

Guideline for quality control in forensic-toxicological analyses

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Purview (Validity)

Forensic-toxicological investigations for the qualitative identification and the quantitative determination of pharmaceutical substances, addictive drugs or other chemical or exogenous substances are particularly carried out in the context of judicature (legally relevant facts regarding crime, offence, insurance and administration), but also in therapeutic care (clinical toxicology).

For investigations in the context of clinical toxicology, primarily the guideline of the German Medical Association on the quality control of medical laboratory testings (Rilibaek) is to be followed. For the toxicological analysis in the context of brain death diagnosis and for the validation of such methods, recommendations were released by the Scientific Committee Clinical Toxicology of the GTFCh.

Standards for forensic-chemical testing are recorded in a separate GTFCh guideline.

General requirements for the competence of testing and calibration laboratories are to be found as basis for accreditation in the respective valid version of DIN norm EN ISO/IEC 17025. These are observed in the present guidelines. The quality standards described in this guideline apply when laboratories collect findings that are to be valid for legal purposes. The results of these investigations must be corroborated with appropriate proven specific methods and the necessary standard should be ensured through regular internal and external quality controls. Basically, toxicological testing may also become relevant in the context of therapeutic care.

Areas of application are in particular:

- General unknown screening in biological matrices when a person may be under the influence of drugs, toxins or addictive substances, e.g. in the context of traffic or criminal offences and criminal acts,

- Targeted forensic-toxicological analyses, particularly quantitative determinations, e.g. of addictive or medical substances in various biomatrices,
- toxicological analyses in investigations on the cause of death (postmortem toxicology),
- toxicological analyses in the assessment of fitness to drive .

Special requirements for the analysis of hair will be discussed in appendix C, those on the analysis of substances other than alcohol (fusel alcohols) with headspace-gas chromatography in biological material will be addressed in appendix E. Recommendations on the collection of autopsy material for forensic-toxicological analyses and special aspects of post-mortem analysis are recorded in appendix D.

1. General measures for quality control

Within the frame of quality control, procedures are to be determined and implemented in a quality management handbook which should contain objectives, fields of duty, responsibilities, competencies, and procedure control of a laboratory. Fields of duty must be defined, responsibilities and competencies should be determined, coordinated, laid down and be evident from an organisation chart.

Laboratories must guarantee that analyses are carried out with state-of-the-art analytical techniques. Methods of analysis should be laid down in Standard Operating Procedures (SOPs). In principle, the laboratory may choose the methods to be applied. Depending on the problem, targeted or non-targeted methods may be used, but it must be guaranteed that the result is reliable.

1.1 Personnel qualifications

The supervisor of the laboratory where the designated tests are carried out should possess a university degree in life sciences or medicine, preferably a PhD, relevant further education and forensic-toxicological experience. This may be demonstrated by e.g. by the GTFCh specialist title in the designated field of work ("Forensic toxicologist GTFCh" or „Forensic chemist GTFCh“ with the work field "forensic-chemical analyses of body fluids and other biological material of living persons"), or by habilitation in forensic toxicology. Otherwise, a comparable qualification is necessary (see the GTFCh Catalogues for the acquisition of a specialist title).

The laboratory supervisor and/or his/her representative should ensure the supervision of all activities. At least one further qualified member of staff should be authorised for supervision and implementation of quality control methods. For the technical personnel a qualified professional education in the field of laboratory work is required. Policies for stand-in or supply personnel for all members of staff should be recorded in a quality management handbook. The laboratory supervisor or his/her representative should also arrange for regular subject-related further education for all members of staff.

1.2 Laboratory layout

It must be guaranteed that unauthorised persons do not have access to the laboratory rooms. Unauthorised persons may only visit the laboratory if accompanied by authorised

personnel. The laboratory space must be large enough to accommodate adequate laboratory equipment for the clear identification and quantitative determination of individual substances. Substance samples and biological material must be processed in separate laboratory rooms; contamination must be excluded. For proper storage of analytical standards and samples and their protection from unauthorised access, sufficient cooling and deep freeze units should be available.

1.3 Requirements for technical appliances

In a forensic-toxicological laboratory appliances must be available which enable an unambiguous identification of individual substances as well as the exact determination of their concentration (qualitative and quantitative analysis).

Apart from the basic equipment, the presently required appliances in an analytic laboratory generally consist of

- Gas chromatography with special detectors such as a nitrogen-specific detector, electron capture and flame ionisation detectors or a mass spectrometer,
- high performance liquid chromatography with special detectors such as a diode array detector, UV- and fluorescence detectors or a mass spectrometer,
- immunochemical and photometric analyses.

Other procedures or equipment which deliver equivalent results may be applied.

1.4 Measures for laboratory and equipment safety

Equipment applied for laboratory tasks must be kept in good working order (fully functional), must be regularly maintained, calibrated, and gauged if necessary. Manufacturers' operating instructions should be observed. It is necessary to keep logbooks of use and maintenance. The laboratory's safety equipment must be available according to regulations and has to be regularly inspected. Safety regulations for any laboratory activity including the potential handling of radioactive substances must be adhered to. Special instruction of technical personnel is necessary regarding the handling of infectious material, narcotics and hazardous substances and their proper disposal. Instructions on safety regulations should be performed regularly and documented.

2. Requirements concerning samples and their handling

2.1 Requirements for sample collection and transport

If not regulated by adequate guidelines or recommended otherwise within the frame of these guidelines, the testing laboratory informs the applicant about the type, quantity, storage conditions and transport conditions of the necessary sample materials, in order to guarantee an adequate investigation.

The sample containers must be suitable for the relevant samples and the sample preparations (clean, sufficient size, glass or synthetic material with adequate lids, adequate diameter for sample removal or, if necessary, for serum separator).

Within Germany (applicant) forensic-toxicological testing is not performed in whole blood, but in serum or plasma (if this can be obtained from the sample). For toxicological testing with or without determination of the blood alcohol concentration, a blood sample without additives as well as a blood sample with fluoride (especially for the determination of cocaine) should be used to obtain serum or plasma. It must be stated in the expert report whether whole blood or serum/plasma was used. The word "blood" is insufficient.

With the collection of urine samples, special measures must be observed, depending on the request, such as collection under visual control in drug abstinence checks (compare also evaluation criteria on fitness to drive diagnostics).

The applicant should be informed that the sample and the application form should be labelled clearly and completely. Date and time of sample collection, type of sample material, type of investigation including research question and case history should be stated in the request.

For transportation, the sample material should be packed in shockproof and tightly sealed containers. Exclusion of heat and light exposure must be guaranteed. The research question and corresponding analyses determine how quickly the sample should be transported and if special transport conditions (e.g. deep freezing) apply. Regulations of the ADR (European treaty on the international transport of hazardous goods on the road) must be observed by the sender.

