TESTS ON SAMPLES OF BIOLOGICAL ORIGIN

Guideline Title	**Test on Samples of Biological Origin**
Legislative basis	**Directive 75/318/EEC as amended**
Previous titles/other references	**None**
Additional Notes	**This document provides basic guidance on the presentation of data validating test procedures carried out for toxicological and pharmacological studies as well as for clinical trials provided for by Directive 75/318/EEC as amended with a view to the granting of a marketing authorisation in respect of a medicinal product.**

CONTENTS

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1. INTRODUCTION

The objective of analytical validation on samples of biological origin (plasma, urine, faeces etc.) is to demonstrate the reliability of results for active substances and metabolites obtained from pharmacokinetic, metabolic and bioavailability studies.

2. CRITERIA FOR VALIDATION OF TEST PROCEDURES

The validation criteria are those currently used in analytical chemistry (Good Laboratory Practice) and consist of:

Specificity

Repeatability

Precision

Reproducibility

Accuracy

Linearity/Range/Sensitivity

Limit of detection

Limit of quantification

Each test procedure should be validated for each type of biological sample and each species (animal, human).

If the same test procedure has been used during the development of the medicinal product (in vitro) and during routine tests (in vivo), a revalidation is necessary.

The degree of validation depends, to a large extent, on the problem posed.

3. RECOMMENDATIONS

3.1 For assays on samples of biological origin, the following specific problems can arise, which may influence both the validation and the interpretation of the results.

3.1.1 The test procedures (assays) carried out are not necessarily done in a single laboratory, but in many and sometimes even outside of those of the manufacturer. Therefore, it is very important – for the same test – to be able to compare the results between the two.

There are two cases to consider:

a) when the same test procedure is always used:

the quality control between laboratories is necessary (reproducibility);

b) using different test procedures:

either:

- it is necessary to have a reference test procedure (control, standard) developed directly in the corresponding biological sample and used for either the assays of substances to be analysed or as a test procedure to which other test procedures can be referred to for validation (correlation between the two test procedures);

or:

- it is recommended proper investigation of recovery in the individual methods using the same reference material.

3.1.2 A significant time-lapse can pass between the moment of sampling and the moment of analysis. For this reason, amongst others, it is necessary to know:

– the stability of the substances being examined in the biological fluid in the precise storage conditions;

– the sorption of the substance by the sampling container and the stopper.

3.1.3 The test procedure may change over time according to the evolution of the problem posed (clinical trials). In that case, a revalidation will be necessary.

3.2 Other recommendations:

3.2.1 A short description of the main principle of the test procedure should be indicated.

3.2.2 The test procedures, including the conditions of sampling, must be described precisely, preferably in the form of a standard operation procedure.

This includes:

– the mode of sampling (type of container, anticoagulant, etc.);

– the conditions of storage before analysis;

– the exact description of the test conditions including precautions, methods of extraction, reagents, reference substances and preparations;

– the exact description of the apparatus used;

– the verification of the test procedure under the defined operating conditions, for example: verification of the separating power of a chromatographic system (system suitability test);

– the detailed formulae of the calculation of results, including statistical evaluation as appropriate.

3.2.3 The reference substances and preparations (in house standards) used for tests - if pharmacopoeial or other official standards are not used- must be precisely described. Their identity, purity and content must be fully established.

3.2.4 In all cases, the complete data which demonstrate validity should be indicated.

ANNEX

Glossary

The annex is a glossary which should give the manufacturer a better understanding of the different validation requirements and definitions. It should again be remembered here, that the criteria which must be satisfied depend very much on the objective of the analysis.

1. TEST PROCEDURE

The test procedure is the total operation necessary to perform the analysis of an analyte: preparation of the sample, of the reference substances or preparations, of the reagents, use of the apparatus, calibration curve, formulae for the calculation, number of replicates and operating procedure for the replicates etc.

