Swiss Guidelines Committee for Drugs of Abuse Testing, SCDAT
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SCDAT

Guidelines for Drugs of Abuse Testing

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Glossary

CSC	**Correctional Service of Canada**
Compliance	**A patient's adherence to a recommended course of treatment**
Cut-off	**Medical and/or Legal Decision Value, positive/negative**
Donor	**Person who donates something voluntarily**
EWDTS	**The European Workplace Drug Testing Society**
GC-MS	**Gas Chromatography with Mass Spectrometric Detection**
HPLC-MS	**High Performance Liquid Chromatography with Mass Spectrometric Detection**
ID	**Identification**
ISO/IEC	**International Organization for Standardization/International Electrotechnical Commission**
IUPAC	**International Union of Pure and Applied Chemistry**
KLV	**Health Care Insurance Benefits Ordinance (Switzerland)**
KVG	**Federal Law about Health Insurance (Switzerland)**
KVV	**Health Insurance Ordinance (Switzerland)**
MS	**Mass Spectrometry**
On Site	**Done or located at the site**
Peak	**Portion of a differential chromatogram recording the detector response when a single component is eluted from the column**
Prodrug	**Inactive (or significantly less active) form of a drug**
QC	**Quality Control**
QUALAB	**Swiss Association on Quality in the Medical Laboratory**
SAMHSA	**Substance Abuse and Mental Health Services Administration (U.S.A.)**
SCDAT	**Swiss Committee for Drugs of Abuse Testing**
Spiker	**Person pretending compliance in a drug substitution program**
Spot	**Spontaneous urine, urine specimen**
Workplace Testing	**Drug Testing Programs in the Workplace**

Contents

Foreword

SCDAT Guidelines were originally published by the work group on Drugs of Abuse Testing (AGSA). The SCDAT, successor to AGSA, was a work group composed of members of the following institutions: Swiss Association of Pharmacists (pharmaSuisse), Swiss Society of Clinical Chemistry (SSCC), Swiss Society of Legal Medicine (SGRM), Swiss Association of the Diagnostic Equipment and Product Industry (SVDI), and University of Bern.

Since 2014, the responsibility of the SCDAT Guidelines have been transferred to the SSCC. This version of the guidelines is the result of a revision commissioned to the working group “Medicaments” of the SSCC.

These guidelines are intended as recommendations for the clinical laboratories and non-legal institutions testing urine and blood samples for drugs of abuse. They are not legally binding in nature. Harmonization in drug analyses is the objective.

Legal samples need to be analyzed by a forensic testing laboratory and samples, which are used for the assessment of driving capability, need to be analyzed by a testing laboratory with a valid authentication by the Federal Roads Office.

The use of drug analysis for the various questions in the therapeutic sectors as well as in specific workplaces can have far-reaching consequences of a professional and social nature for those affected. For this reason, the greatest possible care must be taken when conducting analyses and interpreting the results. These guidelines support analytical laboratories in their adherence to requisite quality assurance measures.

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The guidelines are periodically revised and enlarged.

1. Scope of the Guidelines

The following guidelines encompass the different stages of drug analyses, from the individual to be tested, through the client, to the result. The guidelines specifically deal with sampling, shipping, preanalytical, analytical, and postanalytical aspects, quality assurance, interpretation and documentation of the results of the analyses, as well as the costs (Fig. 1).

Figure 1 Scope of the Guidelines

Preanalytical

Sampling Place

Sampling	
Identity, authenticity, integrity of the donor and/or of the sample, "chain of custody", questioning, range of analyses	**Ch 3,4**
Shipping	
Containers, shipping materials, protection against breakage, "chain of custody"	**Ch 3, 10.1**

Testing laboratory

Storage, sample processing	**Ch 4.1**

Analytical

Analytics	
Quality management, methodology, detection limit, sensitivity, specificity, interferences and adulteration	**Ch 5, 6, 7**
Quality Assurance	
Internal and external quality control	**Ch 9**

Postanalytical

Post-Analytics	
Storage, additional test orders, confirmation	**Ch 7**
Interpretation	
Cut-off values, time of sample collection, pharmacokinetics, significance of a result	**Ch 6.3, 8, 12**
Documentation	
Reports, release of results, handling of positive results, confirmation, recommendation	**Ch 10**

2. Scope of Application of the Guidelines

The guidelines are recommended for use in the clinical and socio-medical areas of application.

Table 1 Scope of Application of the Guidelines

A	**Testing for drugs of abuse associated with differential diagnosis**
B	**Testing for drugs of abuse during substitution therapy, heroin-based therapy, and/or detoxification treatment, educational facilities**

3. Sample Collection, Shipping and Handling ("Chain of Custody")

3.1. Collecting Samples

3.1.1 Objectives of Sampling Strategy

The identity, authenticity, and integrity of the individual and the specimens collected from the individual must be ensured at any time. Sampling strategy should undertake adequate measures to identify and to prevent any medical, chemical or physical tampering with collected specimen.

Urine specimen collection must respect the dignity of the individual whilst ensuring that the collected specimen is freshly voided.

3.1.2 Chain of Custody

The chain of custody must be warranted throughout sample collection, transport and analysis. Standard operation procedures should be in place to guarantee a documented sampling process carried out by dedicated and qualified personnel. At the site of sample collection, a chain of custody form should be used to accompany the sampling process. This form can stay at the collection site, whilst the collected sample is accompanied by the order (request) form. The site of the sample collection is responsible for the correct identification of the sample tube and the order form.

3.1.3 Specific measures for urine specimens

Urine specimens used for screening purposes are known to be target of adulteration attempts. Medical, chemical or physical tampering with collected specimen include issues such as endogenous or exogenous dilution, additives, submission of another's urine specimen or artificial urine, and substitution of an analyte.

Dilution, addition of additives and use of artificial urines can be controlled by various laboratory procedures (see below); submission of another's urine specimen or substitution of an analyte can be controlled by proving the body passage of the received specimen.

To minimize the risk of sample adulteration during the collection process, measures must be taken to avoid items or substances, which might be suited to adulterate the urine specimen to be introduced to the collection site in a concealed manner (e.g. remove coats or jackets, control pockets, leave purses with the garment). Additionally, the place of sample collection should also be prepared in a

similar manner. For example control access to tap water, use coloring of toilet water, and remove soap and other liquids.

To respect the dignity of the individual whilst urinating, observed collection is not necessary if the named precautions are taken and there is not a strong suspicion of sample adulteration and no adulterated specimen has been presented in a previous collection. A measure, which can be taken to ensure the body passage of the collected urine, is the use of suited marker substances, which are ingested, in an appropriate amount and time gap prior to urine sampling.

Prior to urine sample collection:

- Instruct the donor to avoid excessive drinking. Not more than 200 mL liquid per hour should be ingested 2-3 hours prior to sample collection.
- Give oral or written instruction on the collection procedure, which may include the measures outlined in the prior paragraphs.

Urine sample collection:

- Provide labelled and clean specimen container for urine sampling and urine shipment. Make sure that all containers are unequivocally identifiable (e.g. by using bar coding or ID numbers). Make sure that the donor is aware of the identity of the primary and secondary specimen labelling.
- If not enough sample (10 mL) is collected at the first urination, a second collection after additional liquid intake (max. 250 mL) can be undertaken. Samples should not be pooled.

After urine sample collection:

- If possible, a urine temperature measurement should be performed at the sampling site. Timeframe: up to 4 min after collection; acceptable temperature range: 32-38 °C.
- Check for appearance and color. Note any unusual findings on the chain of custody form and transfer them to the order form.
- Transfer the urine from primary to secondary sampling containers. If possible, divide the urine in two or more unequivocally identified portions. Ensure shipment in accordance with the information provided by the testing laboratory.

3.1.4 Specific measures for other sample sources

Generally, any sampling and shipping must be in accordance with the information provided by the testing laboratory. Particularly for hair and saliva, where testing requires very sensitive methods, very specific preanalytical measures are necessary to ensure successful and correct analyses.

3.2 Handling of Samples

3.2.1 Objectives of sample handling

The chain of custody must be ensured over the whole analysis process. The identity, authenticity and integrity of any specimen must be guaranteed at any time from sampling to analysis. Hence, sampling, shipment and storage of any sample must agree with the instructions provided by the testing laboratory. Any evidence of tampering, mix-ups, damage and/or loss of the specimen must be recorded and measures must be taken to minimize the risk of such incidents.

3.2.2 Measures

Whenever possible 10 ml urine should be sampled and divided in at least two separated sub-specimens to allow completely independent secondary / confirmative testing if needed. This is specifically necessary in sample collections with an assumed or known legal background. The minimal urine volume to be submitted for screening and confirmation testing is 5 mL.
Blood samples should be collected as two independent samples in primary tubes whenever a legal background has to be assumed. For all other specimens follow the specific information provided by the testing laboratory.

Sampling containers must adhere to the rules of the testing laboratory. The closure must be unbreakable and leak proof. Tamper proof closures (e.g. by the application of a tape) are not mandatory. All subsamples must bear a legible identification number. The samples must be stored at a secured place before shipment to the testing laboratory.

Order forms must be filled in accordance with the rules of the testing laboratory. Beside the ordered test, the order form should at least contain the following information: sample identification number, last name, first name, date of birth, and gender of the donor and date/time of sampling (see Chapter 10.1 for more details).

3.3 Sample Handling at the Testing Laboratory Site

3.3.1 Objectives of sample handling at the testing laboratory site

The chain of custody must also be ensured at the site of analysis. Traceability of all steps in the analytical procedure is mandatory.

Sample handling and the quality of the analytical methods must comply with written requirements. All testing procedures must adhere at least to the QUALAB requirements. Testing laboratory accreditation (ISO17025, ISO15189) is not mandatory but highly recommended. If such accreditation is not in place, the minimal requirements outlined further below must be documented in a suitable manner.

3.3.2 Measures

Minimal requirements for testing laboratory competency:

- Limited and controlled access to the testing laboratory.
- Reception and analysis of the samples only by authorized personnel.
- Appropriate sample storage facilities: +2 to +8°C pre-analytical, below –18 °C post-analytical. Post-analytical storage and storage of excess samples should be at least 6 months.

4. Specimens

Urine is the most commonly used specimen for drug testing as drug concentrations are usually higher in urine than in other specimens and urine is an easy specimen to work with. Blood is also a commonly used specimen, especially if urine is not available.

Alternative specimen as oral fluid and hair are increasingly being utilized in drug testing. These specimens offer many advantages over blood and urine such as easy sample collection without a need for a special collection facility or personnel, less intrusion in privacy during sample collection, and reduced potential for sample adulteration. Despite these advantages, alternative specimens present unique analytical and interpretive challenges.

4.1 Specimen Stability and Preservation

4.1.1 General Information

A negligible decrease in stability is of little consequence for qualitative testing. In general, for quantitative determinations, conditions that are somewhat more restrictive should be maintained while checking the applicable literature. Depending on the substance, the testing laboratory has to perform its own validations, since only sparse amounts of data might be available in the literature.

4.1.2 Storing and Packaging of Specimens

Practically all common drugs of abuse and their metabolites are stable in urine for 7 days at 4 °C when kept in a dark place (i.e. protected from light).

For further information, please consult the applicable guidelines, literature and websites [e.g. CLSI. Toxicology and Drug Testing in the Medical Laboratory; EWDTS, www.ewdts.org ; SAMHSA, www.samhsa.gov, Gonzales E. et. al. 2013, Dasgupta A. 2019] and Table 2.

Commercially available plastic materials, which are tested and provided by various suppliers, are recommended for sampling and storing samples. Some plastic materials might adsorb analytes and related metabolites and should therefore be tested before use if not being cleared by the supplier.

The stability of standards that cannot be obtained commercially along with a declaration of stability should be verified on a regular basis.

Table 2 Stability and Storage of Urine Samples

Substance or Substance Group	Stability in Urine (≤ 6 months = assured up to 6 months)
6-Acetylmorphine	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Amphetamines, incl. MDMA	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Barbiturates	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Benzodiazepines	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Buprenorphine	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Cocaine + metabolites	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Codeine	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Ethanol	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months; store in gastight containers.
Ethyl glucuronide (EtG)	7 days at +4 to +8 °C (nonbacterial urine), < -18 °C: ≤ 6 months
GHB	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
LSD	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Methadone	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Methadone metabolite (EDDP)	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Nicotine, cotinine	2 days at +4 to +8 °C, < -18 °C: ≤ 2 months
Opiates	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Phencyclidine (PCP)	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months
Psilocybin/psilocin	< -18 °C: ≤ 6 months
THC-carboxylic acid (Cannabis)	7 days at +4 to +8 °C, < -18 °C: ≤ 6 months

5. Factors Interfering with Results of Analytical Testing, Tampering with Urine Specimens

5.1 Objectives

Inaccurate result readings, which complicates the interpretation of test results (see Chapter 8 interpretation), may be caused by substances influencing the testing procedures. The underlying cause for such perturbances can be intentional (adulteration of the sample) or unintentional (endogenous or exogenous interferences).

Most forms of adulteration involve tampering with the urine specimen at the point of sample collection. Other sample materials (for example blood, hair, sweat) are collected by trained staff of the institution ordering the test or performing the analyses, whereas urine sampling involves direct sample handling by the donor.

It must not be overlooked, that in some instances biological effects can be misinterpreted as tampering attempts. This is notably the case for urine dilution, which is generally addressed by the surrogate marker creatinine which can have a low concentration in specific medical conditions.

5.2 Interfering Factors and Adulterations in Urine Drug Testing

Interfering factors alter a test result such that the true test value is not accessible. Interferences may lead to false negative or false positive results.

Interferences occur *in vivo* and are often associated with recent uptake of food / supplements or therapeutically used drugs. In the case of nutritional causes, the ingestion of poppy seeds (which can contain traces of opiates) may for example rarely lead to false positive results in urinary opiate screening tests. In the case of drugs taken for therapeutic purposes, parent compounds or metabolites may interfere with immunochemical screening methods. The trazodone metabolite m-CPP for example may lead to false positive readings in urinary amphetamine screening assays. Care must be taken, that data provided by reagent manufacturers are often not capable to cover all possible interferences and data from literature may not refer to actual assay designs.

An adulteration is present, if the voided urine specimen is deliberately fortified with substances / substance cocktails (xenobiotics) or is replaced or blended with natural or artificial matrices. Adulteration of a specimen usually intends to give false negative testing results. In certain situations also positive results may also occur, for example if a patient on substitution therapy adds the substitution drug (e.g. methadone) to a voided urine specimen free of the drug.

- Adulteration by xenobiotics includes the acidification of the urine or the addition of bleach or detergents. In addition, substances (e.g. enzymes) may be added which modify the addictive drug to be detected, thereby preventing detection of said addictive drug with a confirmation test.
- Blending or replacement of the specimen includes the substitution with another person's drug-free urine sample or commercially available urine and the dilution with water or other liquids to which coloring has been added or that have similar coloring as urine (e.g. apple juice).

5.3 Detection of Interfering Factors and Adulterations in Urine Drug Testing

Key to the detection of interfering factors or specimen adulteration is the unbroken chain of custody.

It is always within the responsibility of the testing laboratory to identify common causes of interfering factors and sample adulteration and to provide information about adequate countermeasures. If care is taken that the sample has not been tampered, damaged or incorrectly transported / stored after specimen collection, any suspicious testing result can be associated with the sampling collection process. For suspicion of sample adulteration at the point of sample collection, see chapter 3.

If interferences from therapeutic drugs, supplements or diet is suspected from screening results, the customer must be informed to provide further anamnestic information, e.g. on drugs taken up to two weeks prior to testing. In addition, adequate measures must be taken to identify the contamination source and to rule out false positive results; for example, by confirmative testing.

