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III. ANALYTICAL VALIDATION 分析方法验证

This section describes the procedures to be carried out to validate the tests that are intended to be described in a Ph. Eur. monograph. These tests include tests for identification, instrumental and non-instrumental tests for the control of impurities, and the assay procedure. The validation requirements vary according to the type of test and the technique employed. This section contains the texts on Analytical Validation adopted by the ICH in 1994, the Extension of the ICH text "Validation of Analytical Procedures" which includes valuable information concerning validation requirements for registration applications and specific guidelines for the validation of pharmaceutical procedures using different analytical techniques.
本节描述了拟收载于欧洲药典各论中的分析方法的验证程序，包括鉴别、仪器和非仪器的杂质检测分析方法以及含量测定方法。验证要求因方法类型和所采用的技术而异。本部分包含 ICH 在 1994 年通过的分析方法验证文本，以及能具体、有效指导注册申请验证和使用不同分析技术的 ICH "分析方法验证"扩展文本。

III.1. DEFINITIONS AND TERMINOLOGY 定义和术语

[ICH document. Text adopted and published by the International Conference on Harmonisation of *Technical Requirements for the Registration of Pharmaceuticals for Human Use* (1994)].
[ICH 文件。人用药品注册技术要求国际协调会议（1994）通过并发布的文本]。

III.1.1. Introduction 简介

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text presentation serves as a collection of terms and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA.
本文件讨论了在欧盟、日本和美国提交的注册申请中，包含的分析方法验证过程中需要考虑的方法特性。本文件并非旨在寻求涵盖世界其他地区注册或出口所要求的测试。此外，本文汇集了术语及其定义，并不旨在为如何完成验证提供指导。这些术语和定义旨在弥合欧盟、日本和美国不同药典和监管机构之间经常存在的差异。

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.
分析方法验证的目的是证明其适用于预期目的。本文以表格的形式汇总了适用于鉴别、杂质控制和含量测定方法的各个项目。未来有望考虑补充其他分析方法。

III.1.2. Types of analytical procedures to be validated 待验证分析方法的类型

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:
分析方法验证的讨论针对四种最常见的分析方法：

- Identification tests;
  鉴别试验；
- Quantitative tests for impurities' content;
  杂质的定量试验；
- Limit tests for the control of impurities;
  杂质控制的限度试验；
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.
  在原料药或制剂中活性成分以及制剂中选定组分的定量试验。

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed herein and may be addressed in subsequent documents.
虽然还有许多其他的分析方法，如制剂的溶出度试验或原料药的粒度测定，但在分析方法验证的初始文本中并未提及。这些额外的分析方法的验证与本文所列的分析方法的验证同样重要，可在后续文件中讨论。

A brief description of the types of tests considered in this document is provided below:
本文中考虑的试验类型的简要描述如下：

- Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g. spectrum, chromatographic behaviour, chemical reactivity, etc.) to that of a reference standard.
  鉴别试验旨在确证样品中的一种被测物的特性。这通常是通过将样品的特性(如光谱、色谱特性、化学反应性等)与标准物质进行比较来实现的。

- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test
  杂质检查既可以是样品中杂质的定量检验，也可以是杂质的限度检查。这两种检验都是为了准确反映样品的纯度特性。定量检验与限度检查的验证要求不同。

- assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g. dissolution).
  含量测定是指测定样品中被分析物的含量。在本文中，含量测定是对原料药中主要成分的定量测定。类似的验证项目也适用于制剂中活性成分或其他指定成分的定量测定。同样的验证项目也可适用于与其他分析方法(如溶出度)相关的定量分析。

III.1.3. Validation characteristics and requirements 验证项目和要求

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics that should be considered are listed below:
应清楚地了解分析方法的目的，因为这将决定需要评估的验证项目。应考虑的典型验证项目如下：

- Accuracy;

准确度；
- Precision;
  精密度；
  - Repeatability;
    重复性；
  - Intermediate precision;
    中间精密度；
- Specificity;
  专属性；
- Detection limit;
  检测限；
- Quantitation limit;
  定量限；
- Linearity;
  线性；
- Range.
  范围。

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.
这些验证项目在附件的术语表中都有定义。下表列出了对不同类型的分析方法中被认为最重要的验证项目。该清单应被视为典型的分析方法，但偶尔的例外情况应根据具体情况逐一处理。应当注意的是，耐用性没有列在表中，但应在分析方法开发的适当阶段加以考虑。

Furthermore revalidation may be necessary in the following circumstances:
此外，在以下情况下可能需要再验证：

- changes in the synthesis of the drug substance;
  原料药合成的变更；
- changes in the composition of the drug product;
  药品成分的变更；
- changes in the analytical procedure.
  分析方法的变更。

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.
再验证的程度取决于变更的性质。一些其他变更可能也需要验证。

TYPE OF ANALYTICAL PROCEDURE 分析方法类型			
IDENTIFICATION 鉴别	**TESTING FOR IMPURITIES** 杂质检查		**ASSAY** 含量测定
	Quantitative test 定量检验	**Limit test** 限度检查	**Dissolution Measurement only Content/potency** 溶出度测定（含量/效价测定）

CHARACTERISTIC 项目				
Accuracy 准确度	—	+	—	+
Precision 精密度				
Repeatability 重现性		+	—	+
Intermediary Precision 中间精密度		+*	—	+*
Specificity** 专属性**	+	+	+	+
Detection Limit 检测限	—	—***	+	—
Quantitation Limit 定量限	—	+	—	+
Linearity 线性	—	+	—	+
Range 范围	—	+	—	+

– signifies that this characteristic is not normally evaluated.
指该特性通常不需要被评价。

+ signifies that this characteristic is normally evaluated.
指该特性通常需要被评价。

* in cases where reproducibility (see Glossary) has been performed, intermediate precision is not needed.
指在评价了重现性（见术语表）情况下，可不需要再评价中间精密度。

** lack of specificity of one analytical procedure, could be compensated by other supporting analytical procedure(s).
指在一种分析方法专属性不足时，可通过其他支持性分析方法予以补充。

***may be needed in some cases.
指在某些情况下可能需要。

III.1.4. Glossary 术语

Analytical procedure. The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the preparation of reagents, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.
分析方法：分析方法是指进行分析的方式。应该详细描述进行每个分析试验所需的步骤。包括但不限于：样品、标准物质和试剂的配制，仪器的使用、校准曲线的绘制、计算公式的使用等。

Specificity. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradation products, matrix, etc.
专属性：专属性是指可能存在某些组分（如杂质，降解产物，基质等）时，对被分析物准确可靠测定的能力。

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

当一种分析方法缺乏专属性时，可由其他辅助分析方法加以补充。

This definition has the following implications:
该定义具有以下含义：

- Identification: to ensure the identity of an analyte.
  鉴别：确证被分析物符合特性。
- Purity tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
  纯度检查：确保采用的分析方法可检出分析物中的杂质的准确含量，如有关物质、重金属、残留溶剂量等。
- Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.
  含量测定（含量或效价）：提供样品中被分析物的含量或效价的准确结果。

Accuracy. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.
准确性：指一种分析方法的测量值与真实值或认可的参考值之间的相近程度。有时也被称为真实度。

Precision. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.
精密度：分析方法的精密度表示在规定条件下对均质样品多次取样进行一系列检测结果的接近程度（离散程度）。精密度可从三个层次考虑：重复性、中间精密度和重现性。

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample, it may be investigated using artificially prepared samples or a sample solution.
应使用均质的、可信的样品考察精密度。如果无法获得，则可以使用人为配制的样品或样品溶液进行研究。

The precision of analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.
分析方法的精密度通常以多次测量结果的变异性、标准偏差或变异系数来表达。

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.
重复性表示在短时间间隔内，同样的操作条件下的精密度。重复性也称为间隙测量精密度。

Intermediate precision expresses variations within laboratories: different days, different analysts, different equipment, etc.
中间精密度表示实验室内部条件改变：不同日、不同的分析人员、不同的设备等情况下的精密度。

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).

重现性表示不同实验室之间的精密度（合作研究，常用于方法学的标准化）。

Detection limits. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
检测限：某一分析方法的检测限是指样品中的被分析物能够被检测到的最低量，但不一定是准确定量。

Quantitation limits. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of substances in sample matrices, and is used particularly for the determination of impurities and/or degradation products.
定量限：某一分析方法的定量限是指在合适的准确性和精密度下，能够定量测定样品中被分析物的最低量。它是样品中含量下，能够定量测定样品中被分析物的最低量。它是样品中含量低的化合物定量测定的参数，特别适用于杂质和/或降解产物的测定。

Linearity. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
线性：分析方法的线性是指在给定范围内检测结果与样品中被分析物的浓度（量）成比例关系的能力。

Range. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
范围：分析方法的范围是样品中被分析物的较高浓度（量）和较低浓度（量）之间的一个区间。并已证实在此区间内，该方法具有合适的准确性、精密度和线性。

Robustness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
耐用性：分析方法的耐用性是指试验参数被故意地发生细小改变时，检测不受影响地能力，用于说明正常使用时地可靠性。

III.2. METHODOLOGY 方法论

[ICH document. Text adopted and published by the *International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use* (1996)].

