AGSA

Drugs of Abuse Testing Guidelines

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Glossary

AGSA	**Swiss Working Group for Drugs of Abuse Testing Guidelines**
ASTRA	**Swiss Federal Roads Authority**
BAP	**Swiss Federal Office for Police Matters**
BSV	**Swiss Federal Office for Social Security**
CAP	**College of American Pathologists**
CSCQ	**Quality Control Center Switzerland**
Cut-off	**Medical and/or Legal Decision Value, pos/neg**
TLC	**Thin Layer Chromatography**
DIN	**German Industrial Standard**
DOD	**U.S. Department of Defense**
EJPD	**Swiss Federal Department of Justice and Police**
EN	**European Norm**
FDHA	**Swiss Federal Department of Home Affairs**
GC-MS	**Gas Chromatography with Mass Spectrometric detection**
GC-NPD	**Gas Chromatography with Nitrogen Phosphor Detection**
HPLC	**High Performance Liquid Chromatography**
HPLC-DAD	**High Performance Liquid Chromatography with Diode Array Detector**
HPLC-ECD	**High Performance Liquid Chromatography with Electrochemical Detection**
HPLC-MS	**High Performance Liquid Chromatography with Mass Spectroscopic Detection**
ID	**Identification**
KBMAL	**Criteria to operate medical-analytical laboratories**
KLV	**Health Care Benefits Ordinance**
KVG	**Swiss Federal Health Insurance Act**
KVV	**Health Insurance Ordinance**
LSD	**Lysergic Acid Diethylamide**
NIDA	**U.S. National Institute on Drug Abuse**
MQ	**Association for Medical Quality Control**
Peak	**Portion of a differential chromatogram recording the detector response when a single component is eluted from the column**
QC	**Quality Control**
QUALAB	**Swiss Commission on Quality in the Medical Laboratory**
s (2s)	**Standard deviation**
SAMHSA	**U.S. Substance Abuse and Mental Health Services Administration**
Spot	**Spontaneous urine, urine specimen**
SULM	**Swiss Union for Laboratory Medicine**
THC	**Delta(9)-tetrahydrocannabinol**
UVEK	**Swiss Federal Department of Environment, Transport, Energy and Communications**

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Foreword

These guidelines were drawn up by a working group comprised of representatives from the following institutions:

- **Swiss Federal Office of Public Health (SFOPH)**
- **Swiss Society of Pharmacists (SSPh)**
- **Swiss Society for Clinical Chemistry**
- **Swiss Society of Forensic Medicine**
- **Swiss Union for Laboratory Medicine**
- **Swiss Association of Diagnostics Manufacturers**
- **Swiss Society for Directors of Clinical Laboratories (FAMH)**
- **University of Berne**

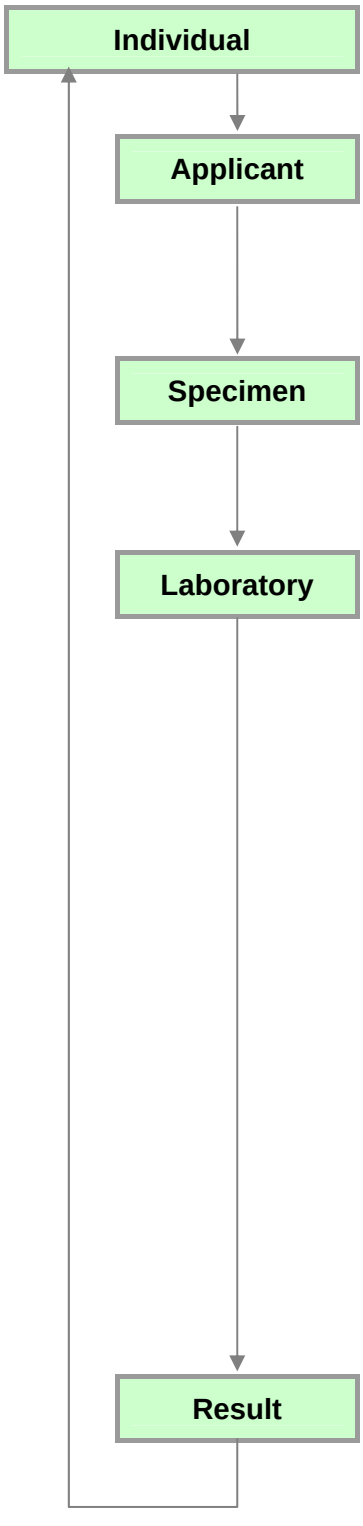
These guidelines are intended as recommendations. They have no legally binding nature. Uniformity in the treatment of drug analyses is the objective.

The use of drug analysis for the various questions in medical and/or psychotherapeutic and forensic sector as well as in specific workplaces can have decisive consequences that are professional and social in nature for those affected. For this reason, it is necessary to take the greatest possible care when conducting analyses and interpreting the results. The guidelines support analytical laboratories in their adherence to the required quality assurance.

The guidelines are periodically revised, updated and amended.

In addition, the working group will consult external quality control scheme organizers and all laboratories that perform drugs of abuse testing.

1. Scope of guidelines



Specimen collection
Identity, authenticity, integrity of donor and urine sample, “chain of custody”, issue, range analyses

Transportation
Containers, material for shipment, fail-safe, “chain of custody”

Pre-analytics
Storage, specimen processing

Analytics
Quality control, methodologies, sensitivity, specificity

Post-analytics
Storage, additional orders, confirmation

Quality Assurance
Internal and external quality control

Interpretation
Cut-off values, timing of specimen collection, pharmacokinetics

Documentation
Reports, release of results, handling of positive results, confirmation, recommendations

Costs
Invoicing, list of analyses, legal aspects

2. Guidelines: Areas of application

A	**Drugs of abuse testing in the clinical sector and in differential diagnosis** • Clinical investigation, intoxication Hospitals with emergency units
B	**Drugs of abuse testing in substitution programs or withdrawal treatment** • Drug substitution program (methadone, heroin, etc.) Psychiatric clinics, dispensing facility, rehabilitation center, etc.
C	**Drugs of abuse testing in forensic investigations** • Drugs in road traffic and prisons, etc.
D	**Drugs of abuse testing in a non-traditional environment** • Workplace testing, examinations by company physicians, military, schools

The terms **A, B, C, D** are used throughout these guidelines.

3. Specimen collection, transport and sample handling ("chain of custody")

Individual

Objectives

- The identity, authenticity and integrity of the individual and of the urine sample must be guaranteed
- Respect private sphere
- Identify and prevent any medical, chemical and/or physical manipulations (endogenous/exogenous dilution, additives, substitutions) of the urine sample

Measures

- Check identity[1]
- Measure temperature (32-38 °C) within 4 min[1] (collection site)
- Check consistency, smell, pH and colour[3]
- Dye rinsing water, washbasin, soap and disinfectant outside toilet[3]
- Conduct visual check[3]
- Instructions and advice on taking urine samples provided by the laboratory[1]

Urine sample

Urine sample of 30 ml or more if possible

- The identity, authenticity and integrity of the urine sample must be guaranteed
- Identify and prevent any chemically- and/or physically-induced changes (decomposition, contamination, breakage, etc.), manipulations, mix-ups and/or loss of the urine sample

- Container (supplied by the laboratory) with safety top[2], leak proof, unbreakable; label with clear ID number[1]
- Order form (uncomplicated, unambiguous): ID number, last name, first name, date of birth, gender, date/time of sampling

Laboratory

- Limited and controlled access to the laboratory[1]
- Receipt of urine sample only by authorized personnel[1]
- Measurement of colour[1], consistency[1], smell[3], pH, creatinine[1], specific weight/density[3] and refraction index[3]
- Storage (under lock and key): +4 °C pre-analytically, -20 °C post-analytically
- Storage times: not defined for A and B (6 months recommended), minimum of 1 year for C and D

Analysis

[1] Compulsory for sectors A – D
[2] Compulsory for sectors C and D
[3] Optional

4. Factors influencing analytically determined results, manipulation of the urine sample

Adherence to the most important pre-analytical steps (described in §3) guarantees the proper approach and can expose any intentional or unintentional influence on the results that would lead to a distortion of the test result and hamper its interpretation (q.v. §12. Interpretation).

4.1 Type of interference (q.v. §12.1 also)

4.1.1 Medicament interference

- Distortions caused by medicaments taken for therapeutic purposes (some of these types of interference are not evident from the data provided by reagent manufacturers since these have not been tested, e.g. neuroleptics, antidepressants).
- Physiological distortion (in-vivo interference), e.g. excessive water intake, alimentary influences and medicaments (e.g. poppy seeds, multivitamin preparations).

4.1.2 Manipulation of urine tests

- Substances added to the urine that are capable of influencing one or several tests.
- Substances that modify the drug to be screened thereby preventing detection using a confirmation process.
- Exchange of urine for other drug-free urine samples, urine for sale or other dyed liquids.

4.2 Manipulation and recognition

Types of manipulation	Laboratory test
Dilution: drinking, diuretics, addition of liquid	Creatinine/density, color
Bleach solutions (toilet cleaner) with hypochlorite	pH, check*, smell, color
Liquid soap	Check*, foam
Aldehyde or glutaraldehyde	Check* and strip tests**
Strong acids and bases	pH, check*
Nitrites	NO_2^- on strip tests**
Ascorbate	pH, check*
Medicaments and vitamins	Chromatography
Chromates	Color test, strip tests**
Peroxide and peroxidase (Stealth)	Check*, strip tests**
Vitamins (multivitamin preparations)	Chromatography
Others (Visine, Maggi, etc.)	Chromatography and others

(q.v. “Further information” for detailed list)

* Check = Checking method specially for the relevant analysis process, e.g. "Sample check"

** Strip tests, e.g. Adultacheck 4 (pH, NO_2^-, creatinine, glutaraldehyde)

4.3 Definitions according to SAMHSA

Urine is considered to be diluted if:
Creatinine <1.8 mmol/L(20 mg/dL), but >0.4 mmol/L (4.52 mg/dL)
In Europe <2 mmol/L(22.6 mg/dL), but >0.4 mmol/L (4.52 mg/dL)
Specific weight <1.003 kg/L, but >1.001 kg/L

No urine matrix, substituted, if:
Creatinine <0.4 mmol/L (4.52 mg/dL)
Specific weight <1.001 kg/L

Urine is considered to be adulterated if:

- The nitrite concentration is >500 mg/L
- The pH value is <3 or >11
- Exogenous substances that lead to adulteration are detectable (q.v. §4.2)
- Endogenous substances are detectable in non-physiological concentrations

5. Specimens

Specimen	Area of application			
	A	B	C	D
Urine	X	X	X	X
Serum, Plasma	X	X	X	X
Whole blood	X	-	X	-
Sweat	-	X	X	X
Post-mortem blood	X	-	X	-
Saliva	X	X	X	X
Stomach contents	X	-	X	-
Punctates and secretions	X	-	X	-
Dialysate	X	-	-	-
Tissue samples	-	-	X	-
Hair	-	X	X	X
Substance samples	X	-	X	X

6. Use of rapid tests: non-instrumental immunoassays for drug screening in urine

6.1 Definition, characteristics

"Rapid tests" for drug screening in urine are non-instrumental immunoassays unsuited to mass screening (q.v. §7) that permit fast (5-10 min) yes/no decisions outside the laboratory (on site). Urine processing is usually unnecessary.

6.2 General remarks

- As is the case with instrumental immunoassays, non-instrumental immunoassays are only indicative and not evidential in nature. All manufacturers' instructions for use point out this fact but many users pay little or no attention to this.
- Despite their simplicity and the fact that equipment can be dispensed with, these non-instrumental immunoassays should only be conducted by trained personnel who also know how to interpret the results and any irregularities.
- If the result is positive, the urine sample must not be disposed of but stored for any confirmation analysis that may be required (q.v. §9 for ruling).
- Most of these assays contain a test area that displays any irregularity in the reaction sequence. Nevertheless, irregularities are possible that are not indicated with internal checks.

6.3 Areas of application

A: Instrumental immunoassays using equipment calibrated according to AGSA guidelines are preferred, particularly in emergency departments. Dependent on requirements, differential and confirmation analyses may be required. Attention should be paid to the fact that qualitative results might lead to false differential diagnoses and quantification will be needed in addition.

B: Mainly in doctors' practices and pharmacies for checking on patients' statements or compliance monitoring (methadone). It is recommended that the rapid test be conducted in the presence of the patient. A result that is disputed by the patient must be verified using a method based on a different analytical principle.

C: Non-instrumental immunoassays are generally not recommended in the forensic sector. For police applications, e.g. during traffic controls, it is vital that the recommendations listed in §6.2 "General remarks" are noted.

D: Non-instrumental immunoassays should only be used in exceptional cases. Instrumental immunoassays using equipment calibrated according to AGSA guidelines are preferable. In the case of workplace testing of urine, positive results require confirmation.

7. Immunochemical analyses

This term applies to all variations of analytical systems that contain an antigen-antibody reaction that is independent of the detection system being used.

7.1 Single substance analyses (S)

Immunoassays of single substances are designed to detect a substance and/or its metabolites.
Examples of single substance analyses are as follows: cannabis (THC carboxylic acid), cocaine (benzoylecgonine), 2-ethyliden-1.5-dimethyl-3.3-diphenylpyrrolidine (EDDP), LSD, methadone, methaqualone, 6-monoacetylmorphine (6-MAM).

7.1.1 Areas of application

Class		A	B	C	D
S	Cannabis (THC carboxylic acid)	X	X	X[2]	X
	Cocaine (benzoylecgonine)	X	X	X[2]	X
	LSD	X	-	X[2]	X
	Methadone	X[1]	X[1]	X[2]	-
	EDDP	X[1]	X[1]	X[2]	X
	Methaqualone	X	X	X[2]	X
	6-Monoacetylmorphine (6-MAM)	X	X	X[2]	-

[1] Negative results need not necessarily be meaningful since most assays detect methadone alone and not the main metabolite EDDP. In urine, EDDP alone may be detected due to fast metabolizers or enzyme induction (interaction with e.g. rifampicin, carbamazepine phenytoin, etc.). In these situations the analysis of EDDP is helpful.
[2] Only usable as a screening test

X = Correct area of application

7.2 Substance group analyses (G)

Substance group analyses using immunoassays are analysis systems that detect a range of (but not all) structure-related substances in one analytical process.
The antibodies react with a more or less large number of structure-related substances or metabolites (q.v. §8). The result in each case only makes a qualitative statement (one up to several substances reacting with the antibody are detectable or not). Dependent on manufacturer, the calibration of substance class test systems is based on different standard substances, which leads to varying specificity in the results.
Examples of such substance group analyses are as follows: methods for detecting benzodiazepines, opiates, amphetamines, barbiturates and tricyclic antidepressants.
Dependent on method, urines with high concentrations (> measuring range) must not be diluted. There is a connection between affinity to the antibody and the concentration of the substance.