To identify sample adulteration by the addition of substances interfering with the test principle, it is highly recommended either to use screening assays, which give strongly positive test results upon sample adulteration due to a competitive assay design, or to employ additional reaction control testing (e.g. the “sample check” principle of CEDIA assays.

If such tests are not available, the urine sample integrity can be checked by urine test strip testing including for example pH (test for acidification / basification), specific gravity (test for urine dilution), nitrite (addition as adulterant), ”leukocytes” (the measurement principle - esterase activity - may give positive results if esterases of different origin (e.g. fruit juice) are present, or if strong oxidizing agents / formaldehyde are present), “erythrocytes” (the measurement principle - peroxidase activity - may give positive results if strong oxidizing agents are present), glutaraldehyde.

Measurement of creatinine, pH and specific gravity are mandatory if no other measures are taken to test the sample integrity. For the interpretation of creatinine and specific gravity measurement results see Chapter 5.4.

A specimen is defined as adulterated (“specimen validity failed”) if the performance of the screening assay is impaired as defined by the test instructions and / or if additional integrity checks are indicating an integrity problem (Table 3).

Table 3 Criteria for sample adulteration based on laboratory testing and specimen inspection

Parameter	**A specimen is defined as adulterated (“specimen validity failed”) if…**
Visual inspection	…an exogenous substance is detectable, which indicates an adulteration (e.g. foam building from liquid soap).
Substance specific urinary testing	… any endogenous substances (e.g. creatinine, glucose) are detectable in grossly non-physiological concentrations without medical evidence of an underlying cause.
Drug testing control reaction	…“sample check” alerts in accordance with the test instructions.
pH	< 3 or > 10
Specific gravity	Outside 1.001 – 1.020
Nitrite	> 500 mg/L

5.4 Detection of Sample Dilution

Creatinine testing is a mandatory element of urine drug testing. A number of factors have to be understood and considered when interpreting its concentration. Its concentration in urine depends strongly on a person’s muscle mass, weight and gender. Due to the water balance of the body, increased drinking leads to an increased water excretion, which in consequence leads to lowered creatinine concentrations but also dilutes urinary drug metabolite concentrations. Hence, excessive drinking must be avoided prior to urinary drug testing (see above).

Urinary creatinine reference ranges provided by assay manufacturers are often defined for a specific urine (e.g. first / second morning urine) but rarely for random sampling. Patients from the emergency department or intensive care unit or pediatric patients may have lower creatinine levels without adulteration. Hence, these ranges must not be used in drug testing to assess the validity of a urine specimen. The following decision limits define lowered creatinine levels as adulteration attempt and are summarized in Table 4:

- Specimen with creatinine levels exceeding 2.0 mmol/L are considered acceptable for drug testing.
- Specimens with creatinine levels less or equal 2.0 mmol/L but above 0.5 mmol/L must undergo additional specific gravity testing. Acceptable gravity results to accept a sample for drug testing range from 1.001 to 1.020.
- Specimens with creatinine levels within the range 0.5 mmol/L to 2.0 mmol/L and acceptable specific gravity: results should be reported as diluted. Since such samples can cause false negative results, a comment should be added to negative drug results. Confirmatory reflex testing must be recommended in such cases. Positive results are not commented.
- Specimens with creatinine levels within the range 0.5 mmol/L to 2.0 mmol/L and not acceptable specific gravity: results are treated equally as specimens with creatinine levels less or equal 0.5 mmol/L.

- Specimen with creatinine levels less or equal 0.5 mmol/L and / or specific gravity results outside the range given above may be unsuited for analysis and should be reported as “specimen validity failed”. If such samples are nevertheless positive in the screening test, confirmative reflex testing must be recommended. Negative results must not be reported.

If specific gravity measurements are not available, urine specimen within the range 0.5 mmol/L to 2.0 mmol/L creatinine must be treated as if the specific gravity test has failed.

Table 4 Summary of urinary creatinine concentration levels for dilution adulteration detection

Creatinine [mmol/L]	Sample status	Specific gravity	Reporting of findings	Comments
>2.0	acceptable for drug testing	not needed	all findings are reported	None
>0.5 - ≤2.0	diluted, subject to specific gravity testing	1.001 - 1.020	all findings are reported	Comment on negative results: dilution can cause false negative results hence confirmatory reflex testing is recommended.
>0.5 - ≤2.0	diluted, subject to specific gravity testing	< 1.001 > 1.020	negative results must not be reported recommend confirmatory reflex testing for positive results	Comment on all results: specimen validity failed
≤0.5		any value	negative results must not be reported recommend confirmatory reflex testing for positive results	Comment on all results: specimen validity failed

6. Immunochemical Screening Analyses in Urine

In general, screening analyses permit only qualitative results. Depending on the consequence of the qualitative screening results, confirmation analyses should be performed. Whatever the case, a chromatographic test method is more conclusive than most immunoassays. However, the latter are the methods of choice for rapid testing, since chromatographic procedures are usually work- and time-intensive. Immunochemical group testing is indicated when rapid detection of presumably ingested substances within a certain substance class (which may cover a large number of substances) and serial analyses are needed. Be aware that there is a substantial probability for false positive and false negative results.

Antibodies used in immunoassays may cross-react with more than one single substance (the main target substance and some of its metabolites or a number of substances of the same class). The amplitude of the cross-reactivity is not homogeneous across the detectable substances. The resulting signal is therefore a cumulative value from all cross-reacting substances. In some cases, this can lead to positive results (according to a defined cut-off concentration) that cannot be confirmed by chromatographic methods (i.e. LC-MS/MS), which detect and quantify single substances with higher individual selectivity. The chromatographic methods are targeted methods that do not necessarily cover all metabolites of a given drug or even all possible drugs from a class, and does not sum up all concentrations of the detected substances. And even so, at similar detection limit to immunoassays, concentrations below this limit will not sum up to a detectable signal. Negative immunoassays may be, in the same reasoning, due to the presence of a low cross-reacting substance, which does underestimate the real concentration.

Since detection with immunoassays normally yields “yes/no” cut-off levels, the results require critical interpretation and, depending on the case, additional confirmative measurements are needed (Chapter 7).

Many immunoassays can produce semiquantitative results, but these values should be cautiously interpreted in urine, because of the cumulative effect of cross-reactivity of multiple substances, as described earlier. Therefore, such semiquantitative results in urine must not be extrapolated to drug dosage or time of ingestion.

6.1 Single-Substance Analyses

Immunoassays of single substances are designed to detect a substance and/or one of its presence proving metabolites.

Examples of single-substance analyses are:

Substance detected:	**Proving the presence of:**
6-Acetylmorphine (6-AM) = 6-Monoacetylmorphine (6-MAM)	Heroin
Benzoylecgonine	Cocaine
Buprenorphine	Buprenorphine
Cotinine	Nicotine
Ethyl glucuronide (EtG)	Ethanol
LSD	LSD
Methadone and/or EDDP	Methadone
THC carboxylic acid	THC

6.2 Substance Group Analyses

Substance-group analyses using immunoassays detect a range of (but usually not all) structurally related substances or metabolites as a group in a single analytical process.

Depending on the manufacturer, calibration of the analytical systems for substance-group testing is based on a different standard substance, leading to varying specificity in terms of the results. The results in each case are only qualitative as the reactivity of the antibodies used with individual substances within a substance class is highly variable. In addition, it remains unknown if one or several substances produce the positive result.

Negative results are not necessarily always conclusive since, depending of the antibody used individual substances of the substance class or their metabolites will not react sufficiently due to their low cross reactivity with the diagnostic antibody.

Examples of such substance-group analyses are:

- Amphetamines
- Barbiturates
- Benzodiazepines
- Opiates
- Tricyclic antidepressants

Urine specimens exhibiting high concentrations of the analyte (above measuring range) should not be diluted as there is no direct correlation between the antibody affinity and the concentration of the substance.

6.3 Recommended Cut-off Concentrations for Instrument Immunoassays of Urine Specimens without Prior Hydrolysis

Table 5 shows the cut-off concentrations from different publications for single substances and substance groups. They apply to instrument immunoassays of urine specimens that have not undergone prior hydrolysis.

Table 5: Different Recommended Screening Cut-off Concentrations for Instrument Immunoassays of Urine Specimens without Prior Hydrolysis (X: No recommendation)

Single Substances		SCDAT (2020)	EWDTS (2015)	SAMHSA (2012)	CSC (2019)
Single	6-Acetylmorphine (6-AM) [µg/L]	**10**	X	10	10
	Buprenorphine [µg/L]	**10**	5	X	X
	Cocaine or cocaine metabolite (benzoylecgonine) [µg/L]	**300**	150	150	150
	Cotinine (nicotine) [µg/L]	**50**	X	X	X
	EDDP (Methadone) [µg/L]	**100**	100	X	100
	Ethyl glucuronide (EtG) [mg/L]	**0.5**[1]	X	X	X
	GHB [mg/L]	**5**[2]	X	X	X
	LSD [µg/L]	**0.5**	1.0	X	0.2
	Methadone [µg/L]	**300**	300	X	X
	THC carboxylic acid (Cannabis) [µg/L]	**50**	50	50	50
Substance Groups					
Group	Amphetamines [µg/L]	**500**	500	500	500
	Barbiturates [µg/L]	**300**	200	X	X
	Benzodiazepines [µg/L]	**100**	200	X	100
	Opiates [µg/L]	**300**	300	2000	300

[1] *A cut-off value of 0.1 mg/L may be appropriate to establish abstinence, patients then need to omit some food before sampling the urine.*

[2] *The endogenous GHB concentration in urine is < 5 mg/L in most cases.*

For non-instrument immunoassays (“rapid tests”) the cut-off concentrations are established by the manufacturers and are therefore constant. When selecting a non-instrument immunoassay, the applied cut-off concentration needs to be considered.

Hydrolysis of the urine prior to analysis permits indirect determination of conjugated metabolites (e.g., morphine glucuronides, benzodiazepine glucuronides), thus improving the likelihood to detect consumption.

6.4 Enzymatic Alcohol Test

In clinical cases, alcohol (ethanol) is determined by enzymatic tests, which convert ethanol by alcohol dehydrogenase (ADH). Other alcohols like isopropyl alcohol (isopropanol, ingredient of many disinfectants) are also converted by ADH and therefore detected. These alcohols can only be found in urine in case of an intoxication with these compounds. It must be considered that after the consumption of ripe fruits alcohol, concentrations of < 3 mmol/L can be found in urine.

In case of a blood sampling for the analysis of alcohol, disinfectants without ethanol or isopropanol should be used (e.g. aqueous solutions of iodine of chlorhexidine).

Due to longer detection windows compared to the parent drug, other biomarkers of ethanol ingestion like ethyl glucuronide are recommended especially for urine samples.

6.5 Use of Non-Instrument Rapid Tests

With few exceptions "rapid tests" for drug screening in urine, oral fluid and sweat are non-instrument immunoassays that permit fast (within 5-10 min) yes/no decisions outside the testing laboratory (“on site”). Saliva and sweat tests have been used for some time, chiefly in forensic cases. Non-instrument immunoassays should only be used as screening tests.

6.5.1 General Remarks

- As with instrument immunoassays, non-instrument immunoassays are merely indicative and not evidential in nature. All manufacturers’ instructions for use point out this fact, but many users fail to or barely pay attention to it.
- Despite their simplicity and lack of reliance on an instrument, these non-instrument immunoassays as well as the instrumental onsite tests should only be conducted by trained personnel who is skilled in the interpretation of the results and any irregularities.
- In the case of a positive result, do not discard the sample. It must be retained for any confirmation analysis that may be required.
- Most of these analytical systems have a test quadrant that displays any irregularity in the reaction sequence. Nevertheless, irregularities that are not indicated through internal checks still may occur (for example, certain methods of tampering, interference with any one of the test quadrants, or irregularities caused by medication).
- Quality control samples generally used for testing the quality of screening tests are artificial. Therefore, discrepancies may be found when comparing the results of individual test quadrants of different manufacturers (e.g., varying cross-reactions with optical isomers, mainly amphetamines). False negative results may also occur due to an antigen excess (High-Dose Hook Effect).
- The cut-off concentrations of non-instrument immunoassays are defined by the manufacturer of the devices and cannot be changed by the user. Non-instrument immunoassays therefore have to be selected carefully.

7. Confirmation and Screening Analyses

7.1 General Remarks

Immunochemical analyses can only provide preliminary results. Because of the risk of false-positive results, confirmation of positive results must be done where sanctions may be imposed on the person involved due to the results obtained, and is strongly recommended in all other situations. Immunochemical analyses may also give false-negative results, e.g., because of poor cross-reactivity of the substance in the sample with the used detection antibody. If the clinical situation is not in accordance with the negative result, samples with negative results should also undergo a confirmation analysis.

The confirmation analytical platform must be a chromatographic analytical platform with a substance-specific detection. Any other immunoassay must not be used for that purpose.

Immunochemical testing includes only few substances or substance classes. Many other substance classes might be of interest for drug screening. E.g., new psychoactive substances (psychoactive compounds that are sold over the internet in many cases) are in most cases not detectable using the “classical” immunochemical testing. Applying a chromatographic screening analysis allows the detection of a much broader spectrum of drugs and drugs of abuse. In contrast to a confirmation analytical platform that only confirms the result of an immunochemical assay by detecting the same substances as the immunochemical test, a chromatographic screening analysis is designed to enable the detection of more substances and substance classes which might not be detectable by immunochemical assays.

It is of utmost importance to store samples subjected to confirmatory or chromatographic screening analysis for an extended period of time to allow possible re-testing e.g. by a forensic testing laboratory. We recommend storage for at least 6 months below -18°C.

7.2 Analytical Platforms

The following analytical platforms are recommended for confirmation testing:

- Liquid chromatography coupled to mass spectrometry (LC-MS)
- Gas chromatography coupled to mass spectrometry (GC-MS)

Both analytical platforms are well established in clinical routine and provide reliable results when a careful method validation process has been performed. We recommend the use of either a multiple reaction monitoring analytical platform or a library-based analytical platform for confirmation or chromatographic screening. Multiple reaction monitoring analytical platforms should include at least two transitions per compound. In order to identify a compound in a sample, library-based analytical platforms search for the best match between the mass spectrum of this compound - acquired after one fragmentation step ("molecular fingerprint") - and the spectra of reference substances stored in the library. We recommend the inclusion of more identification metrics, e.g. retention time. The more identification metrics are used, the higher the specificity of the identification.
GC-MS as well as LC-MS are technically demanding and should be used by well-trained staff only. Analytical platforms must be validated and testing laboratory should be accredited according to ISO 17025 or ISO 15189. Appropriate internal and external quality control measures are mandatory to monitor the performance of the analytical platforms. When interpreting the results obtained with chromatographic confirmation or screening analytical platforms, various pitfalls must be taken into account. Competent staff must do interpretation of the results.

These chromatographic confirmation and screening analytical platforms also have limitations:

- Only substances that are included in the analytical platform can be identified. The rapidly evolving designer drugs of abuse market therefore forces the analytical platform and the reference libraries to be actualized continuously.
- Confirmation and chromatographic screening analytical platforms also have detection gaps. Contact with the testing laboratory in charge of running the analysis is strongly recommended if the clinical situation is not in agreement with a negative result.
- Structurally similar molecules or metabolites might generate similar/identical mass spectra and fragmentation patterns. Careful confirmation and interpretation must be warranted.
- The higher turn-around time compared to immunochemical analytical platforms (usually several hours) must be taken into account. Except at tertiary care centers, the availability is usually not granted for 7d a week.
- Pharmaco- and toxicokinetic aspects still apply: if a substance or its metabolite is completely eliminated out of the body, confirmation and chromatographic screening analytical platforms will not detect the compound in blood or urine. E.g., GHB is eliminated very rapidly from the body.

