A.3 Evaluation of chemical reference substances

The suitability of a substance proposed for use as a chemical reference requires careful evaluation by the issuing body. It is necessary to consider all data obtained from testing the material by a wide variety of analytical methods. When taken as a whole, this will ensure that the substance is suitable for its intended use. The extent of the analyses required depends on the purpose(s) for which the chemical reference substance is to be employed, and may involve a number of independent laboratories.

A.3.1 Use in identification tests For use in identification tests (IR spectrophotometry and/or chromatographic methods), a batch of good quality material selected from the normal production process is satisfactory if it is of acceptable purity. Additional purification by the supplier may be necessary. The most important check is the application of the test(s) for which the substance is intended. It is usual for at least one laboratory to apply all the chemical and physical tests described in the relevant monograph; some tests, such as those for sterility or for bacterial endotoxins, may not be necessary for materials intended as reference standards.

A.3.2 Use in purity tests The characterization of a chemical reference substance for use in the determination of a specific impurity is more extensive, especially when used in a limit test. If the technique employed is thin-layer chromatography (TLC), an acceptable minimum purity is recommended (normally at least 90%), but purer material (at least 95%) may be required for liquid chromatography (LC) or gas chromatography (GC). It is usually enough to involve only one laboratory when the reference substance is used in purity tests. If the proposed reference substance is being prepared or isolated for the first time, appropriate chemical and physicochemical tests, such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis, must be applied to characterize it.

A.3.3 Use in assays If the chemical reference substance is to be used in an assay (colorimetry, LC, GC or UV spectrophotometry), the extent of testing is much greater. Several (a minimum of three) laboratories should collaborate in testing the proposed substance, using a variety of established and validated techniques, including the method used in the pharmacopoeial specification. The relative reactivity or relative absorbance of the impurities present must be checked when a nonspecific assay method is employed, e.g. by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine the quantity of impurities. In such a case, it is best to examine the proposed reference substance by as many methods as practicable including, where possible, absolute methods. For substances that are acidic or basic a titration with alkali or acid is simple, but other reactions which are known to be stoichiometric may be used. Phase solubility analysis and differential scanning calorimetry may also be employed in certain cases.

The total of the determinations of water content, organic solvents, mineral impurities and organic components should amount to 100%. For most chemical reference substances intended for assays, the content may be expressed “as is”. When establishing the chemical reference substance it is, therefore, essential to determine the content of water and residual solvents for a non-specific assay, and also to determine the content of impurities for a selective assay.

A.3.4 Use in the calibration of an instrument Where the chemical reference substance is to be employed as calibration material, the extent of testing is similar to that for a chemical reference substance used in assays. Several laboratories should collaborate in testing the proposed substance using a variety of techniques to check that its purity is adequate. An appropriate number of collaborating laboratories should also participate, after the reference substance has been deemed suitable, to establish a value for the essential property of the substance using an appropriate instrument.