7.2.1 Areas of application

Class		A	B	C	D
G	Amphetamines	X[1,2]	X[2]	X[3]	X[2]
	Barbiturates	X[1,2]	X[2]	X[3]	X[2]
	Benzodiazepines	X[1,2]	X[2]	X[3]	X[2]
	Opiates	X[1]	X	X[3]	X
	Tricyclic antidepressants	X[1,2]	-	X[3]	-

[1] Problems due to the varying reactivity of the antibodies with single substances within a substance class. Quantitative details are therefore not possible.
[2] Negative results need not necessarily be meaningful since, dependent on method; individual substances of the substance class or their metabolites do not react. This also applies to the metabolites of a substance in single tests (e.g. methadone, tricyclic antidepressants).
[3] Only usable as a screening test.

X = Correct area of application.

7.3 Long-term monitoring

If the excretion of a specific drug of abuse requires monitoring, this is only possible by comparing creatinine quotients.
The creatinine quotients of the individual drugs are calculated as follows:

$$\frac{\text{Concentration of drug in the urine (µg/L)}}{\text{Creatinine concentration in the same urine (mmol/L)}} = \frac{\text{Drug (µg)}}{\text{Creatinine (mmol)}}$$

This comparison can prove interim consumption (value sample 1 → consumption → value sample 2) or depict the elimination rate of once-only consumption.

7.4 Comment

In every case, a chromatographic test method is more meaningful than most immunoassays. However, the latter are the methods of choice for rapid results, since chromatographic procedures involve a lot of work. The use of immunochemical group tests is indicated when there is a need for the rapid detection of the possible use of substances within a substance class (which can cover a large number of substances) and for series analyses. It must be noted that false positive and false negative results remain possible.
To some extent, the sensitivity of immunoassays is better than that of chromatographic methods.
Since immunoassays do not permit any quantitative statements (except in the case of specific tests), results have to be interpreted according to application and additional measurements arranged if necessary (§12).

8. Cut-off values, sensitivity and specificity of immunoassays

8.1 Terminology

8.1.1 Cut-off values

By “cut-off” we mean the limits for decisions (yes/no) as to whether a result must be interpreted as positive or negative. In group tests, this value relates to the substance used to calibrate the test procedure.
There are various methods of determining these cut-off values:

1. Sensitivity limit of a test procedure (important for forensic purposes)
2. Determination of the limit at which 95% of the results are still found to be positive after taking specific doses of a substance (e.g. therapeutic dose) after a specific period of time (1 day, 2 days) (procedure formerly used by NIDA/SAMHSA)
3. Experience values based on the limits determined under 2) (NIDA today)
4. Taking on the values determined under 3) and supplementing the limits not determined with values based on experience

In these recommendations, alternatives 1) and 4) are used.

8.1.2 Detection limit/sensitivity

In most of the test procedures commercially available, analytical sensitivity is defined as follows:
The lowest result of a method that can be differentiated from zero with 95% probability (2s confidence limit).
Determining the variability of the results of zero calibrators within the required matrix in series determinations (on 2 different days, n = 20). Under the same conditions, various lower concentrations are analyzed until the value is found for which the 2s confidence limit is valid.
More recent findings indicate that when testing genuine samples with different concentrations of the substance to be defined, the sensitivity of the lowest still quantifiable amount can be defined (detection limit). The global significance of the test procedure refers to different concentrations of the substance under investigation (sensitivity).

8.1.3 Specificity

Substance group tests cannot be specific to single substances. Dependent on substance group, a reaction specific to substance group is required. One exception, for example, is screening for tri- and tetracyclic antidepressants. Most immunoassays conducted for tricyclic antidepressants fail to find effect-related substances (tetracyclic antidepressants) even though this would be desirable from a toxicological standpoint. Dependent on technology, it is possible to determine structurally similar substances that then simulate a positive result.

8.2 AGSA-recommended cut-off concentrations (µg/L) for instrumental immunoassays for urines without pre-analytical hydrolysis

Single substance tests		A	B	C	D*
E	Cannabis (THC carboxylic acid)	S	50	X	50
	Cocaine or cocaine metabolite (benzoylecgonine)	S	300	X	300
	LSD	S	0.5	X	-
	Methadone	S	300	X	-
	EDDP	S	100	X	-
	Methaqualone	S	300	X	-
	6-Monoacetylmorphine (6-MAM)	S	10	X	-

Substance class, substance		A	B	C	D*
G	Amphetamines	S	500	X	1000
	Barbiturates	S	200	X	-
	Benzodiazepines	S	100	X	-
	Opiates	S	300	X	2000*)

S = Sensitivity limit
X = No recommendation
D*= Cut-off concentration in accordance with NIDA/SAMHSA
***)** = Latest SAMHSA recommendation
Comment: No cut-off concentrations can be recommended for non-instrumental immunoassays since these are determined by the manufacturers and cannot be changed. Determination of tricyclic antidepressants in the urine belongs to this category of analysis methods.
Hydrolysis of urine before analysis increases the concentration of non-bonded substances, e.g. unconjugated morphine and benzodiazepines.

9. Chromatographic methods (confirmation analysis)

9.1 Definition

Confirmation analyses in drug analysis involve chromatographic methods (usually using spectroscopic detection) to determine one or several single substances, which are used as secondary back up for an immunochemical result.

9.2 General remarks

Confirmation analyses in drug analysis involve chromatographic methods (usually using spectroscopic detection) to determine one or several single substances, which are used as secondary back up for an immunochemical result.

9.3 Methods

The following methods are suitable:

- Gas chromatography with mass spectrometric detection (all substances) (GC-MS)
- Gas chromatography with nitrogen-phosphorus detection (opiates, cocaine metabolites, amphetamines) (GC-NPD)
- High-pressure liquid chromatography (HPLC)
- High-pressure liquid chromatography with diode-array detection (amphetamines and designer drugs, opiates, etc.) (HPLC-DAD)
- High-pressure liquid chromatography with electrochemical detection (opiates) (HPLC-ECD)
- High-pressure liquid chromatography with mass spectrometric detection (HPLC-MS)
- Instrumental thin-layer chromatography with densitometry (opiates, cocaine and metabolites, THC carboxylic acid, etc.) (TLC)

Gas chromatography with mass–spectroscopic detection is the established method for confirmation analysis. When used correctly, it provides the most reliable results in terms of sensitivity and specificity. Most substances are analyzed in GC-MS as derivatives. Using deuterated internal standards; even variable extraction yields can largely be compensated. The reference spectrum libraries available today facilitate evaluation to a major extent. However, this method must only be used by adequately trained personnel since misinterpretations may very easily occur.

HPLC provides a good alternative to GC-MS confirmation in the case of amphetamines and designer drugs, since these substances can be determined without derivatization including their metabolites. The DAD and MS are detection systems that provide improved reliability for peak identification.

In comparison with the other methods, instrumental TLC is cheaper and faster. Generally, the substances are analyzed after prechromatographic derivatization. However, the UV spectra obtained are only group-specific.

9.4 Areas of application

A: Dependent on requirements, differential and confirmation analyses are required.
B: Confirmation analyses are only required if the immunochemical result is disputed by a patient.
C: Confirmation analyses are necessary for all positive immunotests. Dependent on the case, negative immunoassay results also require confirmation (e.g. urine samples from drug dealers).
D: Positive findings always require confirmation.

10. Cut-off values, sensitivity and specificity of chromatographic methods

10.1 Terminology

10.1.1 Cut-off values

As with immunoassays, the cut-off value means the limit for a decision on whether a result is to be interpreted as positive or negative. The cut-off concentrations for confirmation analysis methods generally differ from those of immunoassays. They always relate to single substances.

10.1.2 Sensitivity

Sensitivity means limit of detection of a method. This limit of detection is dependent on

- The substance under investigation
- The method of analysis used
- The extraction carried out
- Any matrix effects

As a rule, the sensitivity of the confirmation method should be greater than that of the screening test.

10.1.3 Specificity

The specificity of a screening method is understood to mean the ability to measure only the specified substance or substance group.
The specificity of the confirmation analysis should be better than that of the screening test.

10.1.4 Accuracy

The accuracy of a result means its agreement with the true value. It is limited by systematic errors.
The accuracy of the results of the chromatographic methods listed here is influenced by

- Extraction quality
- The choice of stationary phases (columns, TLC plates)
- Calibration of the equipment
- The choice of derivatisation reagents
- The biological matrix
- The quality of the reference spectrum library used
- Interpretation

10.2 AGSA-recommended cut-off concentrations

Drug group	Single substance	GC-MS Cut-off concentration (µg/L)
Amphetamines	Amphetamine Methamphetamine	500[1] 500[3]
Barbiturates	Butalbital Pentobarbital Secobarbital Phenobarbital	200[2] 200[2] 200[2] 200
Cocaine	Benzoylecgonine	150[1]
Opiates	Morphine Codeine	300[1] 300[1]
Cannabis	THC carboxylic acid	15[1]

[1] SAMHSA (NIDA) recommendation
[2] DOD recommendation
[3] Only stated if the amphetamine concentration is > 200 µg/L

In the case of benzodiazepines, methadone, methaqualone and LSD, no cut-off concentrations are set.

11. Blood/serum analysis

11.1 Blood/serum analysis for differential diagnostics (A)

Immunological differential analysis for drug determination in blood, serum and plasma[1]

Substances, substance groups	Sample material in original procedure	Sample material	Sample pretreatment	Test results	Cut-off[2] µg/L
Barbiturates	Serum	Serum or plasma	None (only necessary for whole blood)	Pos/neg	200 - 300 (dependent on test)
Benzodiazepines	Serum	Serum or plasma	None (only necessary for whole blood)	Pos/neg	15 - 300
Tricyclic antidepressants	Serum	Serum or plasma	None (only necessary for whole blood)	Pos/neg	300
Opiates	Urine	Serum or plasma	Necessary	Pos/neg	Limit of sensitivity
Cocaine (benzoylecgonine)	Urine	Serum or plasma	Necessary	Pos/neg	Limit of sensitivity
THC metabolite (THC carboxylic acid)	Urine	Serum or plasma	Necessary	Pos/neg	Limit of sensitivity
Methadone	Urine	Serum or plasma	None (dependent on manufacturer)	Pos/neg oder µg/L	Limit of sensitivity
Amphetamines	Urine	Serum or plasma	Necessary	Pos/neg	Limit of sensitivity
Methaqualone	Urine	Serum or plasma	Necessary	Pos/neg	Limit of sensitivity

[1] Plasma: (lithium, ammonium, sodium heparinate plasma)
[2] (Methods dependent on manufacturer)

Most manufacturers of drugs of abuse analysis reagents also supply reagents for the analysis of barbiturates, benzodiazepines and tricyclic antidepressants in serum/plasma. The other substances or substance groups can be detected by urine detection methods after special sample pretreatment.

The problems relating to immunological detection methods addressed in §8 also apply to most of the serum/plasma determinations (cross-reactions of the antibodies in the group tests vary from manufacturer to manufacturer, different calibration substances).
The results only provide an indication and do not permit any determination of concentration. This can lead to confusion if the substance detected, e.g. in low therapeutic quantities, has already given a positive result and thus cannot be considered as a cause of intoxication.

11.2 Blood/serum analysis for forensic investigations (C)

Where forensic investigations are concerned, the analysis of the urine for drugs is generally insufficient. For example, in the case of road traffic violations or other crimes, to determine the actual impairment of the persons involved or to clarify the cause of death if there are fatalities, a quantitative determination of the drugs in the blood/serum must be carried out subsequent to the qualitative urine analysis. The following methods can be recommended for this:

Opiates	GC-MS, HPLC-ECD, HPLC-DAD or HPLC-MS
Cocaine and metabolites	GC-MS, GC-NPD, HPLC-MS
THC and THC metabolites	GC-MS, HPLC-MS
Methadone	GC-MS, GC-NPD, HPLC-DAD, HPLC-MS
Amphetamines and designer drugs	GC-MS, HPLC-DAD, HPLC-MS
Benzodiazepines	GC-MS, GC-ECD, HPLC-DAD, HPLC-MS
Barbiturates	GC-MS, GC-NPD, HPLC-DAD
Methaqualone	GC-MS, GC-NPD, HPLC-DAD

The use of deuterated internal standards is recommended for GC-MS and HPLC-MS analysis.
'Seite: 18
In forensic investigations, immunochemical procedures for blood analysis (generally after special sample pretreatment) have not evidence character and may not be used for quantification. In this context, no cut-off concentrations can be recommended.

12. Interpretation of the results

12.1 Steps in interpretation

12.1.1 Analytical interpretation (laboratory expert)

- Verification and interpretation of the results with consideration given to any pre-analytical conditions, “chain of custody” documents, quality assurance data, outliers and method specifications (sensitivity, specificity, cut-off, cross-reactivity, etc.)