2. SPECIFICITY

This means for:

IDENTIFICATION:	to ensure the identity of an analyte
TESTS: (Impurity content)	to ensure that all the test procedures performed allow an evaluation of the content of impurities of an analyte i.e. related substances test, heavy metals, organic solvent content etc.
ASSAY: (Content or Potency)	to ensure that the signal measured with the test procedure comes only from the substance being analysed i.e. no interferences from excipients and/or degradation product and/or impurities.

A routine assay may not necessarily comply with the criterion of specificity. This can be compensated by using one or more adequate related substances test(s) (applies mainly to bulk material, see pharmacopoeia).

Specificity is assessed either by a single determination or by the total results of the test procedures.

a) Specific test procedure:

a procedure to measure quantitatively a chemical-physical parameter or functional group of one or even more but different analytes in the sample matrix;

for instance: titration of the carboxylic group of an acid, measure of the specific absorbance, immunoassay.

b) Selective test procedure:

a procedure to detect qualitatively the analyte in the presence of components which maybe expected to be present in the sample matrix;

for instance: chromatography, selective electrode.

c) Absolute test procedure:

a procedure which determines the molar purity of an analyte;

for instance: differential thermal analysis, phase solubility analysis.

Under the heading, the means of satisfying the criteria of specificity may be different:

for instance; identification to ensure the identity of an analyte during:

– development: proof of the structure;

– quality control: comparison to a reference substance.

3. ACCURACY

The accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value (in house standard) or an accepted reference value(international standard, e.g. pharmacopoeial standard) and the value found (mean value)obtained by applying the test procedure a number of times.

The accuracy provides an indication of systematic errors.

Several methods of determining accuracy are available of which the following are two examples:

a) Comparing the proposed test procedure with a second test procedure, the accuracy of which is stated and/or defined (for instance: pharmacopoeial method), (applies normally to starting material).

b) Applying the test procedure to specimens or mixtures of excipients to which a known quantity of the substance to be analysed has been added: the result maybe expressed as percent recovery by the assay of known added amount of analyte (applies normally to finished product).

4. PRECISION

The precision of a test procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

Precision provides an indication of random errors.

4.1 Repeatability

Repeatability expresses the precision under same conditions:

– same analyst,

– same apparatus,

– short interval of time,

– identical reagents.

Results should be expressed as:

– repeatability standard deviation;

– repeatability coefficient of variation (relative standard deviation);

– the confidence interval of the mean value (n 2 6 = 0.05 or P = 95%)

4.2 Reproducibility

The reproducibility expresses the precision under different conditions:

for instance:

– laboratories,

– reagents from different sources,

– analysts,

– days,

– apparatus from different manufacturers,

– etc.

Results should be expressed as:

– reproducibility standard deviation;

– reproducibility coefficient of variation (relative standard deviation);

– the confidence interval of the mean value (n > 6 = 0.05 or P =95%).

5. LIMIT OF DETECTION (LOD)

The lowest amount of analyte in a sample which can be detected but not quantitated as an exact value. The LOD is mostly a parameter of limit tests.

6. LIMIT OF QUANTITATION (LOQ)

The lowest amount of analyte in a sample which can be quantitatively determined with defined precision and accuracy under the stated experimental conditions.

7. LINEARITY

The linearity of a test procedure is its ability (within a given range) to obtain test results directly proportional to the concentration (amount) of analyte in the sample.

8. RANGE

The range of the test procedure is the interval between the upper and lower levels of analyte (including these levels) for which the procedure has been demonstrated as suitable with precision, accuracy and linearity using the method as written.

9. SENSITIVITY

Capacity of the test procedure to record small variations in concentration.

