

**CONTENTS**

Guidelines on the use of reference materials in forensic drug analysis

**FOREWORD**

1 **Requirements of international standards and guidelines**

1.1 General accreditation requirements (European Standard EN/ISO/IEC 17025) concerning reference materials

1.1.1 Reference standards and reference materials (EN/ISO/IEC 17025; Chapter 5.6.3)

1.1.2 Purchasing services and supplies (EN/ISO/IEC 17025; Chapter 4.6)

1.1.3 Test and calibration methods and method validation (EN/ISO/IEC 17025; Chapter 5.4)

1.1.4 Measurement traceability (EN/ISO/IEC 17025; Chapter 5.6.2)

1.1.5 Assuring the quality of test and calibration results (EN/ISO/IEC 17025; Chapter 5.9)

1.2 Specific requirements for reference materials given by ISO-guides 30-34

1.2.1 Terms and Definitions used in connection with reference materials (ISO-guide 30; Chapter 2 and 3)

1.2.2 Reference materials – Contents of certificates and labels (ISO-guide 31, Chapter 6)

1.2.3 Calibration in analytical chemistry and use of certified reference materials (ISO-guide 32, Chapter 3)

1.2.4 Uses of certified reference materials (ISO-guide 33)

1.2.5 General requirements for the competence of reference material producers (ISO-guide 34)

1.3 General requirements (ILAC G19:2002: Guideline for forensic science laboratories) concerning reference materials

1.3.1 Technical requirements (ILAC G19:2002, Chapter 5)

2 **ENFSI DWG recommendations concerning reference materials used for drugs analysis**

2.1 Availability of reference materials

2.1.1 Primary reference material (CRM)

2.1.2 Secondary reference material (RM)
GUIDELINES ON THE USE OF REFERENCE MATERIALS IN FORENSIC DRUG ANALYSIS

2.1.2.1 Analytical standards 11
2.1.2.2 Pharmacopoeial standards and substances 11
2.1.2.3 Precursors and other chemicals 12
2.1.3 In-house or working reference material 12
2.1.3.1 In-house reference materials 12
2.1.3.2 Reference materials prepared from case samples (without purification) 13
2.2 Assessment of the suitability of RMs 13
2.2.1 Stability and retesting 14
2.3 Corrective actions concerning purity of RMs 16
2.3.1 Correction factors 16
2.3.2 Moisture and crystal water 16
2.3.3 Salt form 17
2.4 Uses of reference materials 18
2.4.1 Qualitative analysis 19
2.4.2 Quantitative analysis 20

3 Preparation of in-house reference materials 21
3.1 Material preparation 21
3.2 Homogeneity testing 22
3.3 Stability testing 23
3.4 Measurement of reference value 24
3.5 Documentation 25

4 List of Contributors 26

REFERENCES:

Annex 1 Specification sheet of commercially available reference material 29
Annex 3 A practical guidance to reliable secondary and in-house reference compounds; suggestions for the acceptance and verification of reference compounds 31
FOREWORD

In the ENFSI Working Group (WG) Drugs, quality assurance (QA) and best practice are important topics. QA is an extensive field where a lot of documentation and various guidelines are already available. For this reason, the WG Drugs decided to focus on a number of other topics that were more or less specific for the WG Drugs. In 2003 the first booklet on such specific topics, Guidelines on Representative Drug Sampling has been published (see also http://www.enfsimembers.eu under DOCUMENTS PUBLICATIONS). The positive echo of this document motivated the WG continuing on specific topics. A Reference Material Subcommittee has been appointed with the task to outline practical interpretation of international standards in the purchase, production and use of reference materials in forensic drug analysis.

The availability of good quality reference materials in the field of controlled drugs analysis is poor. Therefore the need for finding acceptable practical solutions for the use of reference materials was evident.

The ENFSI Drugs Working Group cannot guarantee full compliance of these recommendations with accreditation requirements. In unclear cases the reader is advised to rely on EN/ISO/IEC 17025 and relevant ISO Guides.

This document is divided in three chapters. The first chapter summarises the requirements of international standards and guidelines. The second chapter gives practical interpretation to the requirements of the first one. Chapter 3 is dealing with some practical aspects in the verification of secondary reference materials. In the annexes some practical examples are given.

The steering committee is proud of the realisation of this guideline on reference materials. The document has been adopted at the Annual Meeting 2007 in Copenhagen.

We wish to express our thanks to the chairperson and members of the subcommittee on reference material for their excellent work.

The ENFSI WG Drugs,
Dr. Erkki Sippola (Chairman 2002-2006)
Dr. Michael Bovens (Chairman 2006-)

IV
1. Requirements of international standards and guidelines

In this chapter, general accreditation requirements concerning reference materials are summarised according to European Standard EN/ISO/IEC 17025, ISO guides 30-34 and ILAC Guideline for forensic science laboratories (G19:2002). The standard points out that reference materials shall, where possible, be traceable to SI units of measurement, or to certified reference materials.

1.1 General accreditation requirements (European Standard EN/ISO/IEC 17025) concerning reference materials

EN/ISO/IEC 17025 includes a number of requirements regarding the selection and use of reference materials. The relevant requirements are outlined below.

1.1.1 Reference standards and reference materials (EN/ISO/IEC 17025; Chapter 5.6.3)

5.6.3.2 Reference materials
Reference materials shall, where possible, be traceable to SI units of measurement, or to certified reference materials. Internal reference materials shall be checked as far as is technically and economically practicable.

5.6.3.3 Intermediate checks
Checks needed to maintain confidence in the calibration status of reference, primary, transfer or working standards and reference materials shall be carried out according to defined procedures and schedules. (Reference materials: Reference materials shall, where possible, be traceable to SI units of measurement, or to certified reference materials. Internal reference materials shall be checked as far as is technically and economically practicable.)
5.6.3.4 Transport and storage

The laboratory shall have procedures for safe handling, transport, storage and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity.

(NOTE Additional procedures may be necessary when reference standards and reference materials are used outside the permanent laboratory for tests, calibrations or sampling.)

1.1.2 Purchasing services and supplies (EN/ISO/IEC 17025; Chapter 4.6)

4.6.1 The laboratory shall have a policy and procedure(s) for the selection and purchasing of services and supplies it uses that affect the quality of the tests and/or calibrations. Procedures shall exist for the purchase, reception and storage of reagents and laboratory consumable materials relevant for the tests and calibrations.

4.6.2 The laboratory shall ensure that purchased supplies and reagents and consumable materials that affect the quality of tests and/or calibrations are not used until they have been inspected or otherwise verified as complying with standard specifications or requirements defined in the methods for the tests and/or calibrations concerned. These services and supplies used shall comply with specified requirements. Records of action taken to check compliance shall be maintained.