A list with laboratories performing confirmation and chromatographic screening analyses can be found on the website of the Swiss Society of Clinical Chemistry.

The results of confirmation and chromatographic screening analyses are usually qualitative. Recommendations for detection limits for several analytes are published by different scientific societies.

Table 6: Recommended detection limits [µg/L] for confirmation analysis in urine.

Analyte	SCDAT (2020)	GTFCH (2011)	CSC (2019)	SAMSHA (2012)	EWDTS (2015)
THC-COOH	**10**	10[1]	10[1]	15	15
Amphetamine	**200**	200	250	250	200
Methamphetamine	**200**	200	250	250	200
MDMA	**200**	200	250	250	200
MDA	**200**	200	250	250	200
MDEA	**200**	200			200
Benzoylecgonine	**30**	30	100	100	100
Buprenorphine or metabolite	**2.0**				2.0
Methadone	**200**	200	100		250
EDDP	**75**	200			75
6-Acetylmorphine	**10**	10	10	10	10
Morphine	**25**[1]	25[1]	300	2000	300
Codeine	**25**[1]	25[1]	300	2000	300
Oxycodone	**100**		10	100	100
Alprazolam	**50**[1]		50		100
Bromazepam	**50**[1]		50		100
Clonazepam	**50**[1]		50		100
Diazepam	**50**[1]		50		100
Flunitrazepam	**50**[1]				100
Flurazepam	**50**[1]		50		100
Lorazepam	**50**[1]		50		100
Midazolam	**50**[1]				100
Nitrazepam	**50**[1]		50		100
Nordiazepam	**50**[1]		50		100
Oxazepam	**50**[1]		50		100
Temazepam	**50**[1]		50		100
LSD	**0.1**		0.1		1
Ethyl glucuronide (EtG)	**100**				

[1] *after hydrolysis*

7.3. Remarks on Urine Analyses

Concentrations for many substances are higher in urine than in blood, which is – together with the storage function of the bladder – advantageous for longer detection windows. However, as only substances in blood are thought to be pharmacologically active, urine does not necessarily reflect the current state of the patient. Taken together, urine is the preferable matrix for e.g. abstinence monitoring, but not for the assessment of an acute intoxication, where a substance might already be eliminated from blood and still be present in urine. Moreover, analytical procedures for urine must be able to detect drug metabolites, as most substances are excreted in urine as metabolites. For some substance classes, interpretation of the metabolite pattern needs special attention, as several parent compounds share the same or similar metabolites.

7.4 Remarks on Other Matrices

For other matrices (e.g. blood) a chromatographic screening platform based on GC-MS or LC-MS is the preferred analytical platform.

8. Interpretation of Results

The results of the analyses call for interpretation in terms of analytical, toxicological, and medical considerations. In so doing, pharmacokinetic factors, such as significance and consequences of the findings, must be taken into account.

8.1 Stages of Interpretation

8.1.1 Analytical Interpretation (Laboratory Experts)

- Verification and interpretation of the results considering any pre-analytical events, quality assurance data, outliers and, analytical platform specifications (sensitivity, specificity, cut-off, cross-reactivity, etc.).

8.1.2 Toxicological Interpretation (Laboratory Experts)

- Dose, frequency of consumption, route of application, interactions, inter-individual variability, tolerance, pharmacokinetics, pharmacogenetics, and plausibility are taken into account.

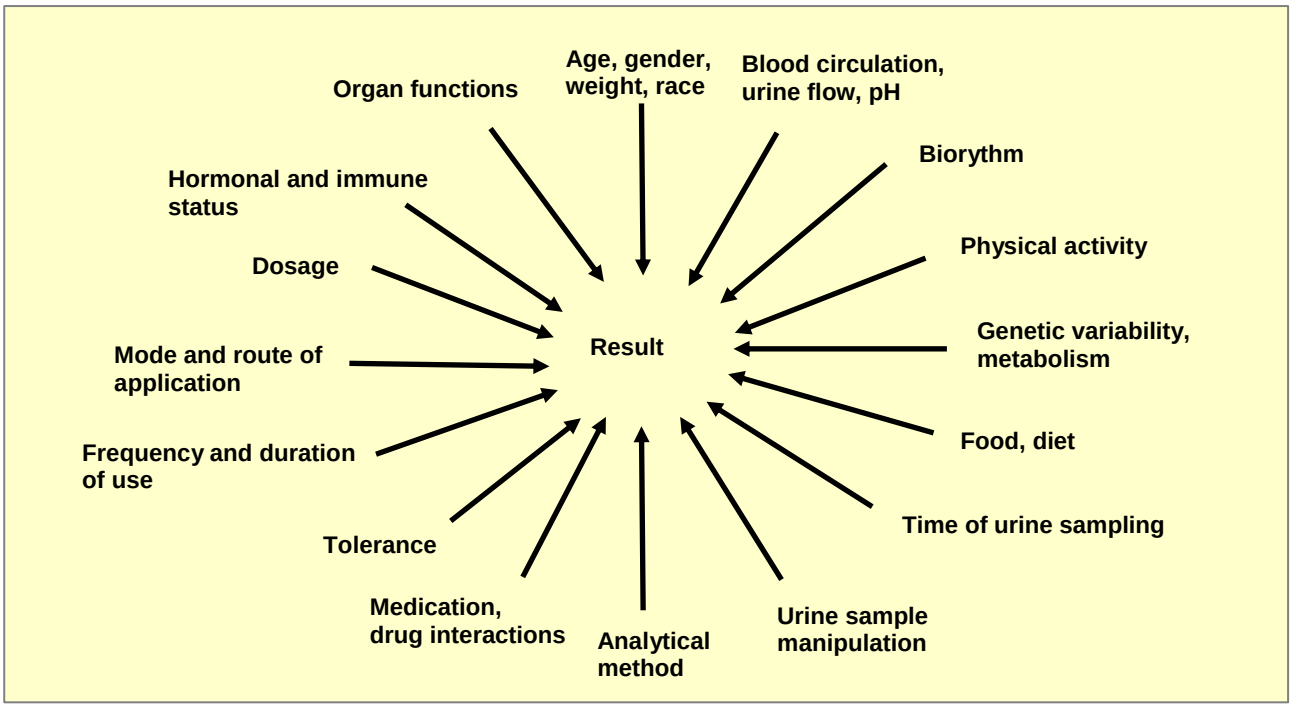
8.1.3 Medical Interpretation (Client, Medical Practitioner, Laboratory Experts)

- Consideration given to the patient's medical history, e.g., any pre-existing conditions (organ function, enzyme deficiency, metabolic disorders, age).
- Evidence of drug influence at the time of the urine sample collection.
- Doctor's prescription? Self-medication? Food?
- Plausibility check.

8.2 Factors Interfering with Pharmacokinetics and the Result of the Analysis

Figure 2 shows the exogenous and endogenous factors influencing pharmacokinetics, metabolism, and the analytical procedure and, ultimately, the result of the analysis.

Figure 2: Factors Influencing Pharmacokinetics and the Result of the Analysis



8.3 Significance of a Result

8.3.1 Questions to Ask When Interpreting a Result

Negative finding:
- Hasn't there been any consumption to date?
- Hasn't there been any new consumption, or is there only occasional consumption?
- No consumption due to announced testing?
- Tampering with urine sample?

Positive finding:
- Necessity for confirmation using chromatographic methods?
- Chronic or occasional consumption?
- Passive inhalation (cannabis, cocaine)?
- Cross-reactions with medication or food?

8.3.2 Answers

The immunochemical test is negative, meaning that the selected drugs of abuse and/or their metabolites are not detectable with the applied method:
- The individual is not consuming drugs of abuse detectable with the method used.
- The individual may possibly be consuming drugs of abuse that are not detectable.
  Potential explanations:
  - Concentrations of the drug of abuse and/or its metabolites are too low:
    - Frequency of consumption too low
    - Wrong time instant for sampling
  - Manipulation of the urine sample or drinking of excessive amounts of liquid (e.g. dilution of the urine)
- The analytical procedure has not been performed correctly:
  - Sample mix up
  - Method not sensitive enough
  - Wrong reagent
  - Faulty analytical method
  - Wrong test requested

The immunochemical test is positive, meaning that the selected drugs of abuse and/or their metabolites are detectable with the applied methods:
- The individual may possibly be consuming drugs of abuse detectable with the method used.
- The individual may possibly be consuming another substance cross-reacting with the method used.
  Next steps:
  - Confirmation of the positive results with a chromatographic method
- The analytical procedure has not been performed correctly:
  - Sample mix up

Positive immunochemical test - Positive confirmation analysis:
- Proof of a minimum of one-time drug consumption.
- Proof of chronic drug of abuse consumption possible only in the case of long-term monitoring (multiple sampling and repeat positive results).

Positive immunochemical test - Negative confirmation analysis:
- The result of the immunochemical test is due to other sample constituents and is therefore a false positive.
- Concentration of each single compound below the limit of identification of the confirmatory method but the sum of all metabolites generates a positive result in the immunoassay

Negative immunochemical test – Positive confirmation analysis:

- The concentration of the drug of abuse established by confirmation analysis is below the cut-off level of the corresponding immunochemical test.
- The result of the immunochemical test is biased by other sample constituents and is therefore a false negative.
- The confirmation analysis was not performed correctly; the analysis has to be repeated.

8.4 Implications of the Finding

The findings obtained from analyses for drugs of abuse may have legal, financial, social, and medical implications. Each individual has the right to be properly tested:

- The quality of the analysis and the reliability of the result are essential not only in forensic testing but also in the clinical and socio-medical fields.
- Critical interpretation of the result by the testing laboratory and quality assurance must be included.

9. Quality Assurance in Testing for Drugs of Abuse

Every lab testing for drugs of abuse either using simple tests or as well implementing more complex ones (such as chromatographic analytical platforms) should follow minimal quality standards. Although formal accreditation is not mandatory, it is highly recommended for labs performing non-simple tests. The corresponding applicable standards are ISO 15189 and ISO 17025. Applying appropriate quality assurance rules ensures that analytical methods are robustly validated or verified, that quality metrics and criteria are defined and followed, and that the whole process between sample collection and results delivery is under control.
Typically, a number of specifications should be documented in order to describe the performance of a test. These include a lower limit of detection or quantitation, a linearity range, the coefficient of variation of the method at a given concentration. Accuracy and precision can be assessed and followed using internal quality controls for each measurement series and by submitting the analytical method to external quality controls as provided by dedicated external quality control scheme providers (Table 7 and 8). For a number of tests, specific criteria are defined by the QUALAB guidelines [http://www.qualab.swiss/Aktuelle-Richtlinien.htm and http://www.qualab.swiss/Aktuelle-Externe-Qualitaetskontrolle.htm]. Those must be followed for the corresponding tests, so that they can be reimbursed by health insurances.

9.1 Metrology Terms for Verification and Validation of Testing Procedures

In the process of implementing a test, a number of measurements have to be performed and qualified. Criteria for acceptance of a test have to be documented and applied.

Guidelines exist that help in that process. Recommended ones include the guidelines of GTFCh, CLSI and FDA. All of them use specific metrology terms that need to be understood. Appendix 1 assembles a list of such terms, which relies on the International Vocabulary of Basic and General Terms in Metrology [JCGM 200:2012], the Guidelines for the Validation of Physicochemical Testing Procedures and for the Determination of Measurement Uncertainty [JCGM 100:2008], and the Guidelines and Recommendations of the GTFCh [www.gtfch.ch; Peters 2007].

9.2 Quality Control

Each testing laboratory must guarantee the quality of the analyses performed in its responsibility. Therefore, special action needs to be taken to fulfil the requirements.

Generally speaking, instruments used for laboratory analyses must undergo maintenance on a routine basis and be kept in good working order at all times. The manufacturers' operating instructions have to be respected. In addition, laboratories must guarantee that their analyses are performed according to the current, recognized state of the art in analytical techniques.

In terms of quality controls, the testing laboratory needs to establish procedures to verify expected results with internal quality controls and to compare its results with other laboratories via external quality controls.

9.2.1 Internal Quality Controls

According to the QUALAB guidelines, an internal quality control must be performed regularly for all medical testing laboratory analyses that are included in the Federal List of Analyses or that can be charged as part of a case-based lump compensation in accordance with the KVG.
Control samples must be analyzed as part of the internal quality control. They must be analyzed using the same reagents and instruments as those used to analyze patient samples.

9.2.2 External Quality Control

According to the QUALAB guidelines, all laboratories being reimbursed by the health assurances must participate at an external quality control scheme. As the drugs of abuse immunoassays are part of the list of compulsory external quality control, testing laboratories have to participate in a scheme of a Swiss provider for external quality controls. For the confirmation and chromatographic screening analyses a participation in a scheme of an international provider for external quality controls is highly recommended.
We also recommend that testing laboratories not being reimbursed by the health assurances participate at an external quality control scheme.

Table 7: SCDAT recommends the following cut-off values in urine for the compulsory external quality controls

Cannabis	50 µg/L	with respect to THC-carboxylic acid
Cocaine (metabolite)	300 µg/L	with respect to benzoylecgonine
Barbiturates	300 µg/L	with respect to secobarbital
Benzodiazepines	100 µg/L	with respect to nordiazepam
Amphetamines	1000 µg/L	with respect to amphetamine or methamphetamine
Opiates	300 µg/L	with respect to morphine
Methadone	300 µg/L	with respect to methadone
EDDP	300 µg/L	with respect to EDDP

9.2.3 Providers of External Quality Control Programs relevant for Drugs of abuse

Table 8 contains a non-exhaustive list of national and international institutions providing external quality control programs.

Table 8: Providers of External Quality Control Programs for Drugs of Abuse

Country	Address	Web Link	QUALAB recognition for drugs of abuse testing
Switzerland	**CSCQ Centre Suisse de Contrôle de Qualité** Chemin du Petit-Bel-Air 2 CH - 1225 Chêne-Bourg	http://www.cscq.ch	Swiss center for external quality control
Switzerland	**MQ Verein für medizinische Qualitätskontrolle** c/o Institut für klinische Chemie Universitätsspital Zürich CH - 8091 Zürich	http://www.mqzh.ch	Swiss center for external quality control
United Kingdom	**UK NEQAS** UK NEQAS Central Office Northern General Hospital Herries Road Sheffield, S5 7AU UK	https://ukneqas.org.uk/	Not applicable for compulsory controls
Germany	**Arvecon GmbH** Kiefernweg 4 D-69190 Walldorf	http://www.pts-gtfch.de	Not applicable for compulsory controls
Germany	**INSTAND Gesellschaft zur Förderung der Qualitäts-sicherung in medizinischen Laboratorien e.V.** Ubierstrasse 20 D-40223 Düsseldorf	https://www.instand-ev.de	Not applicable for compulsory controls
Germany	**Referenzinstitut für Bioanalytik (RfB)** Friesdorfer Straße 153 53175 Bonn	https://www.rfb.bio	Not applicable for compulsory controls
France	**Centre Lyonnais pour la Promotion de la Biologie et du contrôle de Qualité (ProBioqual)** 9 rue Professeur Florence F-69003 Lyon	http://www.probioqual.com	Not applicable for compulsory controls
The Netherlands	**Stichting Kwaliteitsbewaking Klinische Geneesmiddel-analyse en Toxicologie (KKGT)** P.O. Bos 43100, NL - 2504 AC Den Haag	http://kkgt.nl	Not applicable for compulsory controls
United States	**American College of Pathologists (CAP) Laboratory Accreditation program** 325 Waukegan Road, Northfield, IL 60093-2750 / USA	http://www.cap.org	Not applicable for compulsory controls
Germany	**LGC Standards GmbH** Mercatorstrasse 51 46485 Wesel Germany	https://www.lgcstandards.com	Not applicable for compulsory controls

10. Documentation of Order, Results and Reports, Archiving

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

10.1 Order for an Analysis

The testing laboratory must provide a catalog that lists the analyses, specifies the preanalytical conditions (ordering media, specimen, sample collection, sample conservation, shipping information), information on the analytics (methodology) and provides information on the post-analytics (reporting, pricing).