[ICH 文件。人用药品注册技术要求国际协调会议（1996）通过并发布的文本]。

III.2.1. Introduction 简介

This document is complementary to the parent document which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity) the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data which should be presented in a new drug application.
本文是对已有文件的补充，原文已经对分析方法验证过程中应考虑的项目进行了讨论。本文

的目的是就如何考虑每个分析方法的各种验证项目提供一些指导和推荐建议。在某些情况下（例如证明方法的专属性），为了确保原料药或制剂产品的质量，可能需要对几个分析方法的组合进行总体的评价。此外，该文件还对新药申请中应提交的数据提供了指导建议。

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.
在验证期间收集的所有相关数据以及用于计算验证项目的计算公式都应当提交并进行适当的讨论。

Approaches other than those set forth in this guideline may be applicable and acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product. However, it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document.
本指导原则以外的验证方法也可被采用和接受。选择最适合其产品的验证程序和方案是申请人的责任。然而，重要的是要牢记，分析方法验证的主要目的是证明该方法适用于其预期的使用目的。由于生物制品和生物技术的复杂性，在某些情况下，对这些产品的分析方法程序可能会有别于本文的内容。

Well-characterised reference materials, with documented purity, should be used throughout the validation study. The degree of purity required depends on the intended use.
在整个验证研究过程中，应使用经过充分质量研究并附有纯度信息的参考物质。参考物质的纯度取决于其预期用途。

In accordance with the parent document and for the sake of clarity, this document considers the various validation characteristics in distinct parts. The arrangement of these parts reflects the process by which an analytical procedure may be developed and evaluated.
为了与原文内容保持一致并有利于理解，本文将不同的验证项目分为不同的节进行论述。这些节的排列反映了建立和评价一个分析方法的过程。

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity, linearity, range, accuracy and precision.
在实际工作中，可以对实验工作进行充分的设计，使得可以同时考察多个适当的验证项目，提供分析方法科学的、综合的能力情况，例如：专属性、线性、范围、准确性和精密度。

III.2.2. Specificity 专属性

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.
在鉴别试验、杂质测定和含量测定方法的方法学验证中，都应进行方法专属性的考察。用于证明分析方法专属性的程序和步骤取决于分析方法的预期目的。

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.
并不总是能证明一个分析方法对某一特定物质的测定具有专属性(完全的区分能力)。在此种

情况下，推荐联合使用两种或更多分析方法，获得对待测物质所需的区分能力。

III.2.2.1. Identification 鉴别

Suitable identification tests should be able to discriminate between substances of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sensible scientific judgement with a consideration of the interferences which could occur.
一个合适的鉴别试验应具有区分可能存在的结构相关的成分的能力。可以通过从含有被分析物的样品中获得正向结果（可通过与已知参考物质的比较），再加上从不含被分析物的样品中获得负向结果，来确认一个方法的鉴别能力。此外，鉴别试验也可能应用于那些与被分析物结构类似或相关的物质的分析，以确认这些相关物质没有获得正向结果。对这种潜在干扰物质的选择应基于合理的科学判断，并考虑到可能发生的干扰。

III.2.2.2. Assays and impurity tests 含量测定和杂质检查

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques.
对于色谱测定方法，应使用有代表性的色谱图来证明方法的专属性，并对各成分进行适当的标识。采用其他分离技术时也应进行类似的考虑。

Critical separations in chromatography should be investigated at an appropriate level. For critical separations specificity can be demonstrated by the resolution of the two components which elute closest to each other.
在色谱方法中，应在一定程度上考察临界分离。对临界分离，可以采用两个洗脱程度最接近的化合物的分离度来证明其专属性。

In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance, the combination of the assay and a suitable test for impurities can be used.
在使用非专属性含量测定方法时，应使用其他辅助性的分析方法来证明分析方法总体的专属性。例如，在采用滴定法对原料药进行含量测定的情况下，可以结合使用合适的杂质检查方法。

The approach is similar for both assays and impurity tests:
含量测定和杂质检查方法的要求基本相同。

Impurities are available 可以得到杂质

- for the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples);
  对于含量测定方法，应证明在杂质和/或辅料存在的情况下，分析方法能将待测组分与干扰组分区分开来；实际工作中，可以在纯物质(原料或制剂)中加入一定量的杂质和/或辅

料，并与未添加杂质或辅料的样品含量测定结果进行比较，证明其含量测定结果不受这些物质的干扰。

- for the impurity test, the discrimination may be established by spiking the drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Alternatively, for less discriminating procedures it may be acceptable to demonstrate that these impurities can still be determined with appropriate accuracy and precision.
对于杂质检查方法，可以在原料或制剂中加入一定量的杂质，证明杂质能逐个分离，并也能与样品中其他组分分离。对于区分能力较差的分析方法，一个可以接受的替代的证明方法，就是证明该方法仍能以一定的准确度和精密度测定这些杂质的含量。

Impurities are not available 无法得到杂质

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterised procedure, e.g. pharmacopoeial procedure or other validated analytical procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.
当不能获得杂质或降解产物的对照品时，可以将含有杂质或降解产物的样品测定结果与另一种成熟的方法测定结果进行比较，如药典方法或经过验证的其他方法（与该方法不相关的方法）进行比较来证实。如适用，应包括放置在强力破坏实验条件下，光照、加热、湿度、酸/碱水解及氧化情况下的样品测定。

- For the assay, the two results should be compared.
对于含量测定，应进行两种测定结果的比较。
- For the impurity tests, the impurity profiles should be compared.
对于杂质检查，应进行检出杂质情况的比较。

Peak purity tests (e.g. diode array, mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.
峰纯度检查(例如二极管阵列、质谱)是很有用的，它显示被分析物是单个还是多个成分。

III.2.3. Linearity 线性

Linearity should be established across the range (see part III.2.4) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components using the proposed procedure. The latter aspect can be studied during investigation of the range.
应在分析方法的测定范围内建立线性关系（见第III.2.4部分）。可以通过采用拟定的测定方法，直接测定原料药含量(对照品贮备溶液的稀释)和/或药品组分的合成混合物的各组分的含量，来证明分析方法的线性。在范围的考察过程中可对后一种证明方法进行研究。

Linearity should be established by visual evaluation of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.
线性关系应以信号对被分析物的浓度或含量作图，根据图形是否呈线性进行评价。如果有线

性关系，应采用适当的统计学方法对测定结果进行评价，比如，可通过最小二乘法计算线性回归系数。在有些情况下，为了使含量测定结果与样品浓度呈线性关系，在进行回归分析前，可能需对测定数据进行某种形式的数学转换。由线性回归估算所得到的数据本身，又有助于证明线性程度的好坏。

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.
应提交回归曲线的相关系数、Y 轴截距、斜率和残差平方和等参数。还应包括回归曲线的数据图。此外，对实际数据点与回归曲线上数据点之间的偏差进行分析，也可能有助于线性的评价。

Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample.
一些分析方法，如免疫测定法，不管进行何种形式的数学转换，均不能证明线性关系。在这种情况下，可用适当的函数，表述供试品中待测物质的浓度(量)与分析响应值之间的关系。

For the establishment of linearity, a minimum of five concentrations is recommended. Other approaches should be justified.
为了建立线性，推荐至少采用 5 个不同浓度的供试品，也可采用其他经过论证的方法进行线性考察。

III.2.4. Range 范围

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.
分析方法的范围通常来源于线性研究并且取决于其预期用途。可通过对含有分析方法规定范围或极端含量的供试品的测定，确认方法的范围，如果分析方法能够提供可接受的线性、准确度和精密度，证明方法的范围符合规定。

The following minimum specified ranges should be considered:
应考虑下述最小的规定范围：

- for the assay of a drug substance or a drug product: from 80 to 120% of the test concentration;
  原料药或制剂的含量测定：测定浓度的 80%～120%；

- for the determination of an impurity: from the quantitation limit (QL) or from 50% of the specification of each impurity, whichever is greater, to 120% of the specification;
  单个杂质的测定：从定量限或者单个杂质限度的 50%（取较大者）～120%；
- for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled. Note: for validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit;
  已知具有强烈作用或产生毒性或不期望的药理作用的杂质：其检测 / 定量限应与杂质必须控制的水平相适应。注：在方法建立阶段，对杂质检查方法的验证中，可能需要考虑建议(可能)的限度范围；

- if assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from QL or from 50% of the specification of each impurity, whichever is greater, to 120% of the assay specification;
  如果采用同一个分析方法进行含量测定和纯度检查，并且只采用了相当于 100%标示含量的对照品，测定方法的线性范围应涵盖从定量限或者单个杂质规定限度的 50%（取较大者）～120%；

- for content uniformity, covering a minimum of 70 to 130% of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g. metered dose inhalers) is justified;
  含量均匀度测定：除非根据制剂(比如定量吸入剂)的特性，需要更宽的测定范围外，应覆盖测定浓度的 70%～130%；

- for dissolution testing: ± 20% over the specified range, e.g. if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0-110% of the label claim.
  对于溶出度测试：应为规定范围的±20%，比如某控释制剂规定 1h 后的释放度为 20%，24h 后的释放度为 90%以上，那么，验证范围将是标示量的 0～110%。

III.2.5. Accuracy 准确性

Accuracy should be established across the specified range of the analytical procedure.
应在分析方法的测定范围内，确定方法的准确性。

III.2.5.1. Assay 含量测定

Drug substance (Active pharmaceutical ingredient). Several methods of determining accuracy are available:
原料药（活性药物成分）可采用下列方法进行方法准确度的评价：

- application of an analytical procedure to an analyte of known purity (e.g. reference material);
  用该分析方法测定已知纯度的被分析物（比如参比物质）；
- comparison of the results of the proposed analytical procedure with those of a second well-characterised procedure, the accuracy of which is stated and/or defined (independent procedure);
  将拟定分析方法的测定结果与其他规定了准确度的可靠的分析方法（独立的测定方法）的测定结果进行比较；
- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.
  在进行精密度、线性和专属性考察时，可以同时测定方法的准确度。

Drug product. Several methods for determining accuracy are available:
制剂：可采用下列方法进行方法准确度的评价：