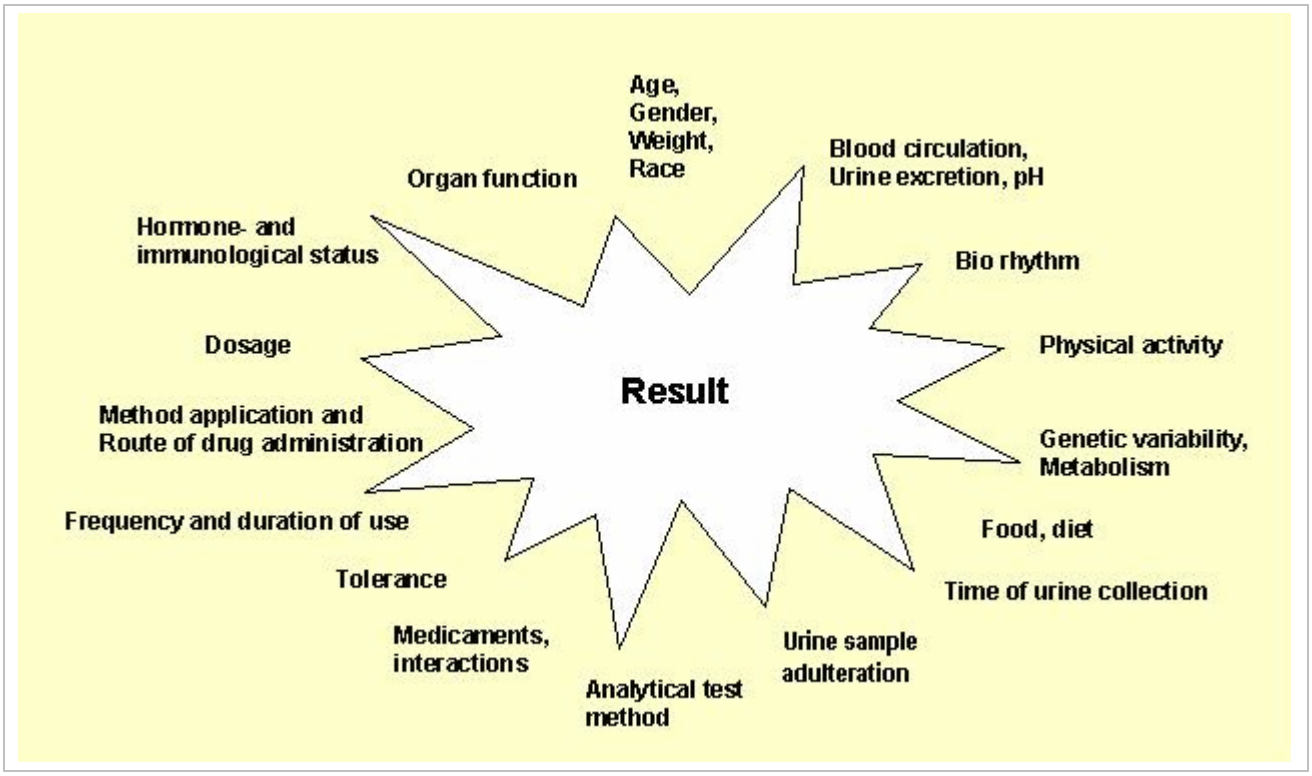
12.1.2 Toxicological interpretation (laboratory expert)

- Consideration given to dose, frequency of consumption, type of application, interactions, interindividual variability, tolerance, pharmacokinetics, pharmacogenetics.

12.1.3 Medical interpretation (client, laboratory expert)

- Consideration given to the individual’s history of health situation, e.g. existing illnesses (organ function, lack of enzymes, metabolic disorders, age)
- Evidence of drug influence at the time of the urine sample collection
- Doctor’s prescription? Self-medication? Food?
- Plausibility check

12.2 Factors that influence pharmacokinetics and analysis result



12.3 Significance of the result

12.3.1 Questions when using immunoassays

With a negative result:

- Has there been no consumption so far?
- Has there been no recent but occasional consumption?
- No consumption due to advance warning of urine sampling?
- Urine sample adulteration?

With a positive result:

- Confirmation using physico-chemical methods?
- Chronic or occasional consumption?
- Passive inhalation? (Cannabis, opiates, cocaine)?
- Cross-reactions with medicaments and food?

12.3.2 Answers

Immunoassay result negative:

The tests used failed to detect any drugs and/or their metabolites:

- The individual is consuming no drugs of abuse detectable with this test.
- The individual is possibly consuming drugs that are not detectable. Reasons:
  - Samples mixed up
  - Concentration too low
  - Consumption frequency too low
  - Wrong time selected for urine sample collection
  - Urine sample manipulation
  - Test not sensitive enough or wrong test, incorrect analytical procedure
  - Wrong investigation requested

Immunoassay result positive:

- Indication of presence of drugs and/or their metabolites in quantities above the cut-off concentration. Evidence only by means of a confirmation analysis.
- No conclusions possible concerning physical and mental condition and behaviour at the time of urine sampling.

Confirmation analysis positive:

- Proof of at least one-time drug consumption.
- Proof of chronic drug consumption only possible in the case of long-term monitoring (multiple urine samples collected or repeated positive result, taking clinical and social facts into consideration).

12.4 Consequences of the results

Results of drug analyses can have legal, financial, social, medical and/or ethical consequences. Each individual tested has the right to a properly conducted investigation:

- The quality of the analysis and the reliability of the result are essential not only in the forensic but also in the socio-medical sectors.
- Critical interpretation of the result by a laboratory, quality assurance must be included.
- Critical interpretation of the result by the client (see §12.1.3).

13. Quality assurance in drug analysis

	A	B	C	D
Internal and external quality control	**X**	**X**	**X**	**X**
Classification of the testing laboratory according to the QUALAB concept, Federal analysis list, KVG, KVV and KLV	**X**	**X**	**-**	**(X)**
In each case, confirmation analysis of positive samples	**-**	**-**	**X**	**X**
Dependent on requirements, confirmation analysis (particularly for positive samples)	**X**	**X**	**-**	**-**

Internal and external quality controls must be conducted with recognized reference material of the known biological matrix. In the case of a method comparison, the sample values should be as close as possible to the cut-off concentration. The analysis spectrum must be covered by external quality checks (q.v. Appendix 3 for materials).
To ensure quality assurance in the laboratory, the criteria for running a medico-analytical laboratory are recommended (KBMAL).
For the compulsory external quality checks (interlaboratory tests), the following AGSA cut-off suggestions are recommended as limits for decisions in accordance with QUALAB:

Cannabis	50 µg/L	(In relation to THC carboxylic acid)
Cocaine (metabolite)	200 µg/L	(In relation to benzoylecgonine)
Barbiturates	300 µg/L	(In relation to secobarbital)
Benzodiazepines	100 µg/L	(In relation to nordiazepam)
Amphetamines	1000 µg/L	(In relation to amphetamine or metamphethamine)
Opiates	300 µg/L	(In relation to morphine)
Methadone	300 µg/L	(In relation to methadone)

14. Documentation of the results and reports, archiving

The documentation serves as information while maintaining safety and confidentiality in the chain of custody. Electronic data media are equal in value to written material for the purposes of information and archiving.

14.1 Analysis order

The analysis order is issued using the form provided by the laboratory. The form should clearly document the analyses to be conducted. The order must contain the following data:

14.1.1 Unequivocal identification of the order[1]

- Name of the client[2]
- Date of the order[1] or date received
- Signature of the client[1]

14.1.2 Reason and/or clinical details[2]

- Poisoning
- Substitution programs or withdrawal treatment
- Forensic (e.g. road traffic)
- Monitoring in the workplace, staff doctor examination
- Physiological factors (e.g. pregnancy, liver or kidney malfunction)
- Biological individuality (e.g. N-acetyl transferase)
- Prescribed and/or consumed drugs of abuse, medicaments or other relevant substances
- Other clinical data (e.g. clinical condition, dialysis, allergies)

14.1.3 Sample data[2] (in forensic investigations[1])

- Date and time of sample collection (control)
- Sample material
- Type of sample (spot, collected urine)
- Special measures (emergency)

14.1.4 Personal data[1]

- Unequivocal identification (last name, first name, date of birth or code[1])
- Gender[2]
- Weight[2]
- Address or locality[2]
- Sample identification by the client[2]

14.1.5 Tests required[1]

- Correct statement of substance or substance group to be analyzed[1]
- Additional information, e.g. confirmation analysis[2]

[1] Compulsory information
[2] Optional information

14.2 Report

The receipt of any irregular orders must be appropriately documented in the report.

14.2.1 Material[1]

- Type of sample material[1]
- Description of the material prior to and subsequent to analysis[2]

14.2.2 Result[1]

Detection by immunochemical methods:

- Name of the single substance or substance group[1]
- Interpretation[1]
- Name of the reference substance[2]
- Cut-off for the reference substance[2]
- Value measured[2]
- Details of the substances screened for but not found[1]
- Details of the substances detected but not listed in the order form[2]

Confirmation analyses (chromatographic methods):

- Name(s) of the single substances found[1]
- Value measured[2]
- Limits of detection[2], cut-off[2]
- Details of measurement inaccuracy[2]
- Findings (q.v. §12)[2]
- Details of the substances detected but not listed in the order form[2]

14.2.3 Administrative data[1]

- Date of sample collection and/or receipt of order[1]
- Date of report (date of transmission)[1]
- Datum of analysis[2]
- Signature of the person responsible for the release of the report (also electronically)[1]
- Type of transmission (e.g. phone, fax)[2]
- Reference to any copies[1]
- Reference to invoicing[2]
- Address of the laboratory (address for queries)[1]

[1] Compulsory information
[2] Optional information

14.3 Archiving

All the data listed under §14.1 and §14.2 must be archived by the client (§14.1) and the laboratory (§14.2).
The data (order forms, extracts from the quality manual, measurement protocols, quality controls, calibrations, reports) must be archived in such a way that it is at all times possible to obtain a copy of the analysis report. Electronic media (e.g. CD-ROM or magnetic media) must be given preference over classic methods of archiving (paper).

14.3.1 Data archiving period

Data of an exclusively clinical nature must be kept for a minimum of 5 years (unless otherwise specified).
Data of a forensic nature must be kept for a minimum of 10 years, unless explicitly directed otherwise by authorities to destroy them or eliminate specific personal references at an earlier date.
The details issued by KBMAL, QUALAB and data protection will also apply.

15. Urgency of the results

15.1 Levels of urgency

Urgency is subdivided into three levels:

Level	Action	A	B	C	D
I:	Result should be available within a maximum of 3 hours	X	-	-	-
II:	Result should be available within a maximum of 24 hours	X	-	X	-
III:	Result should be available within a few days	-	X	X	X

Examples

I: Hospitals with emergency units. In cases of intoxication, it is important to detect toxic substances without any loss of time (emergency situation).
III: For therapists and patients in substitution programs, it is important to be able to detect or exclude the consumption of any other drugs within a useful period of time.

16. Costs, reimbursement, list of analyses

16.1 General remarks

In general, invoicing for drug analyses should follow the tariff system listed in the Federal Analysis List (issued by FDHA, 1994). This tariff is based on CAP data as well as an extensive investigation of public and private laboratories in Switzerland. It is thus representative for our purposes.

16.2 Drugs of abuse testing in the clinical sector and in differential diagnostics (A)

If the paying client is a social insurance, the use of the Federal Analysis List with its tariff is compulsory. Quality assurance (to be provided by the laboratory concerned) is included in this tariff system in accordance with the guidelines worked out by SULM (KBMAL) and QUALAB. The prerequisite for this is the laboratory's accreditation as a medical laboratory both by the canton and the BSV as well as the conclusion of quality assurance agreements between the paying clients and those performing the service or the membership of the laboratory or head of the laboratory in an association which signs these agreements on a collective basis.
Invoices are addressed to the patients.

16.3 Drugs of abuse testing in substitution programs or withdrawal treatment (B)

If the paying client is a social insurance, the use of the Federal Analysis List with its tariff is compulsory (as in §16.2).
Invoices are addressed to the patients.
If the paying client is not a social insurance, individual tariff rates are acceptable.
The tariffs must cover costs. The following basic rates must be applied:

- Material costs (including any sample collection material supplied)
- Equipment costs (equipment amortization, maintenance, power)
- Staffing costs (including insurance)
- Cost of premises
- Administration costs

The application of a special tariff must be set forth in a contract. Invoices are sent to the client or the representatives of the client institution as well as to any federal offices as necessary.

16.4 Drugs of abuse testing in forensic investigations (C)

Ideally, the Swiss Society of Forensic Medicine should issue an appropriate, uniform tariff to meet the requirements of forensic-toxicological investigations. Where possible, it should be closely based on the Federal List of Analyses. Invoices are sent to the client.

16.5 Drugs of abuse testing in the non-traditional sector (D)

The invoicing of analyses of this type must be at least based on the tariff of the Federal List of Analyses. For further clarifications, the tariff in §16.4 will apply. Invoices are sent to the client.

17. Legal aspects, standards, data protection

17.1 General prerequisites (cf. §14)

- In investigations of drugs of abuse, the client must be clearly identifiable.
- His legitimacy to order the investigation must be known.
- The laboratory conducting the investigation must have the relevant qualifications and permits.
- Result traceability must be guaranteed.
- The quality of the results must be provable.
- Results may only be made known to the person under investigation or to persons authorized by him/her or otherwise legally entitled.
- The laboratory conducting the investigation must disclose to the client the names of any subordinate persons placing orders.