TRANSLATION OF SOME IMPORTANT TERMS

FR	DE	EN
Exactitude	Richtigkeit	Accuracy
Fidélité	Präzision	Precision
Spécificité	Spezifizität	Specificity
Sélectivité	Selektivität	Selectivity
Linéarité	Linearität	Linearity
Intervalle linéaire	Linearer Bereich	Linear range
Seuil de détection	Nachweisgrenze	Limit of detection (LOD)
Seuil de quantification	Bestimmungsgrenze	Limit of quantitation (LOQ)
Sensibilité	Empfindlichkeit	Sensitivity
Valeur moyenne	Mittelwert	Mean value
Ecart type/Déviation standard	Standardabweichung	Standard deviation
Coefficient de variation	Variationskoeffizient	Coefficient of variation
Déviation standard relative	Relative Standardabweichung	Relative stand.deviation
Intervalle de confiance de la valeur moyenne	Vertrauensbereich des Mittelwertes	Confidence interval of the mean value
Répétabilité	Wiederholpräzision	Repeatability
Reproductibilité	Vergleichspräzision	Reproducibility
Méthode analytique	Analysenmethode	Analytical method
Procédure d'analyse	Prüfverfahren	Test procedure
Résultat	Ermittlungsergebnis	Result of determination
Erreur systématique	Systematische Ergebnisunsicherheit	Systematic error of result
Erreur due au hasard	Zufällige Ergebnisunsicherheit	Random error of result
Valeur conventionnellement vraie	Richtiger Wert	Conventional true value

TRANSLATION OF SOME IMPORTANT TERMS

IT	PT	NL
Accuratezza	Rigor, exactidao	Nauwkeurigheid
Precisione	Precisao	Precisie
Specificita	Especificidade	Specificiteit
Selettivita	Selectividade	Selectiviteit
Linearita	Linearidade	Lineariteit
Intervallo lineare	Intervalo linear	Lineair interval
Limite di rilevazione	Limite de detecçao	Detectiegrens
Limite di determinazione	Limite de quantificaçao	Bepalingsgrens
Sensibilita	Sensibilidade	Gevoeligheid
Valore medio	Valor médio	Gemiddelde waarde
Deviazione standard	Desvio padrao	Standaardafwijking
Deviazione stand. relativa	Coeficiente de variaçao	Variatiecoëfficiënt
Coefficiente di variazione	Desvio padrao relativo	Relat. standaardafwijking
Intervallo fiduciale del valore medio	Intervalo de confiança do valor médio	Betrouwbaarheidsinterval van de gemiddelde waarde
Ripetibilita	Repetibilidade	Herhaalbaarheid
Riproducibilita	Reprodutibilidade	Reproduceerbaarheid
Metodo di analisi	Método analitico	Analysemethode
Procedimento	Procedimento analitico	Proefopzet
Risultato dell'analisi	Resultado	Resultaat van de bepaling
Errore sistematico	Error sistematico do resultado	Systematische afwijking
Errore casuale	Errore aleatorio do resultado	Toevallige afwijking
Valore reale	Valor verdadeiro convencional /Valor nominal	Conventioneel ware waarde

TRANSLATION OF SOME IMPORTANT TERMS

ES	DA
Exactitud	Nøjagtighed
Precisión	Præcision
Especificidad	Specificitet
Selectividad	Selektivitet
Linealidad	Linearitet
Intervalo lineal	Linearitetsområde
Limite de detección	Detektionsgrænse
Limite de cuantificación	Bestemmlsesgrænse (kvantitativ)
Sensibilidad	Følsomhed
Valor medio	Middelværdl
Desviación estandar	Spredning
Coeficiente de variación	Variationskoefficient
Desviación estandar relativa	Relativ standardafvigelse
Intervalo de confianza del valor medio	Konfidensinterval for middelværdl
Repetibilidad	Repetérbarhed
Reproductibilidad	Reproducerbarhed
Método analítico	Analysemetode
Procedimiento analítico	Afprøvningsmetode
Resultado de analisis	Resultat
Error sistemático del resultado	Systematisk fejl
Error aleatorio del resultado	Tilfældig fejl
Valor verdadero convencional	Sand værdl