- application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;
  用该分析方法去测定按处方量制成的混合物，其中加入了已知量的待测原料药；

- in cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from the second, well-characterised procedure, the accuracy of which is stated

and/or defined (independent procedure);
当不能获得所有制剂组分的样品时，也可在制剂中加入已知量的待测物质，或者将拟定分析方法的测定结果与其他规定了准确度的可靠的分析方法(独立的测定方法)的测定结果进行比较；

- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.
在进行精密度、线性和专属性考察时，可以同时测定方法的准确性。

III.2.5.2. Impurities (quantitation) 杂质（定量）

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.
应在原料药或制剂的样品中加入已知量的杂质，然后通过上述样品的测定来评价分析方法的准确度。

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used.
当无法获得某些杂质和/或降解产物的样品时，可以采用与独立测定方法的结果进行比较。也可采用原料药的响应因子进行准确性评价。

III.2.5.3. Recommended data 申报数据

Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g. three concentrations/three replicates each).
准确性的评价应在规定的线性范围内，使用至少 3 个浓度水平的 9 次测定结果（例如制备 3 种浓度样品，每个样品重复测定 3 次）。

Accuracy should be reported as percent recovery by the assay of a known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.
准确性应在测定样品中加入已知量的被分析物测得的百分比回收率，或以平均值与真实值之间的差值以及置信区间来报告。

III.2.6. Precision 精密度

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.
含量测定和杂质定量测定的方法验证应包括对精密度的研究。

III.2.6.1. Repeatability 重复性

Repeatability should be assessed using:
应使用以下方法对重复性进行评价：

- a minimum of nine determinations covering the specified range for the procedure (e.g. three concentrations/three replicates each), or
涵盖分析方法规定线性范围的至少 9 次测试（例如，3 种浓度，每种浓度重复测定 3 次）；或

- a minimum of six determinations at 100% of the test concentration.
  至少对 100%试验浓度进行 6 次测定。

III.2.6.2. Intermediate precision 中间精密度

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.
中间精密度的考察程度取决于预期在何种情况下使用该方法。申请人应当确定随机事件对于分析方法精密度的影响。需要研究典型变异包括：日期，分析人员，仪器等。没有必要逐个考察每个因素。鼓励使用实验设计（矩阵）的方式进行研究。

III.2.6.3. Reproducibility 重现性

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardisation of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorisation dossier.
通过多个实验室间的试验评价重现性。在分析方法需要标准化时，例如，将分析方法列入药典，则应考虑重现性。申请上市的文档中不需要这些试验数据。

III.2.6.4. Recommended data 申报数据

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.
应当报告每种精密度研究类型的标准偏差，相对标准偏差（变异系数）和置信区间。

III.2.7. Detection limit 检测限

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.
有几种方法可以确定检测限，这取决于该方法是非仪器还是仪器。除了下面列出的方法以外，其他方法也可以接受。

III.2.7.1. Based on visual evaluation 基于直观评价

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.
直观评价可用于非仪器分析方法，但也可用于仪器分析方法。

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
检测限是通过分析已知浓度的样品，并以能准确测得被分析物得最小量来建立。

III.2.7.2. Based on signal-to-noise ratio 基于信噪比

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum

concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally acceptable.
这种方法只能应用于有基线噪声的分析方法。信噪比的测定方法是将已知低浓度分析物的样品与空白样品的测量信号进行比较，并确定被分析物可以被准确地检测的最小浓度。信噪比一般在 3：1 或 2:1 之间是可以接受的。

III.2.7.3. Based on the standard deviation of the response and the slope
基于响应值的标准偏差和斜率

The detection limit (DL) may be expressed as:
检测限（DL）可以表示为：

$$DL = \frac{3.3\sigma}{S}$$

σ = the standard deviation of the response,
σ =响应值的标准偏差，
S = the slope of the calibration curve.
S =校正曲线的斜率。

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:
斜率 S 可以从被分析物标准曲线中估算出来。可以通过多种方式对σ进行估算，例如：

- Based on the standard deviation of the blank. Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.
  基于空白的标准偏差。通过几份空白样品的分析，测量分析背景响应值大小，并计算这些空白样品响应值的标准偏差。
- Based on the calibration curve. A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.
  基于校正曲线。在包含检测限（DL）范围内，通过对含有被分析物地一组样品地分析，建立标准曲线。回归线的残余标准偏差或回归线 y 轴截距的标准偏差都可作为标准偏差。

III.2.7.4. Recommended data 申报数据

The detection limit and the method used for determining the detection limit should be presented.
应当给出检测限和测定检测限的方法。

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.
如果检测限的估计值是通过计算或外推法获得的，可另取一系列接近或等于检测限度地样品进行逐个分析，来验证这一估算值。

III.2.8. Quantitation limit 定量限

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed may be acceptable.
有几种方法可以确定定量限，这取决于该分析方法是非仪器分析的还是仪器分析。除了下面

列出的方法之外，其他方法也可以接受。

III.2.8.1. Based on visual evaluation 根据直观评价

Visual evaluation may be used for non-instrumental methods, but may also be used with instrumental methods.
直观评价既可用于非仪器分析方法，也可用于仪器分析方法。

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
定量限通常是通过对已知浓度的被分析物的样品进行分析，在准确度和精密度都符合要求的情况下，被分析物能被定量测得的最小量即为定量限。

III.2.8.2. Based on signal-to-noise ratio 根据信噪比

This approach can only be applied to analytical procedures which exhibit baseline noise.
这种方法只能应用于有基线噪声的分析方法。

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to noise ratio is 10:1.
信噪比的测定方法是将已知低浓度被分析物的样品与空白样品的测量信号进行比较，以其中被测物能够被准确定量的最小浓度为定量限。一般取信噪比为 10：1。

III.2.8.3. III.2.8.3. Based on the standard deviation of the response and the slope
基于响应值标准偏差和斜率的方法

The quantitation limit (QL) may be expressed as:
定量限（QL）可以表示为：

$$QL = \frac{10\sigma}{S}$$

σ = the standard deviation of the response,
σ =响应值的标准偏差，
S = the slope of the calibration curve.
S =校正曲线的斜率。

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways for example:
斜率 S 可以从被分析物校正曲线中估算出来。可以通过多种方式对σ进行估算，例如：

- Based on the standard deviation of the blank. Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.
  基于空白样品的标准偏差。通过分析适当数量的空白样品，得出分析背景响应值的大小，并计算这些空白样品响应值的标准偏差。
- Based on the calibration curve. A specific calibration curve should be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

基于校正曲线。通过对含有定量限（QL）水平的被分析物的测试，建立校正曲线。回归线的残余标准偏差或回归线 y 轴截距的标准偏差都可作为标准偏差。

III.2.8.4. Recommended data 申报数据

The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.
应当给出定量限和用于确定定量限的方法。应当通过分析一系列接近或等于定量限的样品，来验证该限值。

III.2.9. Robustness 耐用性

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.
对耐用性的评价应当在分析方法开发阶段予以考虑，并应基于分析方法的类型进行研究。该评估应能够表明，在分析方法参数进行有意改变后，分析方法依然是可靠的。

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.
如果测量值容易受到分析条件变化的影响，那么应当适当控制分析的条件，或在分析方法中加入预防性声明。通过耐用性评估，建立一系列系统适用性参数（例如，分离度测试），以确保无论何时使用该分析方法均能保持其有效性。

Typical variations are:
典型的变化有：
- stability of analytical solutions;
  分析溶液的稳定性；
- different equipment;
  不同的设备；
- different analysts.
  不同的分析人员。

In the case of LC, typical variations are:
在使用液相色谱法的情况下，典型的变化有：
- influence of variations of pH in a mobile phase;
  流动相 pH 变化的影响；
- influence of variations in mobile phase composition;
  流动相组成变化的影响；
- different columns (different lots and/or suppliers);
  不同色谱柱（不同批号和/或供应商）；
- temperature;
  温度；
- flow rate.
  流速。

In the case of GC, typical variations are:

在使用气相色谱法的情况下，典型的变化有：

- different columns (different lots and/or suppliers);
  不同的色谱柱（不同批号和/或供应商）；
- temperature;
  温度；
- flow rate.
  流速。

III.2.10. System suitability testing 系统适用性试验

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information.
系统适用性试验是许多分析方法中必不可少的一部分。该试验是把分析设备、电子仪器、实验操作和待分析样品组成了一个整体来进行评估。为特定方法建立的系统适用性参数取决于被验证方法的类型。更多的信息参见药典内容。

III.3. SPECIFIC APPLICATION TO ANALYTICAL PROCEDURES USED IN THE PH. EUR.

欧洲药典中分析方法的特殊应用

The following parts describe a number of points that are important for the validation of analytical procedures employing specific analytical techniques. These guidelines are to be used in conjunction with the general chapters of the Ph. Eur. and the validation requirements given previously in the ICH documents.
下列内容描述了一些重点，对使用特殊分析技术的分析方法的验证有重要意义。这些指导原则与《欧洲药典》通则及前文所述的 ICH 文件中的验证要求配合使用。

III.3.1. Optical rotation (2.2.7) 旋光度 (2.2.7)

III.3.1.1. Introduction 介绍

The solvent should be chosen in order to obtain an angle of rotation that is as great as possible. The stability of the angle of rotation of the solution should be checked over a period of at least 2 hours. If necessary, the use of a freshly prepared solution may be prescribed. In exceptional cases, it may be necessary to prescribe an equilibration period before the measurement is carried out. Whenever possible, the use of a wavelength corresponding to the D-line of sodium (i.e. 589 nm) is prescribed.
为了获得尽可能高的旋转角，应对溶剂进行选择。供试品溶液的旋光度应在至少每 2 个小时检测一次，考察溶液的稳定性。如有必要，应在质量标准中规定，供试品溶液临用前现制。在特殊情况下，在进行测量之前可能需要规定一个平衡时间。在可能的情况下，应规定使用与钠光谱 D 线相对应的波长(即 589nm)来进行旋光度测定。

III.3.1.2. Identification 鉴别

When the substance examined is an enantiomer, the specific optical rotation is used for the identification.