17.2 Data protection

Data protection (raw data and results, patient data) must be guaranteed. The basis for this is the law governing data protection as well as the law governing medical insurance (KVG).
General account must be taken of the following laws and standards:

- Doctors' professional secrecy in accordance with the KVG
- Data protection law
- Criminal law and official secrecy

17.3 Authorized clients

A	**Medical personnel**
B	**Persons authorized by withdrawal and substitution programs or as carers**
C	**Persons or institutions legally authorized**
D	**Anyone who has a justified interest according to civil law standards insofar as the person involved has been informed and is in agreement. The laboratory conducting the investigation bears no responsibility.**

17.4 Laboratories authorized to conduct analyses for drugs of abuse

A	**Cantonal and federally approved medical laboratories in accordance with the KVV and KLV**
B	**As A, with the addition of other investigation agencies approved by the authorities**
C	**Forensic-toxicological departments of the Institutes for Forensic Medicine, investigation agencies specially approved by the authorities**
D	**As B, approval according to A would be desirable**

17.5 Accreditations and permits required by law for laboratories

	According to the Federal List of Analyses KVG	Compulsory quality assurance as laid down by contract (QUALAB concept)	EJPD, UVEK (ASTRA) or cantonal judicial authorities
A	X	X	-
B	X	X	-
C	-	-	X
D	-	-	-

17.6 Confidentiality of unrequested positive results

	Routine check	Additional results that permit conclusions about existing illnesses	In the case of a repeat (same parameters repeatedly positive)
A	X	X	X
B	n	n	X^1
C	X	X^1	X
D	n	n	X^1

X = Disclose
n = Not to be disclosed
X^1 = Only to be disclosed to medical personnel (carrying out treatment)

18. Further information

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- http://www.gifte.de/ketamine.htm
- http://www.jugendinfo.de/party-project/infos/ketamine.html

INTERNET LINKS

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Arbeitsgruppe Suchtstoffanalytik	AGSA	http://www.cscq.ch/agsa
Botanikseite	Botanikus	http://www.botanikus.de
Bund gegen Alkohol und Drogen im Straßenverkehr	BADS	http://www.bads.de/
Bundesamt für Polizeiwesen	BAP	http://internet.bap.admin.ch/
Bundesamt für Sozialversicherungen	BSV	http://www.bsv.admin.ch/
Bundesamt für Strassen	ASTRA	http://www.astra.admin.ch/
Bundeszentrale für gesundheitliche Aufklärung	BZgA	http://www.drugcom.de/
College of American Pathologists	CAP	http://www.cap.org
Deutsche Industrie-Norm	DIN	http://www2.din.de/
Eidg. Departement für Umwelt, Verkehr, Energie und Kommunikation	UVEK	http://www.uvek.admin.ch/
Eidgenössisches Departement des Inneren	EDI	http://www.edi.admin.ch/
Eidgenössisches Justiz- und Polizeidepartement	EJPD	http://www.ejpd.admin.ch/ejpd/de/home.html
European Committee for Standardization	CEN	http://www.cenorm.be/cenorm/index.htm
Giftpflanzen Compendium		http://www.giftpflanzen.com/
Hofmann, Foundation Albert -	AHF	http://www.hofmann.org/
Informationsseiten zum Thema Drogen und Drogenscreening		http://www.drogenscreening.info
Interdisziplinäres Drogenlexikon mit Drogen-Linkliste		http://www.drogen-wissen.de/dr_k.html
International Symposium : 100th Birthday of Albert Hofmann	LSD	http://www.lsd.info/
Krankenpflege-Leistungsverordnung	KLV	http://www.admin.ch/ch/d/sr/c832_112_31.html
Krankenversicherungsgesetz	KVG	http://www.admin.ch/ch/d/sr/c832_10.html
Krankenversicherungsverordnung	KVV	http://www.admin.ch/ch/d/sr/c832_102.html
Krankheiten und Beschwerden von A bis Z: Drogen und Sucht		http://hausarzt.qualimedic.de/Drogen.html
Kriterien zum Betreiben von medizinisch-analytischen Laboratorien	KBMAL	http://www.qualab.ch/KBMAL14.pdf
Party-Project		http://www.party-project.de/
Public education psychoactive drugs and drug use	LYCAEUM	http://www.lycaeum.org
Relationship Between Humans & Psychoactives	EROWID	http://www.erowid.org/
Schweizerische Kommission für Qualitätssicherung im med. Labor	QUALAB	http://www.qualab.ch
Schweizerische Union für Laboratoriumsmedizin	SULM	http://www.sulm.ch/
Schweizerisches Zentrum für Qualitätskontrolle	CSCQ	http://www.cscq.ch/
Society of Forensic Toxicologists	SOFT	http://www.soft-tox.org
Toxikologie in der Notfallmedizin	GIFTE	http://www.gifte.de
U.S. Department of Defence	DOD	http://www.defenselink.mil/
U.S. National Institute on Drug Abuse	NIDA	http://www.nida.nih.gov/
U.S. Substance Abuse and Mental Health Services Administration	SAMHSA	http://www.samhsa.gov/
Verein für Medizinische Qualitätskontrolle	MQ	http://www.mqzh.ch/

19. List of working group members

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20. Pharmacokinetics, detectability

The detection of drugs of abuse is influenced by different factors (q.v. §12.2). In this appendix, we refer to detectability using immunochemical methods.

Amphetamines and Derivates

Metabolism: (Fig 1-4)
The rate of metabolism and excretion is dependent on the urinary pH: acidic urine increases (e.g. amphetamine: up to 78%/24 h, 68% unchanged), alkaline urine decreases excretion in urine (45%/24 h, 2% unchanged).
44% of methamphetamine is excreted unchanged, 6 - 20% as amphetamine and 10% as 4-hydroxymethamphetamine
Medicaments: It should be noted that certain medicaments (e.g. anorectics) are metabolized to amphetamine or methamphetamine (Fig. 4).
MDMA ("Ecstasy") is mainly eliminated as an unchanged substance. Metabolites are formed by N-demethylation, ring cleavage, methylation and glucuronidation (Fig. 3).

Elimination half-life:
10 - 30 h. Amphetamine and methamphetamine appear in urine within 20 min after administration.

Detectability:
Unchanged drug! Up to 48 h, e.g. an oral dose of 5 mg amphetamine: up to 29 h

Fig 1: Metabolism of amphetamines

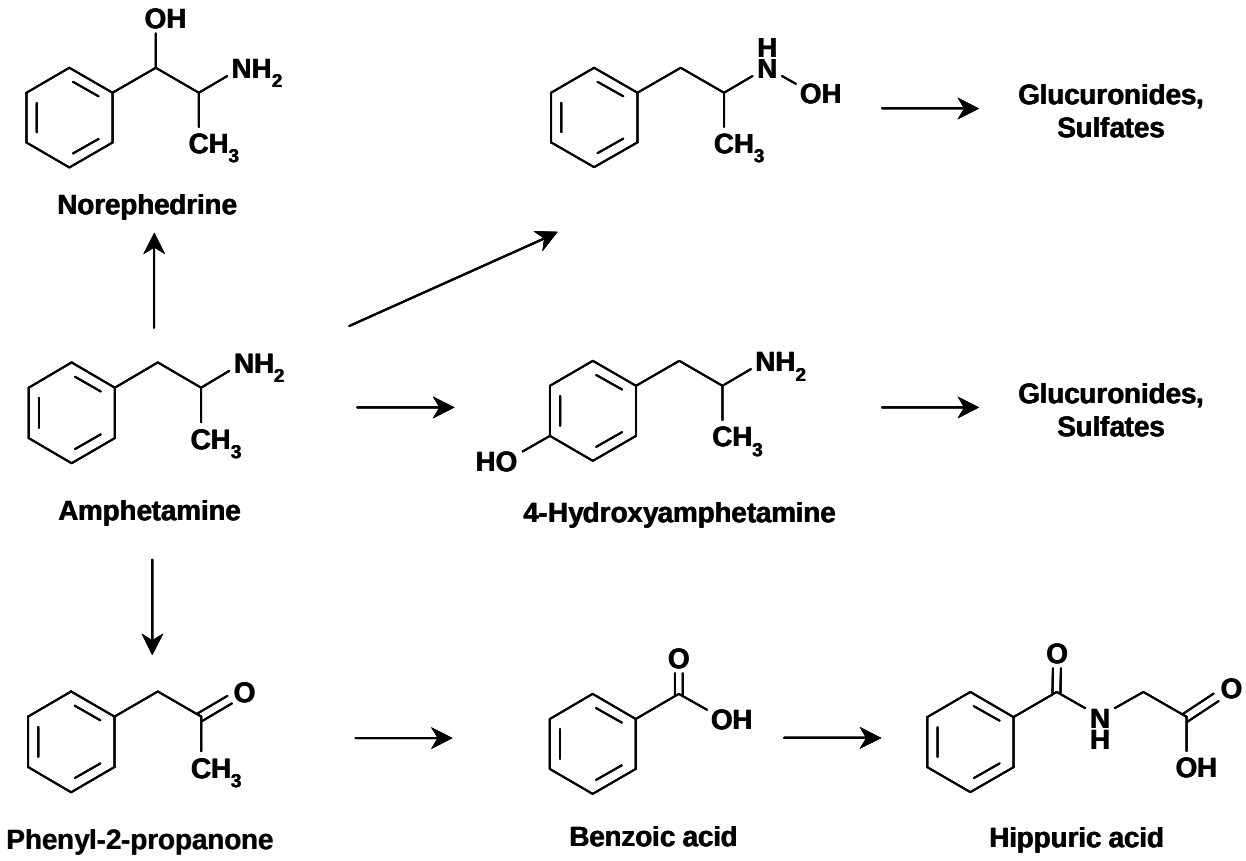


Fig. 2: Metabolism of methamphetamines

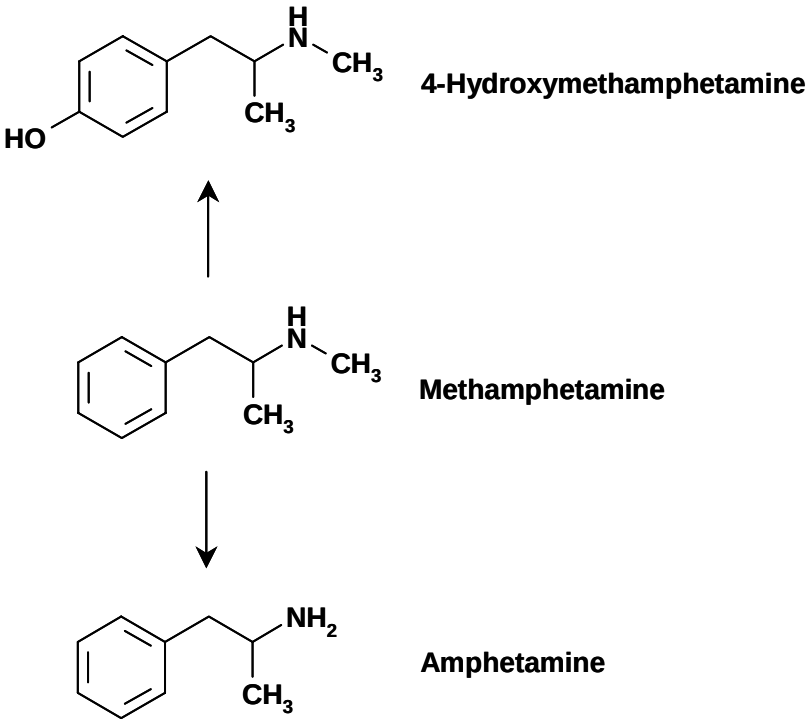


Fig. 3: Metabolism of MDMA

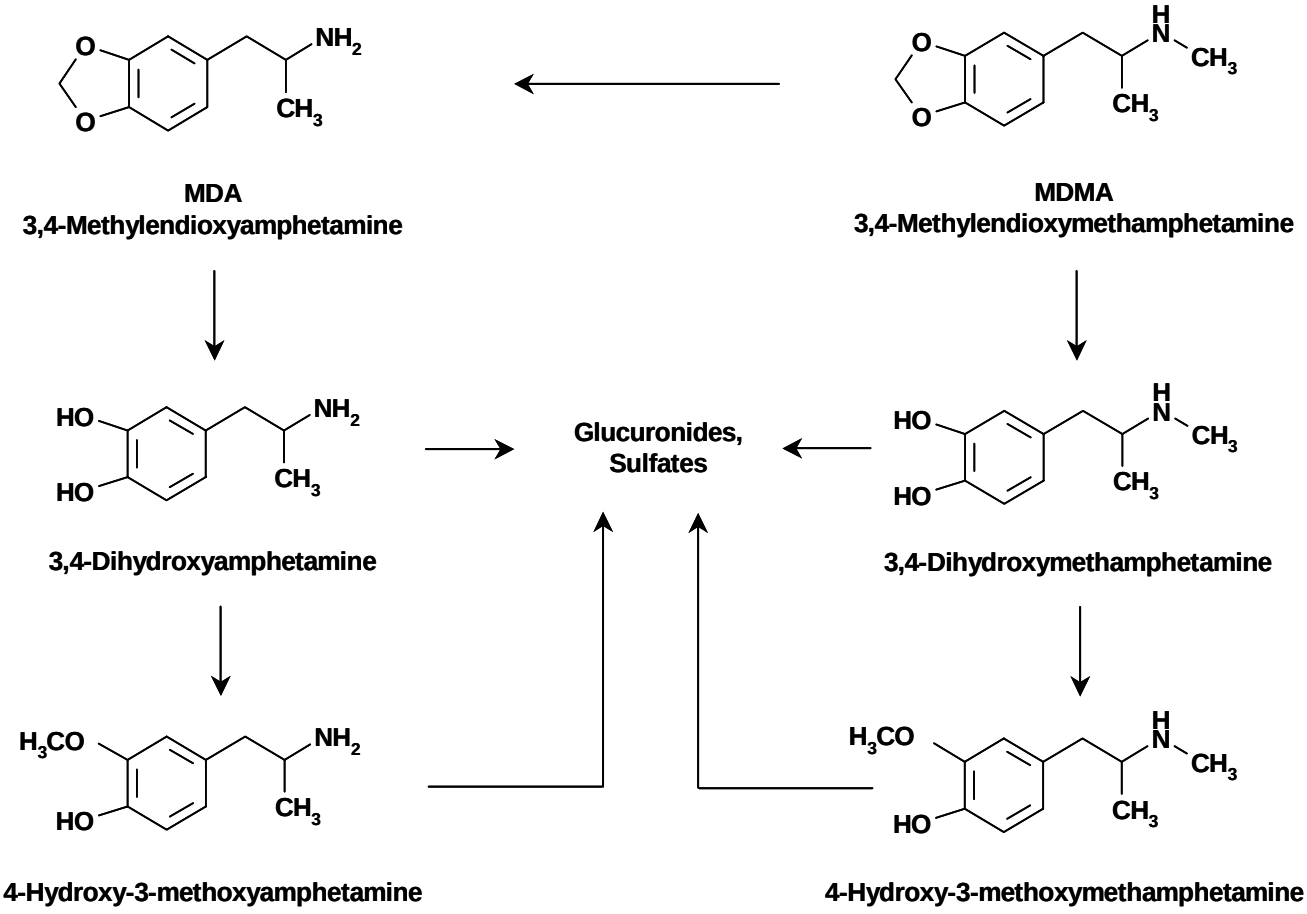
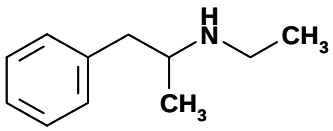
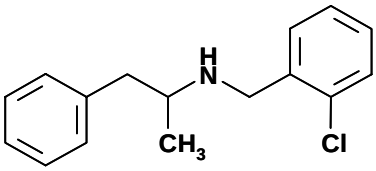