The order for an analysis is issued using the form provided by the testing laboratory. The form should clearly document the analyses to be conducted. The form can be a paper format or an electronic format. The sample(s) must be identifiable and unambiguously associated with the form. The order must include the following data:

10.1.1 Precise Identification of an Order

- Name and coordinates of the client (Client is the institution/institution giving the order)
- Date of the order[1] or date when received
- Designation of the testing laboratory (if the order is not made using the testing laboratory form)[1]

10.1.2 Reason and/or Clinical Details[2]

The following list is not exhaustive and provides examples of reasons and/or clinical details that can be useful for analytical and/or postanalytical treatment of a sample.

- Poisoning
- Substitution program or withdrawal treatment
- Physiological factors (e.g., pregnancy, liver or kidney ailment)
- Biological individuality (e.g., N-acetyltransferase)
- Prescribed and/or consumed drugs of abuse, therapeutic drugs, or other relevant substances
- Other clinical data (e.g. clinical status, dialysis, allergies).

10.1.3 Sample Data[1]

- Date and time of sample collection
- Sample source
- Type of sample
- Special measures (emergency).

10.1.4 Personal Data

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

10.1.5 Tests Requested

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

[1] *Compulsory information*
[2] *Optional information*

The testing laboratory verifies the content of the order, which includes the form and the associated sample(s). In case the order contains a noncompliance, the testing laboratory must have a procedure to deal with it (i.e. collect and document the missing or non-compliant information, contact the client to complete or correct the observed non-compliance, decide to proceed or not with the analysis).

10.2 Report and Results

The testing laboratory must ensure that the report includes sufficient relevant information to communicate and accompany results. This includes:

- Comments on sample conditions that could alter results quality.
- Comments related to sample and / or order form conditions that can be subject to sample rejection.
- Critical results.
- Interpretation comments, if applicable. These can be a verification/repetition status, a comment on a recently changed method, etc.

The report must include the following set of data and meta-data:

10.2.1 Material[1]

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

10.2.2 Result

In all cases, for all requested analytes:

- Name of the requested single substance(s) or substance group[1]
- Identification of all analyses performed by a sub-contractant[1]
- Analytical method (in the report or reference to an external source, such as a vademecum or an analysis catalog)[1]
- Quantitative values with units; qualitative results with unambiguous interpretation[1]
- Reference intervals or cut-off values (decision limits) [2]
- Interpretation comment, if applicable [2]
- Information on sample adequacy and of potential caution to consider for all samples with alteration of the condition that can influence a reading (alternative specimen to the preferred/specified, inadequate volume, sample conservation status, interfering lipidemia, hemolysis, icteric, …)[1]

In addition, in case of detection by immunochemical methods:

- Interpretation: unambiguous description whether test was positive or negative [1]
- Name of the reference substance[2]
- Cut-off for the reference substance[2]
- Details about substances screened for but not found, in case of non-explicit list of substances to screen[1]
- Details about substances detected but not listed on the order form[2] (or on an external resource that lists all the screened analytes in the case of a substance group).

In addition, in case of confirmation analyses (chromatographic methods):

- Limits of detection[2], details of any measurement inaccuracies[2]

10.2.3 Administrative Data

- Identification of the patient
- Identification of the client
- Date of sample collection and/or receipt of order[1]
- Date of report (date when transmitted)[1]
- Date of analysis[2]

- Signature of the person responsible for the release of the report (may be in electronic form as well) [1]
- Mode of report transmission (e.g., by phone, fax, email, electronic patient record system)[2]
- Reference to any copies[1]
- Reference to invoicing[2]
- Identification and address of the testing laboratory (address for queries)[1].
- Information if the report is intermediate or partial[1]

[1] *Compulsory information*
[2] *Optional information*

10.3 Archiving

The testing laboratory must have a documented procedure to identify, collect, index, access conserve, update, modify and eliminate all documents referring to an order, to the preparative, analytical and post-analytical phases of the sample process, as well as to the reporting phase.

In particular, all data listed under sections. 12.1 and 12.2 must be archived by the testing laboratory.

The data (order forms, extracts from the quality manual, measurement protocols, raw data, quality controls, calibrations, reports) must be archived in such a way that it is possible to obtain a copy of the analysis report at all times during the retention period defined by the KBMAL.

10.3.1 Data Retention Period

The laboratory data of an exclusively clinical nature must be kept for a minimum of 5 years (unless otherwise specified). Data protection criteria and the instructions issued by QUALAB shall also apply.

11. Legal Aspects, Data Privacy

General prerequisites are:

- The client requesting the drugs of abuse testing must be clearly identifiable.
- The legitimacy of said client to order the drugs of abuse analysis must be known.
- The testing laboratory conducting the drugs of abuse analysis must have the relevant qualifications and licenses if necessary.
- Traceability of the results must be guaranteed.
- The quality of the results must be provable.
- Results must only be made known to the person having requested the analysis

11.1 Data Privacy

Data privacy (raw data and results, patient data) must be guaranteed based on the Data Privacy Act ("Datenschutzgesetz", DSG) as well as the Federal Law about Health Insurance ("Krankenversicherungsgesetz", KVG).

Overall, the following laws and standards have to be taken into account:

- Doctors' professional secrecy in accordance with the KVG
- Data Privacy Act in accordance with the DSG

11.2 Confidentiality of Unsolicited Positive Results

In accordance with Article 10 of the "Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine 4 April 1997 [European Council 1997]", every human being has the right to information concerning all details gathered about his or her health. All requested results must be transmitted to the person, or as the case may be, agency, person, or office designated by the legal system. In so doing, please note that the interest and welfare of the living human being have priority over the mere interest of society or science. Even results that have not been requested must be treated confidentially.

12. Pharmacokinetics, Detectability

The detectability of drugs of abuse (DOAs) depends on numerous factors, including substance intake, administration route, co-ingestion of other drugs, individual genotypic and phenotypic characteristics, and consumption habits. Detectability is also influenced by the sensitivity of the detection method and by the defined concentration threshold for positivity. Detection windows mentioned here for DOAs should therefore be considered as estimates.

Table 9: Compounds discussed in this chapter

Substances	Compounds with information on detectability
Ethanol	ethanol ethyl glucuronide (EtG) phosphatidyl ethanol (PEth)
Cannabis	∆9-tetrahydrocannabinol (THC) 11-hydroxy-∆9-tetrahydro-cannabinol (11-OH-THC) 11-nor-9-carboxy-∆ 9-tetrahydro-cannabinol (THC-COOH)
Cocaine	cocaine benzoylecgonine ecgonine methyl ester
Opiates	heroin 6-acetylmorphine (6-MAM) morphine codeine
Methadone	methadone EDDP
Oxycodone	oxycodone noroxycodone oxymorphone noroxymorphone
Fentanyl	fentanyl norfentanyl
Benzodiazepines	
Gamma-Hydroxybutyrate (GHB)	GHB
Pregabalin	pregabalin
Ketamine	ketamine norketamine
Lysergic Acid Diethylamide (LSD)	LSD 2-oxo-3-hydroxy-LSD
Psilocybin	psilocin
Amphetamine	amphetamine
Methamphetamine	methamphetamine amphetamine
Methylenedioxymethamphetamine (MDMA)	MDMA methylenedioxyamphetamine (MDA) 4-hydroxy-3-methoxy-methamphetamine (HMMA)
Methylphenidate	methylphenidate
Synthetic cathinones	mephedrone
Synthetic cannabinoids	JWH-018
Piperazines	N-Benzylpiperazine (BZP) 3'-hydroxy-BZP 4'-hydroxy-BZP 1-(3-trifluoromethyl-phenyl)-piperazine (TFMPP) 4-hydroxy-TFMPP

12.1 Ethanol (alcohol)

Pharmacokinetics: Absorption via the gastrointestinal tract is rapid with peak blood concentration reached between 30 and 90 minutes after ingestion when stomach is empty. In the presence of food, both the efficiency and the rate of absorption are reduced and the peak blood ethanol concentration may be decreased by up to 70%. Ethanol is mainly metabolized into acetaldehyde and acetic acid via the alcohol dehydrogenase pathway. Minor non-oxidative metabolic pathways lead to the production of various conjugates: ethyl glucuronide (EtG), ethyl sulfate, free fatty acids ethyl esters (FAAEs), phosphatidyl ethanol (PEth).
T½*: ethanol: 2-14 h.
Elimination rate varies between individuals by a roughly factor 3 (0.1-0.3 g/L/h) depending on factors such as genetic and consumption habits [Winek 1984; Jones 2011].

* *Subsequently always defined as elimination half-life.*

Detectability: Ethanol in blood: a few hours. Blood ethanol concentration, measured by automated enzymatic assays or gas chromatography, is the primary marker of acute alcohol intoxication in a clinical setting. Breath analysis methods are mainly used for roadside controls.
EtG, FAAEs, PEth are direct markers of alcohol consumption that are used to detect alcohol misuse or to control abstinence in addiction and forensic medicine. EtG: up to 1 day in blood, up to 4 days in urine, up to 6 months in hair; PEth: 2 to 4 weeks in blood [Angulo Aguilar A 2019].

12.2 Cannabis

Pharmacokinetics: ∆9-tetrahydrocannabinol (THC) is the main active substance. Oxidation of C-11 results in the formation of 11-hydroxy-∆9-tetrahydro-cannabinol (11-OH-THC; active) and 11-nor-9-carboxy-∆9-tetrahydro-cannabinol (THC-COOH; inactive) metabolites, which are mainly excreted as glucuronides. In addition, fatty acid conjugates have been identified as remaining in the body for an extended period of time. About one-third of the absorbed THC dose is excreted in urine and two-thirds via the feces [Huestis 1999; Iversen 2000; McGilveray 2005; Musshoff 2006].
T½ of THC and its metabolites are difficult to establish since values provided in the literature are not consistent [Verstraete 2004; Musshoff 2006; Baselt 2017]. Values indicated here are therefore estimates: THC: 20-36 h, 11-OH-THC: 12-36 h, THC-COOH: 25-55 h. For regular users, T½ up to 13 days have been reported for both THC and THC-COOH.

Detectability: THC-COOH is the primary urinary marker for the detection of cannabis consumption. This is the target analyte of urine screening immunoassays. Detectability is highly variable:

- single administration (smoking): 30 h on average, up to 4 days;
- single administration (oral intake): up to 6 days;
- occasional use (once or twice a week): up to 30 days;
- regular consumption: up to 3 months.

In blood, THC-COOH is detectable 12 to 48 h for occasional users and up to 1 month for chronic users. The long detection window of THC-COOH can primarily be attributed to multi-compartment kinetics, multi-phase distribution, and multi-phase elimination, as well as the high affinity of THC compounds for adipose tissue.
THC and 11-OH-THC should be used as target analytes, instead of THC-COOH, to detect recent cannabis consumption [Manno 2001; Brenneisen 2010]. The detection window of 11-OH-THC is shorter than that of THC, except in case of oral intake.

Detectability of THC for casual consumption [Niedbala 2001]:
- in blood: 3 to 12 h;
- in oral fluid: 8 to 16 h.

Chronic users exhibit longer detection windows [Karschner 2009]: in blood, THC up to 12 days and 11-OH-THC up to 3 days.

12.3 Cocaine

Pharmacokinetics: The main metabolites of cocaine are benzoylecgonine and ecgonine methyl ester (methylecgonine). They are formed by enzymatic (pseudo-cholinesterase) or spontaneous hydrolysis. Anhydroecgonine methylester is a specific marker for “crack” consumption, while cocaethylene is detectable after the simultaneous consumption of alcohol.
T½: cocaine: 0.5-1.5 h (up to 4h in chronic users); benzoylecgonine: 3.5-8 h; ecgoninemethylester: 3.5-6 h.

Detectability:
In urine:
- cocaine: up to 12 h (single administration);
- benzoylecgonine: 1 to 3 days (single administration), up to 3 weeks (chronic user);
- ecgonine methyl ester: 24 to 48 h (single administration).

In blood:
- cocaine: 4 to 12 h (single administration);
- benzoylecgonine: 1 to 2 days (single administration), up to 8 days (chronic user).

In oral fluid:
- cocaine: 5 to 12 h (single administration);
- benzoylecgonine: 12 to 24 h (single administration), up to 10 days (chronic user).

[Verstraete 2004; Baselt 2017]

12.4 Opiates

Pharmacokinetics: Diacetylmorphine (heroin) is metabolized by esterases into 6-acetylmorphine (6-MAM) then into morphine. Morphine is primarily excreted as 3-O- and 6-O-glucuronide. A minor pathway leads to the production of normorphine and hydromorphone.
Codeine is metabolized into morphine via the CYP2D6 pathway. The rate of morphine production from codeine is therefore influenced by the CYP2D6 genotype. Two other important metabolic pathways are the formation of norcodeine (CYP3A4) and glucuronoconjugation to form codeine-6-glucuronide. A minor pathway leads to the production of hydrocodone, which, in turn is transformed in norhydrocodone, hydromorphone, and dihydrocodeine [Cervinski 2019].
T½: heroin: 2-7 min; 6-acetylmorphine: 6-25 min; morphine: 2-3 h; codeine: 1.5-3.5 h.

Detectability:
In urine:
- 6-acetylmorphine: 2 to 4.5 h (single administration), up to 35 h (chronic user);
- Morphine: 10 to 55 h (single administration), up to 11 days (chronic user);
- codeine: up to 30h (single codeine administration).

In blood:
- heroin: < 10 min (single administration);
- 6-acetylmorphine: 1 to 2 h (single administration);
- morphine: 12 to 24 h (single administration), up to 5 days (chronic user);
- codeine: 6 to 16h (single administration).