如待测物质有对映异构体，可以使用比旋度进行鉴别。

If the specific optical rotation is used for identification only, the result does not have to be calculated on the dried substance or the solvent-free substance. The limits prescribed should take into account any variation in content and purity of samples of different origin that comply with the monograph.
如果比旋度仅用于鉴别，不必以干燥品或无溶剂物计算比旋度值。鉴别项下规定的限度，应考虑到符合各论的不同来源样品的含量和纯度的变化情况。

III.3.1.3. Tests 检查

Specific optical rotation may be used to verify the optical purity of an enantiomer. This method is less sensitive than chiral LC. In the case where one enantiomer is to be limited by the measurement of specific optical rotation, then it is to be demonstrated that under the conditions of the test, the enantiomer has sufficient optical activity to be detected. The result is calculated on the dried substance or the solvent-free substance. Whenever possible, the influence of potential impurities should be reported. Limits for the specific optical rotation should be chosen with regard to the permitted amount of impurities. In the absence of information on the optical activity of related substances and when insufficient amounts of the related substances are available, the limits are usually arbitrarily fixed at ± 5% around the mean value obtained for samples that comply with the monograph. Samples of different origin should be examined whenever possible. It is also worthwhile examining samples that are close to the expiry date to obtain information on the influence of normal ageing.
比旋度可用于确认一个对映异构体的光学纯度。该方法的灵敏性低于手性色谱方法。如果通过测量比旋度规定某一对映异构体的限度，应当证明在测量条件下，该对映异构体有足够的光学活性并可以被检测到。比旋度结果以干燥品或无溶剂物计。在可能的情况下，应报告潜在杂质的影响情况。比旋度的制定应考虑允许的杂质含量。在缺少有关物质旋光信息和无法获得足够有关物质的情况下，通常比旋度的限度一律规定为符合质量标准的药品平均比旋度的±5%。如有可能，应考察不同来源的样品的比旋度。对接近失效日期的样品进行比旋度检查也非常重要，以获得正常贮藏条件下放置时间对比旋度的影响。

Measurement of the angle of rotation may be used to verify the racemic character of a substance. In that case limits of - 0.10° to + 0.10° are usually prescribed.
对旋光度的测定可以用于确认药物的消旋特性。在这种情况下，旋光度的限度一般规定为-0.10°~+0.10°。

If possible, it is to be demonstrated that, under the conditions of the test, the enantiomer has sufficient optical activity to be detected.
如有可能，应当证明在测定条件下，对映异构体有充足的光学活性并能被检测出。

III.3.2. Absorption spectrophotometry (ultraviolet and visible) (2.2.25)

吸收光谱法 (紫外和可见光) (2.2.25)

In all cases, the suitability of the operating conditions (solvents employed and their quality, pH of the solution, etc.), must be demonstrated.
在所有紫外可见光度法进行含量测定中，必须证明操作条件（所用溶剂及其质量情况，溶液 pH 等）的适用性。

In normal use, ultraviolet spectrophotometry is a technique of limited discrimination power. The use of 1 st - and 2nd -order derivative techniques may increase discrimination power.
在通常情况下，紫外分光光度法是一种具有有限区分能力的方法。一阶和二阶导数光谱技术

的使用可以提高方法的区分能力。

III.3.2.1. Identification 鉴别

Ultraviolet spectrophotometry is rarely the only procedure described for identification. When it is included in an identification series, discrimination power must be demonstrated by comparing the spectrum of the analyte with spectra of similar substances. Discrimination power can be increased by using absorbance ratios rather than absorbance values.
紫外分光光度法极少单独作为鉴别方法使用。当该方法作为鉴别项下的一个试验时，必须通过将待测物的光谱与类似物质的光谱进行比较来证明该方法对此类物质的区分能力。与吸光度指标相比，采用不同波长吸光度的比值来作为鉴别指标更有区分能力。

III.3.2.2. Limit test 限度检查

When ultraviolet spectrophotometry is used for a limit test, it is to be demonstrated that at the appropriate wavelength, the related substance to be limited makes a sufficient contribution to the measured absorbance. The absorbance corresponding to the limiting concentration of the related substance must be established.
当紫外分光光度法用于限度检查，应证明在适当的波长下，需要控制的有关物质对测定的吸光度数值有足够的贡献。必须建立与受控的有关物质的浓度与相对应的吸光度值之间的关系。

III.3.2.3. Assay 含量测定

When ultraviolet spectrophotometry is used for the assay, the contribution to the absorbance of the known impurities must be evaluated. The use of specific absorbance values for assays is discouraged, but may be possible in dissolution tests in monographs on medicinal products (see the Technical guide for the elaboration of monographs on medicinal products containing chemically defined active substances). If specific absorbance values are prescribed, they must be evaluated by an inter-laboratory trial using a batch of known purity. Purity is to be estimated by applying a variety of techniques including separation techniques and absolute techniques.
当紫外分光光度法用于含量测定时，必须评估已知杂质对于吸收值的影响。不鼓励使用吸收系数法进行含量测定，但可能在药品各论的溶出度测试中使用该数值(见起草关于含有确定的化学活性物质的药品各论的技术指南)。如果规定了吸收系数，则必须使用纯度已知的样品进行实验室间的评估，对吸收系数数值进行考察。应采用多种技术包括分离技术和绝对法，对样品的纯度进行估计。

III.3.3. Non-instrumental limit tests

非仪器类限度检查

III.3.3.1. Appearance of solution (2.2.1 and 2.2.2) 溶液外观 (2.2.1 and 2.2.2)

These simple visual tests compare the colour (or opalescence) of the test solution against a series of standards. Typically, the test solution should be clear and colourless. These tests are intended to give an assessment of the general purity of the substance. When degrees of colour (or opalescence) are permitted, the impurity and the level to which the degree of coloration (or opalescence) corresponds are often unknown. Validation is based on the examination of batch data supplied by the manufacturer(s). However, when the impurity causing the opalescence or colour is known, it may be possible to validate the visual test by comparison with a more sophisticated analytical technique.
这些简单的目视检查是将供试品溶液颜色（或浊度）与一系列标准溶液进行比较。通常情况下，供试品溶液应澄清、无色。这些检查的目的是对药品的纯度分析提供总体的评价标准。

当允许供试品溶液出现具有一定的颜色（或浊度）时，杂质和相应的色度(或浊度)所对应的关系往往是未知的。方法的验证应基于对生产企业提供的批分析数据的检查。然而，当杂质会导致供试品溶液的浑浊或有颜色变化时，可以通过目视检查法与其他更复杂的分析技术的测定结果进行比较，对目视法进行方法学验证。

III.3.3.2. Acidity or alkalinity 酸/碱度

This is a general test of the purity of a substance. It is a non-specific test used for the control of protolytic impurities. The appropriate use of this test is described above.
本方法是对药品纯度分析的一个总体评价方法。这是一种非专属的方法，用于解离型杂质的控制。如何恰当地使用该检查项目详见前文所述内容。

III.3.3.3. Limit tests for anions/cations (2.4)

阴离子和阳离子的限度检查 (2.4)

These are simple and rapid tests but they are to be shown to be appropriate by recovery experiments and/or comparison with other more sophisticated techniques.
这些是简单而快速的检查方法，但需要通过回收试验和/或与其他更复杂的技术的对比来证明该方法的适用性。

Sulfated ash (2.4.14). The sulfated ash test is intended as a global determination of cations present in organic substances but is obviously not applicable to inorganic salts of acidic organic substances. The limit is normally 0.1%. This gravimetric test controls the content of foreign cations to a level appropriate to indicate the quality of production. This method can be considered to be well established and no further validation is required.
硫酸盐灰分（2.4.14）：硫酸盐灰分检查的目的是对有机药品中的阳离子总体的测定，但显然不适用于酸性有机药品的无机盐中阳离子的检查。硫酸盐灰分的限度通常为 0.1%。该重量分析检查用来控制药品中外来阳离子的含量，在一定水平上反映了药品的质量。这种方法是经过充分考虑而建立的，不需要进一步验证的方法。

Colour or precipitation reactions. Limit tests are also described for individual cations and anions which are based on visual comparison of a colour or opalescence. It is essential that it is demonstrated that:
颜色或沉淀反应。基于颜色或浊度的目视比较，还对各种阳离子和阴离子的限度检查进行论述。测定方法必须满足下列条件：

- the colour or opalescence is visible at the target concentration (limit);
  目标浓度（限度规定浓度）条件下，供试品溶液的颜色或沉淀是目视可见的；
- the recovery of added ion is the same for the test and reference solutions (by visual observation and if possible by absorbance measurement);
  在供试品溶液和对照溶液中加入离子后的回收率相同(通过目视检查法，如果有可能可进行吸光度测定);
- the response is sufficiently discriminating around the target value (50%, 100% and 150% of the target value) by measuring the absorbances at an appropriate wavelength in the visible region;
  测定在适当的可见波长范围处的吸光度，该测定法对目标浓度范围内的离子浓度变化(目标浓度的 50%、100%和 150%)应具有足够的分辨力;
- a recovery experiment at the target value is carried out six times and the repeatability relative standard deviation (RSD) calculated. Recovery should be greater than 80% and the repeatability RSD should be not more than 20%.
  在目标浓度条件下进行 6 次回收率试验，计算重复性相对标准偏差(RSD)。回收率应大于