Fig. 4: Substances with amphetamine or methamphetamine as a metabolite

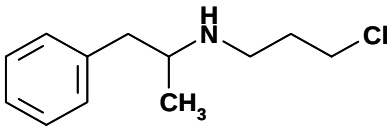
Amphetamine as Metabolite



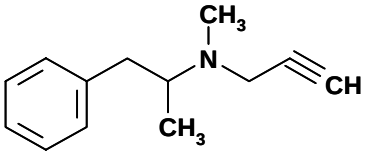
Ethylamphetamine



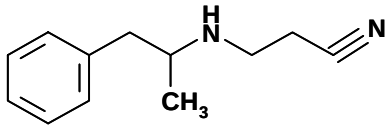
Clobenzorex



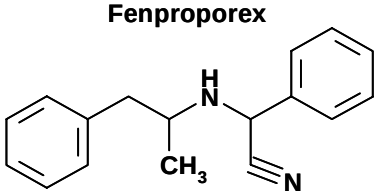
Mefenorex



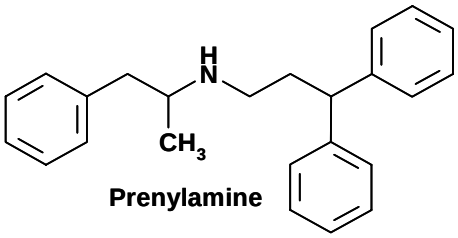
Selegiline



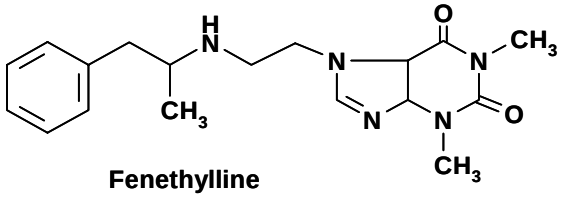
Fenproporex



Amfetaminil

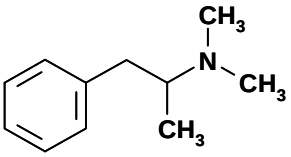


Prenylamine

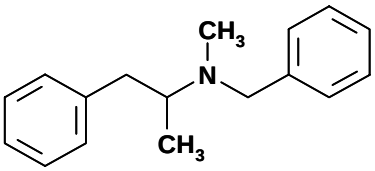


Fenethylline

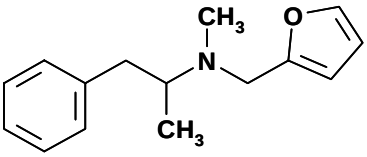
Methamphetamine as Metabolite



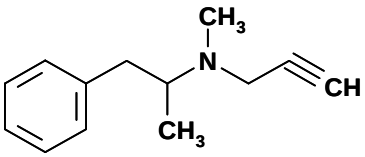
Dimethylamphetamine



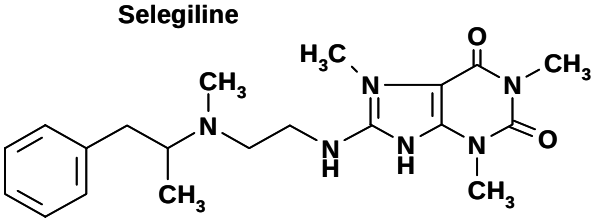
Benzphetamine



Furfenorex



Selegiline



Fencamine

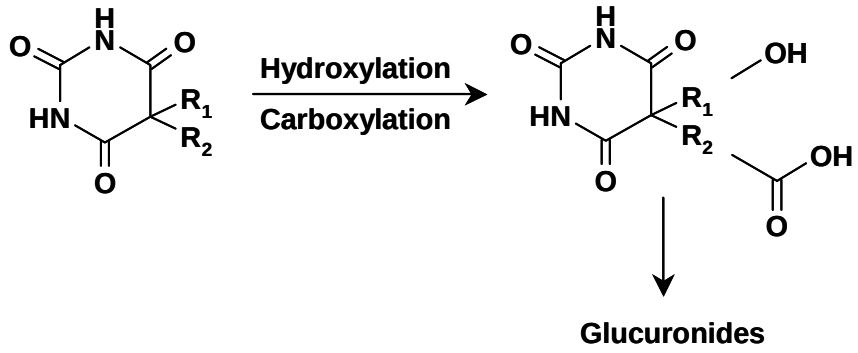
Barbiturates

Metabolism: (Figure 5)
Phenobarbital, pentobarbital, cyclobarbital, etc.: oxidation of the substituents R1 and/or R2 to C-5 → hydroxylation, carboxylation, etc. with subsequent conjugate formation (particularly glucuronides). Phenobarbital is excreted 25% unchanged, pentobarbital 50%.
Thiobarbiturate: → desulphuration S-2.
Methylphenobarbital: → N-demethylation.

Elimination half-life:
20 - 30 h (pentobarbital), 48 - 288 h (phenobarbital) 22 - 29 h (secobarbital).

Detectability:
Up to 5 days (pentobarbital), phenobarbital up to 8 days

Fig. 5: Metabolism of barbiturates



Benzodiazepines

Metabolism: (Figures 6-8)
1,4-Benzodiazepines (diazepam, chlordiazepoxid, etc.): as a result of desalkylation, oxydation and hydroxylation, the main metabolites nordiazepam and oxazepam are formed, which are eliminated renally as glucuronides after 3-hydroxylation.
7-Nitrobenzodiazepines (flunitrazepam, nitrazepam, etc.): metabolism by reduction to 7-amino derivatives, N-acetylation, N-demethylation, 3-hydroxylation and 3-O-glucuronidation. flunitrazepam is excreted less than 1% unchanged.
Triazolobenzodiazepines: 1- and 4-hydroxylation, by ring cleavage also formation of benzophenones (alprazolam).

Elimination half-life:
20 - 40 h (diazepam), 40 - 100 h (nordiazepam), 10 - 30 h (flunitrazepam), 8 - 20 h (bromazepam), 1 - 30 h (triazolam).

Detectability:
Days to months (after long-term consumption).

Fig. 6: Metabolism of 1,4-benzodiazepine

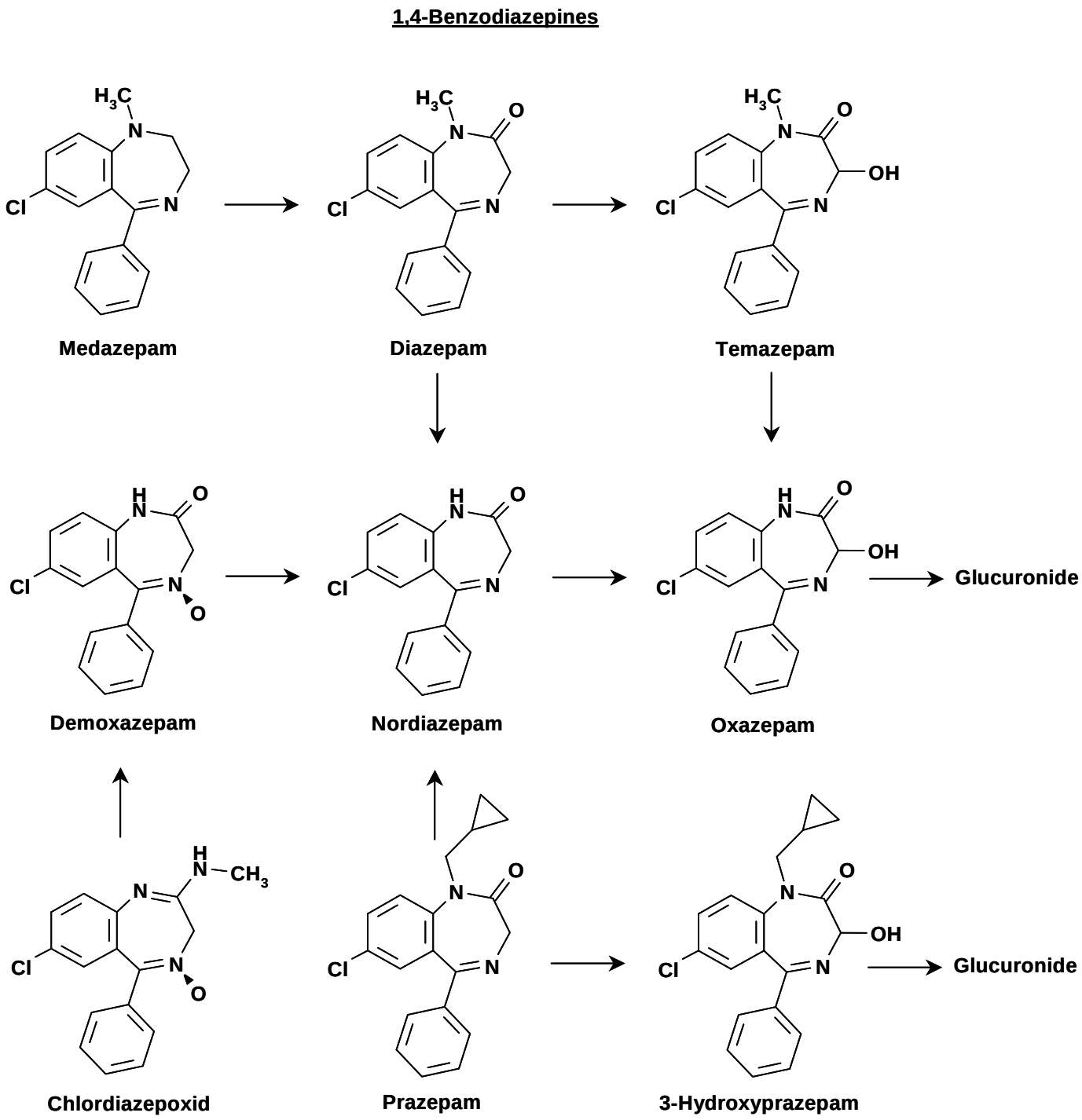


Fig. 7: Metabolism of 7-nitrobenzodiazepine

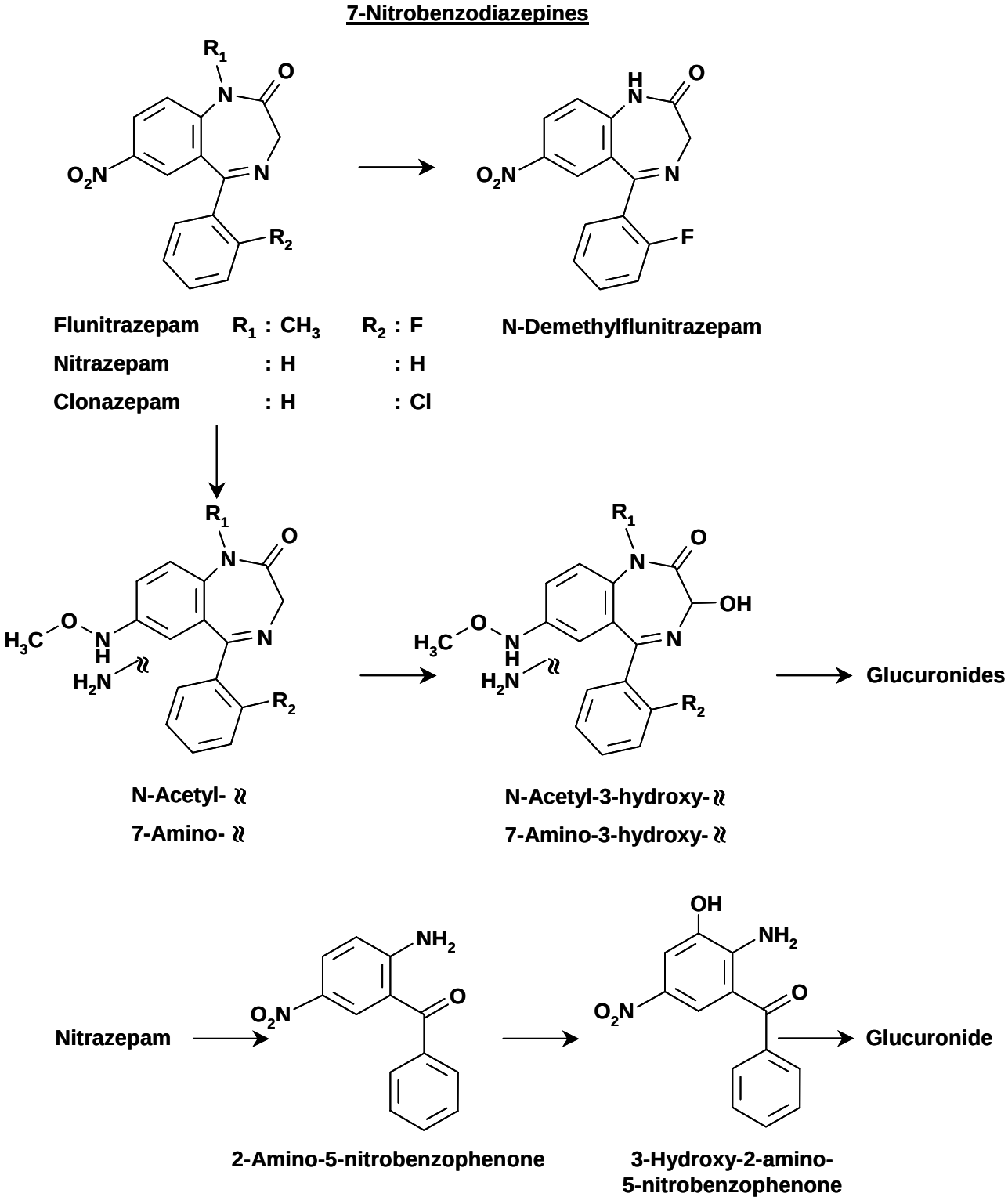
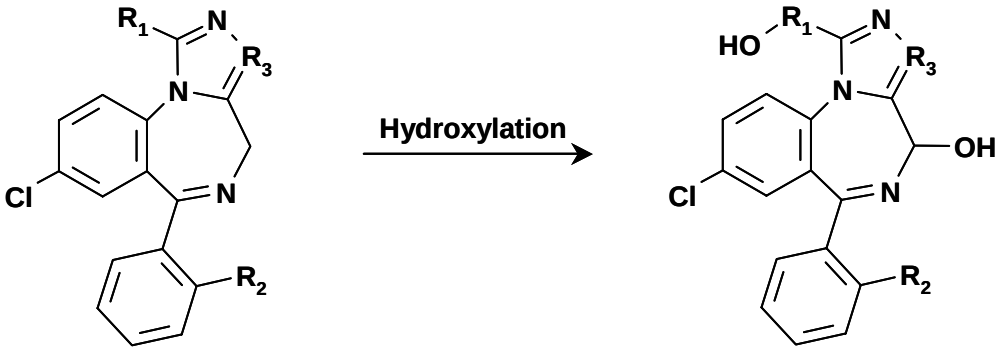


