B.4 Interlaboratory testing to establish the assigned content

Having demonstrated the suitability of the substance, the content value is assigned on the basis of the results generated by an interlaboratory trial. At least three laboratories participate in testing the proposed substance (*10*).

B.4.1 Competence of the participating laboratories Participating laboratories will have demonstrated their adherence to the concepts of an appropriate quality management system (*9*–*12*).

B.4.2 Dispatch of the candidate materials The proposed secondary reference substance is packaged in appropriate unit quantities. The quantity of each unit is dependent on the intended use. The proposed substance and the primary reference substance are dispatched to the participating laboratories in sufficient amounts for replicate analysis as required by the test protocol. The participating laboratories are instructed to record any abnormalities observed with the proposed substance. The packaging facilities are adequate and environmental conditions are controlled to ensure the integrity of the material throughout the packaging process.

The following documents should be supplied with the material:

– test protocol;
– test result report form;
– health and safety information; and
– information on the primary chemical reference substance.

B.4.3 Test protocol While the testing of primary chemical reference substances employs different analytical methods in a collaborative study, an alternative approach is normally applied to the testing of a secondary chemical reference substance. Since most secondary reference substances are established to determine the content of the drug substance itself (for which a pharmacopoeial monograph exists) and/or the amount of the drug substance contained in a pharmaceutical preparation, it is essential to use the method specifi ed in the relevant pharmacopoeia to obtain the assigned value.

The coordinating laboratory prepares the testing protocol, including predefined acceptance criteria of the results. The protocol clearly describes each step of the procedure and includes data reporting sheets The experimental design of the interlaboratory study is such that the results are statistically evaluated to assign a content with an acceptable confi dence interval in relation to the permitted limits of content as set in the defi nition. Both the number of independent replicate determinations to be performed and acceptance criteria to be applied are predefi ned.

B.4.4 Evaluation of test results Test results submitted by the participating laboratories are evaluated in accordance with the criteria set out in the protocol. The data submitted by each laboratory are tested statistically for “outliers” and for conformity with the system suitability criteria. Apparent “outliers” are investigated by the laboratory concerned, remedial action taken, and the analysis repeated. If a valid reason is discovered for the “outliers” then these are excluded from the statistical evaluation.

The mean and confidence interval are then calculated. The reference value is assigned using the mean of the laboratory means.

B.4.5 Traceability The term for “traceability”, for the purposes of this document, is defined as the property of a result of measurement which can be related to the appropriate standards, generally international or national standards, through an unbroken chain of comparison. In other words, when the result of a measurement is described as traceable, it is essential to specify to what (value of) “appropriate standards” traceability has been established.

The assigned value of a secondary chemical reference substance is traceable to the relevant primary reference substance. In the context of WHO quality specifications the relevant primary chemical reference substance is usually the ICRS established for use with *The International Pharmacopoeia*. In other contexts the relevant primary chemical reference substance will be the reference substance established for use with another internationally recognized pharmacopoeia (e.g. the European Pharmacopoeia chemical reference substances (Ph.Eur CRS), British Pharmacopoeia chemical reference substances (BPCRS), or the United States Pharmacopeia reference substances (USPRS)).