Special recommendations for the collection of hair are covered in appendix C, those on collection of autopsy material for forensic-toxicological analyses are covered in appendix D.

2.2 Receipt of samples

All incoming orders and samples must be registered by the laboratory. Incoming samples are immediately checked regarding completeness, intactness and suitability for testing. Unlabelled or insufficiently marked samples should be sufficiently labelled (identified). Samples not clearly assignable will not be processed and sent back where appropriate. This should be recorded in the laboratory files and the applicant must be informed. Each order and the accompanying samples must get a laboratory-internal code and be clearly marked (e.g. with a barcode). A mix-up of samples in the laboratory must be ruled out. Amounts and characteristics of samples must be documented. In case of body fluids, syringes etc. the material should be regarded as potentially infectious.

The regulations of the data protection act should be observed. All persons dealing with the samples or working in the laboratory should be instructed regarding confidentiality. These instructions should be set down in writing.

The laboratory immediately informs the applicant in case the sample is damaged, the sample is unsuitable for testing, the sample amount is too small for the research problem, or in case the required analysis cannot be carried out by this laboratory.

2.3 Sample storage

Measures must be taken to prevent unauthorized persons from gaining access to samples

and to guarantee that samples cannot be stolen, tampered with or manipulated.

After receipt, samples should be stored so that analytes do not decompose and samples are not contaminated.

The identity of the sample and derivatives obtained by processing (extracts) should be ensured by correct labelling at all stages of the analysis. In every stage, the person in charge of dealing with the sample material must be sure to use the correct internal code labelling, e.g. when issuing work lists or results protocols. Documentation must clearly show which persons were involved at which stage of the process.

After completion of the analysis and the final report, remains of sample material and original containers (blood withdrawal systems, tubes, containers etc.) should be stored according to the applicable administrative regulations, however at least for six months, and blood samples for two years. In general, a storage period of 2 years is advised. If other laws apply, these should be followed. The applicant should be informed of the storage period. Original containers including all sample remains must be presented on demand.

Body fluids should always be refrigerated. For requests originating from Germany, blood samples should be centrifuged as soon as possible after receipt in the laboratory. Suitable serum separators can be employed. If testing is not performed immediately, one or more parts of the serum or plasma should be transferred to appropriate containers. This should be done in the presence of two persons ("four-eyes-principle") and should be recorded in a written document. If no fluoride blood withdrawal system was used, then - at least for the determination of cocaine - the separated part should be mixed with e.g. sodium or potassium fluoride (at least 0.25 % w/v, equates to 2.5 mg/mL). By addition of fluoride, the in vitro degradation of cocaine, flunitrazepam and other substances is counteracted.

The separated serum or plasma sample or – if no serum or plasma can be extracted – a part of the full blood sample (homogenised beforehand) should immediately be deep frozen (at least – 15°C) in order to avoid ageing of the sample matrix and loss of analytes. The original blood withdrawal system containing the remaining blood should be kept refrigerated. It should be traceable if and how much serum or plasma has been removed, for instance by a mark on the container.

Urine samples or aliquots thereof should be deep frozen and stored after receipt. All other testing materials should also be adequately preserved.

3 Requirements for immuno-assays

Investigations can be divided into indicative and confirmatory (evidential) analyses. Indicative analyses are immuno-chemical test procedures and simple chromatographic techniques. In itself, positive results of indicative analyses cannot be used as evidence in court and should be confirmed by a second independent specific confirmatory method with at least equivalent sensitivity. An immuno-chemical test result cannot be confirmed by a second immunoassay.

Results of monospecific immunoassays e.g. on pharmaceutical substances like paracetamol and valproic acid in plasma/serum may constitute an exception in certain questions. With appropriate calibration, quantitative values can also be accepted here.

Calibrators and controls should be established with the authentic matrix if available. In principle, calibrators should be produced using whichever analyte (original substance or metabolite) of the substance class to be tested is expected in the relevant matrix.

In some substance classes, an (enzymatic) hydrolysis of the phase II-conjugate should be performed prior to analysis, in order to obtain the necessary sensitivity. Particularly for the determination of benzodiazepines in urine a hydrolysis of the phase II-conjugation is required.

Cut-off-values (defined decisive values regarding the measurement result) are used to discriminate between positive and negative immunoassay findings. In this way, false positive results due to matrix effects would be avoided. Since preliminary immunochemical tests serve as a selection for confirmation by chromatographic methods, in particular false negative results should be avoided, by respecting the applicable cut-off-values to be set for the relevant analytes or substance class. An immunoassay result is false negative in terms of these guidelines when it is below the cut-off, though relevant analytes can be detected above defined limits with the confirmatory method. (also see appendix A).

Cut-off values should be set fairly low, since apart from proof of an acute influence, proof of less recent consumption or evidence of abstinence can also often be the issue. Thus, the cut-off values suggested by producers of immuno-chemical tests are often too high for forensic analyses. From a scientific point of view it is fundamentally not possible to stipulate general fixed cut-off values for immuno-chemical methods, since they depend strongly on the test employed, relevant antibodies and cross-reactivities of structure-related substances.

Every laboratory must check whether the cut-off values of the immuno-chemical methods are adequately chosen to discriminate between "positive" and "negative". Authentic samples should show a positive result in the immuno-chemical pre-testing procedure at analyte concentrations at the forensically required limit of the identifying chromatographic method, in the appropriate matrix. More precise requirements can be found in appendix B of the guideline (validation). For certain analytes, the maximum detection and quantitation limits in various biological matrices are given in appendix A.

4 Confirmatory and quantitative analyses

Confirmatory methods should give evidence on the structure of the analyte. The confirmatory analysis should lead to a sound and at least qualitative identification of each substance. This is generally effected by a gaschromatographic (GC) or by a high performance liquid chromatographic (HPLC) method coupled with a spectrometric procedure. The performance of analytical systems (sensitivity, stability of retention times, mass accuracy) should be checked regularly by injection of a suitable mixture of test substances in order to exclude false negative results (see also chapters 4.2.1 and 4.2.2). Requirements on the validation of analytical methods should be observed (see appendix B).

When performing a systematic-toxicological analysis (STA) in biomatrices, the substance identification should take place within the context of the case. This identification has to be performed or checked by qualified persons with analytical and toxicological knowledge. These persons should respect the possibilities and limits of the procedure and should be able to critically evaluate the results, in connection with results of other methods and the

respective problem definition. Generally, phase I metabolites should be included in urine analyses since the mother substances in urine are often present in very low concentrations. An enzymatic or acidic hydrolysis for cleavage of phase II conjugates should be performed prior to the STA in urine unless glucuronides and sulphates are detected as such.

4.1 Sample preparation

The sample material should be used economically in order to enable follow-up examinations when the problem definition is extended or when counter-expertise is required.