Fig. 8: Metabolism of triazolobenzodiazepine

Triazolobenzodiazepines



Alprazolam	R_1 : CH_3	R_2 : H	R_3 : N
Brotizolam	: CH_3	: Cl	: N
Midazolam	: CH_3	: F	: CH
Triazolam	: CH_3	: Cl	: N

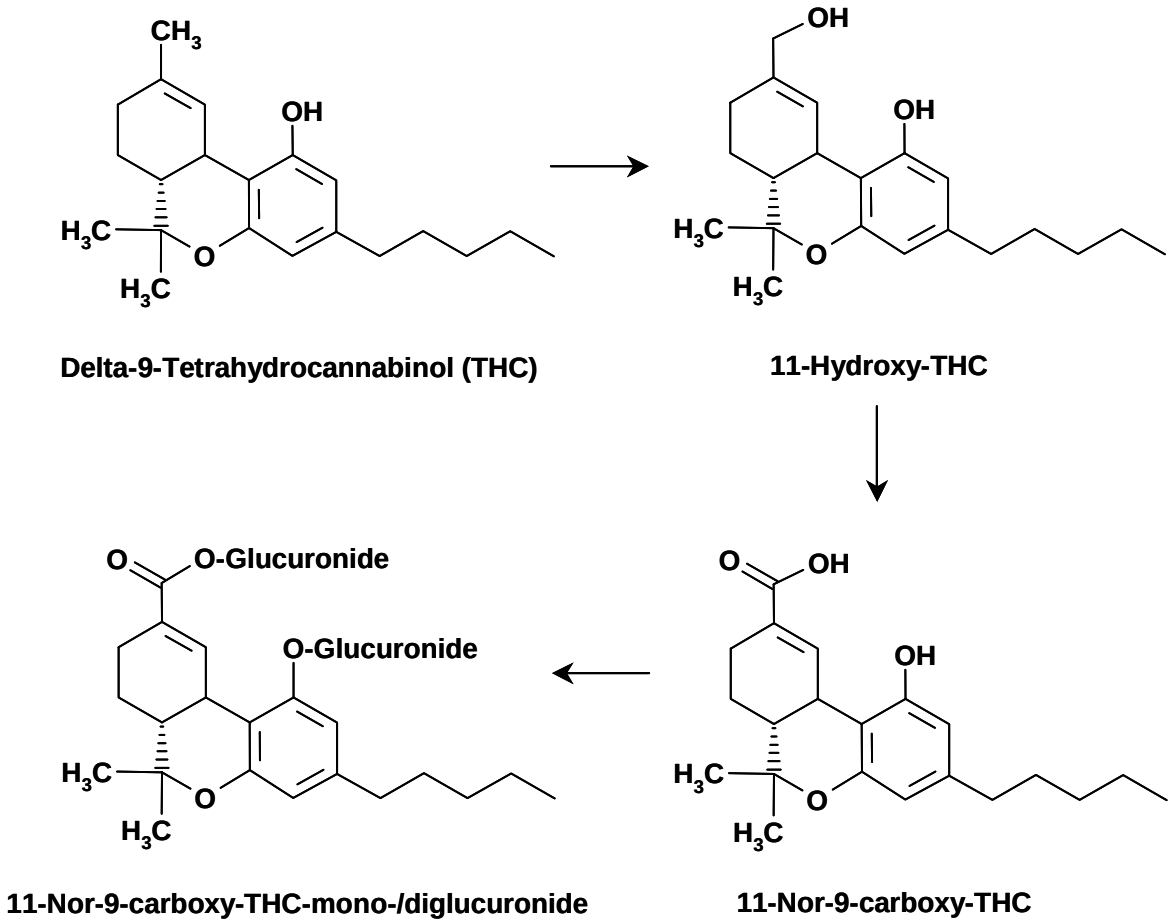
Cannabis

Metabolism: (Figure 9) Due to oxydation of C-11 (and also in the side chain), several hydroxy- and carboxy metabolites are formed, which are mainly excreted as glucuronides.

Elimination half-life: 20 - 30 h (THC carboxylic acid).

Detectability: Up to 3 days (once-only consumption), up to 30 days (occasional consumption, once a week), up to 80 days (continuous consumption).

Fig. 9: Metabolism of delta-9-tetrahydrocannabinol (THC)



Cocaine

Metabolism: (Figure 10)

The main metabolites are benzoylecgonine and ecgonine methyl ester (methylecgonine). They are formed by enzymatic (pseudocholinesterase) or spontaneous hydrolysis. Anhydroecgonine methyl ester is a specific marker for “crack” consumption, while cocaethylene is detectable after the simultaneous consumption of alcohol.

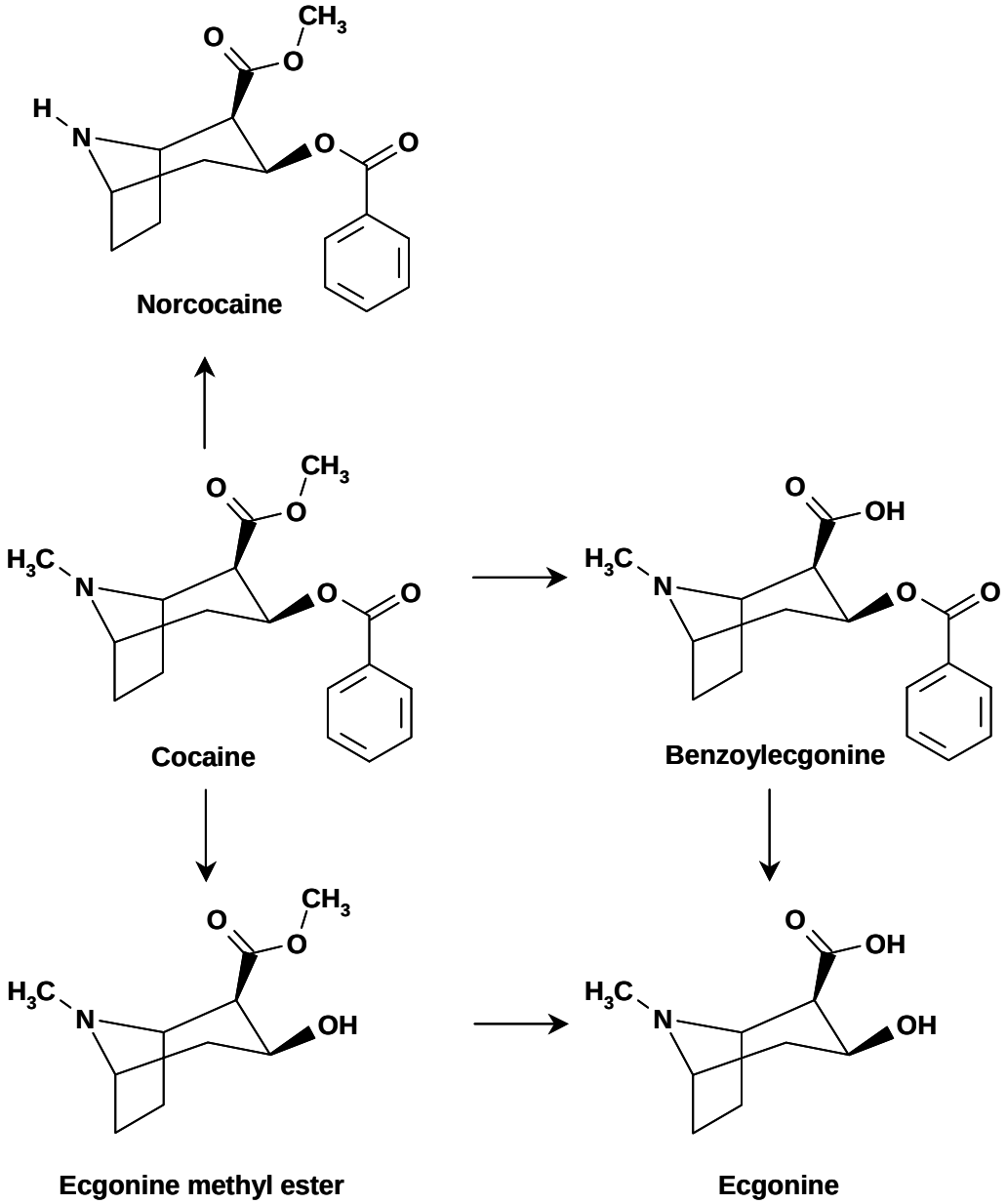
Elimination half-life:

0.5 - 1.5 h (cocaine), 3.5 - 8 h (benzoylecgonine), 3.5 - 6 h (ecgonine methyl ester).

Detectability:

4 - 12 h (cocaine), 1 - 4 days (benzoylecgonine), up to 5 days (benzoylecgonine, long-term consumption)

Fig. 10: Metabolism of cocaine



Gamma-hydroxy-butyrate (GHB, „Liquid Ecstasy“)

Metabolism: (Figure 11)
GHB is almost completely metabolized by alcohol dehydrogenase to unidentified oxidation products. Thus generally less than 5% of the dose of GHB is excreted in the urine unchanged (e.g. only about 1% after 25 mg/kg GHB).

Elimination half-life:
30 - 60 min.

Detectability:
After oral administration of 25 mg GHB per kg around 1% will appear in urine, resulting in a detection window of 12 h.

Pharmacokinetic parameter:
Plasma (after administration of 25 mg/kg GHB): t_{max} = 20-45 min, C_{max} = ca. 40 µg/L.
Urine: maximum concentration after 30 - 60 min
.

Detection method:
Currently no immunological methods are available. Analysis in plasma or urine requires GC, GC/MS or CE-UV/MS.

Literature:
Brenneisen R, Elsohly MA, Murphy TP, Passarelli J, Russmann S, Salamone SJ, Watson DE. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects (Im Druck).

Baldacci A,Theurillat R, Caslavska J, Pardubska H, Brenneisen R, Thormann W. Determination of g-hydroxybutyric acid in human urine by capillary electrophoresis with indirect UV detection and confirmation with electrospray ionization ion-trap mass spectrometry. J. Chromatogr. A 2003; 990: 99-110.

Baselt, RC. Gamma-Hydroxybutyrate. In: Disposition of Toxic Drugs and Chemicals in Man, 6th ed., Biomedical Publications, Foster City, CA, 2002; ISBN 0-9626523-5, S. 472-5.

Fig. 11: Gamma-Hydroxy-Butyrate

There are no known gamma-hydroxy-butyrate metabolites.

Formula: $HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$

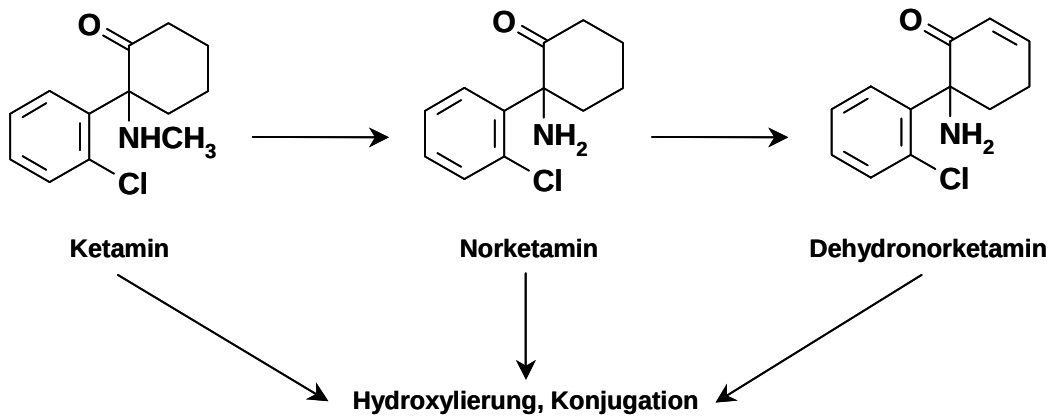
Ketamine

(2-(2-Chlorophenyl)-2-(methylamino)-cyclohexanon [HCl])

Ketamine hydrochloride is an anaesthetic used in hospitals, where it is administered by the intravenous or intramuscular route. Ketamine is available only on prescription, but is not subject to the Betäubungsmittelgesetz [law relating to controlled drugs/narcotics]. It was first synthesised by the Dupont Company in 1962 and is structurally and pharmacologically related to phencyclidine.
In the Techno music scene, ketamine is known under the particular name "Special K". In this environment it is obtainable in liquid form or as a white crystalline powder and is either swallowed, injected or sniffed.