4.6.3 Purchasing documents for items affecting the quality of laboratory output shall contain data describing the services and supplies ordered. These purchasing documents shall be reviewed and approved for technical content prior the release.

(NOTE The description may include type, class, grade, precise identifications, drawings, inspection instructions, other technical data including approval of test results, the quality required and the management system standard under which they were made.)
1.1.3 Test and calibration methods and method validation (EN/ISO/IEC 17025; Chapter 5.4)

5.4.5 Validation of methods

5.4.5.2 ….

NOTE 2 The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:

a) calibration using reference standards or reference material;

b) comparison of results achieved with other methods;

c) interlaboratory comparisons;

d) systematic assessment of the factors influencing the result;

e) assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

1.1.4 Measurement traceability (EN/ISO/IEC 17025; Chapter 5.6.2)

5.6.2.1 Calibration

5.6.2.1.1 For calibration laboratories, the programme for calibration of equipment shall be designed and operated so as to ensure that calibrations and measurements made by the laboratory are traceable to the International System of Units (SI) (Système international d’unités).

5.6.2.1.2 There are certain calibrations that currently cannot be strictly made in SI units. In these cases calibration shall provide confidence in measurements by establishing traceability to appropriate measurement standards such as:

a) the use of certified reference materials provided by a competent supplier to give a reliable physical or chemical characterisation of a material;

b) the use of specified methods and/or consensus standards that are clearly described and agreed by all parties concerned.
Participation in a suitable programme of interlaboratory comparisons is required where possible.

5.6.2.2 Testing

5.6.2.2.1 For testing laboratories, the requirements given in 5.6.2.1 apply for measuring and test equipment with measuring functions used, unless it has been established that the associated contribution from the calibration contributes little to the total uncertainty of the test result.

5.6.2.2.2 Where traceability of measurements to SI units is not possible and/or not relevant, the same requirements for traceability to, for example, certified reference materials, agreed methods and/or consensus standards, are required as for calibration laboratories (see 5.6.2.1.2).

1.1.5 Assuring the quality of test and calibration results (EN/ISO/IEC 17025; Chapter 5.9)

5.9.1 The laboratory shall have quality control procedures for monitoring the validity of tests and calibrations undertaken. ... This monitoring shall be planned and reviewed and may include ... the following:

a) regular use of certified reference materials and/or internal quality control using secondary reference materials;

b) participation in interlaboratory comparison or proficiency-testing programmes;

c) replicate tests or calibrations using the same or different methods;

d) retesting or recalibration of retained items;

e) correlation of results for different characteristics of an item.

NOTE The selected methods should be appropriate for the type and volume of the work undertaken.
1.2 Specific requirements for reference materials given by ISO-guides 30-34

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies. ISO-guides 30-34 are dealing with terms and definitions, use and production of reference materials and contents of their certificates.

1.2.1 Terms and Definitions used in connection with reference materials (ISO-guide 30; Chapter 2 and 3)

2.1 Reference material (RM): material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

NOTE: Many RMs are accompanied with a certificate or other documentation, but this should not be taken to mean that they are a true CRM, or even equivalent to a CRM in the context of the ISO definition of a CRM below.

2.2 Certified reference material (CRM): reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

2.3 Primary standard: standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to other standards of the same quantity, within a specified context.

2.4 Secondary standard: Standard whose value is assigned by comparison with a primary standard of the same quantity.
3.8 Traceability: Property of the result of a measurement or the value of a standard whereby it can be related, with a stated uncertainty, to stated references, usually national or international standards, through an unbroken chain of comparisons.

1.2.2 Reference materials – Contents of certificates and labels (ISO-guide 31, Chapter 6)

6 Summary of the essential contents of a certificate
To assist producers of CRMs, those contents of a CRM certificate which are considered essential are summarized in the list below. This checklist is also intended to promote harmonization of practice in those organisations with responsibility for accrediting producers of CRMs.

   a) name of the material;
   b) producer and producer’s code for the material;
   c) general description of the material;
   d) intended use;
   e) instructions for proper use;
   f) instructions for appropriate conditions of storage;
   g) certified property value(s), each accompanied by a statement of uncertainty; method(s) used to obtain property values (with full details where values are dependent on the method of measurement);
   h) period of validity, if appropriate.

1.2.3 Calibration in analytical chemistry and use of certified reference materials (ISO-guide 32, Chapter 3)

3 Basic considerations
Any measurement, particularly any quantitative chemical analysis, shall employ reference elements to ensure demonstrated traceability to the relevant basic quantities. This is an essential condition for the accuracy of the results.

The metrological quality of the calibration performed depends on:
• the uncertainty of the reference used (set of calibration masses, titrated solutions, gas mixtures, composition CRMs², etc.),
• ....
2 Remarks:

a) … They (i.e. RMs) may also be used to check the drift with time, and possibly to correct an instrumental drift. The also serve as a basis for a conventional scale (e.g. octane index)…

b) The analytical chemist is often a user of analytical materials or reagents. These products shall not be confused with CRMs. In fact, a CRM corresponds to an identified batch of material of which the certified characteristics have been determined with an optimised and defined accuracy. An analytical reagent is only characterised by a nominal value, determined with a large uncertainty. It is the user’s duty to observe all necessary precautions to ensure, when used, that an analytical reagent meets his/her needs

1.2.4 Uses of certified reference materials (ISO-guide 33)

Introduction
Certified reference materials should be used on a regular basis to ensure reliable measurements. However, in doing so, the magnitude of the supply of that CRM, its relative cost, its availability (accessibility) and the measurement technique, be it destructive or non-destructive, should be considered. Also important to the user is in fact the misuse of a CRM may not provide the intended information.

Misuse of CRMs differs from incorrect use. The user of a CRM is expected to be familiar with all information pertinent to the use of the CRM as specified in its certificate. He should comply with such factors as the period of validity of the CRM, the prescribed conditions for storage of the CRM, instructions for the use of the CRM, and specifications for validity of the certified properties of the CRM.

1.2.5 General requirements for the competence of reference material producers (ISO-guide 34)

Introduction
The demand of new for new reference materials of higher quality is increasing as a consequence of both the increased precision of measuring
equipment and the requirement for more accurate and reliable data in the scientific and technological disciplines. Some previously acceptable reference materials may not meet those more stringent requirements. It is, therefore, not only necessary for reference materials producers to supply information about their materials in the form of reports, certificates and statements, but also to demonstrate their competence in producing reference materials of appropriate quality.

1.3 General requirements (ILAC G19:2002: Guideline for forensic science laboratories) concerning reference materials

The use of reference materials in validation and calibration is recommended in the ILAC Guideline for forensic science laboratories. The relevant chapters are outlined below.