In oral fluid:

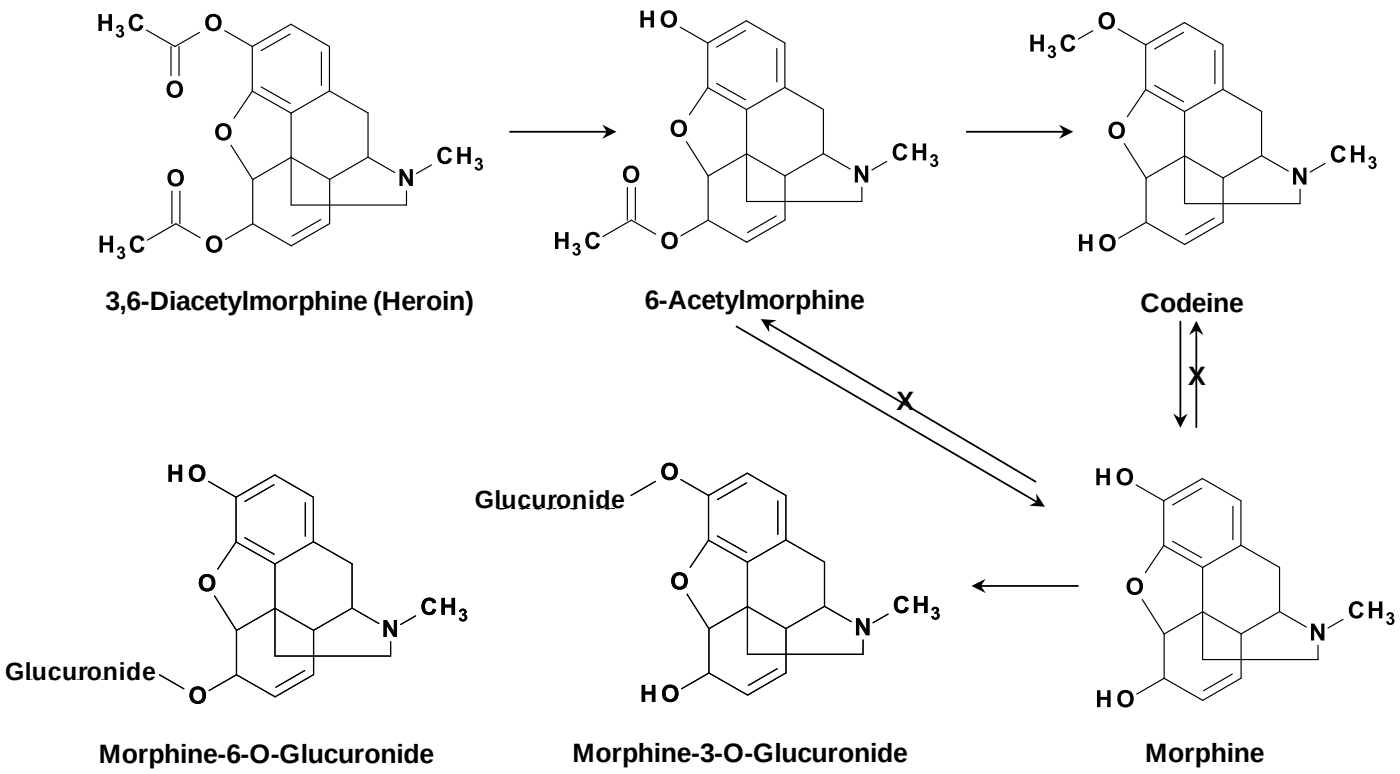
- 6-acetylmorphine: < 1 hour (single administration)
- Morphine: 12 to 24 h (single administration)

[Verstraete 2004; Baselt 2017)]

Differentiation of opiates use: determining whether a subject has taken heroin or a prescription drug containing codeine is difficult since both compounds are metabolized to morphine. In addition, illicit heroin preparations contain as impurities codeine and acetylcodeine, which is deacetylated to produce codeine. Evidence of heroin use is thus only possible via the determination of the specific metabolite 6-acetylmorphine. However, since 6-acetylmorphine has a short half-life, its detection window in blood is narrow. In case of 6-acetylmorphine negative samples, authors have proposed to use the morphine/codeine ratio: a ratio M/C > 1 in plasma, serum or whole blood being indicative of heroin use [Ceder 2001]. Nevertheless, the metabolism of codeine into morphine is subject to a high inter-individual variability and morphine/codeine ratios must be interpreted with caution.

Heroin-assisted treatment: the parallel consumption of street heroin can only reliably be confirmed by the detection of the specific marker 6-acetylcodeine, formed during preparation of heroin from raw opium [Staub 2001; Brenneisen 2002]. The presence of alkaloids from *Papaver somniferum*, such as thebaine, noscapine, and papaverine, suggests the use of illicit heroin but cannot be considered as a conclusive proof [Trafkowski 2006].

Figure 3: <u>Metabolism of diacetylmorphine (heroin)</u>



12.5 Methadone

Pharmacokinetics: Methadone is metabolized by mono-, di-N-demethylation and subsequent spontaneous cyclization into 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) followed by glucuronidation. The main metabolite is EDDP [Baselt 2017].
T½: methadone: 15-55 h.

Detectability: In urine:
- Methadone: 1.5 to 3 days,
- EDDP: 3 to 4 days.

The additional determination of EDDP is recommended for compliance testing as the metabolism of methadone may be strongly accelerated by interaction with co-medications as well as in case of fast metabolizers. This also enables to detect urine adulteration by addition of methadone:
- methadone and EDDP negative: no methadone consumption,
- methadone and EDDP positive: methadone consumption,
- methadone negative, EDDP positive: methadone consumption (fast metabolizer, interaction with co-medication),
- methadone positive, EDDP negative: spiked urine.

12.6 Oxycodone

Pharmacokinetics: The main metabolization pathway of oxycodone is N-demethylation to form noroxycodone. There is also, to a lesser extent, formation of oxymorphone via O-demethylation. Both metabolites are then transformed into noroxymorphone. Only 10% of the dose is excreted unchanged in urine
T½: oxycodone: 3.2-5.6 h (immediate-release formulations), 4.5-8 h (controlled-release formulations); noroxycodone: 5.8 h; oxymorphone: 8.8 h; noroxymorphone: 9 h [Cone 2015].

Detectability: In urine (single administration) [Cone 2013]:
- oxycodone: 24 to 36 h,
- noroxycodone: 32 to 52 h,
- oxymorphone: up to 28 h,
- noroxymorphone: up 36 h.

In blood (single administration):
- oxycodone: 12 to 14 h,
- noroxycodone: 10 to 32 h.

In oral fluid (single administration) [Cone 2015]:
- oxycodone: 14 to 36 h,
- noroxycodone: 8 to 24 h.

12.7 Fentanyl, fentanyl analogs, and other synthetic opioids

Pharmacokinetics: The main route of metabolism of fentanyl is N-dealkylation into norfentanyl, an inactive metabolite. Other minor pathways account for less than 1% of the metabolic process. Less than 10% of the dose is excreted unchanged in urine or feces.
There are more than 30 fentanyl analogs. Sufentanyl (Sufenta®), alfentanyl (Rapifen®), and remifentanil (Ultiva®) are used clinically. The others are illicit drugs. All these substances are characterized by their high potency (carfentanyl: 10’000 times the potency of morphine). Sufentanyl main metabolites are N-desalkylsufentanyl and O-desmethylfentanyl. About 80% of a dose is excreted in urine in 24h with only 2% as unchanged drug. Carfentanyl is primarily metabolized by N-dealkylation and monohydroxylation of the piperidine ring.

U-47700 is a non-fentanyl-based synthetic opioid. It is mainly metabolized into N-desmethyl-U-47700 and N,N-didesmethyl-U-47700.
T½: fentanyl (highly variable depending on the administration route): IV: 2-4 h; transdermal: 13-22 h; nasal spray: 15-25 h, oral: 3-36 h (single dose), 11-45 h (multiple dose); sufentanyl: IV: 2.7 h, sublingual 7-12 h; carfentanyl: 5.7 h, norcarfentanyl: 11.8 h.
[Baselt 2017; Krotulski 2018; Jannetto 2019]

Detectability: In urine (single administration) [Silverstein 1993]:
- fentanyl: 12 to 48 h;
- norfentanyl: 2 to 4 days.

12.8 Benzodiazepines

Note: Beside established prescribed benzodiazepines, a number of new benzodiazepines have appeared in Europe in the recent years. They are sold on the internet or on the illicit drug market as replacements for prescribed drugs or as fake versions of licit drugs. The most frequently seized molecules in Europe in 2017 were etizolam, clonazolam, norfludiazepam, diclazepam, phenazepam [EMCDDA - EU Drug Markets Report 2019].

Pharmacokinetics: Metabolic pathways of benzodiazepines vary depending on the molecule structure. Chlordiazepoxide (Librax®, Limbitrol®, Libricol®), clorazepate (Tranxilium®), diazepam (Valium®), ketazolam (Solatran®), prazepam (Demetrin®), temazepam (Normisom®) are metabolized to nordiazepam and/or oxazepam (Anxiolit®, Seresta®) by desalkylation, oxydation, and hydroxylation. Metabolites are eliminated via the kidneys, mainly as glucuronides.
Clonazepam (Rivotril®), flunitrazepam (Rohypnol®), nitrazepam (Mogadon®) are metabolized by reduction to 7-amino derivatives, N-acetylation, N-demethylation, 3-hydroxylation and glucuronidation.
Bromazepam (Lexotanil®) is metabolized by 3-hydroxylation and cleavage of the 7-membered ring followed by hydroxylation. Both hydroxylated metabolites are conjugated to glucuronic acid.
Clobazam (Urbanyl®) is primarily metabolized to desmethylclobazam by N-demethylation.
Alprazolam (Xanax®) and triazolam (Halcion®) are metabolized by 1- and 4-hydroxylation and conjugation. Alprazolam also leads to the formation of benzophenones by ring cleavage.
Flurazepam (Dalmadorm®) is metabolized by oxidation, N-deethylation, and conjugation. The main urinary metabolite is conjugated N-1-hydroxyethylflurazepam.
Midazolam (Dormicum®, Buccocalm®) is mainly metabolized by CYP450 to 1-hydroxymidazolam.
Lorazepam (Temesta®, Sedazin®, Somnium®) and lormetazepam (Noctamid®, Loramet®) are primarily metabolized by glucurono-conjugation.
Etizolam is extensively metabolized by hydroxylation at the α- and 1'- positions. α-OH and 1'-OH metabolites are then conjugated.
T½: 1-30 h (triazolam), 8-20 h (bromazepam), 10-30 h (flunitrazepam), 20-40 h (diazepam), 40-100 h (nordiazepam).