Metabolism: (Figure 12)	Ketamine is metabolized in the liver primarily via N-demethylation and hydroxylation, followed by conjugation. The main pathway consists of N-demethylation by cytochrome P_{450} to norketamine, an active metabolite with one third of the anesthetic potency of ketamine (Baselt 2002).
Active dosis:	200 - 450 mg (oral), 50 - 150 mg (nasal), 30 - 120 mg (i.v., i.m.)
Onset of action:	15 - 20 min (oral), 5 min (nasal), 2 - 5 min (i.m.), less than 1min (i.v.)
Duration of action:	1.5 - 2 h (oral), 1 - 1.5 h (nasal), 40 - 80 min (i.v., i.m.)
Elimination half-life:	80 – 190 min (ketamin) 240 min (norketamine)
Detectability:	1 d in urine
Detection method:	no immunological methods available, only detectable in urine (ketamine, norketamine, dehydronorketamine and conjugates) or blood with GC-MS or LC-MS
Literature:	Baselt, RC. Disposition of Toxic Drugs and Chemicals in Men, 6th Edition, Chemical Toxicology, Institute, Foster City, California 2002, ISBN 0-9626523-5-0 http://www.drogen-wissen.de/dr_k.html http://www.drogenscreening.info/ketamin.htm http://www.gifte.de/ketamin.htm

Fig. 12: Metabolism of Ketamine



LSD

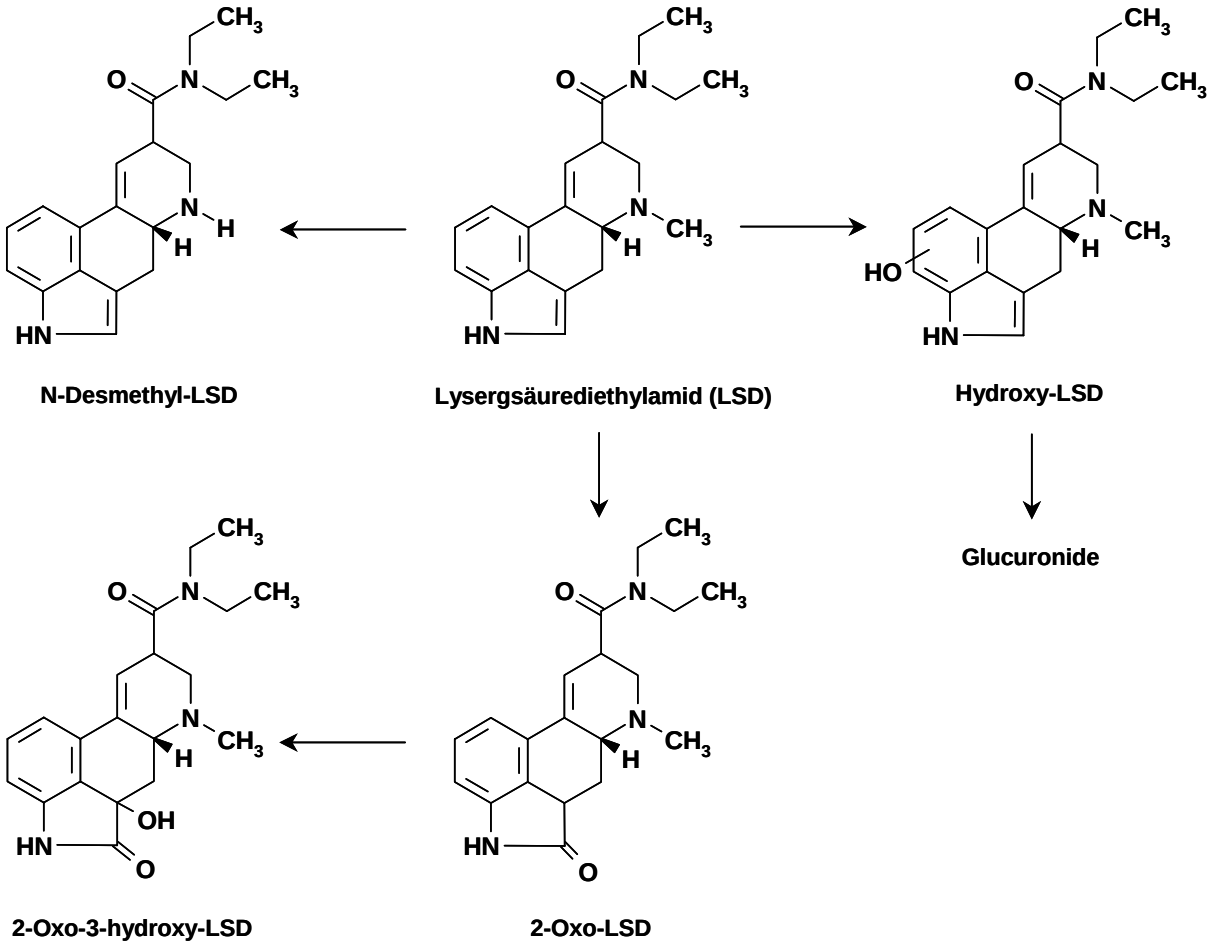
Metabolism: (Fig 13) Metabolism has been studied in animals. Probable route: N-demethylation, N-deethylation, hydroxylation and glucuronidation. Main metabolite, established after illicit ingestion, is 2-oxo-3-OH-LSD

Elimination half-life: 3 – 4 h

Detectability: 1 – 2 d

Literature: Baselt RC. Disposition of Toxic Drugs and Chemicals in Men, 6th Edition, Chemical Toxicology, Institute, Foster City, California 2002, ISBN 0-9626523-5-0

Fig. 13: Metabolism of LSD



Methadone

Metabolism: (Figure 14)
Methadone is metabolized by mono-, di-N-demethylation followed by spontaneous cyclization to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) and subsequent glucuronidation. The main metabolite is EDDP.

Elimination half-life:
15 - 55 h

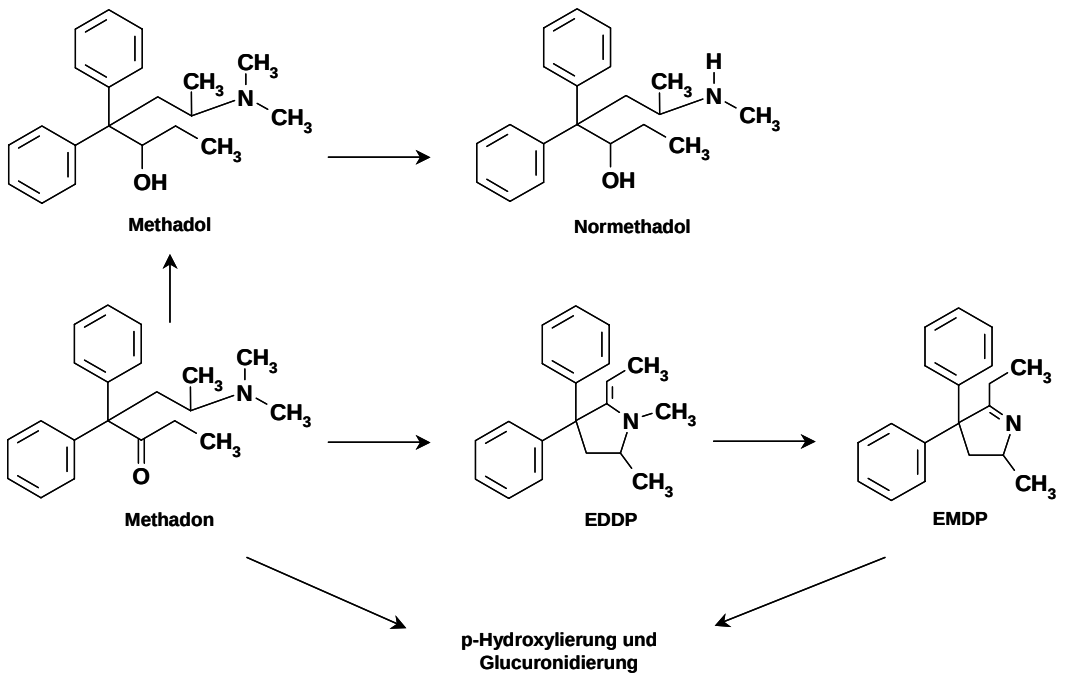
Detectability:
Methadone 1.5 – 3 d; EDDP 3 – 4 d

EDDP-detection:
Since the metabolism of methadone is greatly accelerated by interaction with concomitant medication and rapid metabolism (see also Chapter 7.8), for testing compliance, EDDP should also be measured. This additional determination also enables any spiking of urine with methadone with manipulative intent to be detected (sale of leftover methadone/spikers).

Methadone	EDDP	
neg	neg	No methadone intake
pos	pos	Methadone intake, normal case
neg	pos	Fast metabolism, interaction with therapeutic drugs
pos	neg	Spiker

Literature:
Baselt RC. Disposition of Toxic Drugs and Chemicals in Men, 6th Edition, Chem. Toxicology, Institute, Foster City, California 2002, ISBN 0-9626523-5-0

Fig. 14: Metabolism of Methadone



Methaqualone

Metabolism: (Figure 15)
Methaqualone is metabolized by hydroxylation at various positions, which gives rise to numerous metabolites, including a dihydroxy- and N-oxidized derivative.

Main metabolites:
Methaqualone-N-oxid, 4'-Hydroxymethaqualone-glucuronide, 2'-Hydroxy-methyl-methaqualone-glucuronide, 3-Hydroxymethaqualone, 2-Hydroxy-methyl-methaqualone-glucuronide, 6-Hydroxymethaqualone-glucuronide

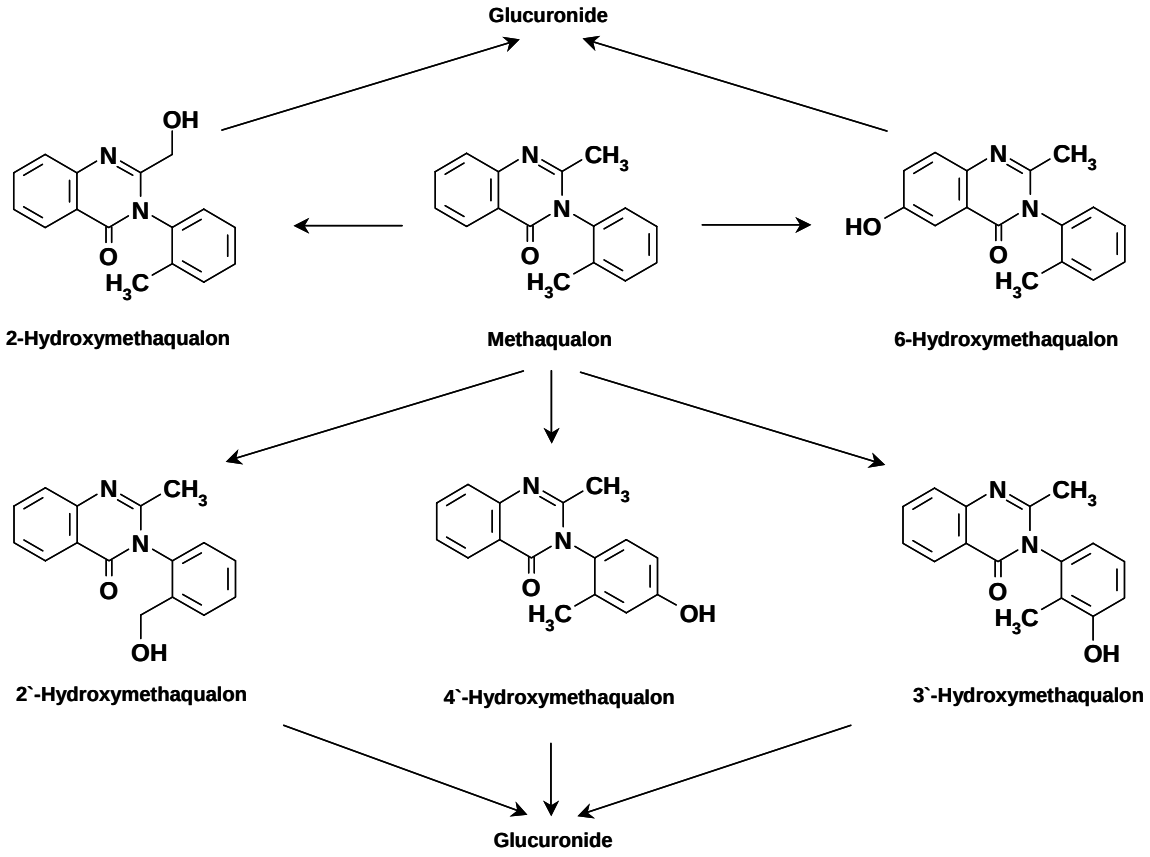
Elimination half-life:
20 - 60 h

Detectability:
3 – 4 d

Literature:
Baselt RC. Disposition of Toxic Drugs and Chemicals in Men, 6th Edition, Chem. Toxicology, Institute, Foster City, California 2002, ISBN 0-9626523-5-0

Brenner C, Hui R, Passarelli J, Wu R, Brenneisen R et al; Comparison of Methaqualone Excretion Pattern Using Abuscreen ONLINE and EMIT II Immunoassays and GC/MS ; Forensic Science International 79 (1996) 31-41

Fig. 15: Metabolisms of Methaqualone



N-Benzylpiperazin (A2) and related substances

New group of designer drugs with a central serotoninomimetic action, which includes inhibition of serotonin uptake and 5-HT_1 antagonistic effects. Other representatives of this class are 1-(3,4-methylenedioxybenzyl) piperazine (MDBP), 1-(4-methoxyphenyl) piperazine (MeOPP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and 1-(3-chlorophenyl) piperazine (mCPP). Methaqualone-N-oxide, 4'-hydroxymethaqualone glucuronide, 2'-hydroxymethyl-methaqualone glucuronide, 3-hydroxymethaqualone, 2-hydroxymethylmethaqualone glucuronide, 6-hydroxymethaqualone glucuronide

Metabolism: (Fig. 16)
Staack et al. postulated the metabolism shown in Figure 17 on the basis of animal studies and analysis of human urine.