1.3.1 Technical requirements (ILAC G19:2002, Chapter 5)

5.4 Test and calibration methods and method validation

5.4.1 All methods should be fully documented including procedures for quality control, and, where appropriate, the use of reference materials.

5.4.5.1 All technical procedures used by a forensic science laboratory must be fully validated before being used on casework. Methods may be validated by comparison with other established methods using certified reference materials (where available) or materials of known characteristics.

5.6 Measurement traceability

5.6.2.2 For many types of analysis, ‘calibration’ may be carried out using synthetic standards containing the analytes under test, prepared within the laboratory from chemicals of known purity and composition, or matrix matched standards. Alternatively, ‘standard’ solutions may be purchased. Many chemicals can be purchased with manufacturer’s statements or certificates. Wherever possible, laboratories should obtain supplies of chemical standards from competent suppliers.
5.9 Assuring the quality of test and calibration results

5.9.1 a) Analytical performance should be monitored by operating quality control schemes which are appropriate to the type and frequency of testing undertaken by a laboratory. The range of quality control activities available to laboratories includes the use of:

- reference collections;
- certified reference materials and internally generated reference materials;
- ...
2. ENFSI DWG recommendations concerning reference materials USED FOR DRUGS ANALYSIS

2.1 Availability of reference materials

Two classes of materials are recognised by ISO, namely ‘certified reference materials’ (CRMs) and ‘reference materials’ (RMs) as described in Chapter 1. The following classes of reference materials are also encountered:

**Primary reference material (CRM)**
Certified by nationally or internationally recognized institutes (NIST, BCR, NIME)

**Secondary reference material**
Traceable back to Primary reference materials or otherwise verified, for example through independent test method Decreasing Uncertainty with certificate from manufacturer

**In-house or working reference material**
Prepared by user with traceability to Primary or Secondary standards or otherwise verified, for example through independent test method

2.1.1 Primary reference material (CRM)
Generally the demand for primary reference materials exceeds supply in terms of the range of materials and availability. It is rare to have a choice of primary RMs, i.e. certified reference material (CRM), and therefore the user must choose the most suitable material available, i.e. secondary reference material. It is important therefore that users understand any limitations of reference materials employed.
2.1.2 Secondary reference material (RM)

2.1.2.1 Analytical standards
A secondary reference material, in forensic drug analysis, is often considered as a pure material which is purchased from a reliable manufacturer together with a certificate. Even, that the manufacturer provides a certificate, the material is not certified according ISO-standards. These materials are often referred to as analytical standards with purity more than 98%.

There are also commercially available analytical standards which are in solution and/or in matrix.

Pure substances are preferred over mixtures and solutions since:
- verification of the reference material is easier to carry out without matrix
- using a pure substance, the risk of matrix effects or formation of artefacts can be avoided
- concentration of solutions can vary over time by degradation or evaporation.

Therefore, ENFSI DWG recommends that whenever possible, laboratories should try to obtain pure, i.e. more than 98%, reference materials. If this is not appropriate for a specific application solutions of pure substances may be used.

2.1.2.2 Pharmacopoeial standards and substances
In the absence of specific guidelines for forensic drug analysis, it seems reasonable to refer to pharmacopoeias, since pharmacopoeial standards and substances are established and distributed by pharmacopoeial authorities to give the user the information on the composition of the substance. Usually this is following the general principles of ISO guide 34. It should be noted, however, that they are not considered as CRMs. A different approach is used by the pharmacopoeias; they describe the minimum quality that is needed for the pharmaceutical use of the drug. Pharmacopoeial authorities also refer to primary standards, i.e. CRMs, which can be purchased as references.
Nowadays when obtaining a ‘pharmaceutical’ compound not only reference is made to the Pharmacopoeia which specifications are met but also a specification of analysis is provided. And usually the expiration date is also mentioned. Then it can be considered if the purity is sufficient for analytical purposes; e.g. the European pharmacopoeia suggests for cocaine hydrochloride a number of purity tests and requires a minimum content of 99.0% cocaine hydrochloride. The uncertainty of their assigned values is not stated since it is negligible in relation to the defined limits of the method-specific assays of the pharmacopoeias for which they are used.

Therefore, ENFSI DWG recommends using pharmacopoeial standards and substances as secondary reference materials for analytical purposes when they comply with the requirements of the (European) Pharmacopoeia.

2.1.2.3 Precursors and other chemicals
Occasionally precursors and other chemicals are used as reference materials. Often these compounds are of ‘analytical’ grade or compounds ‘for synthesis’. In many cases the purity is given as a minimum concentration.

ENFSI DWG recommends that also these standards can be considered as secondary reference materials, but only after a verification confirming the claimed values.

2.1.3 In-house or working reference material

2.1.3.1 In-house reference materials
Sometimes reference standards must be made in forensic laboratory e.g. due to their poor availability. This can be carried out by synthesising or by isolating them from seized material. One or more purification steps (distillation, extractions, salt formation and/or recrystallisations) may result in material of sufficient purity. Some aspects of the preparation of in-house reference materials are described in chapter 3.
ENFSI DWG recommends that in-house RM can be used for calibration when in-house RM is well characterised by appropriate physical and/or chemical properties.

An internal Certificate of Analysis describing the relevant properties should be made in this case. An example of an internal certificate is presented in Annex 1.

2.1.3.2 Reference materials prepared from case samples (without purification)

These materials are often used as so-called control samples for quality assurance purposes. Typical example of this type of in-house or working reference material is a case sample which content has been determined against a secondary reference material. The advantage of such material is that it can be made available in large amounts and that it bears no costs. The disadvantages of reference materials prepared from case samples include:

- a) matrix effects
- b) homogeneity aspects
- c) increased uncertainty
- d) expiration date is difficult to define

ENFSI DWG advises to avoid the use of case samples as secondary standards for quantitative purposes.

2.2 Assessment of the suitability of RMs

Three situations can be recognised:

1) reference standards supplied with a certificate (so-called CRMs);
2) reference standards with a specification sheet (often called as certificates; many RMs are accompanied by a certificate or other documentation, but this should not be taken to mean that they are a true CRM, or even equivalent to a CRM in the context of the ISO definition of a CRM) and
3) those without certificate or specification sheet.

Almost per definition content verification is not necessary for CRMs. Therefore verification of reference materials in the situations 2 and 3 will be discussed.
If the reference standard is supplied with a specification sheet, the choice will be made between acceptance and verification of the content. The choice may be influenced by various factors including:

a) former experiences with the supplier  
b) general impression about the material and packaging  
c) level of information given in the certificate  
d) time between certificate issue and delivery time of compound at the laboratory  
e) type of material  
f) purpose of use (qual/quant, accuracy, accredited method,....)