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

Figure 4: Metabolism of 1,4-Benzodiazepines

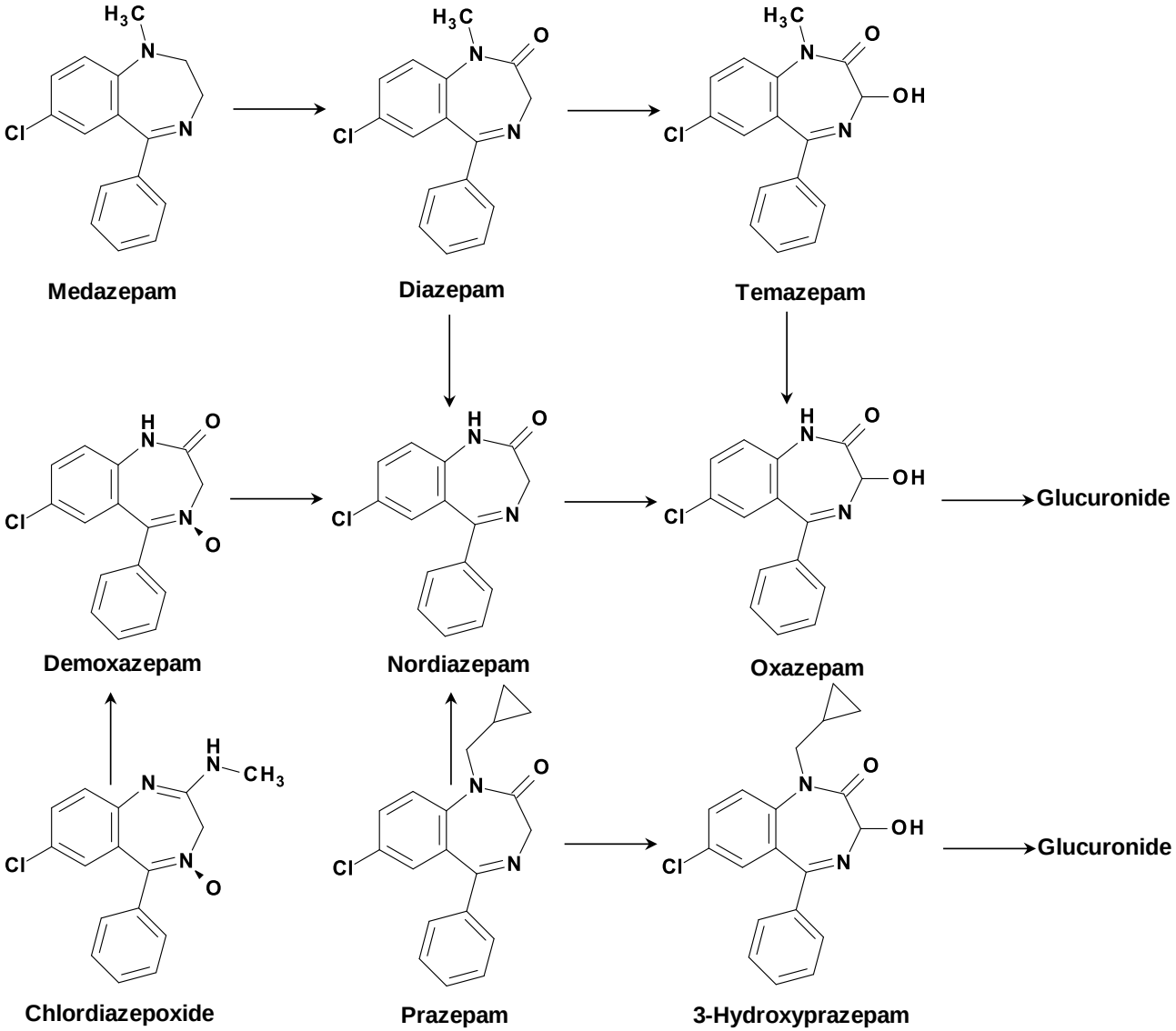


Figure 5: Metabolism of 7-Nitrobenzodiazepines

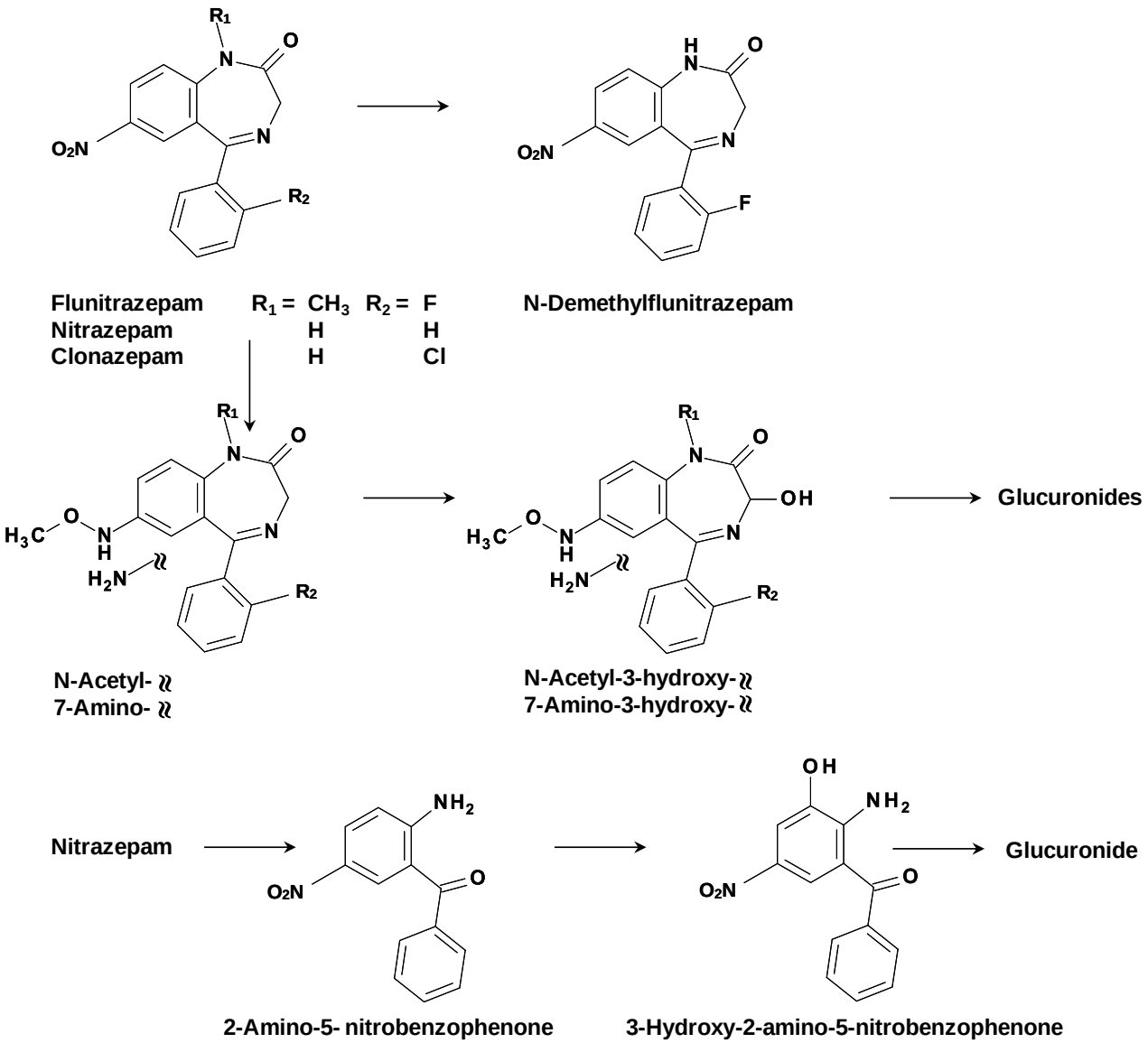
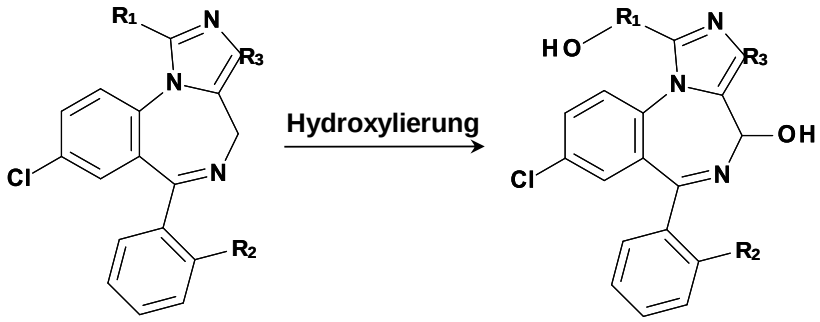


Figure 6: Metabolism of Triazolobenzodiazepines



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**

12.9 Gamma-Hydroxybutyrate (GHB)

Pharmacokinetics: GHB is almost completely metabolized to succinate by the alcohol dehydrogenase pathway. Less than 2% of a GHB dose is excreted unchanged in urine [Baselt 2017].
Gamma-butyrolactone (GBL) and 1,4-butanediol (BD) are substances rapidly metabolized to GHB after oral ingestion. The psychoactive effects of GBL and BD are based on their transformation into GHB. Gamma-valerolactone (GVL) is metabolized into gamma-hydroxy-valeric acid (GHV, 4-methyl-GHB).
T½: GHB 20-60 min.

Detectability: In urine:
- 6 to 12h

note: endogenous GHB concentrations found in urine from healthy subjects ranged from 0.1 to 6.6 mg/l.

In blood:
- up to 6 h

In oral fluid:
- up to 6 h

[Brenneisen 2004; Haller 2006; Baselt 2017]

12.10 Pregabalin

Pharmacokinetics: Pregabalin is predominantly excreted unchanged in urine (90% of the dose). The main metabolite, N-methylpregabalin account for less than 1 % of the dose [Baselt 2017].
T½: pregabalin 4.6-6.8 h [Bockbrader 2010].

Detectability: In urine (single administration) [Spigset 2013]:
- pregabalin: 2.5-4 days;

In blood (single administration) [Bockbrader 2010]:
- pregabalin: 36-48 h;

12.11 Ketamine

Pharmacokinetics: Ketamine is metabolized in the liver into norketamine via N-demethylation, followed by subsequent hydroxylation to dehydronorketamine and conjugation. Norketamine is an active metabolite with one third of the anesthetic potency of ketamine.
T½: ketamine 2-4 h (IV) [Dinis-Oliveira 2017].

Detectability: In urine (single administration) [Adamowicz 2005]:
- ketamine: 1 to 4 days;
- norketamine: 1 to 5 days.

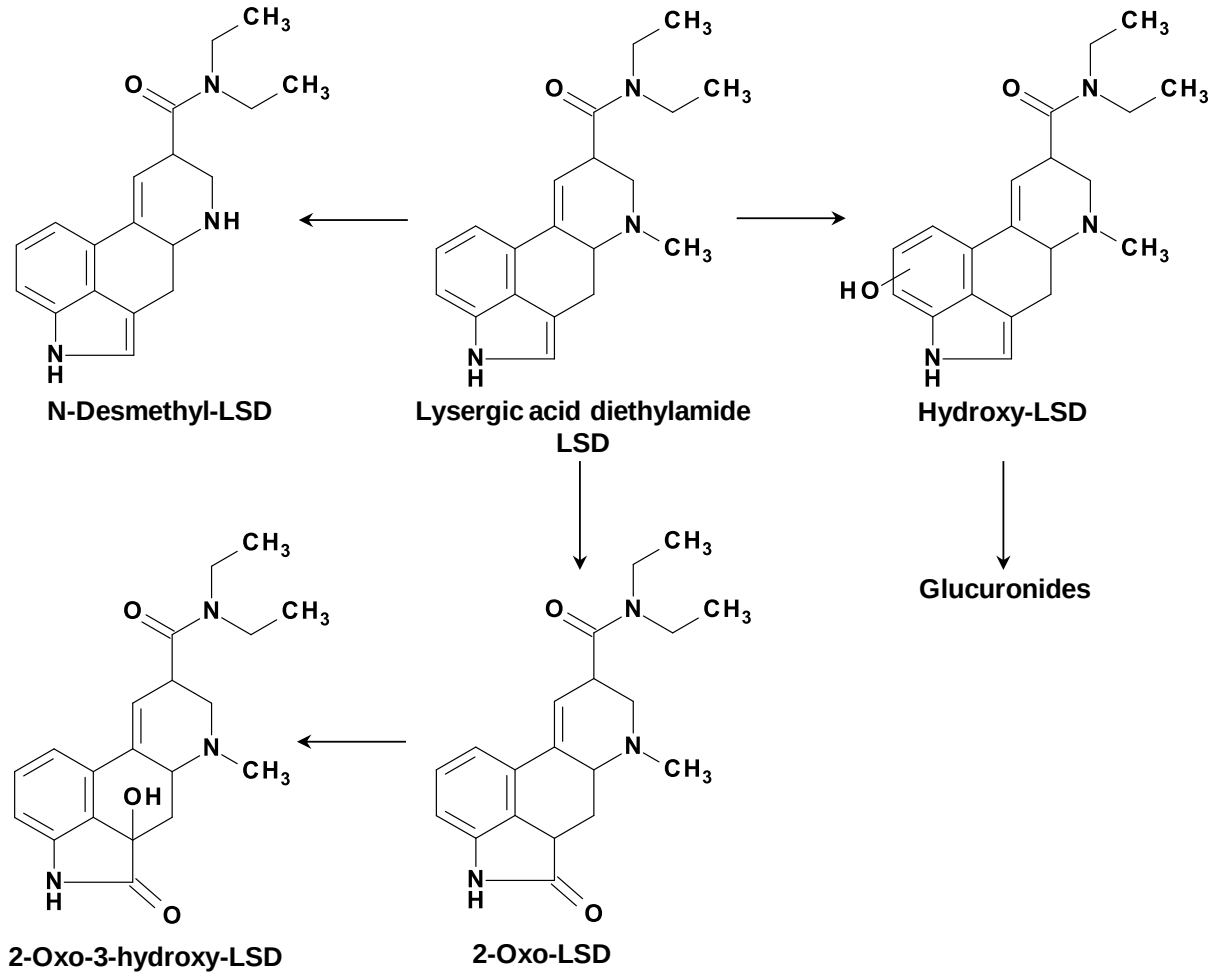
12.12 Lysergic Acid Diethylamide (LSD)

Pharmacokinetics: N-dealkylation, hydroxylation and glucuronidation are the main LSD metabolic pathways. The predominant metabolite in urine is 2-oxo-3-hydroxy-LSD. Additional metabolites are nor-LSD, lysergic acid ethylamide, trioxylated-LSD, lysergic acid ethyl-2-hydroxyethylamide, and 13-/14-hydroxy-LSD as well as their glucuronides [Canezin 2001].
T½ LSD: 2.2-3.4 h [Dolder 2017].

Detectability: In urine (single administration) [Verstraete 2004]:
- LSD: 24 to 36 h;
- 2-oxo-3-hydroxy-LSD: up to 96 h.

In blood (single administration) [Passie 2008; Dolder 2016]:
- LSD: 6 to 16 h.

Figure 7: Metabolism of Lysergic Acid Diethylamide (LSD)



12.13 Psilocybin

Metabolism: Psilocybin is an alkaloid occurring in many *Psilocybe* species, such as *P. mexicana*, *P. cubensis*, and *P. semilanceata* (“Magic Mushrooms”). It is a phosphate derivative of N,N-dimethyltryptamine (DMT) [Hofmann 1959]. Psilocybin acts as a prodrug and is rapidly converted by intestinal esterases to psilocin, which is the pharmacologically active substance. Psilocin is then metabolized into an inactive metabolite, 4-hydroxyindol-3-yl-acetic acid (HIAA). HIAA is the dominant urinary metabolite. Psilocin also undergoes glucurono-conjugation. Within 24 h, only 3% of the dose is excreted as free psilocin.
T½: psilocin: 1.8- 4.5 h, HIAA: 1-4 h [Hasler 1997]

Detectability: In urine (single administration) [Hasler 2002]:
- psilocin: 24 h

In blood (single administration) [Hasler 1997]:
- psilocin: 7 h.

12.14 Amphetamine

Pharmacokinetics: Amphetamine is metabolized by several oxidative pathways leading to various metabolites (phenylacetone, benzoic acid, hippuric acid, norephedrine, p-hydroxynorephedrine, p-hydroxyamphetamine) followed by conjugation. Metabolites are primarily excreted in urine. Rates of metabolism and excretion depends on urine pH: 30-40% of the ingested amphetamine is excreted unchanged at normal urine pH, but an acidic pH increases excretion (up to 78% / 24 h, 68% unchanged) while an alkaline pH lowers excretion (45% /24 h, 2% unchanged).
T½: 7-14 h [Baselt 2017].

Detectability: In urine:
- amphetamine: 1 to 3 days (single administration); up to 9 days (chronic users).

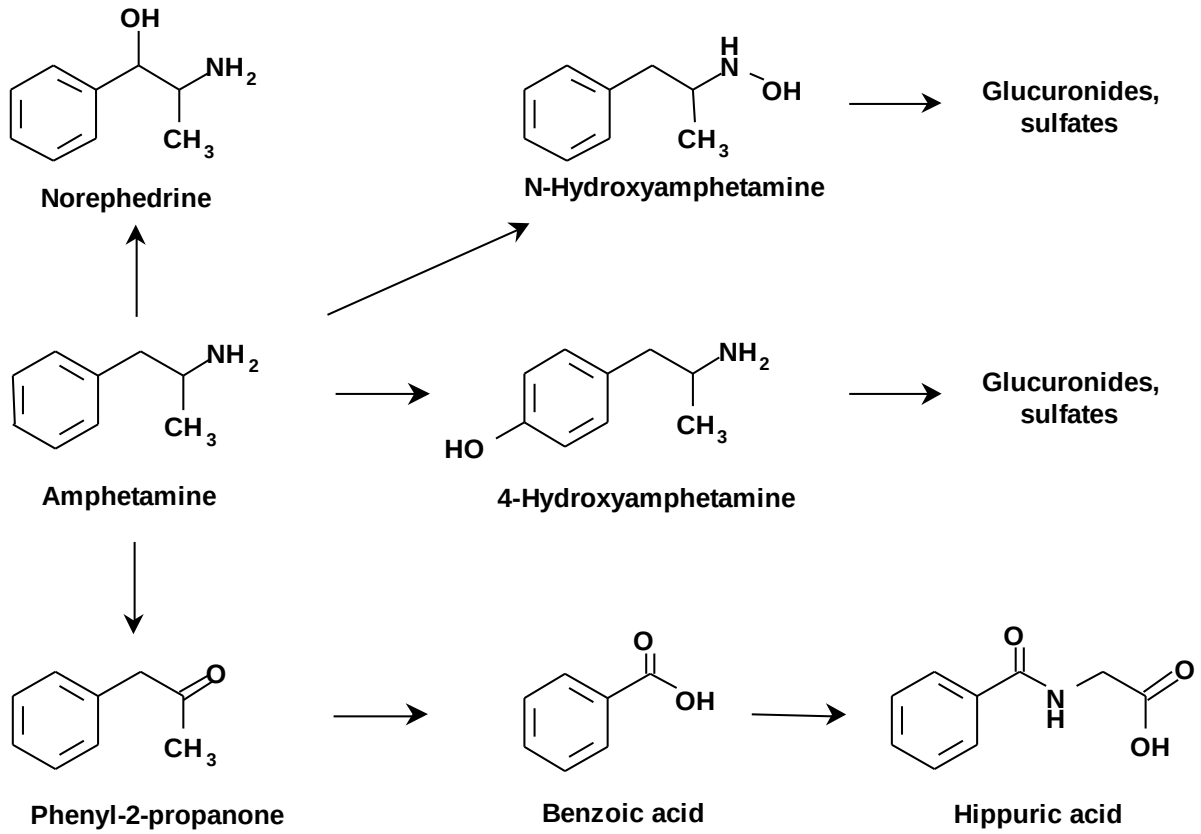
In blood:
- amphetamine: 48 h.

In oral Fluid:
- amphetamine: 20 to 50 h (single administration); up to 6 days (chronic users)

[Verstraete 2004; Andås 2016]

Several prescription drugs, most of which are now prohibited in Europe, are metabolized to amphetamine and may positive detection assays [Cody 2002]: amphetaminil (brand name Aponeuron), benzphetamine (brand name Didrex), clobenzorex (brand names Asenlix, Dinintel, Finedal), dimethylamphetamine (brand name Metrotonin), Lisprodexamphetamine, d-amphetamine (brand name Elvanse), ethylamphetamine (brand names Apetinil, Adiparthrol), famprofazone (brand names Gewodin, Gewolen), fenethylline (brand names Captagon, Biocapton, Fitton), Fenproporex (brand name Perphoxene), Furfenorex (brand name Frugalan), mefenorex (brand names Pondinil, Rondimen), prenylamine (brand name Segontin), selegiline (brand names Selegiline Mylan, Deprenyl).

Figure 8: Metabolism of Amphetamine



12.15 Methamphetamine

Pharmacokinetics: Methamphetamine is N-demethylated into amphetamine, the major active metabolite. Under normal urine pH conditions, up to 43% of methamphetamine is excreted unchanged within 24 h and 4-7% as amphetamine. In acidic urine, up to 76% are excreted unchanged within 24 h and 7% as amphetamine. In basic urine, 2% of methamphetamine is excreted unchanged within 24 h and < 0.1% as amphetamine. Other metabolites are p-hydroxymethamphetamine (free or conjugated), accounting for about 15%, and metabolites of amphetamine in minor amounts [Baselt 2017].
T½ methamphetamine: 10-33 h [Baselt 2017].