For the quantitative proof of narcotics, amongst others with GC-MS (mass spectrometry) or LC-UV or LC-MS (e.g. narcotics in blood, serum or plasma), these are generally isolated from the biological sample material, e.g. by liquid-liquid or solid phase extraction, and derivatised if applicable.

4.1.1 Selection of internal standard

Especially for quantitative analyses, internal standards should be used. These are to be added to the assay before extraction in a sufficient but not too high concentration.

A great advantage of mass spectrometry is the opportunity to use deuterated analogues of the analytes to be quantified, which have physical-chemical characteristics that are very similar to those of the relevant analytes. If possible, deuterated standards should be used for all substances to be quantified. It should be guaranteed that deuterated substances do not contain a non-deuterated part of the molecule that could falsify the result. The number and position of hydrogen atoms in the molecule replaced by deuterium should lead to mass fragments which are unequivocally distinguishable from those of the undeuterated compound. In order to avoid a false result, ions which are present in the mass spectrum of the deuterated standard should not be selected as target of qualifier ions of the analyte and vice versa. The degree of deuteration should be at least 3. These specifications are valid in a similar way for the use of other isotope-marked Internal Standards.

When using non-deuterated standards (i.e. in HPLC-DAD (diode array detection/UV), these should have similar physical-chemical properties as the analytes. However, a chromatographic separation should be possible, at least in case of UV-detection. Substances that may be present in real samples (e.g. pharmaceutical substances, narcotics and nutritional components) should not be used as internal standards. In addition, screening procedures (STA) should contain an Internal Standard in order to check e.g. extraction and derivatisation.

4.1.2 Extraction and derivatisation

For sample preparation, methods such as liquid liquid extraction (LLE) or solid phase extraction (SPE) as well as protein precipitation can be applied. Directions and requirements for recovery and extraction efficiencies can be taken from appendix B (validation).

All reagents, sample containers etcetera should be checked for interfering signals. Regularly occurring, matrix-related interfering signals (endogenous substances, putrefactive products and decomposition products) should be eliminated as much as possible, since they may mask the analytes to be detected (UV-detection) or alter the signal intensity of the analyte (ion suppression/enhancement, adduct formation).

Derivatisation can be carried out in order to improve the analytical properties. The possible formation of artefacts that could falsify the result should be taken into account.

4.2 Chromatographic separation: gas chromatography (GC) and high performance liquid chromatography (HPLC)

The identification of substances or substance mixtures from biological matrix normally requires the primary application of a chromatographic separation. Methods available are primarily gas chromatography and liquid chromatography.

Capillary gas chromatography offers a high separation performance and is therefore very well suited for separating chemically similar substances of a substance class or metabolites. Chromatographic separation on the HPLC-column can be performed isocratically or with a mobile phase gradient.

Great emphasis should be placed on a high chromatographic resolution and low signal width without tailing. The signals of each ion- and especially UV-chromatogram should be separated chromatographically from other substances and matrix components.

One criterion for the positive identification of a substance via chromatographic analysis is the comparability of retention times (see table 1) and peak forms (symmetry, width) of all signals belonging to this unknown substance with the characteristics of a reference sample. The chromatographic retention time (RT) of the analyte or the quotient of the retention times of the analyte and the Internal Standard, i.e. the relative retention time (RRT) of the analyte should comply with that of a reference solution analysed in the same analytical run (matrix sample spiked with pure substance or external reference sample). Table 1 describes the accepted tolerance values. Deviations from retention times and/or altered peak forms (shoulder, tailing) are acceptable as long as these changes can be explained e.g. by a recognisable co-elution of matrix components, sample overload or other means.

Analytical methods with a high identification character (i.e. full scan MS-analyses) are excluded from these strict regulations; however, the RT or RRT should be in the same range as in a reference sample or as stated in a database.

If the retention data given in an HPLC-DAD spectrum library are used for identification, all chromatographic conditions (composition of the mobile phase (especially pH-value), temperature stability, stationary phase, flow, retention-standard substances) should be observed. Due to fluctuating analysis conditions, a certain deviation of the retention time (e.g. $\pm 15\%$) from that given in the database can be tolerated in the substance search. For such large deviations, a direct comparative measurement of the respective reference substances should be done for reliable identification.

Table 1: Requirements on the repeatability of the absolute (RT) or relative (RRT) retention time for analytical methods with limited identification character

Chromatographic separation	Acceptable tolerance	
	Δ RRT*	Δ RT**
Liquid chromatography	$\pm 2.5\%$	$\pm 5\%$
Gas chromatography	$\pm 1\%$	$\pm 2\%$

* Relative retention time of the substance relative to the Internal Standard compared to a reference sample

** Retention time of the substance compared to a reference sample measured shortly before or after under similar conditions

Regular maintenance of the gas chromatographs and HPLC-equipment and checking the chromatographic separation is necessary. For targeted analyses, functionality can be tested with a QC-sample. For screening procedures a standard test mix with substances of different chromatographic properties is recommended, which permits the localisation of sources of error upon regular injection per measurement day..

4.2.1 Performance control of GC equipment

The following standard test mixture can be used e.g. for GC-screening procedures:

A test mix consisting of valproic acid, metamfepramone, phenobarbitone, pentobarbitone, methaqualone, nalorphine, codeine, morphine, quinine, strychnine, diphenhydramine, haloperidol and acetylated amphetamine as well as C₄₀, 0.05 mg/mL respectively, injection volume 1 μ l (see Maurer, Pflieger, Weber)

Evaluation criteria are:

- valproic acid should not be in solvent delay
- the intensity of the morphine peak should not be less than 15% of the codeine peak
- C₄₀ must be present in the chromatogram
- all peaks must be base-line separated from each other
- all peaks should preferably show a symmetric peak form (except valproic acid)

4.2.2 Performance control of HPLC equipment

The following standard test mixture may be used for HPLC-DAD screening methods (e.g. in the acidic isocratic mobile phase (acetonitrile/phosphate buffer pH 2.3, 37:63 v/v) and, when using an RP8-column, as follows (injection 10 μ l):

- Histaminedihydrochloride (0.1 mg/mL) for dead time control
- Caffeine (0.1 mg/mL) for peak area control

- 5-p-methylphenyl-5-phenylhydantoin (MPPH; 0.1 mg/mL) as retention time standard
- Benzene (1 mg/mL, addition of 11.4 µl per 10 mL test solution via micro litre syringe) for control of the wavelength accuracy of the DAD (see chapter 4.4.2)

The composition of the solution should be such that the obtained chromatogram provides information on the accuracy of the following parameters:

- Flow velocity of the mobile phase (HPLC-pump)
- Injected volume of the sample solution (auto injection)
- Dead time and chromatographic separation capability (HPLC-column)
- Transparency of the mobile phase in the complete wavelength area (mobile phase and degassing)

With regard to following parameters, the chromatogram should be compared with set values:

- Retention times of peaks (daily)
- Half width value and symmetry of the peaks (weekly)

Results should be documented.