In cases where no specification is available, the contents should always be verified.

**ENFSI DWG recommends verifying the claimed contents, except in cases where a specification sheet with sufficient relevant data is available and the supplier has a good reputation or/and is known to be reliable.**

**Note:** It is recognised that some of the ENFSI laboratories have a QA policy to verify purchased secondary standards.

An example of an acceptable specification is presented in Annex 2.

### 2.2.1 Stability and retesting

When a supplier has given an expiration date, the reference standard cannot be used after that date. However, if the standard is tested and meets the original or other clearly defined criteria, the standard can be accepted again for a new period.

When doing retesting, the results should be compared, if possible, with those obtained at the first verification test upon receiving the reference compound, or otherwise compared to analysis result before the expiring date.

Some compounds are known to be very stable, an example being amphetamine sulphate. That means that a retesting after one year can be
considered as overdoing. On the other hand, if no data are available about the stability of the compound, an acceptance for 10 years is too long period.

**When no documentation about the stability is available, ENFSI DWG advises to retest all solid substances at least every 5 years. Substances known to be unstable must be checked with a higher frequency, the frequency being dependent on the nature of the instability and storage conditions.**

As an example, it is reasonable to retest heroin base on an annual or bi-annual basis to check for the formation of 6-monoacetylmorphine, and MDMA hydrochloride to check frequently for the absence of crystal water.

The stability of reference compounds dissolved in water or methanol is not always well defined. Therefore these solutions should be checked carefully.

**ENFSI DWG advises to retest solutions at least every year.**

Testing and/or retesting do not necessarily require a whole set of chemical tests. The minimum form of (re)testing can be a visual inspection. In other cases more specific investigations are required for instance for compounds forming degradation products. The purpose of the reference compound will also influence the choice, e.g. formation of 6-monoacetylmorphine in heroin is not a reason for rejecting the reference compound for qualitative purposes. And for quantitative work, the reference compound can be accepted as long as the percentage is under a defined limit. For other compounds, a number of other tests will be necessary. And often, water determinations are necessary for the correct characterisation of the reference material.

Some examples for a strategy for testing, verification and acceptance of secondary and in-house reference materials are given in annex 3.
2.3 Corrective actions concerning purity of RMs

2.3.1 Correction factors

In relation with the purity of the reference material, the question arises whether correction factors should be applied. In other words, if it is known that an MDMA standard is 98.5 % pure, should the result of the analysis be corrected (multiplied) by a factor 0.985 in order to avoid a too high value. Proper action is dependent on the reliability of the concentration, if the standard is specified by the manufacturer or if the concentration can be confirmed by other reliable means, correction factor can be used.

Another possibility is to take the uncertainty of the standard into consideration in the measurement uncertainty estimation. Then the results are corrected but the laboratory is aware of uncertainty caused by the standard impurity.

ENFSI DWG recommends using

1. No correction factors when the purity meets the pharmacopoeial standards (usually > 98 or>99%)
2. Correction factors when the purity of the reference material is known and less than 98%

2.3.2 Moisture and crystal water

Water

Many reference materials are not always completely dry. So, in many assays, heating till constant weight, mostly at 105 °C is performed. The European Pharmacopoeia ed. V mentions for accepted loss of weight:

- Amphetamine sulphate 1.0 %;
- Cocaine hydrochloride 0.5%

and the British Pharmacopoeia 2005 for

- Heroin hydrochloride 4.5%

(the latter may involve the removal of 1 crystal water which is theoretically 4.2%)

ENFSI WG Drugs advises to apply no corrections for the moisture content as long as this is within the given pharmacopoeial specification.
Crystal water
Crystal water has a major influence. Heroin hydrochloride is defined as heroin + hydrochloride + 1 crystal water. So, when a quantitation is based on heroin hydrochloride reference compound and this compound is known to contain 1 crystal water, the conversion factor into the base is 0.8715 instead of 0.910 and the other way around, if the quantitation is based on heroin base reference compound and the contents must be expressed as hydrochloride, the crystal water should be taken into consideration in the calculations as well.

Note: It is realized that in at least one European country other agreements have been made concerning the conversion from heroin base to heroin hydrochloride; they ignore the crystal water.

MDMA hydrochloride can contain crystal water which varies over time (between 0 and 1) depending on the atmospheric humidity. For a small molecule, such as MDMA hydrochloride, content of crystal water has a high impact (about 8%). For instance, during the CTS' proficiency test 2000-501, most participants reported values of about 36%, whereas CTS mentioned a MDMA content of 41%. It is quite probable that the crystal water played a role here. In the case of MDMA hydrochloride the most practical solution seems to be drying and storing under such conditions that the reference material is free of crystal water.

ENFSI DWG recommends that crystal water should be taken into consideration unless in conflict with national rules.

2.3.3 Salt form
Sometimes the reference material is in another salt form than the sample to be analysed. It is also possible that the reference material is in base form and the sample is in salt form or the other way around.

1 Collaborative Testing Services, Inc.
Theoretical conversion factor is not the main risk, but the potential different behaviour of salts and bases in the analysis, e.g. due to different solubility. Often the analytical system can be optimised to cope with this matter. In gas chromatography special attention has to be paid to a possible different volatility of bases and salts in the injector.

**ENFSI DWG recommends that the salt form should be taken into consideration and conversion factors should be used when appropriate. ENFSI advises to report in the form of the base. Other forms are also possible but then the report should clearly mention the salt form in which data is reported.**

### 2.4 Uses of reference materials

Reference materials shall be used, where appropriate, for

- a) method validation and measurement uncertainty
- b) method verification
- c) calibration
- d) quality control and quality assurance

For method validation and measurement uncertainty determination usually primary or secondary RMs are required. In-house or working RMs (case samples) are used when the effect of matrix is studied during the determination of selectivity and specificity. Studies of repeatability and reproducibility should be carried out by using case samples since they represent “authentic samples”.

In method verification, it is not essential to use primary and secondary RMs and thus, in-house or working RMs can be used.

For calibration purposes case samples should not be used, but the use of in-house RMs, which are properly characterised, is acceptable. Use of RMs are summarised in table 1.
2.4.1 Qualitative analysis

In most analytical measurements, the identity of the material needs to be confirmed by reference material or reference data. Reference materials will often serve for identity confirmation where available in sufficient quantity.

Comparison with reference data, for example in the form of spectroscopic data, is normally acceptable evidence of identity and complies with SWGDRUG guidelines. In this case, however, it is important to ensure that

a) reference data are obtained under closely similar conditions to those used in the laboratory

b) reference data are traceable to appropriate references (for example, wavelength standards)

c) the equipment used to generate data for the test items is traceable to the same references.
ENFSI DWG recommends using reference materials instead of reference data for qualitative purposes but recognizes the use of spectroscopic data as an acceptable alternative.

