Appendix A
of the Guidelines for Quality Control in Forensic-Toxicological Analyses

Quality requirements for the determination of special analytes from biological matrices including annex of tables (prevailing specifications for detection limits)

Authors: L.D. Paul, München; F. Mußhoff, Bonn; Subgroup "Determination of Guidelines" of the Scientific Committee Quality Control (F.T. Peters, Jena; G. Skopp, Heidelberg; T. Krämer, Homburg; B. Aebi, Bern; V. Auwärter, Freiburg); D. Thieme, München; G. Schmitt, Heidelberg; S. Tönnes, Frankfurt, and authors of the replaced guidelines (see chapter Final Clause); Members of the Scientific Committee Quality Control

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1. Quality requirements for the quantitative determination of special analytes

For the determination of target analytes in serum, plasma, whole blood, urine, hair or other biological matrices, validated methods for clean-up and measuring are to be applied. Chromatographic procedures should have identifying (confirmatory) power.

In forensic casework, the limit of quantification as determined according to the GTFCh guidelines (appendix B, validation), concerning evidential analysis in serum/plasma, urine or hair, should be less than or equal to the maximum permissible value.

For forensic problems, chapter 2 outlines the limits of detection (LOD) and quantification (LOQ) that should be met in case of special analytes in common matrices. If no other regulations exist (as laid down by law or external parties) that contain legal limits or other cut-off values (e.g. recommendations by the Grenzwertkommission on limiting values for substances from the appendix of § 24a (2) of the road traffic law (StVG) in Germany), the GTFCh limits (LOD, LOQ) according to this appendix (table 1) are valid.

For special forensic-toxicological problems, e.g. analyses in cases of fitness to drive (see criteria for the evaluation of fitness to drive) or workplace drug testing, the corresponding criteria regarding limits and cut-off values must be followed.

Working ranges should be chosen in such a way that the concentration range that is needed for the evaluation of the majority of the samples is covered.

1.1 Determination of cannabinoids

Apart from Δ9-tetrahydrocannabinol (THC), the determination of cannabinoids in serum, plasma or blood should at least include the metabolite 11-Nor-Δ9-tetrahydrocannabinol carboxylic acid (THC-COOH); the additional determination of 11-hydroxy-Δ9-THC (11-OH-THC) is recommended for more balanced assessments.

When determining THC-COOH one should consider that the concentration of the analyte may be increased by release from its glucuronide.

It should be taken into account that whole blood and serum/plasma concentrations for THC and its metabolites are not directly comparable. Whole blood concentrations are lower than those in serum or plasma.

When determining THC-COOH in urine, a hydrolysis step should be carried out, at least when fitness to drive has to be assessed.

The detection of the main active cannabinoid,THC, serves as proof of previous cannabis exposure in the context of a hair analysis. Detection of other cannabinoids (cannabinol (CBN), cannabidiol (CBD)) serves as plausibility control, i.a. because of the higher stability as compared to THC. Detection of THC-COOH can provide further information in individual cases. In that case, however, very low detection limits for THC-COOH (0.001 ng/mg) have to be attained.

1.2 Determination of amphetamine, methamphetamine and methylenedioxyamphetamine

A suitable and validated method should be able to detect (qualitatively) as well as determine (quantitatively) at least amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA). The possibility of losses through evaporation, especially of amphetamine during sample pretreatment, should be observed. The GC-injection of underivatised primary amines such as amphetamine should never be done in methanol, since conversion of methanol to formaldehyde may lead to the formation of formyl artefacts. When using deuterated internal standards, the standards with deuterium in the isopropyl side chain generally produce the most characteristic mass spectra. In this way, the finding of three different mass fragments per substance is facilitated.

Exclusive occurrence of methamphetamine in a blood sample is very rare. Normally the metabolite amphetamine is also found. A targeted assay regarding amphetamine should definitely be carried out after a positive methamphetamine result. The possibility that amphetamine or methamphetamine appear as metabolites of pharmaceuticals should be

taken into account. In case of doubt, these pharmaceuticals should also be tested. If appropriate, an enantiomeric separation may contribute to clarification.

Ecstasy tablets generally contain MDMA or MDEA. MDA can also be a component, but MDA is also a metabolite of MDMA and MDEA. Since MDA is rarely found alone in blood samples, the possible parent substances (MDMA, MDEA) should also be searched for.

1.3 Determination of cocaine and benzoylecgonine

The extraction and analysis should be such that cocaine and benzoylecgonine (BE), and if possible also ecgoninemethylester (EME), ecgonineethylester (EEE), and/or cocaethylene are extracted from the sample and, where applicable, are suitably derivatised and qualitatively detected as well as quantitatively determined.

Cocaine has a relatively short half-life, so its sole presence without the typical metabolite BE is most unlikely.

When cocaine is not detected in cases of serum, plasma or blood samples without added fluoride, the presence of cocaine at the time of blood withdrawal cannot be excluded. If fluoride was not added, a statement should be made, especially in the results report, that degradation of cocaine before receipt and/or in the laboratory cannot be ruled out. Usually, EME concentrations in blood are low.