80%，重复性 RSD 不大于 20%。

It would be desirable, when appropriate, to compare the results obtained from a recovery experiment using the proposed limit test procedure with a quantitative determination using a different technique (e.g. atomic absorption spectrophotometry for cations or ion chromatography for anions). The results obtained by the two techniques are to be similar.
在条件适当时，最好将拟定限度检查的回收率实验所得结果与使用不同技术(例如，采用原子吸收分光光度法进行阳离子测定或采用离子色谱法进行阴离子的测定)进行的定量测定结果进行比较。两种方法所得结果相似。

III.3.4. Atomic absorption spectrometry (2.2.23)

原子吸收分光光度法 (2.2.23)

Atomic absorption spectrometry is exclusively employed in tests to determine the content of specific elements that are present in substances as impurities. The following validation requirements are particularly pertinent to atomic spectrometric methods. More validation requirements are given in the general chapter.
原子吸收光谱法专门用于测定以杂质形式存在于物质中的特定元素的含量。以下验证要求对原子光谱法是必要的。在通则中给出了更多的验证要求。

III.3.4.1. Specificity 专属性

In principle, this technique is specific, using the appropriate source and wavelength, for the element to be determined since the atom emits or absorbs radiation at discrete spectral lines. However, interferences may be encountered due to optical and/or chemical effects. Thus it is important to identify the interferences and, if possible, reduce their effect by using appropriate means before starting the validation programme.
原则上，因为原子在不连续的光谱线上发射或吸收辐射，该技术是使用适当的光源和波长来确定要测定的元素含量，因此该技术是专属的。然而可能由于光谱或化学因素对原子吸收光谱法产生干扰因素。因此重要的是能够识别干扰因素，如有可能，在方法验证开始之前，通过使用适当的方法降低这些干扰因素的对测定的影响。

Such interferences may result in a systematic error if a direct calibration procedure is employed or may reduce the sensitivity of the technique. The most important sources of error in atomic spectrometry are associated with errors due to the calibration process and to matrix interference (care must be taken to avoid memory effects).
如果采用直接标准曲线方法，这些干扰因素可能导致系统误差或降低该技术的灵敏性。原子分光光度法中最重要的误差来源于标准曲线的操作和基质干扰（应注意避免记忆效应）。

III.3.4.2. Calibration 标准曲线

Aqueous standards are prepared and analysed at different concentration levels, spread over the calibration range.
制备水溶性对照品溶液，并对分布在标准曲线范围内的不同浓度水平的对照品溶液进行分析。

The number of concentration levels at which standards must be prepared depends on the calibration model used. To demonstrate the applicability of a straight-line regression model, standards should be prepared at a minimum of four concentration levels. A parabolic regression model also requires at least four concentration levels. Preferably, the concentration levels are evenly distributed over the calibration range.

制备不同浓度水平的对照品溶液的数量取决于所使用的标准曲线方法。为了证明标准曲线符合线性回归模型，应至少制备四份不同浓度水平的对照品溶液。抛物线回归模型也需要至少制备四份浓度水平的对照品溶液。首选的对照品溶液的浓度水平在标准曲线范围内均匀分布。

Generally, it is recommended to perform at least five measurements at each concentration level.
通常情况下，推荐在每个浓度水平下至少进行五次测定。

Calibration problems can often be detected visually. However, these plots alone cannot be used as proof of the suitability of the calibration procedure.
通常通过目视检测就可以发现标准曲线的问题。然而，单独这些曲线不能用来证明标准曲线的适用性。

- The measured absorbances are plotted as a function of the concentration, together with the curve that describes the calibration function and its confidence interval. This curve should fit the data points.
  测得的吸光度值为纵坐标，相应的浓度值为横坐标，绘制标准曲线并给出回归方程及置信区间。测定的数据点应与拟合曲线相适配。
- The residuals (i.e. the difference between the measured and the estimated absorbance) are plotted as a function of the concentration. When a suitable calibration procedure is applied, the residuals are randomly distributed around the x-axis.
  残差(即测定的吸光度值与标准曲线上估算的吸光度值之间的差值)，绘制该参数与浓度的关系。当建立了适当的标准曲线时，残差应沿 x 轴成随机分布。

When the variance of the signal increases with the concentration, as is often the case with atomic spectrometry and shown from either a plot of the residuals or with a one-tailed t-test, a weighted calibration model is better suited. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed.
原子吸收光光度法经常发生检测信号的变异随着浓度的增加而增大这样的情况，通过残差-浓度曲线，是残差图或是单侧 t-检测都可以表明这种情况，采用加权的标准曲线模型可以进行最精密的估计。将数据代入线性和二次加权方程，找到最适合的关系曲线。

For a weighted model, the weighted residuals (i.e. the weight multiplied by the residual) are plotted as a function of the concentration:
对于加权模型，可以绘制加权残差，即残差与加权重系数的乘积与浓度的关系曲线：

- the measured absorbances are plotted as a weighted function of the concentration, together with the curve that describes the calibration function and its confidence interval;
  将测量的吸光度值绘制成浓度的加权标准曲线并给出回归方程及其置信区间；
- the weighted residuals are plotted as a function of the concentration.
  加权残差作为浓度的一个函数被绘制出来。

It must be demonstrated that the data accurately fit the model. Application of a straight-line regression model implies that the linearity of the calibration line is investigated.
必须证明测定的数据与模型能够准确地匹配。采用线性回归模型的意味着已经对标准曲线的线性进行了研究。

III.3.4.3. Matrix effects 基质效应

When aqueous reference solutions are used to estimate the calibration function, it must be ensured that the sensitivities obtained with the sample solution and the aqueous solutions are similar. When a

straight-line calibration model is applied, differences in sensitivity can be detected by comparing the slopes of standard addition and aqueous reference solutions calibration graphs. The quality of the estimation of the slopes of both regression lines depends on the number and distribution of the measurement points. Therefore, it is recommended to include sufficient measurement points (always > 5) in both regression lines, and to concentrate these points mainly on the extremes of the calibration range.
当以水溶性对照品溶液为标样进行测定，对标准曲线方程进行估计时，必须确保供试品溶液和水溶性对照品液具有相似的灵敏度。当采用线性回归模型时，可以通过标准加入法和水溶性对照品标准曲线的斜率的比较，来确定标准品溶液与供试品溶液间的检测灵敏度的差异。对两种线性回归曲线斜率估算值得精密程度，取决于测定数据点的数量及其在拟合曲线上得分布情况。因此，两种回归曲线应有足够的测量点（通常多于 5 个点），并且这些标样的浓度应主要分布在标准曲线的测量范围内。

The slopes of the standard addition line and the aqueous calibration line are compared, by applying a t-test, to check whether slopes of both regression lines are significantly different. If that is the case, then Method II (standard additions) is to be applied; if it is not the case, Method I (direct calibration) can be applied.
采用 t 检验法，比较标准加入法和水溶性标样法的标准曲线的斜率，检查两条回归线的斜率是否有显著差异。如果两种方法的斜率间有显著性差异，则采用方法二(标准加入法);如果两种方法的斜率间没有显著性差异，则可采用方法一(直接标准曲线法)。

III.3.4.4. Detection and quantitation limit (based on the standard deviation of the blank)

检测限及定量限（基于空白的标准偏差）

To estimate the detection and quantitation limit, representative blanks are prepared and analysed. Preferably, matrix blanks are used, which contain every component of the sample except the analyte. However, when no matrix blanks are available, reagent blanks, containing all reagents and prepared in the same manner as the sample solution, can be used.
为了估算检测限和定量限，制备并检测有代表性的空白溶液。最好使用基质空白，因为该溶液中包含了样品中除被分析物外的所有成分。然而，当不能获得基质空白时，可以按照与供试品溶液制备的相同步骤，制备样品溶液中所含全部试剂的空白溶液。

Other aspects of the validation programme are covered above.
验证方案的其他方面已在前文进行了介绍。

III.3.5. Separation techniques 分离技术

The different chromatographic procedures (TLC, GC and LC) may be employed in the IDENTIFICATION section, in the TESTS section for the limitation of related substances and in the ASSAY section to determine the content of the active substance. The analytical procedures are to be validated according to the principles described previously, but there are specific aspects of the different chromatographic techniques that are to be taken into consideration. These are described below.
不同的色谱方法（如薄层色谱、气相色谱和液相色谱）可用于活性物质的鉴别、有关物质限度以及含量测定等质量标准项下的测定。需根据上文描述的原则进行方法学验证，还需考虑不同色谱技术的特点。下文将进行描述。

III.3.5.1. Thin-layer chromatography (2.2.27) 薄层色谱法（2.2.27）

This chromatographic technique is widely employed in the Ph. Eur. for identification using a reference substance and for the limitation of impurities with or without the use of a reference substance. When impurities are to be determined quantitatively, appropriate instrumentation must be employed. For the most part, silica is employed as the stationary phase but reverse-phase stationary phases (e.g. silanised silica gel) or cellulose stationary phases are also employed. Nonetheless, the following points are common to the application of thin-layer chromatographic techniques whether used for identification or for a test for related substances.
该色谱技术在欧洲药典中广泛应用于鉴别检测（对照品法），以及使用或不使用对照品情况下的杂质限度检查。需进行杂质定量检测时，必须使用适当的仪器。大多数情况下使用二氧化硅作为固定相，有时也可使用反相固定相（如硅烷化硅胶）或纤维素固定相。无论用于鉴别还是用于有关物质检查，都需要关注以下几点共同的事项。