2.4.2 Quantitative analysis

Chromatographic methods are commonly used for the quantitative analysis of drugs and these methods rely heavily on a reference material to provide accurate data. Therefore the quality and purity of the reference material is very important. Verification of the reference materials is essential as explained in chapter 2.2.1. It can be carried out by spectroscopic and chromatographic methods, titration, elemental analysis, melting point etc. An acceptable and common chromatographic method is to use indirect reference standards. This is based on the principle that a flame ionisation response is linear to the number of carbon atoms in a molecule. The big advantage of this method is that

a) it is also suitable for reference compounds which are available in a aqueous or methanolic solution
b) it can be used for ‘new’compound which are not available
c) it can be used for any compound that is not commercially available

Example: Methamphetamine can be checked / determined by using the readily available homologue phentermine as the reference standard.
3. Preparation of in-house reference materials

Reference materials (RMs) which are produced in-house (often referred to as working reference materials, check materials, quality control standards or secondary working standards) are required for use by laboratories on a day to day basis. The attractiveness of using such RMs is that they
a) can be made available in relatively large amounts for a relatively low price
b) can be made for compounds not available on the market like ‘new’ synthetic drugs

It is of paramount importance that the RMs are appropriately prepared to ensure that they are fit for the purpose for which they are intended.

Before producing in-house RMs, the following points should be taken into account:

a) RMs can be used in day to day quality control systems
b) RMs should wherever possible be calibrated against a CRM or certified measuring device or instrument being used, when the RM is first prepared and thereafter on a regular basis
c) The decision on whether RM is fit for its intended purpose and the frequency of checking its assessed value(s) needs to be carefully considered by the analyst depending on:
   - Intended use of the RM
   - Level of homogeneity
   - Stability of material
   - Precision of the method for which the RM will be controlling

3.1 Material preparation

The material should be prepared/processed in such a way as to ensure that its final form is fit for its intended use and that it is sufficiently stable. The amount of material depends on the ideal sample size, stability of material, storage capacity, the frequency of its use and the processing equipment available.
The procedures for processing the material need to be carefully chosen in order to achieve the required homogeneity and maximise the materials shelf-life. Typical operations include drying, grinding and sieving, etc.

Good container and appropriate labelling is essential. Wherever possible each container should include the following:
- A unique reference name or number
- A batch and sample name number
- Reference or nominal value
- Weight or volume of contents
- Any safety warning signs
- Storage requirements
- Date of expiry or re-test

### 3.2 Homogeneity testing

The RM should be homogenous, that is the difference between representative sample measurements should be smaller than the overall uncertainty limits of measurements. Thus it is necessary to assess whether any variation between representative samples is fully accountable to the uncertainty of the measurement method being used. The object of the test for homogeneity is mainly to determine the distribution at a constant level of any impurities, interferences or irregularities. The degree of homogeneity is dependent on the quantity of material taken i.e. a RM can be only be said to be homogenous at or above the weight/volume of the representative samples analysed. Thus, the quantity of material chosen for testing should not be greater than that used in the routine analysis for which RM will be employed.

When testing for homogeneity the following issues need to be considered in the context of the degree of homogeneity required:
- Selection of homogeneity test(s): It is important that relevant methods be used to detect for all possible inhomogeneity.
- Sample selection: This is normally carried out by either random or
systematic selection, normally on the final packed units.
c) Number of samples selected: There are no definitive rules that can be
given since it does depend on the type of material, the preparation
process, the end use and organisation’s own QA requirements.
d) Sample size: The quantity of material used to assess the homogeneity
should not be greater than the quantity that will be used when
subsequently using the RM for calibration or validation purposes.
e) Statistical tests to apply: A recommended test is the F-test which
should be used to:
f) Compare the standard deviation of the measurement results of the RM
with the method repeatability, obtained by replicate analysis on one of
the samples
g) Compare the relative standard deviation of the measurement results of
the RM with the method repeatability, obtained by replicate analysis
on a CRM
h) Compare the relative standard deviation of the measurement results of
the RM with a quoted method error (repeatability) published for
measurement method used.

The degree of homogeneity required for a particular RM will depend on its
intended purpose, but it is normal to conduct the F-test at the 95% or 99%
confidence level.

3.3 Stability testing

The aim of the stability testing is to determine whether the RM maintains all
its reference values from the time of production until the moment it is used.
The frequency of stability testing depends on the risk of any change in the
reference value with time. When selecting the material from which to produce
the RM it is beneficial if stability data is already available to indicate the
suitability of the material. Where such data is available it may not be
necessary to determine the initial stability. However, in all cases ongoing
stability checks are required if the material is to be used beyond the time
span of the initial stability determination or stability data already available.
When testing for stability the following issues need to be considered with respect to the material to be tested, the parameters being measured and the minimum shelf life required:

Selection of measurement method(s): it is necessary to select a method that provides precise measurements over a long period of time i.e. it is necessary to ensure that variations between one measurement and another made at a later time on the RM are not due to the calibration system used. Two different approaches could be adopted:

a) The system could be calibrated against a calibrant that can be reproduced at some time in the future. This may take the form of a standard calibration solution that is gravimetrically prepared or, if available, a CRM that is sufficiently stable could be used.

b) Where it is not possible to reproduce a calibrant over the time span required, the RM can be compared to a sample of that RM that has been stored at low temperatures, preferably refrigerator. The latter is assumed to remain stable.

3.4 Measurement of reference value

When measuring the reference value the following issues need to be considered in the light of the material to be tested, the parameters being measured, the end use of the material and the measurement approach to be used:

Selection of measurement method(s): a number of alternative approaches are available depending on the end use of the material:

a) if the RM is to be used as a quality control check sample, the methods that will be controlled by the RM should be used. In this case it may not be necessary to obtain an accurate reference value since the RM will be used to compare the value obtained using the method for each subsequent batch of analyses.

b) if the RM is to be used for assessing the performance of an analytical system then a more rigorous approach is required in order to obtain an accurate reference value. In this case it should be referenced against a CRM or the calibrated measuring device or instrument being used
c) if a CRM does not exist to which the RM can be referenced or the measurement method or measuring device is not certified then the reference value needs to be obtained by:

- a definitive (primary) method
- two or more methods and preferably include some independent check
- an interlaboratory exercise involving a reasonable number of participants

If a number of laboratories already take part in some form of regular interlaboratory exercise this may offer a mechanism for measuring the RM using a number of methods and laboratories. This would be particularly relevant where no appropriate CRM exists, the measurement method or measuring device is not certified or there are no better methods available.