For LC-MS equipment a suitable test mixture should be applied in the relevant investigation.

The mobile phase(s) employed in the HPLC should have a constant composition. For each run, especially the pH-value should be checked and adjusted by a pH-meter since it has considerable influence on the UV-spectra.

4.3 Mass spectrometric (MS) detection

For quality control of mass spectrometric analyses it is necessary that the user is aware of the limits and possibilities of the analysis and the equipment and operates the equipment accordingly. This includes regular adjusting and checking of the equipment parameters (tuning): regular check and, if necessary, calibration of the mass axis, setting up the required mass spectrometric resolution and controlling the mass spectrometric sensitivity. With the help of the test mixture used as a control of chromatographic separation, the sensitivity of the mass spectrometer can also be tested.

4.3.1 Ionisation techniques

The type of ionisation is not subject to basic restrictions and should be adjusted to the analyte's requirements in view to ionizability and stability (polarity, ionisation energy, fragmentation). The ionisation process should lead to the reproducible formation of diagnostic ions in sufficient quantity, selectivity and intensity.

At present, electron impact ionisation (EI) is the most frequently used ionisation technique in GC-MS for identification of the indicated substance groups in biological matrices. For special investigations, the use of chemical ionisation with negative or positive ions (NICI or PICI) can be advantageous.

One benefit of the liquid chromatography-mass spectrometry (LC-MS) is the simplified sample preparation since generally no derivatisation step is necessary. The most common types of ionisation are electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). When using LC-MS techniques, particular attention should be paid to the matrix effects which can suppress or enhance the signal of the analyte.

4.3.2 Identification criteria in MS-detection

As a minimum requirement for identification, each diagnostic ion should form a chromatographic peak with a height that is at least three times the background (signal-to-noise-ratio $\geq 3:1$).

The mass spectrometric identification of a substance should relate to a uniform species, e.g. molecule ion and associated fragment ions. As an alternative to the molecule ion, adducts (e.g. NH_4 , Na) or multiply charged ions can be analysed. Principally, an alternative method with respect to derivatisation or type of ionisation should supply *qualitatively* different diagnostic ions in order to be accepted as additional information.

4.3.2.1 Full scan MS detection

The full scan MS-modus is employed above all for broad screening analyses. These include database searches. When recording complete spectra, all measured ions that have a relative intensity of more than 10% in the reference spectrum should basically be present in the substance spectrum (molecule ion, characteristic adducts of the molecule ion, characteristic fragment ions and isotope ions). As a minimum requirement, four diagnostic ions from the reference spectrum, having at least 10% intensity (relative to the most intense ion), should be present in the MS. This should include the molecular ion, if this is present in the reference spectrum with a relative intensity of $\geq 10\%$. If the substance does not supply such an informative MS-spectrum, additional plausibility tests should be carried out (inclusion of retention indices, search for artefacts or metabolites, inclusion of preliminary test results etc.).

The occurrence of additional ions (missing in the reference spectrum) should be explainable by co-elution with matrix components.

When using a computer-aided database search (e.g. with the Maurer, Pflieger, Weber-database or Roesner, Junge, Westphal, Fritschi-database), potential hits should be checked by an experienced analyst for plausibility. Performing a background subtraction or deconvolution should be comprehensible and documented.

4.3.2.2 Single ion detection

When using a quadrupole mass spectrometer in quantitative analyses, single ion detection is superior to full scan analysis, due to a higher sensitivity and better statistical certainty.

The substance identification by means of single ion detection requires three structure-specific (diagnostic) ions per analyte with the corresponding signal-to-noise-ratio and the same retention time.

Where possible, the diagnostic ions should represent the total molecule and should therefore not originate solely from the same molecular fragment. Preferably, the molecular ion should be one of the diagnostic ions. “A+2” element isotope peaks (Cl, Br) are acceptable as diagnostic ions. “A+1” isotope peaks and fragments resulting from unspecific fragmentation reactions (e.g. M-H₂O, M-TMS) may be used only in addition to characteristic ions. Only in analytically justified exceptions the identification can be accomplished with only two ions.

One ion is used for identification and quantification (so-called target ion or quantifier), two further ions are used for identification only (so-called qualifiers). The target ion is usually, but not necessarily the ion with the highest signal. The ion signals used for quantification should not be prone to interference from foreign substances nor from the respective analogous (deuterated/undeuterated) compounds (observe isotope distribution!). When using deuterated Internal Standards, recording two SIM-traces or one mass transition is sufficient for the Internal Standard.

The intensity ratio of the selected ions is an important criterion for identification. The relative ion intensities (fragment ion-peak area or peak height ratios) should be the same as in a reference sample (QC-samples run in parallel, calibration standards, or spiked matrix sample in comparable concentrations, measured under the same analytical conditions). They are expressed as a percentage of the intensity (peak area or peak height) of the most intense ion (= 100%) or transition. Accepted tolerances can be found in table 2.

Table 2: Accepted tolerances of the relative intensities of diagnostic ions in different MS-techniques

Relative ion intensity	GC-EI-MS (relative*)	GC-CI-MS, GC-MS ⁿ LC-MS, LC-MS ^{n**} (relative*)
>50%	20%	20%
>20-50%	20%	25%
>10-20%	25%	30%
≤10%	50%	50%

* relative = referring to the value of the relative ion intensity

** n>1

In the case of larger deviations, the analysis should be repeated or it should be pointed out why a larger deviation in a single mass is acceptable. A possible plausibility control would be e.g. the presence of metabolites (with similar half-life periods) or the inclusion of results of other analyses.

If the ion ratios of both qualifier ion do not meet the requirements, the identity of the substance in that case is not verified, and, if necessary, the validity of the analysis method should be examined.

4.3.2.3 Tandem MS

Product ion scans

At least four diagnostic ions from the reference spectrum, having at least 10% intensity (relative to the most intensive ion) should be present in the MS. Exceptions should be justified. The occurrence of additional ions (missing in the reference spectrum) should be explainable by co-elution with matrix components.

When using a computer-aided database search, potential hits should be checked for plausibility by an experienced analyst. Background subtraction or deconvolution should be traceable.

Detection of multiple fragmentation reactions:

In multiple reaction monitoring mode (MRM), two mass transitions are regarded as sufficient for identification, provided that the relative fragment ion intensities are within the acceptable range (see table 2). The precursor ion (e.g. the pseudo molecular ion) can be identical as long as the product ions are sufficiently different with respect to their fragmentations.

If the ion intensity ratios show larger deviations, the analysis should be repeated or it should be stated why a higher deviation of a single mass is acceptable. A possible plausibility control is e.g. the presence of metabolites (with similar half-life periods) or the inclusion of results of other analyses.

