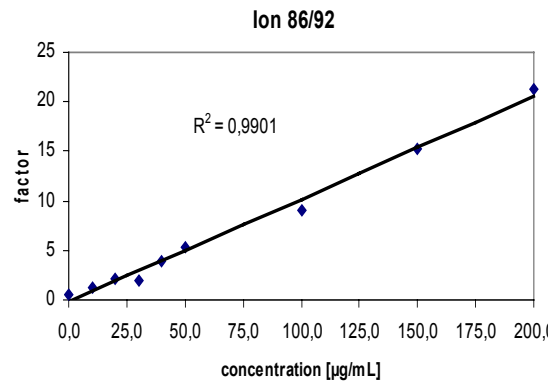
SPDE-Syringe:

PDMS/AC, 50 µm x 56 mm (2.5 mL)

Calibration Curve (Urine)

Urine was spiked with GHB (10 - 200 µg/mL). Ion ratio 86/92 was used for quantification. Ion ratios 42/44 and 56/60 were used as qualifiers.

Linear Range: 10 µg/mL – 200 µg/mL
LLOQ: 10 µg/mL
LOD: < 2 µg/mL
(detection of endogenous GHB-levels possible)



Conclusions

The described method offers a time and labour-saving possibility for the analysis of urine samples for the illegal party and rape drug GHB without derivatisation, without liquid-liquid extraction and without injection port contamination due to the headspace SPDE-technique.

The SPDE-technique is a promising method for screening of body fluids for volatile compounds.The same SPDE-headspace technique has been used for detection of trichloroethanol (metabolite of chloral hydrate) and propofol in subtherapeutic concentrations in blood in scan-mode.