3.5 Documentation

It is essential that records are kept for each stage of the in-house production of a RM. Such records should at least contain the following information:

a) Name(s) / Reference number(s) /Batch number(s) of the materials to which the documentation applies
b) Origin/source of the material(s)
c) Details on the material preparation techniques used
d) Dates of all measurements made
e) Details of analytical methods used including calibration i.e. CRMs used
f) Raw/primary data
g) Reasons for rejection of any data on technical grounds
h) Results of statistical analysis and the methods used
i) The reference value(s) to use
j) Name(s) of analysts who were responsible for the material preparation, analytical measurements and the calculation & interpretation of the data
k) Expiration date
l) Signature / name of the responsible chemist

In addition, details on stability, homogeneity, storage requirements and instructions on the correct use of the reference material should be noted. All documents should be retained as specified in your own Organisation’s quality system.
4 List of Contributors

Dr. Laura AALBERG (Chairman of the Reference Material Subcommittee)
National Bureau of Investigation, Crime Laboratory, Finland
e-mail laura.aalberg@poliisi.fi

Dr. Henk HUIZER
Netherlands Forensic Institute, Netherlands
e-mail h.huizer@nfi.minjus.nl

Dr. Anna STENFELDT HENNINGS
SKL-Swedish National Laboratory of Forensic Science, Sweden
e-mail anna.stenfeldt@skl.polisen.se

Dr. Jiri ZAPLETAL
Ministry of Interior, Police Institute of Criminalistics, Slovakia
e-mail zapletal@minv.sk

Dr. Erkki SIPPOLA
National Bureau of Investigation, Crime Laboratory, Finland
e-mail erkki.sippola@poliisi.fi

Dr. Michael BOVENS
Scientific Forensic Service, City Police of Zurich, Switzerland
e-mail michael.bovens@stp.stzh.ch

Dr. Scott OULTON
Scientific Working Group Drugs, DEA
e-mail scott.r.oulton@usdoj.gov

Note: The document is an ENFSI WG Drugs document, which means that all the rights are given to the WG steering committee; the committee has the right to propose changes and to have the document updated at any time. However, the booklet contains, in this chapter, the names of all colleagues and their laboratories that have contributed in writing.
REFERENCES:


ISO/IEC 17025 (2005), General requirements for the competence of testing and calibration laboratories. ISO, Geneva, Switzerland

ISO Guide 30 (1992), Terms and definitions used in connection with reference materials. ISO, Geneva, Switzerland


EA-4/14 (2003), The selection and use of reference materials.


SWGDRUG Guidelines, Recommended minimum standards for forensic drug identification, Microgram 33 (9), pp. 260-263 and www.swgdrug.org

H. Huizer, A. J. Poortman - van der Meer and H. E. van Egmond, Quantitation of amphetamine-type compounds for which no reference compound is available: the validation of a theoretical model, Science and Justice 41(3),(2001), pp. 185 - 192


European Pharmacopoeia, Edition 5.0 (2006)

United States Pharmacopoeia, edition 29

British Pharmacopoeia 2005

Kommentar zur Ph. Eur 4.00, 18.Lfg. 2004


ANNEX 1 : Specification sheet of commercially available reference material

**SPECIFICATIONS AND CERTIFICATE OF ANALYSIS**

Lipomed GMP-Document QA-F-20.1  30.06.1996  

Chemical name: Heroin (3,6-Diacetyl-morphine)  
Chemical formula: C_{21}H_{23}NO_{3} hydrochloride-hydrate  
Molwt: 369.42

Lot No: 29.3B31.1  
Art. Nr.: M-29-HC  
Release date: 28.11.2001  
Retest date: 11/2006

<table>
<thead>
<tr>
<th>TEST</th>
<th>SPECIFICATIONS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Appearance</td>
<td>white fine crystals</td>
<td>conforms</td>
</tr>
<tr>
<td>2. Identity</td>
<td>IR</td>
<td>IR identical to reference</td>
</tr>
</tbody>
</table>
| UV: in bidist. H_{2}O    | \( \lambda_{\text{max}} = 278.0 \pm 1 \text{ nm} \)  
| \( \varepsilon_{\text{mol}} = 1700 \pm 500 \) | UV: in bidist. H_{2}O  
| \( \lambda_{\text{max}} = 278.7 \text{ nm} \)  
| \( \varepsilon_{\text{mol}} = 1792 \) | |
| 3. Melting point         | 239 ± 3 °C (dec.)       | 238.7-241.8 °C (dec.) |
| 4. Purity HPLC           | > 98.5 %                | 98.94 ± 0.05%         |
| 5. Optical rotation      | \([\alpha]_{D}^{25} = -152 \pm 2^* \)  
| (c = 1.0 in bidist. H_{2}O) | \([\alpha]_{D}^{25} = -152.3^* \)  
| (c = 1.0 in bidist. H_{2}O) | |
| 6. Free base content     | > 88.5 %                | 88.87 %                |
| 7. Water content         | ≤ 4.25 %                | 1.54 %                 |
| 8. Calculated hydro-     | 8.87 %                  | 8.87 %                 |
| chloride content         |                         |                        |

QC - Officer: Dr. R. Gasio  
Date sign: Arlesheim, den  
\( \frac{\text{vol}}{\text{mol}}; 10^3 \)
ANNEX 2 : Specification sheet of in-house reference material

SPECIFICATION SHEET REFERENCE MATERIAL

Compound: 3,4-methylendioxymethamphetamine.HCl

= MDMA.HCl

Batch number: 050623LW
Origin Isolation and purification case 2004.04.13.065 – 1.028
Date of isolation: 4 May 2005
Received at NFI: 9 April 2004
Amount: 115.6 g (after isolation and purification)
Identity: IR consistent with lit [1]
Identification in case work was established over GC-MSD consistent with lit [1]

Literature reference:

[1] CND Analytical, Analytical profiles of substituted 3,4-methylenedioxyamphetamine: Designer drugs related to MDA, Alabama, USA, 1988, p.36(IR), p.54(MS)

Purity: Gas chromatographically pure: 99.6 % MDMA.HCl
Content: Titration at WINAP: 100.4% MDMA.HCl (rsd : 0.04%) 
HPLC: determination on previous reference standard 900205/VDA: 84.28% MDMA base; 100.2% MDMA.HCl
Expiration date: Qualitative: 1 May 2025

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Date + init</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 06</td>
<td>Absence of crystal water Yes / No</td>
<td></td>
</tr>
<tr>
<td>May 07</td>
<td>Absence of crystal water Yes / No</td>
<td></td>
</tr>
<tr>
<td>May 08</td>
<td>Absence of crystal water Yes / No</td>
<td></td>
</tr>
<tr>
<td>May 09</td>
<td>Absence of crystal water Yes / No</td>
<td></td>
</tr>
</tbody>
</table>

Storage: In desiccators
Approved: 9 September 2005, Dr. H.Huizer
ANNEX 3: A practical guidance to reliable secondary and in-house reference compounds; suggestions for the acceptance and verification of reference compounds

1. General

The basic idea is that secondary reference compounds should be verified upon reception in the laboratory and before being used in the analytical process. The verification must be adequately documented together with relevant dates and origin of the compounds.