- Specificity: it is accepted that for an identification test, specificity cannot be attained using this technique alone but good discrimination can be expected. It must be accompanied by other tests which together assure specificity. Selectivity may not be attainable for a limit test, in which case one or more additional tests must be described to control the impurities not separated. Discrimination power is to be demonstrated. For an identification test, improvement in discrimination power can sometimes be achieved using a spray reagent that differentiates similar substances by colour.
  专属性：对于薄层色谱用于鉴别试验，被认可的共识是仅使用该技术无法获得方法的专属性，但希望获得良好的分离能力，必须结合其他测试以确保专属性。当限度检查试验可能无法达到方法的专属性要求时，必须增加一个或多个检验方法，对未能获得分离的杂质进行控制。必须证明薄层色谱的分离能力。对于鉴别试验，采用根据颜色能够区分待测类似物的显色剂，来提高薄层色谱法鉴别试验的区分能力。

- Stationary phase: it is to be demonstrated that the test is applicable using plates of the same type but of different origin. Separations that can only be achieved on one particular type of plate are to be avoided, if possible.
  固定相：必须证明可采用不同来源的同一类型的薄层色谱板进行试验。如有可能，应避免出现只有特定类型或品牌的薄层色谱板才能满足试验要求的情况发生。

- Performance test (SST): such a test is generally performed to verify the separation of two closely eluting substances, the substance itself and a similar substance (critical pair). It is to be demonstrated that the separation of the chosen substances will guarantee the suitability of the chromatographic system. This performance criterion is essential for a for related substances.
  系统适用性试验：这种测试通常是为了证明两种保留性质接近的物质，即物质本身和类似物（关键物质对）能够在该试验条件下获得分离。需要证明所选物质的分离能够保证色谱系统的适用性。这一性能标准对于有关物质的检查至关重要。

Additional aspects that require further documentation when TLC is applied to a test for related substances include:
当采用薄层色谱法进行有关物质检查时，还需要提供下列参数的相关资料：

- Detection: the use of specific spray reagents must be avoided when applying a related substances test unless the test is designed to limit a named impurity using a reference substance.
  检测：除非采用对照品进行已知杂质的限度检查，否则必须避免在有关物质检查中使用指定的显色剂。

- Detection limit: when applying a quantitative instrumental procedure, one of the described methods for the calculation of the DL applies. When a visual method is applied, it is to be demonstrated that the quantity corresponding to the specified limit is detectable.
  检测限：当采用定量仪器进行分析时，应提供所用方法的检测限度计算方法。当采用目视检查方法时，必须证明规定限度的杂质(自身对照)斑点能被检出。

- Response factors: if the known impurities are available, then the similarity of response factors (relative to the substance itself) is demonstrated using the given detection conditions. For a limit test, differences in response can be shown by comparison of the visual detection limits.
  响应因子：如果已知杂质可用，可使用给定的条件证明响应因子（相对于物质本身）的相似性。对于限度检查，可通过目视法比较检测限条件下的响应差异。如果有已知杂质的杂质对照品，应给出测定条件下的各杂质响应因子的接近程度(与主成分的相对响应因子)。对于限度检查，可通过目视法比较各杂质检测限条件下的响应差异(斑点颜色深浅)。

- Quantitation limit, linearity, range and repeatability: data are also required when an instrumental quantitative TLC procedure is applied.
  定量限、线性、范围和重复性：当采用薄层色谱法进行仪器定量分析时，应提供相关数据。

III.3.5.2. Liquid chromatography (2.2.29) 液相色谱（2.2.29）

LC is usually applied to limit the content of impurities in a substance (employing an external standard, usually an appropriate dilution of the test solution), to determine the content of a substance (employing an external standard), and occasionally as an identification by cross-reference to one of the aforementioned procedures. Attention is to be paid to a number of aspects peculiar to LC.
液相色谱常用于药物中杂质限度检查（可采用外标法，常用的方法是主成分自身稀释对照法），主成分的含量测定（采用外标法），有时鉴别项下也会交叉引用检查或含量测定项下的色谱法作为主成分的鉴别方法。需要在下列内容中对液相色谱技术的特点进行关注。

III.3.5.2.a. Identification 鉴别

It is accepted that for an identification test, specificity may not be attained using this technique but good discrimination can be expected. It must be accompanied by other tests that together ensure specificity. Discrimination power must be demonstrated with retention times, relative retentions or mass distribution ratio of similar substances, and the substance itself, being reported. Such information is to be supplied for a variety of stationary phases of a similar type.
普遍认为，对于鉴别试验，使用液相色谱法可能不具有专属性，但可以通过该技术获得良好的分离能力。必须同时进行其他测试来共同保证鉴别试验的专属性。必须用保留时间、相对保留时间或主成分以及相似物质的质量分配系数，来证明方法的区分能力。应提供类似类型的多种固定相的相关信息。

III.3.5.2.b. Limit test 限度检查

- Specificity:专属性
  - Discrimination power of the separation: separation of known and potential impurities from the substance itself and if possible, from each other, must be demonstrated. Specificity may be ensured by detection by mass spectrometry. Impurities not separated from the substance must be controlled by another procedure. The retention times, relative retention times or

mass distribution ratio of the substance and the impurities must be reported. Such information is to be supplied for a variety of stationary phases of a similar type.
分离度：必须证明已知和潜在的杂质与主成分能够良好分离，如果可能，还必须证明杂质间良好分离。还可用质谱检测器来确保色谱鉴别方法的专属性。必须报告类似物与药物之间的保留时间、相对保留时间或质量分配比。应提供相似类型的多种固定相的相关信息。

- Discrimination power of the detection system: the choice of the detector or the detector conditions employed must be justified (e.g. change in the detection wavelength when using UV detection) while specificity can be ensured by the use of detection by spectrometry.
检测系统的分离度：检测器的选择或所采用的检测器条件的合理性必须被证明（例如使用紫外检测器检测波长的改变），同时还要用质谱检测器来保证该方法的专属性。

- Response factors: it is essential to demonstrate the similarity of response of the substance and known impurities (at the wavelength of detection for UV detection but applies also to other detection systems, e.g. conductimetry). A response factor of a known impurity that is greater than 1.2 or less than 0.8 compared to that of the substance to be examined may require the use of either CFs or of that individual impurity as an external standard when the proposed limit is 0.1% or greater.
响应因子：必须证明主成分和已知杂质的响应因子接近是必要的（紫外检测器的检测波长下的响应，但也适用于其他检测器，例如电导检测器等检测系统）。如果一个已知杂质与主成分的响应因子之比大于 1.2 或小于 0.8，当拟定的限值为 0.1%或更高时，可能需要使用校正因子或对每个杂质进行外标法检测。
- Detection and quantitation limits: these limits must be determined for the external standard, which is either a dilution of the substance to be examined or a known impurity. When a peak of an impurity elutes close to the peak of the substance, particularly if it elutes after the peak due to the substance, detection and quantitation limits are to be determined on that impurity. One of the methods for calculation of both the DL and the QL is applied.
检测限和定量限：当以供试品溶液的稀溶液或已知杂质对照溶液作为对照溶液时，必须使用外标法确定检测限和定量限。当杂质的色谱峰与主成分色谱峰接近，特别是杂质在主成分之后出峰时，应测定该杂质的检测限和定量限。可采用计算检测限与计算定量限中的一种方法进行确认。
- Stability: data should be provided demonstrating the period of use of reference and test solutions.
稳定性：应提供供试品溶液和对照品溶液在使用期间的稳定性证明数据。
- Recovery: when an extraction procedure is employed, a recovery experiment using known and available impurities is to be carried out under optimal conditions and the results reported. It is to be demonstrated that the recovery shows an acceptable accuracy and precision.
回收率：当检测程序中有提取步骤时，应使用已知的、可以获得的杂质，在优化的条件下进行回收率试验并报告试验结果。需要证明回收率数据显示出可接受的准确度和精密度。
- Derivatisation: when pre- or post-column derivatisation is employed, it is important to establish the optimal reaction conditions (time and temperature) and also to investigate the stability of the derivative under normal conditions of use.
衍生化反应：当采用柱前或柱后衍生化处理操作时，重要的是确定最佳反应条件（反应时间和温度），并且要考察衍生物在正常测定条件下的稳定性。
- System suitability test: as described for TLC. The use of the S/N ratio is only required when the DL and the specified limit are similar.

系统适用性：如薄层色谱所述。只有在杂质的检测限和规定的限度接近时，才需要计算信噪比（S/N）。

III.3.5.2.c. Assay 含量测定

- Specificity: this is preferable but not essential provided that the interfering impurity is present at a low level and is controlled by another test.
  专属性：如果干扰测定的杂质含量低并且已经用其他的试验进行了杂质含量的控制，提供方法的专属性更好，则不是必须提供该杂质的专属性资料。

- System suitability test: as described in general chapter 2.2.46. Chromatographic separation techniques. Table 2.2.46.-1 can be extended as follows:
  系统适用性测试：如一般鉴别 2.2.46.色谱分离技术所述。表 2.2.46.-1 可扩展如下：

	Number of individual injections 进针数				
	3	**4**	**5**	**6**	**10**
B (%)	**Maximum permitted relative standard deviation** 允许最大相对标准偏差				
1.0	0.21	0.30	0.37	0.42	0.60
1.5	0.31	0.44	0.55	0.64	0.90
2.0	0.41	0.59	0.73	0.85	1.20
2.5	0.52	0.74	0.92	1.06	1.51
3.0	0.62	0.89	1.10	1.27	1.81
3.5	0.72	1.04	1.22	1.48	2.11
4.0	0.83	1.19	1.46	1.70	2.41
4.5	0.93	1.33	1.65	1.91	2.71
5.0	1.04	1.48	1.83	2.12	3.01

Limit tests and assays must be validated as described above (see part III.2) for linearity, repeatability and reproducibility.
必须按照上述线性、重复性和再现性要求（见 III.2 部分）对限度检查和含量测定方法进行验证。

III.3.5.3. Gas chromatography (2.2.28) 气相色谱法（2.2.28）

III.3.5.3.a. Identification 鉴别
Specificity: as described for LC.
专属性：参见液相色谱法。

III.3.5.3.b. Limit test 限度检查

Specificity: as described for LC.
专属性：参见液相色谱法。

- Response factors: as described for LC; response factors relative to the substance itself must be provided. This is particularly important when using selective detectors (ECD, NPD, etc.).