Detectability: In urine:
- methamphetamine ± amphetamine: 1 to 4 days (single administration), up to 5 days (chronic users);

In blood:
- methamphetamine: 48 h (single administration);

In oral Fluid:
- methamphetamine: 24 h (single administration), 36 to 72 h (repeated administration), up to 8 days (chronic users).

[Verstraete 2004; Andås 2016]

Several prescription drugs, most of which are now prohibited in Europe, are metabolized to methamphetamine and may positive detection assays [Cody 2002]: benzphetamine (brand name Didrex), dimethylamphetamine (brand name Metrotonin), famprofazone (brand names Gewodin, Gewolen), fencamine (brand names Altimina, Sicoclor) Furfenorex (brand name Frugalan), selegiline (commercialized in France, brand name Selegiline Mylan, Deprenyl).

12.16 Methylenedioxymethamphetamine (Ecstasy)

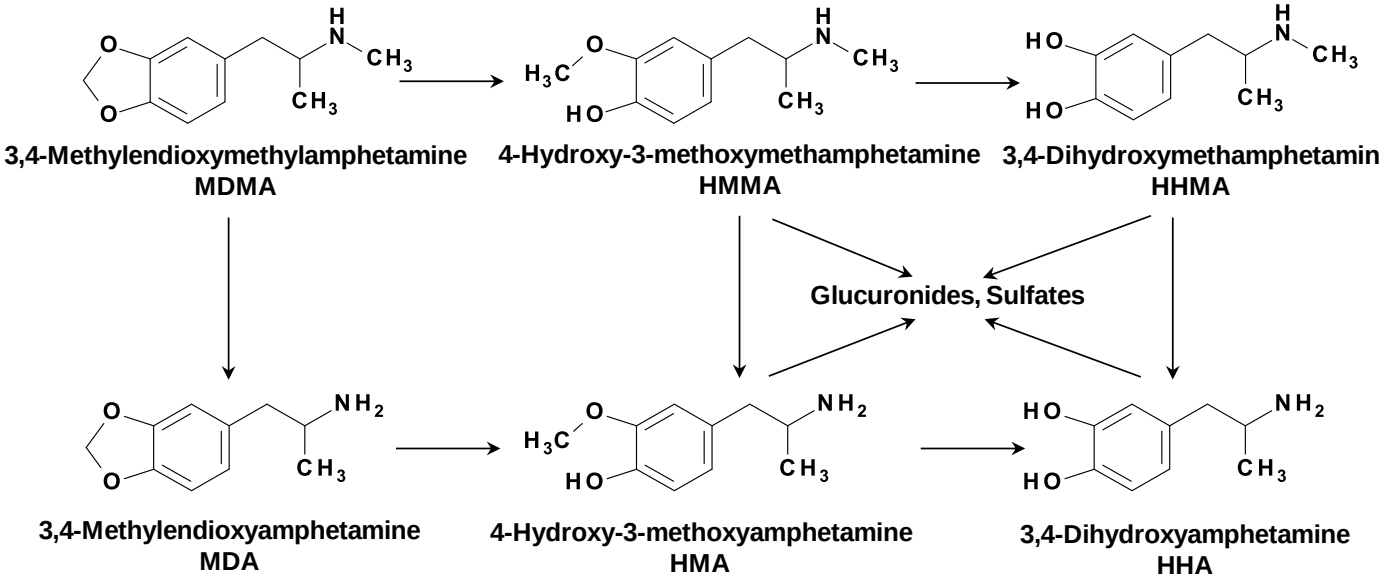
Pharmacokinetics: Metabolization of 3,4-methylenedioxymethamphetamine (MDMA) involves N-demethylation to form 3,4-methylenedioxyamphetamine (MDA) and oxidative ring cleavage to form hydroxylated metabolites, 4-hydroxy-3-methoxy-methamphetamine (HMMA), 4-hydroxy-3-methoxy-amphetamine (HMA), 3,4-dihydroxymethamphetamine, and 3,4-dihydroxyamphetamine, which are then conjugated with glucuronic acid [Baselt 2017]. MDA is pharmacology active.
3,4-Methylenedioxyethylamphetamine (MDEA, "Eve") is metabolized by ring cleavage, conjugation, N-deethylation and deamination.
T½: MDMA: 7-8 h, MDA: 11-16 h, HMMA: 10-12 h

Detectability: In urine (single administration) [Schwaninger 2011]:
- MDMA: 1 to 5 days,
- MDA: 1 to 3 days
- HMMA: 1 to 4 days

In blood (single administration):
- MDMA: 48 h
- MDA: 24 h

In oral fluid (single administration) [Barnes 2011]:
- MDMA: 1 to 3 days
- MDA: 1 to 2 days

Figure 9: Metabolism of 3,4 Methylenedioxymethamphetamine



12.17 Methylphenidate and ethylphenidate

Pharmacokinetics: Methylphenidate (Ritalin) is rapidly metabolized to ritalinic acid, an inactive metabolite. Other metabolites are formed by hydroxylation, methylation, oxidation, and conjugation. Ethylphenidate can be detected in small amounts after co-consumption of ethanol. 80% of a methylphenidate dose is excreted within 24 h, 60-81% as ritalinic acid and 5-12% as 6-oxo-ritalinic acid. Less than 1% is excreted unchanged; however, the percentage can be higher at acidic urine pH [Baselt 2017].

Ethylphenidate is a close analog of methylphenidate sold on the recreational drug market for its stimulant effects. Ritalinic acid and methylphenidate were identified *in vitro* as the main metabolites of ethylphenidate [Negreira 2016].
T½: methylphenidate 2.1-3.5 h (normal release), 3.8-5.7 h (extended release), 3-5 h (transdermal system); ritalinic acid: 4 h (normal release).

Detectability: In urine, methylphenidate and ritalinic acid: at least 24 h (20 mg oral therapeutic dose) [Solans 1994].

12.18 New Psychoactive substances

New psychoactive substances represent a vast group of chemical compounds, which appeared in recent years on the recreational drug market as legal replacements of traditional controlled drugs. Since 2008, the NPS market has been characterized by an unprecedented rate of appearance of new substances with several dozens of new molecules identified each year [EMCDDA - EU Drug Markets Report 2019]. The NPS market is characterized by its high dynamic and constant changes with a rapid turnover of substances. Accordingly, only a few compounds remain on the market for several years. The first consequence is that, for most NPS, no or very limited data are available on pharmacokinetic properties and detectability. The second consequence is that detection of these substances and/or their metabolites represent an analytical challenge for clinical laboratories. The main categories of NPS are presented below with examples of typical substances, keeping in mind the impossibility of providing an accurate and comprehensive picture of the actual market in a given place. It should be noted that examples of some NPS categories (synthetic opioids, benzodiazepines and ethylphenidate) have been presented in specific sections above.

12.18.1 Synthetic cathinones

Note: Synthetic cathinones are structural analogs of cathinone, one of the main psychoactive substances of khat (*Catha edulis*). More than 130 different molecules have been described and new ones are regularly appearing on the market. Substances most commonly seized in Europe in 2017 were N-ethylhexedrone, 4-CMC/clephedrone, 4-CEC, 3-CMC, 3-CEC, ephylone, dibutylone/bk-MMBDB [EMCDDA - EU Drug Markets Report 2019]. Only some examples of synthetic cathinones are presented below.

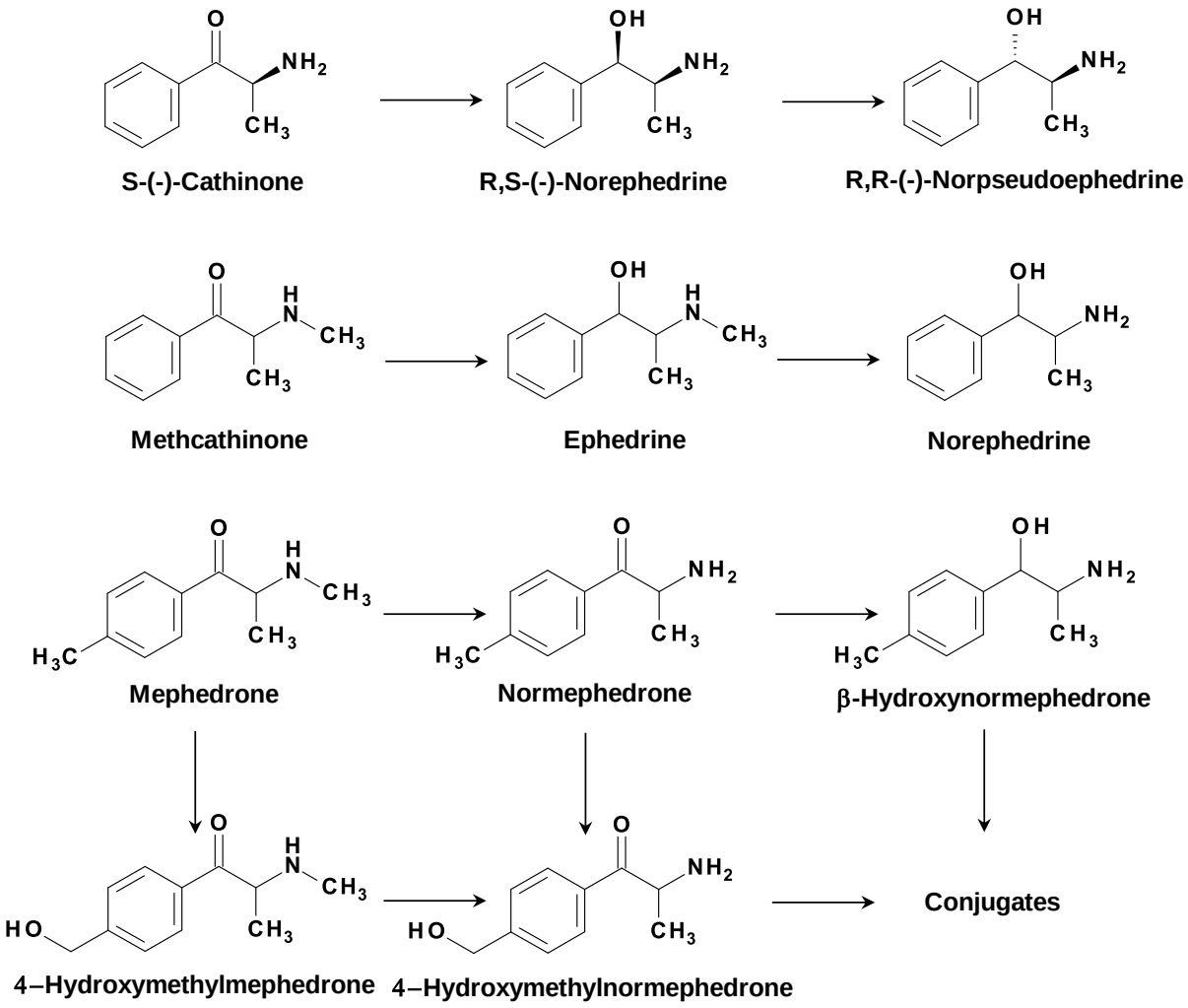
Pharmacokinetics: Mephedrone (4-methylmethcathinone) is metabolized by demethylation, reduction, hydroxylation, and conjugation of the hydroxylated metabolites. The parent drug, as well as normephedrone, hydroxynormephedrone, 4-hydroxymethylmephedrone and 4-hydroxymethylnormephedrone are detected in urine [Baselt 2017].
3-MMC (3-methylmethcathinone) is structurally related to mephedrone. Its metabolism is not fully elucidated but 3-methylephedrine and 3-methylnorephedrine have been identified as probable metabolites [Ferreira 2019].
Methcathinone is metabolized into ephedrine and pseudoephedrine [Paul 2001].
Methylone (3,4-methylenedioxy-N-methylcathinone) is metabolized into 3,4-methylenedioxycathinone, 4-hydroxy-3-methoxymethcathinone, and 3-hydroxy-4-methoxymethcathinone. The parent drug and the metabolites are found in urine as free compounds and conjugates [Baselt 2017].
4-MEC (4-methylethcathinone) is metabolized by N-deethylation, reduction, hydroxylation, and conjugation. The parent drug and various metabolites, including nor-4-MEC, are detected in urine [Helfer 2015].
α-PVP (α-pyrrolidinovalerophenone) has a complex metabolism, which involves several oxidation pathways. Main urinary excretion products include α-PVP, 2'-oxo-PVP (α-PVP lactam), 1-hydroxy-N,N-bis-dealkyl-α-PVP, and 1-hydroxy-PVP [Nóbrega 2018].

MDPV (3,4-methylenedioxypyrovalerone) is metabolized by demethylenation, oxidation of the pyrrolidine ring, and aromatic and side-chain hydroxylation. Metabolites are sulfated or glucuronated and subsequently excreted in urine. 4-hydroxy-3-methoxypyrovalerone is the major metabolite found in urine [Baumann 2017].
T½: mephedrone: 2.2 h; α-PVP: 4.3 h

Detectability: In urine (single administration) [Olesti 2017]:
- mephedrone: 12 - 48 h

In blood (single administration):
- mephedrone: 8 -18 h

Figure 10: Metabolism of cathinone, methcathinone and mephedrone



12.18.2 Synthetic cannabinoids

Note: Synthetic cannabinoids are cannabinoid receptor agonists mimicking the effect of natural cannabis. They are usually sprayed or mixed with herbal products to be smoked as a joint but may also be available as a powder. More than 190 different molecules have currently been described and new ones are regularly appearing on the market. Substances most commonly found on the European market in 2017 were 5F-MDMB-PINACA/5F-ADB, MDMB-CHMICA, AMB-FUBINACA, AB-CHMINACA, ADB-FUBINACA, CUMYL-PeGACLONE, CUMYL-4CN-BINACA, and JWH-018 [EMCDDA - EU Drug Markets Report 2019].

Pharmacokinetics: Synthetic cannabinoids often undergo extensive biotransformation processes producing numerous metabolites (≥ 13 metabolites for JWH-018, ≥ 15 for AB-CHMINACA or MDMB-CHMICA).
T½: A wide range of elimination half-lives has been observed for these compounds ranging from a few hours to several weeks in chronic users [Baselt 2017].

Detectability: With a few exceptions, data on detectability of synthetic cannabinoids in human body fluids are scarce. It is noteworthy that very long detection windows over several months have been described for some synthetic cannabinoids metabolites in case of extensive consumption [Franz 2020].
In urine:
- JWH-018 pentanoic acid: up to 4 weeks (single administration) [Toennes 2018],
- JWH-018-COOH: up to 6 weeks [Hegstad 2015]

In blood:
- JWH-018: up to 4 weeks (single administration) [Toennes 2017]

In oral fluid:
- JWH-018: < 12h (single administration) [Toennes 2018]

12.18.3 Piperazines

Pharmacokinetics: N-Benzylpiperazine (BZP) is mainly metabolized by hydroxylation, N-desalkylation, O-methylation, and conjugation [Baselt 2017]. In 24h urines, unchanged BZP accounts for about 6% of the dose while two metabolites, 3'-hydroxy-BZP and 4'-hydroxy-BZP, are detected as free compounds (0.1%) and O-sulfate and N-sulfate conjugates (about 10%) [Antia 2009]. A number of other piperazine derivatives have been synthetized such as 1-(3-trifluoromethyl-phenyl)-piperazine (TFMPP), 1-(3-chlorophenyl)-piperazine (mCPP), para-methoxyphenylpiperazine (MeOPP), and 1-(3,4-methylenedioxybenzyl)-piperazine (MDBZP). TFMPP is metabolized by hydroxylation, cleavage of the piperazine ring and conjugation. The main urinary metabolite is 4-hydroxy-TFMPP [Staack 2003; Antia 2010]. mCPP is the metabolite of the antidepressant drug trazodone (Trittico®).
T½: BZP: 4.3-5.5 h; TFMPP: 2-6 h, 4-hydroxy-TFMPP: 6.6 h; mCPP: 4.3 h

Detectability: In blood (single administration):
- BZP, 3'-hydroxy-BZP, and 4'-hydroxy-BZP: 24 h [Antia 2009].
- TFMPP: 24h, 4-hydroxy-TFMPP: 8 h [Antia 2010].