Elimination half-life:
The only information available is non-published values (female patient from Case Report (2): approx. 5 hr

Pharmacokinetic parameter:
unknown

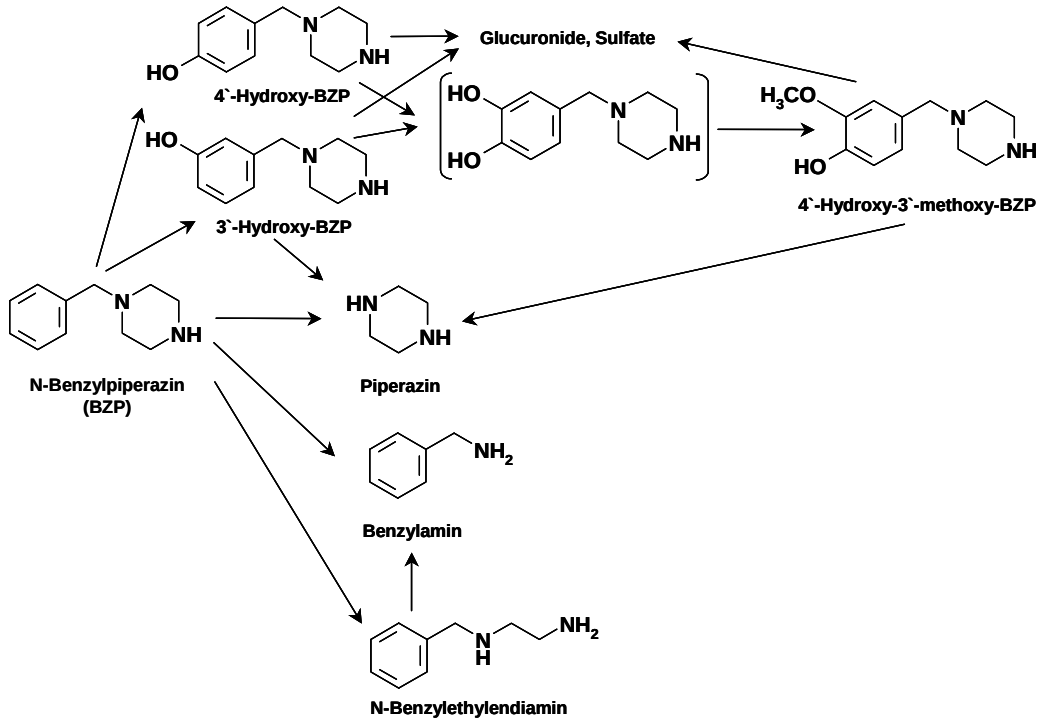
Detection method:
No immunological methods available, only chromatographic methods e.g. HPLC, HPLC-MS or GC-MS

Literature:

Staack RF, Fritschi G, Maurer HH. Studies on the metabolism and toxicological detection of the new designer drug N-benzylpiperazine in urine using gas chromatography – mass spectrometry. J. Chromatogr. B 773 (2002) 35-46

Balmelli C, Kupferschmidt H, Rentsch K, Schneemann M. Fatal brain oedema after ingestion of ecstasy and benzylpiperazine. Dtsch. Med. Wschr. 126 (2001 809 – 11)

Fig. 16: Metabolism of N-Benzylpiperazine



Opiates

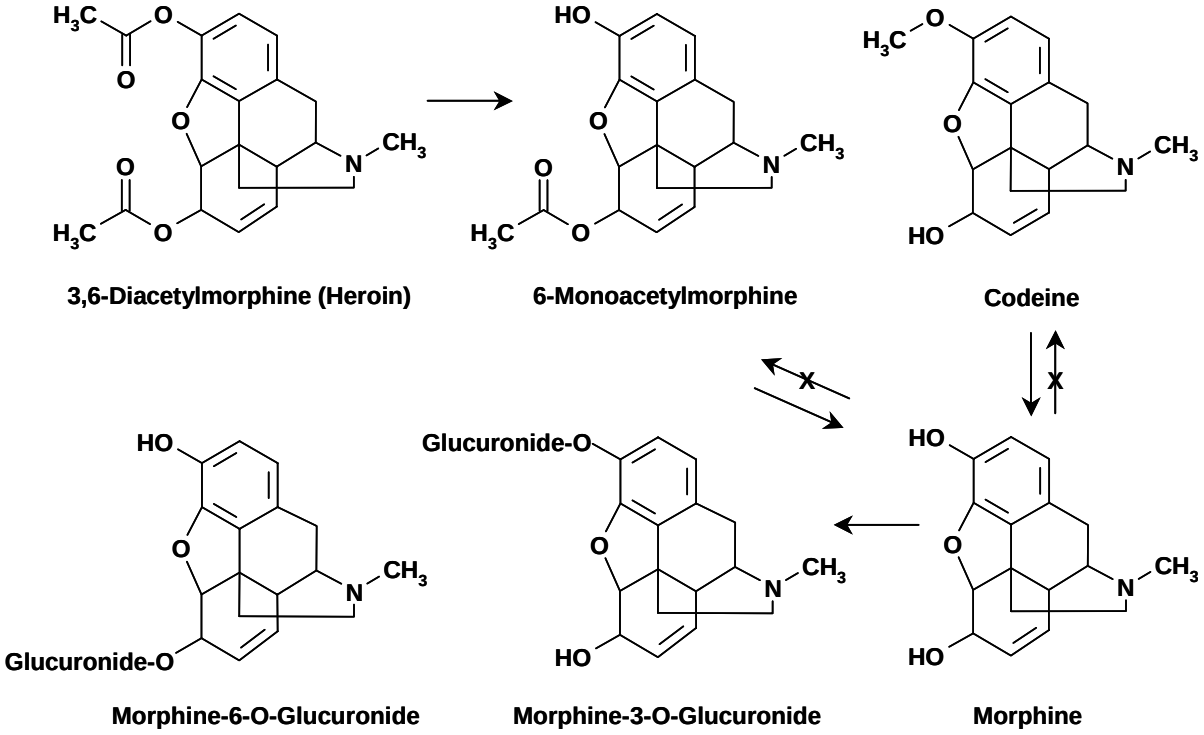
Metabolism: (Figure 17)
Diacetylmorphine (heroin) is metabolized by acids and enzymes (esterases) to 6-monoacetylmorphine and morphine and is primarily excreted as 3-O- and 6-O-glucuronide.

Elimination half-life:
3 - 20 min (diacetylmorphine), 9 - 40 min (6-monoacetylmorphine), 1 - 7 h (morphine).

Detectability:
Up to 48 h (in individual cases up to 72 h)

Differential detection of opiate intake:
The consumption of poppy seeds as well as medicaments containing codeine can lead to detectable concentrations of opiates in urine. A reliable differentiation is immunochemically not possible. The metabolism of codeine to morphine in particular is subject to a high level of interindividual variability. Codeine-morphine ratios must therefore be interpreted with caution.
Heroin consumption: 20 - 200 mg daily doses result in an urine level of 2 - 150 mg/L morphine, 0.05 - 10 mg/L codeine and 0 - 10 mg/L 6-monoacetyl-morphine (specific marker for heroin consumption).
Codeine consumption: 60 - 240 mg daily doses result in a urine level of 1 - 10 mg/L morphine and 5 - 50 mg/L codeine. Codeine-morphine ratios > 0.5, if morphine > 0.2 mg/L (cf. morphine consumption: codeine-morphine ratio < 0.5, if morphine > 0.2 mg/L).
Poppy seed consumption: 1 - 10 g daily doses result in an urine level of 0.1 - 18 mg/L morphine and 0 - 2 mg/L codeine.

Fig. 17: Metabolism of opiates



Psilocybine

Indole alkanamine (related to serotonin). From the sacred mushroom (Psilocybe mexicana, etc), synthesis by Hofmann et al.

Metabolism: (Fig 18)
Psilocybine acts as a *prodrug* and is converted by intestinal esterases to psilocin, the actual active substance (dephosphorylation). Psilocin is converted via an intermediate product (4-hydroxyindol-3-yl acetaldehyde) to 4-hydroxytryptophol and the main end product 4-hydroxyindol-3-yl acetic acid.

Elimination half-life:
1.5 – 4.5 h

Detectability:
App. 12 h

Pharmacokinetic parameter:
t_{max} ~ 30 min, C_{max} – 19 ng/mL in plasma for psilocin,
in urine 3 – 10 % psilocin, otherwise 4-hydroxyindole acetate acid: 1.5 – 4.5 h

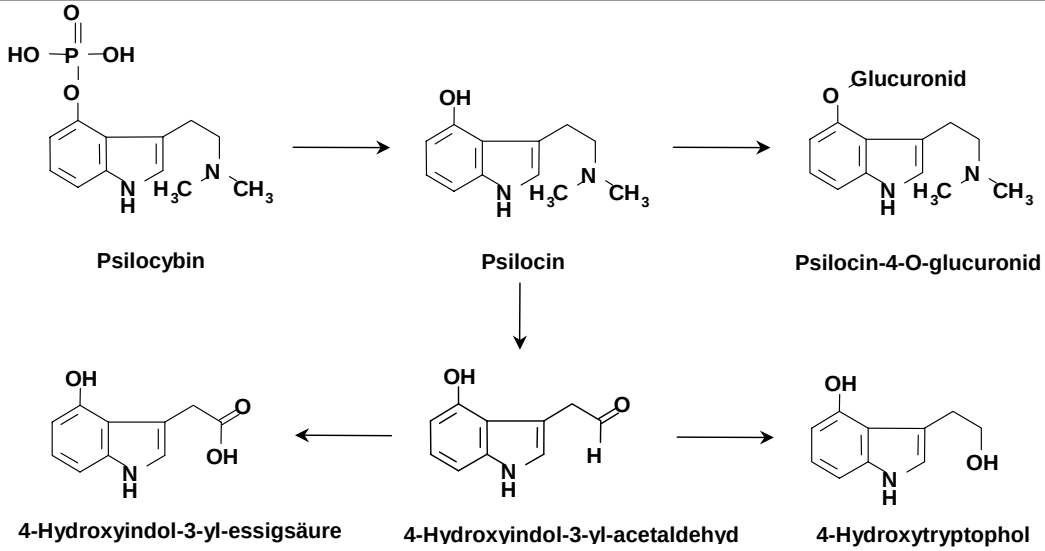
Detection method:
No immunological methods available, only chromatographic methods e.g. GCMS or LOMS

Literature:
Hoffmann A, Heim R, Brack A, Kobel H, Frey A, Ott H, Petrzilka Th. Psilocybin und Psilocin, zwei psychotrope Wirkstoffe aus mexikanischen Rauschpilzen. Troxler F. Helv. 42 (1959)1557-70

Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. Pharmaceutica Acta Helvetiae 72 (1977) 175 – 84

Hasler F, Bourquin D, Brenneisen R, Vollenweider FX. Renal excretion profiles of psilocin following oral administrataion of psilocybin: a controlled study in man. J. Pahrm. Biomed. Anal. 30 (2002) 331-9

Fig. 18: Metabolism of Psilocybine



Appendix 3: **External quality control**

Medicaments Drugs of Abuse	CH[1]	CH[2]	DE[1]	DE[3]	DE[4]	US	FR[2]	IT[1]	NL	ES[1]	GB[1]	FI
Drugs of Abuse												
Amphetamines	WSU	U	WSU	U	U	SU			SU	X	U	U
Barbiturates	WSU	U	W	SU	SU	SU			SU	X	U	U
Benzodiazepines	WSU	U	WSU	SU	SU	-	X		SU	X	U	U
Cannabis	WSU	U	WSU	U	U	SU			SU	X	U	U
Cocaine metabolites	WSU	U	WSU	U	U	SU			SU	X	U	U
Ethanol	WSU	U	WSU	SU	SU	SU			SU		WSU	
GHB												
Ketaminee												
LSD	U	U	SU	U		U					U	
Methadone	WSU	U		U	U	U			U	X	U	U
Methaqualone	WSU			U	U	U			SU		U	
Opiates	WSU	U	WSU	U	U	SU			SU	X	U	U
Psilocybin												
Others												
Trace elements			WSU					X	X	X	WSU	
Volatile substances* and CDT	S											
Toxic substances**	S		WSU	WSU		SU		X	X	X	WSU	

*) Acetaldehyde, acetone, ethanol, isopropyl alcohol, methanol and carbohydrate-deficient transferrins
**) including specific drugs of abuse and medicaments

S = Serum/Plasma W = Whole blood U = Urine A = Aqueous Standard X = Status 1996		CH[1], CH[2], D[1], D[2], SF, UK, USA: Status 2003 All other programs: Status 1996

Appendix 4: **External quality control schemes**

Country	Address	Field
Switzerland **CH[1]**	**Schweizerisches Zentrum für Qualitätskontrolle (CSCQ)** Chemin du Petit Bel-Air, CH - 1225 Chêne-Bourg	TDM, Drugs of abuse, Forensic
Switzerland **CH[2]**	**MQ Verein für medizinische Qualitätskontrolle** Universitätsspital Zürich, CH - 8091 Zürich	Drugs of abuse
Germany **DE[1]**	**GTFCH Institut für Rechtsmedizin und Verkehrsmedizin** Prof. Dr. Rolf Aderjan, Voss-Strasse 2, D - 69115 Heidelberg	TDM Forensic Toxic substances
Germany **DE[2]**	**Deutsche Gesellschaft für Arbeits- und Umweltmedizin** Schillerstrasse 25, D - 91054 Erlangen	Metals Toxic substances
Germany **DE[3]**	**Deutsche Gesellschaft für Klinische Chemie e.V. (DGKC)** **Referenzinstitut für Bioanalytik** Im Mühlenbach 52a, D – 53127 Bonn	TDM Drugs of abuse
Germany **DE[4]**	**Institut für Standardisierung und Dokumentation im medizinischen Laboratorium e.V. (INSTAND)** Postfach 250211, D - 40093 Düsseldorf	TDM
Finland **FI**	**Labquality** Ratamestrinkatu 11, SF - 00520 Helsinki	Drugs of abuse
France **FR[1]**	**Laboratoire des services de réanimation** rue H. Leguiloux, F - 35033 Rennes-Cedex	
France **FR[2]**	**Agence du Médicament**, Département de Biologie Médicale, 25, Boulevard Saint Jacques, F - 75680 Paris-Cedex 14	TDM
Italy **IT[1]**	**Fondazione Clinica del Lavoro**, Laboratorio di Igiene Industriale I - 27100 Pavia	Metals Toxic substances
Italy **IT[2]**	**Ospedale CTO** via Palagi 1, I - 50139 Firenze	TDM
The Netherlands **NL**	**Stichting Kwaliteitsbewaking Klinische Geneesmiddel-analyse en Toxicologie** P.O. Bos 43100, NL - 2504 AC Den Haag	Drugs of abuse TDM
Spain **ES[1]**	**Asociacion Española de Farmaceuticos Analistas (AEFA)** AEFA C/ Condado de Treviño, E – 28033 Madrid	Drugs of abuse
Spain **ES[2]**	**Comision de Control de Calidad**, Sociedad Española de Quimica Clinica Liansa 51, E - 08015 Barcelona	TDM
United Kingdom **GB[1]**	**Cardiff Bioanalytical Services Ltd.** Cardiff Medicentre, Heath Park, UK Cardiff CF4 4UJ, Wales	TDM Drugs of abuse
United Kingdom **GB[2]**	**UK-NEQUAS OFFICE Toxicology**, UK Sheffield S5, England	Headquarters
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