响应因子：参见液相色谱法，必须提供各杂质与主成分相关的响应因子，在选择检测器时尤为重要（电子捕获检测器、氮磷检测器等）。

- Detection and quantitation limits: as described for LC.
  检测限和定量限：参见液相色谱法。
- Stability: as described for LC.
  稳定性：参见液相色谱法。
- Derivatisation: as described for LC.
  衍生化处理：参见液相色谱法。
- Internal standard: it is to be demonstrated that under the chromatographic conditions employed, the peak due to the internal standard does not interfere with the impurity peaks or that due to the substance itself.
  内标：必须证明在采用的色谱条件下，内标物的色谱峰不干扰主成分峰及其杂质峰的测定。
- Recovery parameters: as described for LC.
  回收率参数：参见液相色谱法。

III.3.5.3.c. System suitability test 系统适用性试验

Details that are to be provided of chromatographic criteria to which a user must conform to successfully apply the test are as follows.
为保证试验的顺利进行，应提供给操作人员必须遵守的详细色谱条件或判断标准：

- The S/N ratio is usually determined for a signal that is equal to or greater than the DL.
  通常在系统适用性项下要求测定检测限浓度或更高浓度样品溶液的信噪比（S/N）。

- Resolution between the peak due to the substance and a closely eluting peak of an impurity or between the peak due to the substance and the peak due to the internal standard. It is also useful to give the acceptable range of values for the symmetry factor when it is different from the accepted range of 0.8-1.8 as given in general chapter 2.2.46. This is particularly important when employing packed columns and when the peak of an impurity to be controlled elutes immediately after the principal peak. Verification of performance using a similar column, when possible, is recommended.
  主成分峰与相邻杂质峰或内标物峰的分离度要求。当对称因子超出了 2.2.46 章中规定的 0.8-1.8 的范围时，给出可接受的对称因子的范围是有用的。当采用填充柱并且需要控制的杂质峰紧随主成分出峰时，给出对称因子的范围特别重要。如有可能，推荐使用性质类似的色谱柱进行方法确认。

- Head-space injection technique: this type of injection is employed for highly volatile substances. It is important to demonstrate that the temperature and time of pre-heating the injection vial results in equilibrium conditions. The presence or absence of a matrix effect should also be demonstrated. One way of validating head-space injection conditions is to carry out multiple head-space extractions (after each injection, the head space is vented and the vial is re-equilibrated before re-injection of the gaseous phase). The pre-requisite for good conditions is that the relationship of the logarithms of the areas of the analyte peak to the number of extractions is linear with a coefficient of regression of 1.0. Matrix effects can be overcome by the use of the standard addition technique.
  顶空进样技术：这种进样技术适用于高挥发性的物质。重要的是要证明进样瓶预热温度和时间能达到平衡状态。还应证明是否存在基质效应。还应进行多次顶空提取（每次进样后，顶空气体被排空，平衡被破坏，再次进样前要对进样瓶进行再平衡）。获得良好

的平衡条件的前提条件是待测物色谱峰面积的对数与提取次数呈线性关系，回归系数为1.0。可以通过标准加入法消除基质效应。

III.3.5.3.d. Assay 含量检测

- Specificity: as described for LC.
  专属性：参见液相色谱法。
- System suitability test: as described in general chapter 2.2.46. Chromatographic separation techniques (see also part III.3.5.2.c).
  系统适用性：参见一般鉴别 2.2.46 色谱分离技术（见 III.3.5.2.c 部分）。

Limit tests and assays must be validated as described above (see part III.2) for linearity, repeatability and reproducibility.
限度检查和含量测定方法必须按照验证部分的要求进行线性、重复性和再现性考察（见 III.2 部分）。

III.3.5.3.e. Identification and control of residual solvents (2.4.24)
残留溶剂鉴别及控制的测定方法(2.4.24)

The sample preparation and GC systems employed are to be validated for the substance to be examined by applying the criteria given above with particular respect to:
供试品测定的样品制备方法和气相色谱系统进行验证，并且需要特别关注下列因素：

- specificity; 专属性
- detection and quantitation limits; 检测限及定量限
- recovery; 回收率
- repeatability; 重复性
- linearity, when employed quantitatively. 线性（定量检测）

III.3.6. Semi-micro determination of water (2.5.12)

半微量水分测定(2.5.12)

A number of commercial Karl Fischer reagents are available so it is important to ensure their suitability for use by means of a validation procedure such as standard addition.
因有多种商业化的卡尔费休试剂可供选择，所以通过标准加入法等验证程序确保试剂的适用性是十分重要的。

Standard addition 标准加入法

Determine the water content of the sample under the proposed conditions. Then, under airtight conditions, add a suitable volume of a standardised solution of water in methanol R and determine the water content m_{H2O} as mg water. Repeat this step at least five times.
在拟定的操作条件下对供试品中的水分（m_{H2O}）进行测定。然后在密闭条件下，加入水分含量已知的标准甲醇溶液适量并测定加入后的水分，以 mg 计。至少重复此步骤五次。

Calculate the regression line of the cumulative water determined against the water added. Calculate slope b, intercept with the ordinate a and intersection of the extrapolated calibration line with the abscissa d.

根据水分的累积测定结果，与加入的已知水分含量，获得直线回归方程，计算方程的斜率 b、与纵坐标的截距 a、标准曲线外推后与横坐标的相交点距原点的数值 d。

The slope b is to be between 0.975 and 1.025 (deviation ± 2.5%) to be acceptable. The percentage errors e_1 and e_2 are lower than ± 2.5%.
斜率 b 的数值在 0.975~1.025 之间（偏差± 2.5%）是可以接受的。误差百分比 e_1 和 e_2 的绝对值应不超过 2.5%.

$$e_1 = \frac{a - m_{H_2O}}{m_{H_2O}} \times 100 \qquad e_2 = \frac{|d| - m_{H_2O}}{m_{H_2O}} \times 100$$

Calculate the recovery of each standard addition step. The mean recovery is to be within 97.5% and 102.5% to be acceptable.
计算每次标准加入操作的回收率。标准加入法的平均回收率应在 97.5%和 102.5%之间。

III.3.7. Volumetric titrations (2.5.11, 2.2.19, 2.2.20) 容量滴定法

When developing a new volumetric assay procedure, it is recommended to titrate at least seven different quantities under the prescribed conditions in a randomised order to give end-point volumes in the range of 20-90% of the volume of the burette employed. Subsequently, the data are treated statistically and a number of criteria are to be fulfilled to permit acceptance of the titration procedure.
当建立一个新的含量测定用容量分析方法时，推荐在规定的测定条件下，按照随机原则，对至少 7 份不同重量的供试品进行滴定，滴定终点消耗的滴定液体积应为所用滴定管体积的 20%~90%，然后对数据进行统计学处理，必须满足多个评价指标的要求才能获得对滴定方法的认可。

The relative error in reading of the mass on the balance and of the volume at the end-point is to be less than 0.5% of the values found.
供试品称重的天平的读数、终点时消耗的滴定液体积的读数的相对偏差不得超过观察值的 0.5%。

The results, as end-point volumes V_i in dependence of mass m_i , are evaluated by linear regression. The regression line is calculated and characterised by the slope b_{obs}, the extrapolated intercept a_{obs} and the precision as $\sigma(V)$.
通过线性回归方程对基于供试品重量“m_i ”的滴定终点体积“V_i ”进行评价，获得线性回归方程并用斜率“b_{obs}”、外推法获得截距“a_{obs}”以及精密度（标准偏差，用σ(V)表示）。

1st Criterion – Proportional Systematic Error (Bias)
第一个判断标准—成比例的系统误差（偏倚）

The calculated slope b_{obs}, taking into account the titre of the standardised volumetric solution, is within 0.3% for potentiometric titrations (0.5% for visual titrations) compared to the theoretical value given as titration constant btheor.
考虑到标准滴定液的滴定度，对于电位滴定法，计算获得斜率“b_{obs}” 与理论值（给定的滴定常数）相比，“b_{theor}”之间的相对偏差应不得超过 0.3%，如果是采用指示剂法。测定值与理论值相对偏差不得超过 0.5%。

$$\left(\frac{b_{obs} - b_{theor}}{b_{theor}}\right) \times 100 \leq 0.3\% \ (0.5\% \ for \ visual \ determination)$$

where $b_{theor} = \frac{Z}{M_r C_r}$

M_r is the relative molecular mass, Z is the stoichiometric factor of the chemical reaction and C_r is the molar concentration of the titrant.
M_r 是相对分子质量；Z 是化学反应的计量系数，C_r 是滴定液的摩尔浓度。