12.18.4 Other NPS

Phenylethylamines:

Phenylethylamines family encompasses a range of substances that share a common basic structure corresponding to an amino-group being attached to a benzene ring through a two-carbon, or ethyl group. Depending on chemical substitutions, phenylethylamines have stimulant, entactogen or psychedelic effects [King 2014, Tyrkkö 2016]. In addition, even small changes in the chemical structure may lead to great variations in potency. Examples of hallucinogenic phenylethylamines are compounds from the 2-Cx series (2C-B, 2C-E, 2C-I, etc.), the Dox series (DOB, DOC, DOI, etc.), the 25x-NBOMe series (25B-NBOMe, 25C-NBOMe, 25I-NBOMe, etc.), and Bromo-DragonFLY. 4-fluoromethamphetamine (4-FA), *para*-methoxymethamphetamine (PMMA), 4-methylthioamphetamine (4-MTA) are structural analogs of amphetamine and methamphetamine with stimulant properties. Substances such as 6-(2-aminopropyl)benzofuran (6-APB, benzofury) and 5-(2-Aminopropyl)indole (5-API, 5-IT) are phenylethylamines with empathogenic effects.

Arylcyclohexylamines:

These compounds are structural analogs of phencyclidine (PCP) and ketamine [Morris 2014]. They exert dissociative effects by, at least in part, antagonism of the NMDA receptor. Examples of this category of NPS are 3-MeO-PCP, 3-MeO-PCPr, and Methoxetamine (MXE).

Tryptamines:

Substituted tryptamines are psychedelic substances structurally related to psilocybin. This group of NPS contains compounds such as dimethyltryptamine (DMT), alpha-methyltryptamine (AMT), 5-MeO-DMT, and 5-MeO-DALT

Aminoindanes:

Aminoindanes have entactogenic and empathogenic effects similar to MDMA. This group contains substances such as 5,6-methylenedioxy-2-aminoindane (MDAI) and 2-aminoindane (2-AI).

Plant extracts:

Extracts or parts of tropical plants have recently gained popularity thanks to their psychoactive properties and have hence been classified as NPS. Kratom (*Mitragyna speciosa*) is a tropical tree whose leaves (and sometimes other parts of the plant) are used for their narcotic effects. Various alcaloids including mitragynine, mitraphylline and 7-hydroxymitragynine are responsible for the pharmacologic properties. Seeds of the climbing vine *Argyreia nervosa* (hawaiian baby woodrose) contain ergoline alkaloids, such as ergine ergometrine, lysergic acid, that produce psychedelic effects. The beans of the tree *Anadenanthera peregrine* (yopo) are used for their hallucinogenic properties due to the presence of tryptamine-related compounds, particularly bufotenin, dimethyltryptamine and 5-MeO-DMT.

13. Literature

13.1 Originals

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ISBN 978-3-410-20070-3. English-French version:
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13.3 Websites

13.3.1 Guidelines of Other Institutions:

Substance Abuse and Mental Health Services Administration (SAMHSA), www.samhsa.gov

Forensic Toxicology Laboratory Guidelines, Society of Forensic Toxicologists (SOFT):
http://www.soft-tox.org

Gesellschaft für Toxikologische und Forensische Chemie (GTFCh): http://www.gtfch.org

Mandatory Guidelines for Federal Workplace Drug Testing Programs, Substance Abuse and Mental Health Services Administration (SAMHSA): https://www.samhsa.gov/workplace/resources

Substance Abuse and Mental Health Services Administration (SAMHSA): https://www.samhsa.gov/

Kriterien zum Betreiben von med.-analyt. Labors: https://www.sulm.ch/d/qualitaetssicherung/kbmal-3-0

Schweizerische Kommission für Qualitätssicherung im medizinischen Labor (QUALAB): http://www.qualab.ch

Correctional Service of Canada (CSC): https://www.csc-scc.gc.ca/index-en.shtml

Correctional Service of Canada (CSC), Urinalysis Testing: https://www.csc-scc.gc.ca/politiques-et-lois/566-10-cd-eng.shtml

European Guidelines for Workplace Drug Testing in Urine (EWDTS):
http://www.ewdts.org/13.3.2General Information About Drugs of Abuse and Drugs of Abuse Testing

Infos Drugs and Drug Screening: http://www.drogenscreening.info

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). .
http://www.emcdda.europa.eu/

Obervatoire Français des Drogues et des Toxicomanies: https://www.ofdt.fr

Party Project: http://www.party-project.de

Drugs: http://www.drogen-wissen.de/

Erowid: http://www.erowid.org

Streetwork: https://www.saferparty.ch/allgemein.html

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Annex 1 : Metrology terms for verification, validation and qualification of testing procedures

The following terms are to be understood as quality metrics for the methods and tests implemented and, as such, serve to document the suitability of analysis procedures for their intended purpose. References to the International Vocabulary of Basic and General Terms in Metrology (JCGM 200:2012) are mentioned in parentheses.

Trueness (VIM 2.14)

Trueness describes the closeness of agreement between the average of a infinite number of replicate measured value and a reference value. The trueness of the results of the immunochemical methods covered in these guidelines is influenced by various factors:

- Biological matrix
- Interference (documented as “selectivity”)
- Cross-reactivity (documented as “specificity”)
- Differing reactivity responses as a function of antibody concentration and affinity in the case of substance-group testing.

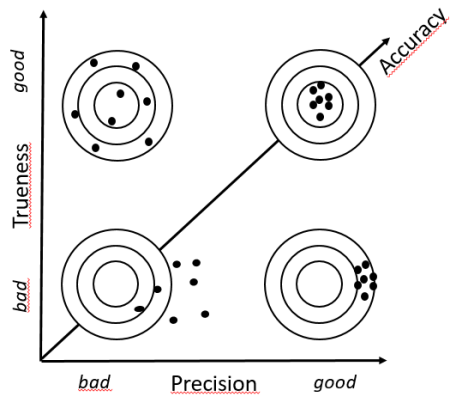
Precision (VIM 2.15)

Precision describes closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. It quantifies the random deviation of the values that are near the mean. Precision is usually expressed in terms of “imprecision” and is calculated as a standard deviation, or as a coefficient of variation of the readings obtained. High imprecision is expressed as a large standard deviation. A distinction is made between repeatability, laboratory precision, and comparative precision

Repeatability (in a series) describes the extent of agreement of repeated measurements of one and the same quantity, performed under the same experimental conditions. It is a measure of the random error component in a quantitative experiment.
Laboratory precision is obtained from a determination of one and the same sample within a laboratory, with the intentional change of one parameter (for instance, a person, an instrument, time of analysis, internal quality control on a day- to-day basis to the next).
Comparative precision describes precision under conditions such that readings are obtained by different individuals using different instruments, the same method, and identical sample sources in different laboratories (external quality control).

Accuracy (VIM 2.13)

Accuracy (measurement accuracy) describes
closeness of agreement between a measured quantity value and a true quantity value of a measurand. It is qualified by a systematic (trueness) and a random (precision) error.
(See also diagnostic accuracy)



Measurement error (VIM 2.16)

Measurement error describes the difference between the measured quantity value and a reference value. It can be used to express the difference between a measured quantity and an expected value, for instance in the case of a measurement of a calibrator for which one suppose a negligible measurement uncertainty. In this case, the error is known.

Systematic measurement error (VIM 2.17)

Component of measurement error that in replicate measurements remains constant or varies in a predictable manner. In this case, the reference value can be a true (and known) quantity value, a measure quantity value of a measurement standard or a conventional value.

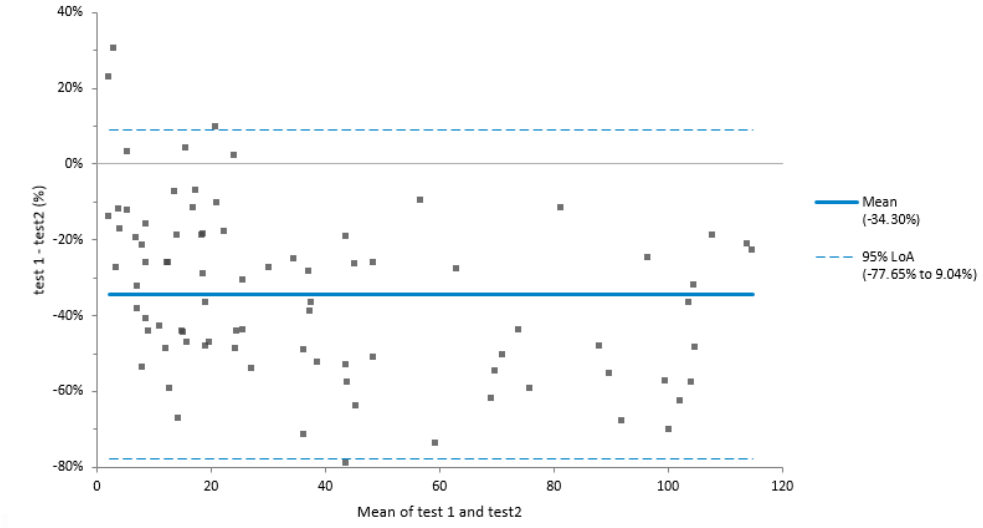
Bias

Estimate of a systematic measurement error:

A Bias can be highlighted and characterized using a Bland-Altman plot (or difference plot).
This type of graph displays a scatter diagram of the differences plotted against the averages of the two measurements.

The mean difference and limits of agreement (LoA) are represented as horizontal lines.

The limits of agreement (LoA) are commonly calculated as the mean difference ± 1.96 (or two) Standard deviations (SD, see also Precision) of differences. They represent the borders of the 95% confidence interval of the agreement between the measurements.



Measurement Uncertainty (VIM 2.26)

Measurement uncertainty is a parameter of a result and signifies the dispersion of the values attributed to a measured quantity.
It can comprise the uncertainties in the different steps of an analysis:

- Sample collection
- Condition of the sample
- Sample preparation
- Size of an aliquot of the sample
- Calibration
- Reference materials
- Equipment and instruments
- Environmental conditions and tampering.

Estimation of measurement uncertainty can be specified, e.g., by way of inter-laboratory tests or with the aid of the laboratory precision computed from control samples. A standard measurement

uncertainty results from the standard deviation for the measurement of quality control material across measurement days.
Measurement uncertainty constitutes an important parameter for all analyses. The narrower the range of values for a correct measurement happens to be, the more powerful is the analysis procedure [DIN 13005, Eurachem Guidelines, International Vocabulary of Metrology].

Selectivity (VIM 4.123) (Interference)

Selectivity is the capability of a measuring system to discriminate and unequivocally identify different analytes without interfering with each other or without interference due to other endogenous or exogenous substances (metabolites, contamination, degradation products, matrix).

Limit of Detection (VIM 4.18)

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample for which the defined probability criteria are met. Neither qualitative nor quantitative results that are below this concentration are to be reported.
The limit of detection depends on:

- the analyte being sought
- the analysis method used
- the extraction that has been performed
- any possible matrix effects
- the noise level of the instrument.

Lower Limit of Quantification

The lower limit of quantification (LLOQ) is the lowest concentration of an analyte in a sample matrix that can be determined with an acceptable measurement uncertainty (bias and imprecision), with a predefined performance uncertainty Values below the lower limit of quantification can only be interpreted on a qualitative basis.

Sensitivity (VIM 4.12)

Sensitivity is defined as the quotient of the change in the signal of a measuring system and the change in the concentration of the substance measured. In a linear relationship, this corresponds to the sensitivity of the slope of the calibration curve. Sensitivity can be a function of the concentration of the measured substance.

The “Cut-off”

So-called cut-off values (defined decision limits with respect to a measured quantity) are established in order to distinguish a positive result from a negative one. In group tests, a cut-off value applies to the substance used to calibrate the test procedure. The cut-off is usually set several times higher than the detection or measuring limit in order to prevent “false positive” results.

Classification in a binary diagnostic test

Figure 11: Distribution of population descriptives in a diagnostic test. TP are true positives, TN are true negatives, FP are false positives and FN are false negatives

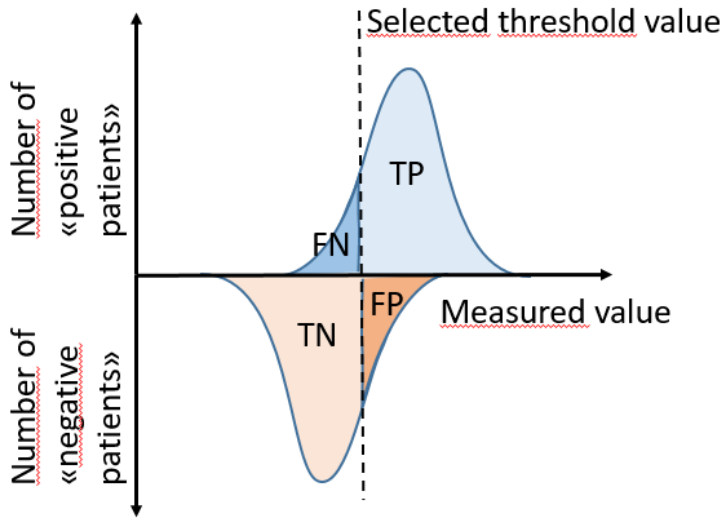


Figure 11 describes the distribution of TP, TN, FP, FN in a diagnostic test based on a quantitative measured value, as function of a given threshold value. The blue distribution represents the measured value for the positive test (affected patients for instance) and the orange distribution represents the measured values for the negative test (healthy or non-affected individuals for instance).

The performance characteristics of the test (diagnostic sensitivity, diagnostic specificity, diagnostic accuracy, etc.) can be measured from these four populations.

Diagnostic Sensitivity

Diagnostic sensitivity is a statistical quantity that describes the probability according to which a true positive fact will be recognized as being positive. It is also called recall or true positive rate.

Diagnostic Sensitivity =TP / (TP + FN)

Where TP and FN are the number of true positives and false negatives, respectively.

Analytical Specificity

Specificity is the ability of a method to determine an analyte or a substance class without being influenced by any adulterating compounds in the sample, thus identifying them conclusively.
Depending on the technology, different compounds sharing structural similarities can mimic positive results.
The lower limit of quantification in a quantitative confirmation method should therefore be lower than the one in the preceding test (screening test).

Diagnostic Specificity

Diagnostic specificity is a statistical quantity (hit rate) describing the probability with which a truly negative factor will be recognized as being negative in a test.

Diagnostic specificity =TN / (TN + FP)

Where FP and TN are the number of false positives and true negatives, respectively

Diagnostic Accuracy

Diagnostic accuracy = (TP + TN) / (TP + TN + FP + FN)

Where TP, FN, FP and TN are the number of true positives, false negatives, false positives and true negatives, respectively.

Stability

The chemical stability of an analyte in a given matrix under specific conditions should be ensured from the time instant of sample collection until the conclusion of the analysis.
Stability during storage and during possible refreezing and repeated thawing phases is independent of the method. Therefore, corresponding stability data can be carried over from the literature. The data must be collected in conjunction with method validation, should this information be unavailable.