4.4 UV-VIS detection diode array detection

4.4.1 General requirements

Identification is accomplished by using the retention time or a suitable related retention parameter (also see chapter 4.2) and the UV-spectrum. The UV-spectrum should match that of a reference substance analysed under the same conditions. There is also the option of comparison with a data base.

The HPLC-DAD ((photo)diode array-detection) -unit should be regularly serviced according to the manufacturer's guidelines. This necessarily includes a wavelength calibration and checking the DAD lamp energy.

4.4.2 Compound identification using DAD-detection

If the HPLC-DAD-unit is used for systematic toxicological analysis, the full functionality of all components should be checked and documented at the beginning of each workday, by measuring a suitable test solution (also see chapter 4.2.2).

The composition of the test solution should be such that with respect to UV-detection, the obtained chromatogram provides conclusive information on the accuracy of the following parameters:

- wavelength allocation/-accuracy (DAD)

- spectroscopic resolution (DAD)

Here, the use of benzene is recommended (see 4.2.2). Despite its toxicity, benzene was selected due to the distinct resonance structure of the longest wavelength aromatic absorption band. With the quantities used, risks are low.

The chromatogram has to be compared with target values regarding following parameters:

- correspondence of the UV-spectra with the reference spectra (spectrum overlay, similarity-index > 0.999)
- correctness of the λ_{\max} -values: the four strongest resonance bands of benzene should be around 242-243 nm, 247-248 nm, 253-254 nm, and 259-260 nm
- resolution of the resonance structure of benzene bands: the fine structure of the spectrum should be distinct. The extinction quotient E_{258}/E_{254} should be more than 2. This is often not achieved by older detectors. The crucial issue is a constant resolution.
- noise level within the entire wavelength range at high sensitivity. For this, a spectrum is evaluated at a peak free zone of the chromatogram, e.g. at 1 mAbs./full scale (1 mAbs. = 0.001 extinction units). The noise should be less than 0.05 mAbs. in the entire wavelength range.

The results should be documented.

In case of gradient elution, an analogous functionality test can be carried out with test solutions of alkaline and acidic drugs, according to a method of Bogusz and Erkens. In case of deviations, the sources of error must be eliminated immediately. The test chromatograms should be filed.

Prior to library search (e.g. with the Pragst data-base), each peak should be checked for consistency (purity) with the options offered by the DAD-software. If no consistent spectrum can be obtained from a peak, or if there are justified doubts regarding consistency, the analysis may have to be repeated under altered chromatographic conditions, in order to separate the superimposed peaks. In certain cases however, the evaluation of the spectrum at the flanks of the peak may be appropriate.

UV-spectra show very good reproducibility. Therefore identification requires a complete match of the whole spectrum (sample vs reference). Apart from a high similarity index (match angle) of the search result, this should always be confirmed by the analyst by visual control of the superimposed spectra. At very low concentrations, the sample spectrum may be distorted by noise. The concentration where the noise reaches 10% of the maximum extinction of the spectrum can be regarded as the lower identification limit. In cases of rather much noise, a higher substance amount can be injected for clarification.

UV-spectra have different specificities, depending on the spectral expansion in the wavelength range and the incidence of the underlying chromophor. If necessary, especially for spectra with low specificity, the identity should be confirmed by using other (different) methods, especially mass spectrometry.

If for library search a commercially available spectral library is used, it should be confirmed

beforehand that the mobile phase used, especially its pH-value, is the same as that of the library.

Generally the correlation of measured spectra should be compared with the library spectra using selected examples. For frequently occurring substances it is recommended to create one's own database, parallel to the commercially available libraries.

5 Aspects of quality assurance and quality control in quantitative determinations

Every peak to be quantified must be clearly identified beforehand; e.g. one single wave length is insufficient for identification. Quantification is normally performed on the basis of peak areas and peak heights.

In the case of UV-detection, a chromatographic separation of the analyte from other analytes and matrix components is a prerequisite for quantitative determination. For quantification, a wavelength is chosen where on one hand a high sensitivity is reached around the absorption maximum, and on the other hand interference from coeluting compounds is excluded.

In the case of mass spectrometric detection, care must be taken of the possible falsification of a quantitative result by influences on the ionisation process, particularly when analysing complex matrices. By using deuterated or structurally analogous Internal Standards with approximately equal retention times, these effects can partly be compensated.

Principally, a quantitative method must be validated according to the current guidelines (appendix B). In case of infrequently occurring substances, it is not always necessary to perform a complete validation. For semi-quantitative measurements, a matrix-based one-point-calibration is sufficient.

Particularly in the case of postmortem analyses, a directly comparable reference matrix is often not available, which means that validation is impossible. In these cases, the standard addition method can be used to obtain a semi-quantitative result. In this procedure, the calibration curve is generated directly in the present sample matrix, enabling an individual correction for matrix characteristics (including post mortem changes in postmortem analyses). With this semi-quantitative method, the sample is processed and measured as such and after adding defined amounts of the substance to be determined, using the same procedure. An added concentration should approximately match the highest sample concentration to be expected. If the sample amount is sufficient, several different concentrations should be added. The original analyte concentration in the sample can be deduced by using linear regression. Prerequisites for the application of the standard addition method are a linear response of the analyte for the entire concentration range of the procedure, reliability of the added analyte concentrations, and homogeneity of the sample material.

5.1 Series of measurements and intra-laboratory quality control

Within a series of measurements, so-called quality control (QC) samples (external or internal control samples), blank samples and, if necessary, calibration standards should be

run in parallel along with the real samples.

In summary, per series of measurements the following is the minimum requirement: one blank sample, one low QC-sample and one high QC-sample. At least after 20 real samples a further QC-sample should be measured.

Forensic-toxicological analyses in biological matrices are normally carried out using a single determination.

5.1.1 Alternate injections of real samples and zero samples

A carry-over of the analyte from sample to the other should be excluded by appropriate measures. A carry-over from the injection of the first sample to the next can be avoided e.g. by injection of pure solvent or preferably a blank (zero) sample (processed matrix without analyte and Internal Standard), before each analysis of a real sample. A reduced program can be applied if the elution times of the respective analytes are known.

5.1.2 Calibration

A new calibration should be performed with at least five calibrators which cover the relevant concentration range. The zero sample should not be included in the calculation of the calibration curve, except in photometric methods. None of the calibrators are permitted below the quantitation limit. Calibrators are prepared by spiking the respective matrix, unless the validation (appendix B) showed that a calibration using solutions in solvent leads to the same results. The use of pure substances as reference material should be ensured. If injection of calibration standards is done in ascending order, an injection of solvent or of a zero sample should follow after the highest calibrator.

To verify the calibration, a retrospective calculation is performed for each of the calibrators by using the linear calibration functions (plot of measured signal such as e.g. peak area ratio versus specified concentration). The concentrations thus obtained should not deviate more than $\pm 15\%$ (or 20% at the quantification limit) from the nominal value. Calibrators with a higher deviation are eliminated. 75% of the calibrators - but a minimum of five - should be within the limits.