2nd Criterion – Additional Systematic Error (Bias)
第二个判断标准—额外的系统误差（偏倚）

The extrapolated intercept a_{obs} is less than 0.4% for potentiometric titrations and 0.6% for visual titrations of the expected or target titration volume. This criterion may not be fulfilled if the titration is carried out too rapidly (potentiometric titration) or an unsuitable indicator has been employed (visual titration).
对于电位滴定法，外推法获得的截距"a_{obs}"应小于预期或目标滴定体积的 0.4%，对于指示剂目视法滴定法，则小于 0.6%。如果滴定速度过快（电位滴定）或使用了不合适的指示剂（指示剂目视滴定），测定结果则可能无法满足该标准。

$$\left(\frac{a_{obs}}{V_T}\right) \times 100 \ < 0.4\% \ (0.6\% \ for \ visual \ determination)$$

where a_{obs} is the extrapolated intercept of the regression line at zero and V_T is the expected or target titration volume.
式中，a_{obs} 为线性方程外推至消耗的滴定液体积为零时所得截距；V_T 为预期或目标滴定体积。

3rd Criterion – Precision (Statistical Error)
第三个判断标准—精密度(统计学误差)

The remaining estimated standard deviation σ(V) is less than 0.3% for potentiometric titrations (0.5% for visual indicator titrations) of the mean titration volume of end-point using the titration procedure to be introduced in the monograph.
对于电位滴定法，标准偏差 σ（V）的估计值应小于各论项下规定滴定方法平均体积标准偏差的 0.3%（目视指示剂滴定，σ(V)估计值应不得超过 0.5%）。

$$\left(\frac{\sigma(V)}{V_T}\right) \times 100 \ < 0.3\% \ (0.5\% \ for \ visual \ determination)$$

where σ (V) is the estimated standard deviation.
式中，σ(V) 是标准偏差的估计值

$$\sigma(V) = \sqrt{\frac{\sum(V_i - a_{obs} - b_{obs} m_i)^2}{n-2}}$$

where V_i is the titration volume, m_i is the mass of the substance and n is the number of titrations performed.
式中，V_i 是消耗的滴定液体积；m_i 是供试品质量；n 是滴定次数。

4th Criterion – Practical Relative Error 第四个判断标准—相对标准偏差

Some titration procedures may not fulfil the first and second criteria but exhibit low and acceptable bias at the target titration volume (8 mL±1 mL for a 10 mL burette). Thus, if the first and/or the second criteria given above are not met, then calculate the relative accuracy at the target titration

volume.
某些滴定方法可能不能满足第一个和第二个判断标准，但与目标滴定体积相比，显示出较小且可接受的偏倚（对于 10mL 滴定管，消耗的体积为 8 mL±1 mL）。因此，如果不满足上述第一和/或第二判断标准，则可计算目标滴定体积的相对准确度。

$$\left|\left(\frac{a_{obs}}{V_T}+\frac{b_{obs}-b_{theor}}{b_{theor}}\right)\right|\times 100$$

However, when the volumetric titration procedure is well established, it is sufficient to verify that the repeatability and accuracy of the titration (minimum 6 replicates) are not greater than the limits given in the table and decision tree below.
然而，当容量滴定方法建立后，应充分验证滴定方法的重复性和准确性（至少平行滴定 6 次）不超过下表和决策树中给出的限值。

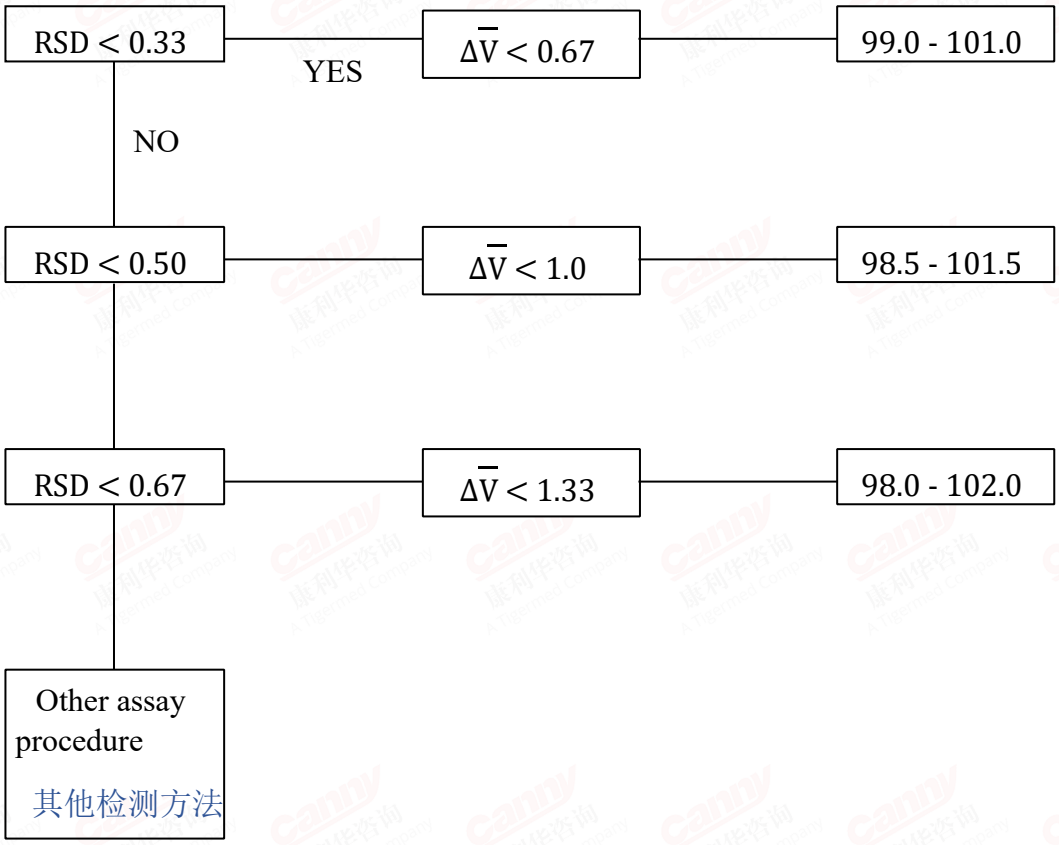
VOLUMETRIC TITRATION 容量滴定	CONTENT LIMITS (%) 含量限度（%）	REPEATABILITY (RSD) 重复性（RSD）	RELATIVE ACCURACY (%) 相对准确度（%）
Acid/base 酸/碱滴定	±1.0	0.33	±0.67
Non-aqueous 非水滴定	±1.0	0.33	±0.67
Conjugate acid of base 碱的共轭酸滴定	±1.0	0.33	±0.67
Redox 氧化还原法	±1.5	0.5	±1.0
Argentometric 银量法	±1.5	0.5	±1.0
Complexometric 络合滴定法	±2.0	0.67	±1.33

The figures in the table are given as guidance and it may be demonstrated that stricter limits can be applied. The use of volumetric titrations is applicable only when it has been demonstrated that impurities are present at low levels, otherwise other assay methods are to be introduced.
表中所给的数据仅作为指导值，更严格的限度规定也可能被证明是合适的。只有在药品中的杂质含量较低时才可以采用容量滴定法，否则应引入其他的含量测定方法。

Decision tree for validation of volumetric titrations 容量滴定方法验证决策树
Repeatability: Relative standard deviation (RSD) over six replicate measurements (n = 6)
重复性：6 次（n=6）滴定的相对标准偏差（RSD）

Relative accuracy:
相对准确度

$$\Delta\overline{V}=\frac{\overline{V}-V_{theory}}{V_{theory}}$$



III.3.8. Peptide identification by nuclear magnetic resonance spectrometry (2.2.64)

核磁共振波谱法鉴定多肽（2.2.64）

The following factors should be addressed in procedure validation.
方法验证中应考虑以下因素

- Spectral consistency, to demonstrate that, within reasonable ranges, the spectrum obtained is independent of sample quantity, sample pH, analysis temperature (calibration error or recalibration changes) or mis-setting of spectral acquisition parameters such as pulse width. The effects of small changes in sample preparation procedures, such as deuterium exchange, should be considered. Analysis of a number of different batches of the test product should be included to demonstrate consistent spectra.
  光谱一致性，证明在合理范围内，获得的光谱不受样品数量、样品 pH 值、分析温度（校准误差或重新校准的变化）或光谱采集参数（如脉冲宽度）的错误设置影响。应考虑样品制备过程中的微小变化（如氘交换）的影响。应包括对多个不同批次测试产品的分析，以证明光谱一致性。

- Specificity: the spectrum of the test sample should be compared with those of other similar products handled on the same manufacturing site and shown to be distinctive, with notes of obvious spectral differences. The spectra of potential impurities could be assessed (especially specified impurities). These may be deamidated forms, variants containing a “wrong” amino acid enantiomer, or forms with an incorrect sequence. This approach should be similar to that used when assessing the specificity of chromatographic identity tests.
  专属性：将供试品的光谱与一同处理的其他类似产品的光谱进行比较，应显示其特异性，并注明明显的光谱差异。应评估潜在杂质的光谱（特别是特定杂质）。这些杂质可能是脱酰胺形式、含有“错误”氨基酸对映体的变体或具有错误序列的形式。这种方法应与评估色

谱特性测试的专属性时使用的方法类似。

- Other variability: 其他变异性
  - operator-to-operator variability, expected to be small; it should be confirmed if more than one operator will undertake the test;
    不同操作员之间的变异性，预计很小；如果有多个操作员进行检测，应确认该变异性；
  - spectrometer drift over time, probably negligible.
    光谱仪随使用时长的漂移，可能可以忽略不计。

Minor revalidation will be required after probe servicing or console servicing, software upgrades or purchase of new spectrometer components; this can often be achieved using reference samples supplied with the spectrometer.
探头或控制台维修、软件升级或购买新光谱仪组件后，需要进行部分项目的再验证；通常可使用光谱仪自带的标准品来进行测试。