

A.4 Chemical and physical methods used in evaluating chemical reference substances

It is important to establish by individual testing that a substance proposed for use as a chemical reference is suitable for that purpose.

The methods used to establish the suitability of such a substance fall into two broad groups: those intended primarily to identify the substance and those used to establish its purity. With most methods, the percentage purity of a chemical reference substance cannot be expressed as an absolute value if the impurities have not been identified. The quoted purity is then an estimate based upon the data obtained by the various analytical methods.

A.4.1 Methods used to verify the identity of chemical reference substances Where a proposed reference substance is a substance whose structure has been satisfactorily defined, its identity may be confirmed by matching the IR spectrum of the substance to that of an authentic specimen. Particular care should be taken when polymorphism exists (8). Other highly specific techniques, such as NMR spectroscopy, MS, or X-ray diffraction crystallography, may also be used for such comparisons. The identity of a substance that is intended to replace an established chemical reference substance of the same molecular constitution must be verified, to determine that the characteristic properties of the two specimens are identical. For this purpose it is often sufficient to compare their IR absorption spectra.

However, where no authentic specimen of the proposed substance is available for comparison, and definitive data about its properties are lacking, it may be necessary to verify its identity by applying several of the analytical techniques currently used to characterize new compounds. Such analytical methods may include elemental analyses, crystallographic studies, MS, NMR spectroscopy, functional group analyses, and IR or UV spectrophotometry, as well as other supplementary tests, as required, to establish that the proposed substance is fully characterized.

A.4.2 Methods used to determine the purity of chemical reference substances The analytical methods to be employed in examining a substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories:

- those that require comparison with an external chemical reference substance (e.g. chromatographic or spectrophotometric methods);
- those that depend solely on an intrinsic dynamic property (e.g. phase solubility analysis and differential scanning calorimetry); and
- other methods.

A.4.2.1 Separation techniques

The methods used for the determination of purity should be established and validated with system suitability requirements as appropriate.

Chromatographic methods. Methods of analysis based on chromatographic separation are especially useful for detecting and determining impurities in chemical reference substances. High-performance liquid chromatography (HPLC) is the most widely used chromatographic method, but TLC and GC are also used. The individual components separated by chromatographic methods may sometimes be recovered for characterization.

The selectivity of HPLC and of GC usually exceeds that of TLC. Both of the first two methods also have the advantage of being readily applicable on a quantitative basis, but they require more complex equipment. HPLC, employing a spectrophotometric method of detection, is of particular value in the examination of chemical reference substances intended for use in UV spectrophotometric assays. The UV wavelength of detection employed for determining the impurity content of the chemical reference substance should be chosen so that the detection responses of the substance and its impurities are similar. When the response factors are significantly different at the optimal wavelength of detection, appropriate corrections must be made to estimate the content of impurities. LC with diode-array detection is very useful for recording the UV spectra of both the main peak and the impurities. LC with MS detection is used for identification of separated impurities as well as for the main component, and is particularly important for use with chemical reference substances for which no other reference standards or IR reference spectra are available.

In a GC method used for an assay, as with LC, the detection responses of the impurities are determined. Generally, monograph methods using GC are of particular value in detecting and determining volatile impurities, including solvent residues, in chemical reference substances.

TLC uses apparatus that is simple and inexpensive; the technique is easy to carry out and is readily applicable even in the microgram range. It can separate closely related compounds, such as geometric isomers and the members of a homologous series. All the constituents of a substance subjected to chromatography appear somewhere on the chromatogram. However, some constituents may remain on the starting line, some may move with the solvent front, some may migrate at the same rate as the main component and some may remain undetected. For this reason, the usefulness of the method may be greatly enhanced by performing two-dimensional chromatography and by using a number of different solvent systems and a variety of detection methods. In some cases the method may be used quantitatively with acceptable accuracy by using a densitometer.

Capillary electrophoresis. Capillary electrophoresis (CE) is an increasingly common method. It may be considered as complementary to LC for detecting impurities.

A.4.2.2 Methods based on intrinsic thermodynamic properties

Methods in this group measure total impurity levels in absolute terms.

Differential scanning calorimetry. This technique is used to check for the presence of different polymorphic forms and to determine the total amount of solid impurities. Purity estimation is based on determination of the heat of fusion of the sample and of the change in its melting point caused by the presence of impurities. This analytical method can be performed rapidly and with high precision. However, it is not applicable if the substance decomposes on melting. This limits its value as a general procedure for estimating the purity of chemical reference substances. It is also inapplicable if solid solutions are formed.

Phase solubility analysis. The method has occasionally been used but its value is limited and the procedure is time consuming. It may be employed to detect contaminating substances, including isomeric species, and to estimate their concentration. Some factors that may make the method inapplicable are degradation of the substance during the course of analysis, formation of a solid solution and polymorphism in the main component.

A.4.2.3 Other methods

Spectrophotometric methods. UV spectrophotometry is occasionally used to determine purity. Since it depends upon the presence of a characteristic chromophore, it can detect impurities that contribute excessively to the absorbance value and may indicate the presence of impurities that have a negligible or distinctive absorbance.

However, the utility of the method is limited by the small number of absorption maxima in the UV range, the large numbers of compounds containing similar characteristic chromophores, and the need for an external chemical reference substance.

IR spectrophotometry may be used to identify and determine the proportions of geometric isomers. NMR spectroscopy, a powerful spectroscopic identification tool, is also occasionally useful in the determination of purity.

Titrimetric methods. Titrimetric methods provide a valuable means of confirming the identity and purity of a proposed chemical reference substance and are useful in confirming purity values obtained by other methods.

Optical rotation methods. Many chemical reference substances are optically active and the relative proportion of optical isomers can sometimes be determined by an optical rotation method, but generally such methods lack specificity and sensitivity. However, the quantitative use of these techniques is well established and can yield results of high precision, depending on the solvent and the wavelength chosen for measurement, provided that pure substances of individual isomers are available. Chiral chromatography, NMR and CE are becoming increasingly important.

Determination of water and organic volatiles. It is essential that an accurate assessment of the moisture content and the content of volatile substances be made. These total values may often be obtained by drying under defined conditions that are appropriate to the proposed substance. Sometimes this may not be possible or may yield misleading results. In such cases, thermogravimetric analysis may be used to determine the content of water and organic volatiles. Alternatively, the water content may be determined by Karl Fischer titration and the content of volatile solvents by GC. Without an accurate assessment of these values at the time that other determinations are being made, judgements of the acceptability of the proposed chemical reference substance will be invalid.