If the calibration meets the acceptance criteria, and the QC-samples of the respective sequence are within the limits (see below), a stored calibration can be used to calculate the concentrations of the samples.

After maintenance work that affects the system, such as a new column, cleaning of the source or complete retuning, the analyst should check that the quality control samples are within the acceptable limits, before using the stored calibration.

By using the calibration standards or the QC samples, the actual retention times and fragment ion ratios can be determined at different concentrations, for verification of the analyte identity in the samples.

5.1.3 Zero samples

These are processed matrix samples without analyte, but with added Internal Standard. This sample should be measured at least once within a series of measurements (sequence). It cannot contain any traces of analyte. In order to obtain evidence of analyte

carry-over, it is recommended to analyse a zero sample after the highest calibrator or the highest QC-sample.

5.1.4 Quality control (QC-) samples

Quality control samples should monitor and document the quality of measurements over the complete measurement series. Combined, they monitor the bias as well as precision.

5.1.4.1 Internal QC-samples

This refers to matrix spiked with known concentrations of analyte. If possible, certified weighed analyte solution should be employed for preparation of QC-samples. Preparation must be performed independent of the preparation of calibration samples. A preferably large pool (depending on the stability of the analyte) should be prepared and frozen after aliquotation. The homogeneity of the pool should be shown by measuring six different aliquots. The same acceptance criteria as for validation (see appendix B) are applicable, i.e. the average of the six measurements may deviate $\pm 15\%$ or $\pm 20\%$ (limit of determination) from the spiked target value (bias), the coefficient of variation RSD of the six values should also be $\leq 15\%$ (20% at the limit of determination).

Furthermore, the total error (combination of bias and precision), expressed as so-called 95% β -tolerance interval, should be within an acceptance interval of $\pm 30\%$. If bias and repeatability were done in a six-fold-determination on one day as suggested above, an estimation of the relevant β -tolerance interval can be obtained with the following approximation:

$$\begin{aligned}L_u[\%] &= \text{Bias} [\%] - 2.57 \times \text{RSD}_r [\%] \\L_o[\%] &= \text{Bias} [\%] + 2.57 \times \text{RSD}_r [\%]\end{aligned}$$

L_u lower limit of the 95% β -tolerance interval
 L_o upper limit of the 95% β -tolerance interval
(signs and symbols see appendix B, validation guideline)

If possible, the prepared QC-sample pool should also be tested by means of external reference material.

The storage life and/or usability of the frozen QC-pool must be determined by the laboratory and has to be indicated.

Sample preparation and measurement of the QC-samples is performed analogously to the calibration samples and real samples. The QC samples are prepared at two concentration levels at least. A sample close to the lowest calibrator should be injected at least once, e.g. at the beginning of the sequence, in order to test the sensitivity of the device. To recognise deterioration over the measurement period, it can be measured again towards the end of the sequence. Repeat measurements of a QC-standard should be done by injecting the same extract. However, splitting the standard into separate autosampler vials is recommended in order to avoid evaporation and thus concentration effects in the punctured (broached) vials. At least one further QC-sample must be in the higher calibration area (around 75% of the highest calibrator or higher). This QC-sample should also be measured at least once. At least after every 20 real samples, another QC-sample should be measured (alternating low and high). The analyte concentrations of the QC-

samples should not be the same as the calibrator concentrations. QC-samples as well as calibration samples may contain several analytes, as long as they do not interfere with each other.

The results should be documented on control charts. Acceptance criteria are defined in chapter 5.2.

5.1.4.2 External QC-samples

External control samples (certified reference material with known concentration, stability and confidence interval), if available, should be run at regular intervals (at least every fourth sequence). They are evaluated (analysed) the same way as the internal QC-samples (see chapter 5.2).

5.2. Control charts for QC-samples

A control chart must be issued per analyte, concentration, and measuring device. Any recalibration should be noted on the control chart. If QC-samples of the same concentration are measured by repeated analysis, it is sufficient to keep record of one sample on the control chart. It must, however, be determined beforehand which sample will be recorded. The results of the remaining QC-samples should be examined and documented.

5.2.1 Acceptance criteria

For the deviation of the measured value (actual value) from the reference value (target value) a maximum of $\pm 30\%$ (or $\pm 40\%$ at the detection limit) is tolerable since it contains systematic as well as random errors. This also applies to certified material.

The result of controls within routine analysis is immediately to be recorded on a laboratory control chart and is subject to a graphic-statistical test. The following parameters are recorded in tabular form and graphically on the laboratory control chart (see graphic example fig. 1):

- the target value (central line)
- warning limits ($\pm 30\%$ deviation from the target value or $\pm 40\%$ at the detection limit (DL))

The maximum tolerated deviation of the measured value must not be exceeded. All QC-samples of a measurement series should meet the requirements. If the deviation is higher, its cause should be identified, corrective action must be taken and, if necessary, the test series has to be repeated.

The analysis should also be checked if seven successive values increase or drop monotonously.

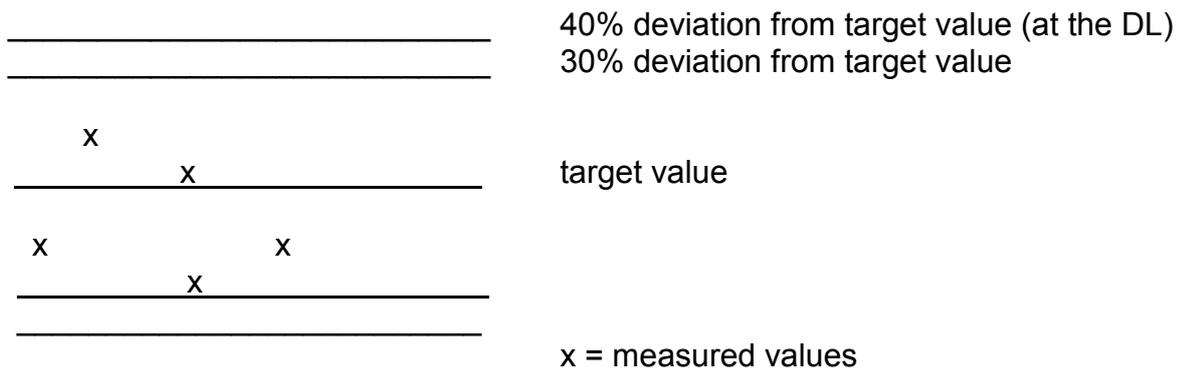


Fig. 1: example picture of a control chart

5.3 External quality control (collaborative testing)

External quality control is done by collaborative testing. Collaborative testing complements the laboratory-internal accuracy monitoring and simultaneously guarantees the objective supervision of accuracy or bias of the results of qualitative and quantitative forensic-toxicological analyses.