The number of tests to be run for verification will vary depending on the source of the reference material. When the material has been bought as being of pharmacopoeial quality usually reference is made to the requirements mentioned in the monograph in a specific pharmacopoeia. Also it is common that a specification is provided referring to the requirements and mentioning the acceptance by the seller’s QA department. In these cases, especially when supplier has a good reputation, a minimum inspection seems acceptable. Under these conditions, most of the ENFSI laboratories finds reasonable that minimum inspection comprises the single check of the specification and the good condition of the package only. On the other hand, some ENFSI laboratories have such a QA policy, which requires also verification of secondary standards which have been purchased from “reliable supplier”.

A different situation is where reference compounds have been synthesized or isolated and purified at the own laboratory, so called in-house reference materials. In this situation, it has to be demonstrated that the material meets certain specifications and is fit for end use. ENFSI WG Drugs has discussed these requirements during the meeting in Riva in 2004. It was agreed to follow the specifications of the European Pharmacopoeia whenever possible. In case that compounds are not included there other well-recognized Pharmacopoeias could be referred to. Further, it was decided to apply a similar approach as much as possible for compounds that were not
described there. In this annex some Pharmacopoeia based requirements will be suggested starting with four important compounds. It was considered that the properties of the various drug substances vary and that specific approaches would needed for each compound.

No matter what is the source of reference material, after a certain period of storage the question arises whether the reference material still fulfils the requirements, or needs retesting.

In principle, pharmaceutical primary standards exist. They are available through European and national pharmacopoeial organisations. WHO has compiled a listing with available pharmaceutical standards (WHO/EDM/QSM/2001.2). However, as stated before, the availability of the specific compounds in practice is minimum due to very high prices and export limitations. Therefore, often forensic drug laboratories will have to use pharmaceutical quality standards as secondary reference standards. In this annex a number of approaches and criteria are suggested.

## 2. Qualitative analysis

**Verification upon acceptance:**

1) Compounds with a specification referring to a pharmacopoeia. Their identity can be accepted. In practice laboratories will often perform one GC-MS or one IR analysis for an (extra) confirmation of the identity.

2) There is usually no discussion about the identity of a synthesised compound. In these cases a single GC-MS or IR analysis will be sufficient for establishing the identity. NMR can be applied in very specific cases. Also tests for stereoisomers will only be necessary in very specific cases where such aspects are relevant.

3) For compounds isolated from case samples an analysis with GC-MS and IR or NMR is advised.

**Retesting**

It is unlikely that the identity of a compound changes. Retesting is not considered as necessary unless there is a serious risk that the substance degrades to a substantial degree, or that periods over 10-20 years are considered.
3. Quantitative analysis

The next paragraphs will describe a specific approach per compound. In these paragraphs, we assume the availability of relatively large amounts of these compounds, related with their commercial availability and/or availability in relation with in-house synthesis or isolation.

3.1. Amphetamine sulphate

General
Amphetamine is available in the form of the sulphate salt. Since it is still used in pharmacy, it is commercially available in some countries. It is a very stable compound and no problems with crystal water exist. Under normal dry, dark shelf conditions stability is expected to be over 20 years.

Pharmacopoeia specifications [Ph.Eur ed. V]

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (on dried material)</td>
<td>&gt; 99.0 % and &lt; 100.5 %</td>
</tr>
<tr>
<td>Loss on drying (2 h at 100 - 105° C)</td>
<td>&lt; 1.0%</td>
</tr>
</tbody>
</table>

Verification for amphetamine (sulphate) provided with a certificate, mentioning to comply with the PhEur:

1. Upon reception:
   - check the certificate
   - check labelling and good condition of the package and the contents
   - perform an impurity profile(*)(**)

2. Retesting:
   - Is advised after 5 years
   - check good condition of powder and package
   - perform an impurity profile; if possible check for changes since reception

(*) optional (**) advised if material is to be kept for a longer period
- check the control charts for the results of the control sample over the last 5 years
- perform a quantitative determination by the pharmacopoeia method (*)
- compare the content with that of a sample of the same reference compound taken upon reception and stored at 4-6°C.

Verification and acceptance of amphetamine (sulphate) from other sources:

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
<th>Comment/Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Character</td>
<td>White powder</td>
<td></td>
</tr>
<tr>
<td>Package and labelling</td>
<td>Good condition</td>
<td>Preferably PhEur standard</td>
</tr>
<tr>
<td>Infrared</td>
<td>conform library</td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td>&lt; 0.1% per impurity and &lt; 0.5 % total impurities</td>
<td>1) Chromatographically pure by GC or HPLC, based on area, or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) USP 29 method</td>
</tr>
<tr>
<td>Impurity profile</td>
<td>-</td>
<td>For reference purposes only; European harmonized method for amphetamine profiling. (SMT)</td>
</tr>
<tr>
<td>Moisture</td>
<td>&lt; 1.0 %</td>
<td>1) By loss on drying at 100 - 105°C for 2 h or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) Karl-Fisher determination</td>
</tr>
<tr>
<td>Content</td>
<td>&gt;99.0 and &lt; 100.5 %</td>
<td>1) Titration according to European pharmacopoeia, or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) no test, accepts material when all other tests are OK</td>
</tr>
</tbody>
</table>

Retesting:
As for reference material with a specification.
3.2. Heroin hydrochloride

The British Pharmacopoeia 2005 includes a monograph on heroin hydrochloride; in some countries this compound is prescribed in medical programs and thus commercially available. Storage of the compound in the refrigerator is recommended by the supplier for reducing its breakdown to 6-monoacetylmorphine. The life time of heroin hydrochloride is specified as 5 years under these conditions. The life time of heroin base was specified as 2 years.

A point of concern with heroin hydrochloride is the crystal water in the molecule.