Often cocaine and alcohol are consumed together. By transesterification in the presence of carboxylesterase 1 (hCE-1), cocaethylene is produced which is degraded to EEE. Thus, the presence of EEE or cocaethylene proves that cocaine and alcohol were consumed concomitantly.

For proof of previous consumption of cocaine, the detection of the parent substance cocaine is leading in hair analyses and, if the cocaine concentration is not in the range of the LOQ, the additional detection of BE or further metabolites. The metabolites BE and EME, which may be formed through hydrolysis, do not necessarily prove that systemic exposure to cocaine occurred.

1.4 Determination of opiates/opioids

The extraction and analysis should be such that morphine and codeine, possibly also 6-monoacetylmorphine (6-MAM) and dihydrocodeine are extracted from the sample material and, where applicable, are suitably derivatised and qualitatively detected as well as quantitatively determined.

In blood, morphine is also partly present in glucuronidated form. Depending on the case, not only free morphine, but also total morphine (free and conjugated) should be determined (after hydrolysis), or the individual glucuronides should be determined directly. This should be mentioned in the report. Codeine is not a metabolite of morphine and thus cannot be detected in body fluids after intake of pure morphine or pharmaceutically pure diacetylmorphine (heroin). Acetylcodeine, however, is a component of illegal heroin products. This means that after systemic exposure to street-heroin, morphine as well as codeine (the metabolite of acetylcodeine) will be detected in blood, in free as well as in conjugated form. Upon intake of illegal heroin, the codeine concentration is clearly lower

than the morphine concentration. In case of recent heroin consumption, the sample (serum, plasma or urine) should be tested for the presence of 6-MAM..During sample pre-treatment, the risk of hydrolysis of 6-MAM to morphine must be taken into account.

After codeine intake, morphine is found in blood as a metabolite, partly free, but primarily conjugated, and about 10% of a single dose is excreted as morphine or morphine conjugates in urine. In the final elimination phase, the morphine and codeine concentrations may become equal or reverse.

Methadone is mainly metabolised to EDDP, which however can also originate during GC-MS analysis.

Other opioids such as buprenorphine, tilidine, tramadol, and fentanyls may also be relevant. In hair analyses, the detection of the characteristic metabolite 6-monoacetylmorphine (6-MAM) is leading as a proof of recent heroin consumption. Morphine and, where indicated, heroin itself should also be tested. During sample pre-treatment there is the risk of hydrolysis of heroin to 6-MAM and of 6-MAM to morphine, especially under acidic, but also under alkaline conditions. A metabolite ratio of 6-MAM to morphine that is less than 1.3 suggests hydrolysis.

2 Specifications for forensic cases

Table 1: GTFCh list regarding the limits of detection and quantification that should be met for drugs of abuse and their metabolites according to forensic requirements in different matrices, determined via confirmatory chromatographic methods.

Substance class	Serum/Plasma [µg/L]	Urine [2] [µg/L]	Hair [2] [ng/mg]
Cannabinoids			
Delta-9-Tetrahydrocannabinol (THC)	1	-	0.02
Delta-9-Tetrahydrocannabinol-9-carboxylic acid (THC-COOH)	10 [1]	10 (after hydrolysis)	-
Amphetamine and Derivatives			
Amphetamine	25	200	0.1
Methamphetamine	25	200	0.1
MDMA	25	200	0.1
MDA	25	200	0.1
MDEA	25	200	0.1
Cocaine and Metabolites			
Cocaine	10	-	0.1
Benzoylecgonine	30	30	0.1
Opiates/Opioids			
Morphine	10	25 (after hydrolysis)	0.1
Codeine	10	25 (after hydrolysis)	0.1
6-Monoacetylmorphine	2 [2]	10	0.1
Methadone	50	200	0.1
EDDP	-	200	0.1

[1] Lower in certain problems, [2] detection limit, semi-quantitative value

The serum/plasma values comply with the specifications of the Grenzwertkommission for substances from the annex of § 24a (2) StVG (road traffic law) that are to be used in cases of regulatory offense, from the date of legal validity, or alternatively the last amendment of this appendix.

In the context of fitness to drive, specifications apply according to the evaluation criteria in their relevant current version.

Table 2: Legal limits in whole blood for driving inability as laid down in the Swiss Federal Roads Office (FEDRO) regulations for road traffic control

Substance class	Full blood [µg/L]
Cannabinoids	
Delta-9-Tetrahydrocannabinol (THC)	1.5
Amphetamine and Derivatives	
Amphetamine	15
Methamphetamine	15
MDMA	15
MDEA	15
Cocaine and Metabolites	
Cocaine	15
Opiates/Opioids	
Free Morphine	15

3 Legal validity

This appendix was passed by resolution of the GTFCh executive committee on the 1st of April 2009 and takes effect on the day of its publication in Toxichem + Krimtech.

Transitional periods apply until 31st of March 2011.