5.4 Measurement uncertainty

Measurement uncertainty is an important parameter for each analytical method. With its calculation according to a standardised procedure it is expressed that the degree of confidence (e.g. 95%) characterises the value range that can be ascribed to the value obtained by the measurement performed. The smaller the value range at a correct (accurate) measurement, the more powerful the analytical method (DIN 13005, Eurachem guide, Int. vocabulary of metrology).

The general basic principles for the determination of measurement uncertainty ensue from the "Guide to the Expression of Uncertainty in Measurement" (GUM), on which the EURACHEM/CITAC guide for chemical as well as the Nordtest Technical Report for environmental-analytical methods are based (DIN 13005, Eurachem Guide, Nordtest Technical Report). In these documents, the different methods for the detection of measurement uncertainty are described. These are all based on the principle that components of measurement uncertainty are determined or estimated and subsequently added to the total uncertainty of measurement according to the law of error propagation. In contrast to the "bottom-up approach" where all uncertainty components are determined one by one, in the "top-down approach" several uncertainty components can be determined together by using statistical methods.

Due to the complexity of bioanalytical methods and given the fact that with these, bias and precision offer by far the biggest contribution to the total uncertainty of measurement, the use of the "bottom-up approach" seems to make little sense. Thus, it is obvious to apply the alternative „top-down approach“ with such methods. Here, care must be taken to ensure that all relevant influencing factors are taken into account. While the precision component can be easily determined with self-prepared precision controls, the accuracy

component can only be determined by means of suitable reference samples or in collaborative testing. Based on these considerations, the estimation of measurement uncertainty by means of collaborative testing data and precision data, as suggested in the Nordtest-document and as specified below, offers the most practicable solution for toxicological analytical procedures

5.4.1 Estimation of measurement uncertainty via collaborative testing and intermediate precision determined from control samples

As an estimation, the averaged uncertainty values obtained via collaborative testing that contribute to the accuracy (Bias) are combined with the uncertainty of the target values that are established in collaborative testing (C_{ref}) and the precision of the control samples measured in the laboratory (Nordtest Technical report). The data for the precision control samples may be taken from the control charts. Reference material with preset target values is not required to estimate the contribution of intermediate laboratory precision to the total uncertainty. This procedure schematically corresponds to the cause-effect diagram shown below (fig. 2).

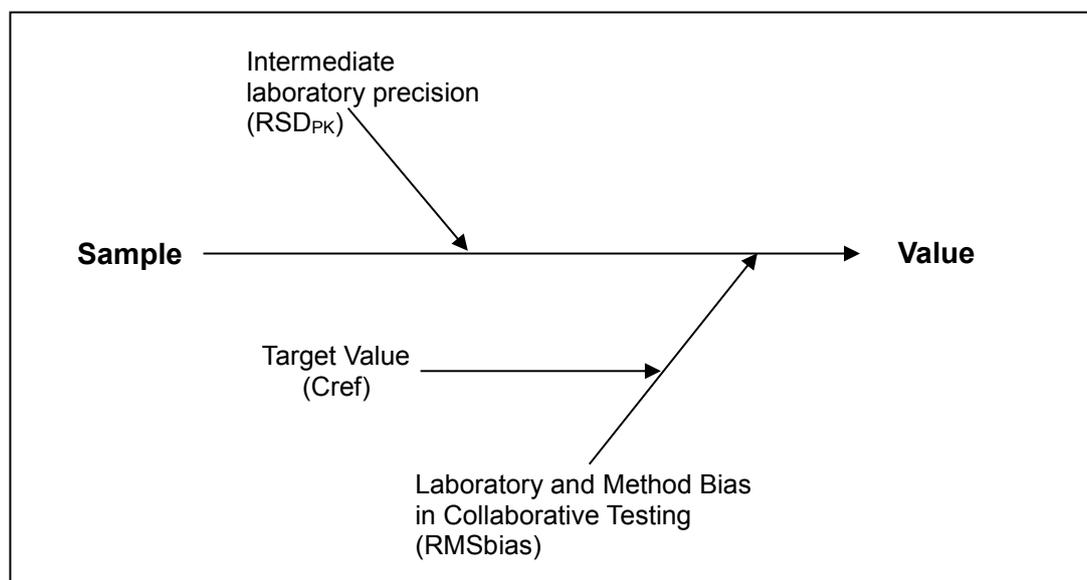


Fig. 2: Cause-effect diagram for estimation of measurement uncertainty by using the precision and accuracy obtained from collaborative testing results and repeatedly measured control material (Nordtest Technical Report)

5.4.1.1 Minimum requirements

Due to the practical fact that the measurement uncertainty is highest in the lower concentration range, it should at least be determined around the detection limit. For statistical purposes, a minimum number of values are required for determination. This means that a minimum of five consecutive collaborative testing results and at least eight consecutive single determinations of QC-samples from different measurement series should be used. With these data, measurement uncertainty can be determined (F. 1-5 in the formula index, chapter 5.4.3). Statistically significant outliers ($P=95\%$) can be eliminated if argued and substantiated.

5.4.1.2 Alternative approach

In the case that five collaborative testing results are not available, data can alternatively be replaced by measuring former collaborative testing material or certified reference material. Here it should also be observed that the measurements are carried out in different measurement series.

5.4.2 Estimation of measurement uncertainty in other cases

Should estimation of measurement uncertainty fail due to lack of collaborative testing material or precision control material, it is permitted to proceed according to the general guidelines EURACHEM/CITAC.

In analyses which are only carried out as individual determinations, an estimation according to the Horwitz-function (Horwitz, 1982) seems justifiable.

5.4.3 Formula index

$$U = k \cdot u(y) \quad \text{F. 1}$$

$$u(y) = \sqrt{(\text{RMSbias})^2 + u(\text{Cref})^2 + u(\text{RSD}_{\text{PK}})^2} \quad \text{F. 2}$$

$$\text{RMSbias} = \sqrt{\frac{\sum \left(100 \cdot \frac{(\text{bias})}{\text{SW}} \right)^2}{m}} \quad \text{F. 3}$$

$$u(\text{cref}) = \frac{\left(\frac{\sum 100 \cdot \frac{\text{SD}_{\text{RV}}}{\text{SW}}}{m} \right)}{\sqrt{p}} \quad \text{F. 4}$$

$$u(\text{RSD}_{\text{PK}}) = 100 \cdot \frac{\text{SD}_{\text{PK}}}{\text{MW}} \quad \text{F. 5}$$

5.4.4 Abbreviations

Bias	Deviation of the participant's result from the collaborative testing target value
k	Coverage factor A coverage factor of 2 corresponds to a confidence level of about 95%
m	Number of collaborative tests
p	Average number of participating laboratories
RMSbias	Uncertainty contribution by inaccuracy of measurement, corresponding with the average bias of several collaborative testing series
SD_{PK}	Standard deviation of the quality control results over the measurement days
SD_{RV}	Standard deviation in collaborative testing (relative reproducibility standard deviation)
SW	Target value in collaborative testing
U	Expanded measurement uncertainty, calculated from the combined measurement uncertainty and the coverage factor k
u(Cref)	Uncertainty contribution from target values determined in collaborative testing
u(RSD_{PK})	Uncertainty contribution from intermediate precision (RSD _{PK}) This comprises all variable factors in a laboratory such as e.g. operator and/or equipment and/or time and/or calibration. The between-day intermediate precision as stated in appendix B of the GTFCh guideline for quality control may be used here.
u(y)	Combined measurement uncertainty

6. Reporting

The client should be informed of the test results either by a report or an expert report. The report is laid out according to the research question.