Pharmacopoeial specifications [BP 2005]

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
<th>Comment/Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Character</td>
<td>White powder</td>
<td></td>
</tr>
<tr>
<td>Content (on dry substance)</td>
<td>&gt; 98.0% and &lt; 102.0 %</td>
<td></td>
</tr>
<tr>
<td>Loss on drying</td>
<td>&gt; 3.0 and &lt; 4.5 %</td>
<td>Theoretically the crystal water content is 4.2%</td>
</tr>
<tr>
<td>6-Monoacetylmorphine</td>
<td>&lt; 2%</td>
<td></td>
</tr>
<tr>
<td>Related substances</td>
<td>&lt; 0.5 %</td>
<td></td>
</tr>
</tbody>
</table>

Verification for heroin (hydrochloride) provided with a specification, mentioning to comply with the BP 2005:

1. Upon reception:
   - check the certificate
   - check labelling and good condition of the package and the contents
   - determine the amount (or ratio) of 6-monoacetylmorphine (*)
   - determine the amount (or ratio) of related compounds; for methods see table (*)

2. Retesting:
   - is advised every two years
   - check good condition of the package and the contents
   - check the control charts for the results of the control sample over the last two years

(*) optional
- determine the 6-monoacetylmorphine content (or ratio to heroin)
- determine the moisture content
- compare the composition and content with that of a sample of the same reference compound taken upon the reception and stored at -18°C

For suitable methods, see below

Verification and acceptance of heroin (hydrochloride) from other sources:

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
<th>Comment/Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR spectrum</td>
<td>conform library</td>
<td></td>
</tr>
<tr>
<td>6-Monoacetylmorphine content</td>
<td>&lt; 2.0%</td>
<td>1) pharmacopoeial HPLC test or 2) GC on silylated sample or 3) any other method capable of indicating the 6-monoacetyl-morphine content or ratio to heroin</td>
</tr>
<tr>
<td>Other alkaloids</td>
<td>&lt; 0.5%</td>
<td>1) pharmacopoeial HPLC test or 2) GC on silylated sample or 3) any other method capable of indicating the content or ratio to heroin of noscapine, papaverine, acetylcodeine and morphine</td>
</tr>
<tr>
<td>Moisture</td>
<td>&gt;3 and &lt; 4.5 %</td>
<td>1) Drying to constant weight at 105°C or 2) Karl Fisher determination</td>
</tr>
<tr>
<td>Content (of dry material)</td>
<td>&gt;98.0% and &lt; 102.0 %</td>
<td>1) Method BP 2005 or 2) Perchloric acid titration with mercury acetate (BP 1968 method)</td>
</tr>
</tbody>
</table>

3.3 MDMA hydrochloride

General
MDMA hydrochloride is commercially available at few chemical companies. However, it is extremely costly and almost impossible to buy on the grams level. Therefore, many laboratories prefer to do their own isolations and purifications from seized material. The purification is not complex but quite a few steps may be necessary before a rather ‘pure’ and white reference material is obtained.
MDMA is usually available as the hydrochloride salt. This salt can be present as a water free salt, but also as the monohydrate. These forms can interchange rather easily dependent on the atmospheric humidity. Because of the large influence of one molecule of crystal water on this small molecule it is strongly advised to store it under controlled, water-free conditions and/or to perform a frequent retesting on this point.

The specifications for an in-house reference standard MDMA hydrochloride chosen by ENFSI 2006:

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria/requirements</th>
<th>Method/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>White or off-white powder</td>
<td></td>
</tr>
<tr>
<td>Infra red</td>
<td>Conform MDMA x HCl waterfree</td>
<td>Ref Microgram</td>
</tr>
<tr>
<td>GC-MS</td>
<td>conform library</td>
<td></td>
</tr>
<tr>
<td>GC-MS (acyetylated)</td>
<td>conform library</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>&lt; 1.0 %</td>
<td>1) By drying at 105°C for 2 h or 2) Karl Fischer determination</td>
</tr>
<tr>
<td>Chromatographic purity</td>
<td>&gt; 99 %</td>
<td>By GC or HPLC based on area</td>
</tr>
<tr>
<td>Content (on dried material)</td>
<td>&gt;98.0 % and &lt;102.0 %</td>
<td>Perchloric acid titration</td>
</tr>
</tbody>
</table>

Verification upon acceptance:
Product must comply with the ENFSI specifications

Limited retesting:
- Check for crystal water by running an IR every 6 months and/or with every refill from a stock

Retesting:
- retesting is advised every 5 years
- check good condition of the package and the contents
- check the control charts for the results of the control sample over the last 5 years
- perform a quantitative determination by perchloric acid titration (*)
- compare the content with that of a sample of the same reference compound taken upon reception and stored at 4-6°C.

(*) optional
3.4. Cocaine hydrochloride

General
Cocaine hydrochloride is still used in medical practice and therefore readily available in some countries. It is a relatively stable compound, however, it is an ester and therefore a check for the presence of its hydrolysis compound benzoylecgonine is advised. Cocaine hydrochloride is mentioned as a hygroscopic compound. This means that appropriate (dry) storage conditions should be maintained and a retesting should preferably involve a check for moisture.

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (on dried material)</td>
<td>&gt; 98.5% and &lt; 101.0%</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>0.5%</td>
</tr>
<tr>
<td>Related substances: benzoylecgonine</td>
<td>&lt; 0.5% (all) and &lt; 0.1% (per compound)</td>
</tr>
</tbody>
</table>

Pharmacopoeial specifications [Ph.Eur Ed. V]

Verification for cocaine hydrochloride provided with a certificate, mentioning to comply with the PhEur:

1. Upon reception:
   - check the certificate
   - check good condition of the package and the contents
   - check for benzoylecgonine by a GC analysis after silylation(*) (**)  

2. Retesting:
   - is advised after 5 years
   - check good condition of the package and the contents
   - check for benzoylecgonine; for methods see table
   - determine water content; for methods see table
   - check the control charts for the results of the control sample over the last 5 years
   - perform a quantitative determination
   - compare the content with that of a sample of the same reference compound taken upon the reception and stored at 4-6° C

(*) optional  (**)advised if material is to be kept for a longer period
Verification and acceptance of cocaine hydrochloride from other sources:

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
<th>Comment/Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR spectrum</td>
<td>White crystalline powder or colourless crystals</td>
<td></td>
</tr>
<tr>
<td>Infrared</td>
<td>conform library</td>
<td></td>
</tr>
<tr>
<td>Related compounds</td>
<td>&lt; 0.1 % per impurity</td>
<td>1) HPLC according to PhEur, or 2) GC on silylated sample</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.5 % total impurities</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>&lt; 0.5 %</td>
<td>1) drying over silica gel for 3h (USP 29 method) or 2) drying at 100-105°C or 3) Karl-Fischer determination</td>
</tr>
<tr>
<td>Content (of dry material)</td>
<td>&gt; 98.5 and &lt; 101.0%</td>
<td>1) titration according to PhEur or 2) a titration with perchloric acid according to USP 29</td>
</tr>
</tbody>
</table>