According to ISO 17025, at least the dates and times of sample collection and sample receipt as well as the period of analysis (beginning and end of analysis) must be given as header data in the results report. If known, the time of the incident must be included. The name of the person responsible for the analysis and external representation must be indicated.

If statements are made, all relevant connecting facts should be included in the expert report if no other arrangement was made with the client.

When analysing biological material, the result must explicitly be assignable to the person from whom the sample was taken as well as to the blood withdrawal system in case this was sent in along with the material. The methods used should be indicated. The nature of the test material analysed (e.g. full blood, serum, plasma, remaining blood etc.) should be stated. The use of unsuitable withdrawal or sampling systems should be indicated.

The possible loss of analyte due to delayed or inadequate storage of e.g. serum or plasma samples (required are freezing conditions of minimum -15°C) or to unfavourable analytical conditions when determining whole blood or remainders of blood samples

should be mentioned in the report. The significance of the result should be elucidated if applicable (with analytical and, if necessary, toxicological assessment).

If a result below the calibration range is obtained, it must be termed “ca.” or “positive (less than..)”. A remark that this value is below the ~~detection~~ limit of quantification (corrected 25.01.2018) or below the calibration range should be included. Results above the calibration range will be designated analogously.

The measured value is presented after rounding. Independently of the concentration unit, a maximum of two significant digits (i.e. one more digit after the first that is not zero) should be given, if not required otherwise.

7. Documentation

The head of the laboratory is responsible for the written documentation of all methodological instructions used by the laboratory, such as standard operation procedures (SOPs) and procedures for all important operations in the laboratory, as part of the quality management handbook. The instructions should comply with approved quality criteria and must be audited, e.g. within the framework of an accreditation. Methods and procedures must be set up and described in such a way that technical personnel is able to attend to these after adequate training. Any change of regulations should be documented. It must be ensured that the work is carried out exactly according to the actual directives. Methods must be validated. The result of the validation should be documented with the instructions.

Analyses for which no instructions are laid down in the context of the quality management system may be carried out if the method used is carefully documented.

Application forms, status protocols, and all documents such as evaluations of measurement results and analyses, measurement protocols, calibrations, chromatograms, spectra, analysis reports or expert reports as well as the assay procedure must all be filed and stored in a way that they may be presented to an expert authorised by court at any time.

On the basis of the documents, the correct analytical procedure and the expert opinion on the results must be deducible. It should be clear which person(s) performed the analysis and which expert is responsible for the final result. The person responsible for the analysis warrants that the research was performed according to the documents in force. Documentation may also be electronic if the accessibility is guaranteed over the relevant period of storage. The head of the laboratory or the quality control representative takes care of staff training concerning correct documentation.

Documents must be retained for at least six years unless the relevant administrative regulations stipulate longer retention periods.

8. Literature and co-applying regulations

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9. Appendices

The following guidelines are regarded as appendices to the “GTFCh Guidelines for Quality control in Forensic-Toxicological Analyses“

Nr.	Title	Replaces guideline with title
A	Requirements for the assay of special analytes in biological matrices including an appendix with a list of detection limits	Appendix B: Quality standards for special analytes T+K 67 (3), S. 78-80
B	Requirements for the validation of analytical methods	Appendix C: Requirements for the conduction of analyses 1. Validation T+K 71 (3), S. 146-154; 13.11.2004
C	Requirements for the analysis of hair samples	Appendix B: Quality standards for special analytes 2. Analysis of hair samples T+K 71 (3), S. 140-145; 13.11.2004
D	Recommendations on the collection of biological samples during autopsy for forensic toxicological investigations (final part in process)	Recommendations of the GTFCh on the collection of biological samples during autopsy for forensic toxicological investigations T+K 71 (2), S. 101-107; 05.06.2004
E	Analysis of fusel alcohols with headspace gas chromatography in biological samples	New compilation (in process)

Current versions available on the GTFCh website (www.gtfch.org)

10. Final clause

These GTFCh guidelines for quality control in forensic-toxicological analyses were approved by the GTFCh executive committee on the 1st of April 2009 and replace the following previous regulations and appendices:

Nr.	Title	published
	GTFCh guidelines for quality control in forensic-toxicological analyses <i>R. Aderjan, Heidelberg; T. Briellmann, Basel; Th. Daldrup, Düsseldorf; U. Demme, Jena; K. Harzer, Stuttgart; M. Herbold, Heidelberg; H. Käferstein, Köln; G. Kauert, Frankfurt/M.; L. v. Meyer, München; M. Möller, Homburg; F. Mußhoff, Bonn; G. Schmitt, Heidelberg; W. Weinmann, Freiburg.</i>	T+K 65 (1), S. 2-8; 17.01.1998
A1	Appendix A: Requirements for individual analytical methods 1. Analyses with gas chromatography-mass spectrometry through electron impact ionisation <i>R. Aderjan, Heidelberg; B. Babel, Würzburg; T. Briellmann, Basel; T. Daldrup, Düsseldorf; U. Demme, Jena; J. Hallbach, München; M. Hartung, Homburg/Saar; K. Harzer, Stuttgart; M. Herbold, Heidelberg; L. von Meyer, München; M. Möller, Homburg/Saar; F. Mußhoff, Bonn; G. Schmitt, Heidelberg; W. Weinmann, Freiburg i.B.</i>	28.03.2000
A2	Appendix A: Requirements for individual analytical methods 2. Analyses via high performance-liquid chromatography with photodiode array detector (HPLC-DAD) <i>F. Pragst in cooperation with the GTFCh scientific committee quality control</i>	04.06.2004
B1	Appendix B: Quality standards for special analytes <i>F. Mußhoff, T. Daldrup, M. Herbold, L. v. Meyer</i>	T+K 67 (3), S. 78-80
B2	Appendix B2: Quality standards for special analytes <i>F. Mußhoff, Bonn; T. Daldrup, Düsseldorf; R. Aderjan, Heidelberg; L. v. Meyer, München</i>	T+K 69 (1), S. 32-34; 14.03.2002

11. Legal validity

This guideline is legally valid from the day of publication in Toxichem + Krimtech.

Transitional periods apply until 31st of March 2011.