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Traditional Chinese medicine — Simplified accelerated stress simulation methods

Médecine traditionnelle chinoise — Méthodes simplifiées de simulation accélérée des contraintes

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Foreword

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This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Stability is the most important quality criterion for pharmaceutical products after production. So typically for all pharmaceutical products an accurate expiry date is established based on scientific measurement data. In addition to long-term storage tests, accelerated stability tests under specific stress conditions can be carried out at the same time in order to reproduce possible degradation reactions. The aim of the stability tests should be to obtain the most accurate possible assessment of the effects of packaging, closure, dosage, batch, temperature, humidity, pH value and light, as well as other potential factors influencing decomposition processes.

Phytopharmaceuticals are substances from plants, plant parts and plant components in processed or unprocessed condition. Like all other medicinal products, they are subject to authorisation; and the quality, efficacy and harmlessness of the respective preparations must be demonstrated. In contrast to chemically defined drugs, the active ingredient of a phytopharmaceutical is not a single substance, but usually an extract.

The extract as a whole is regarded as the active substance. Depending on the state of knowledge on the active principle of medicinal plants, extracts can be classified as follows:

— extracts for which the efficacy-determining ingredients are known and for which a clear dose-response relationship can be established (e.g. anthraquinone drugs);

— extracts for which co-efficacy-determining ingredients are known, but other (possibly unknown) ingredients are responsible for the overall effect (e.g. St. John's wort);

— extracts which show pharmacological effects but for which the effect cannot be assigned to specific substances.

All pharmaceutical manufacturers must guarantee the consistent, high quality of their products in the whole shelf life. According to international health laws, quality means the nature of a drug, which is determined by the identity, content, purity and other chemical, physical and biological properties or by the manufacturing process as well as the stability.

Herbal medicinal products in the sense of rational therapy are regarded as real remedies. In this respect, the same legal requirements regarding efficacy and harmlessness apply as for chemically defined substances. Nevertheless, there are significant differences in the composition of the two forms of medicine. While chemically defined substances are usually considered as single substances or combinations of a few substances, herbal medicinal products are highly complex multi-component mixtures with hundreds of ingredients. Therefore, a much greater analytical effort is required to satisfy the qualitative demands on the finished product.

According to the national and international requirements on the quality of herbal medicinal products, the drug or the single herbal finished product as a whole is regarded as the active substance to be investigated. National or international guidelines for new or existing active substances and final products are taken into consideration in the process of stability control. However typically the guidelines already indicate that they are not intended to apply for biologically or biotechnologically manufactured medicinal products; and the guidelines do not take into account the specificities of herbal medicinal products.

Quantitative studies of the content of efficacy-determining ingredients ensure, that under defined storage conditions there are no changes in content of typically more than ±5 % compared to the initial value over the proposed shelf life. If no defined active substances are present, a deviation of ±10 % from the content of main substances can be accepted. Other "significant changes" mentioned in the guidelines are changes in pH value, solubility and appearance which would lead to a failure of the approval.

The stability of a few ingredients is the basis for the stability of the whole herbal preparation.

To monitor the qualitative and quantitative composition, high pressure liquid chromatography (HPLC) with ultra violet (UV)-diode array detection is mostly used, because the highest measurement accuracy can be expected from this method. In addition, the extracts are examined by thin layer chromatography (TLC) with various mobile phase systems as a further fingerprint method for qualitative changes in the ingredient

spectrum. In order to avoid the problem of repeatability in TLC, the samples should not be measured immediately but should be collected as deep-frozen samples.

In addition to the monitoring of the ingredients by chromatographic methods, the physical changes of the drugs and extracts should also be documented at the respective sampling times. Other quality features include a change in appearance and organoleptically measurable changes.

International regulations define the framework conditions for accelerated stress simulation without making detailed proposals for its realization in the laboratory.

This document defines simple and detailed methods for the technical implementation of the required stability tests.

NOTE Stress testing of the active substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability and validate the stability indicating power of the analytical procedures used.

When no data are available in the scientific literature, including official pharmacopoeias, stress testing should be performed.

Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures.

Since the herbal substance or herbal preparation in its entirety is regarded as the active substance, a mere determination of the stability of the constituents with known therapeutic activity does not suffice. The stability of other substances present in the herbal substance or in the herbal preparation should, as far as possible, also be demonstrated, for example, by means of appropriate fingerprint chromatograms. It should also be demonstrated that their proportional content remains comparable to the initial fingerprint. If a herbal medicinal product contains combinations of several herbal substances or herbal preparations, and if it is not possible to determine the stability of each active substance, the stability of the medicinal product should be determined by appropriate fingerprint chromatograms, appropriate overall methods of assay and physical and sensory tests or other appropriate tests.

Traditional Chinese medicine — Simplified accelerated stress simulation methods

1 Scope

This document specifies the application of simplified accelerated stress simulation methods for stress tests of finished products, used in and as Traditional Chinese medicine (TCM). Testing for stability or degradation under the influence of daylight or sunlight is outside the scope of this document.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
accelerated stress simulation
exaggerated conditions, such as gas, chemical and storage, to increase the rate of chemical degradation or physical change of a product

3.2
centrifuge tube with plug seal screw cap
standardized plastic test tube with a screw top cap

3.3
fermenting tube
fermentation tube
glass tube with two spheres, which allows the escape of emerging gas and prevents entering of ambient air into a closed system

3.4
marker compound
chemical constituent that can be used to verify the potency or identity of a medicinal product

3.5
out-of-specification
OOS
examination, measurement or test result that does not comply with defined acceptance criteria

[SOURCE: ISO 22716:2007, 2.21, modified — The abbreviated term "OOS" has been added.]

3.6
SPE cartridge
short column (generally an open syringe barrel) containing porous metal or plastic frits

4 Simplified accelerated stress simulation methods

4.1 General

Drug stability and the course of changes in a product from manufacture to consumption by the patient are critical parameters in the product development. So far, only theoretical mathematical relationships or experimental test series for the simulation of longer storage conditions exist.

Valid test procedures therefore must be critically questioned as to whether they can also be applied over the entire stability period – usually 36 months. The test procedures shall coincide with those of the approval or registration procedure and cannot simply be subsequently adapted to any changed external conditions. If the products do not meet the stability criteria, i.e. out-of-specification (OOS) results occur in the testing laboratory, the causes for this shall be identified and risk assessment processes shall be established.

It is therefore imperative to make appropriate stability predictions by means of a bundle of analytical investigations and simulated stress conditions, without additionally extending the often long development process by the prescribed 36 months.

It is therefore necessary to establish a cost-effective and fast simulation for finished products with a relatively large chance of being able to make a realistic stability prediction by suitable accelerated stress simulations. This proves to be an obstacle that is difficult to overcome, especially in the case of herbal preparations, since the concrete active substance in the chemical sense is often not known in comparison to the chemically defined active substance in synthetic preparations. The mechanism of action of herbal extracts is usually known, so analytics shall be focused on one or more meaningful lead substances (marker compounds). To ensure the reliability of the results, each test approach should be carried out with three parallel test samples.

4.2 Different types of accelerated stress simulation

4.2.1 Types of accelerated stress

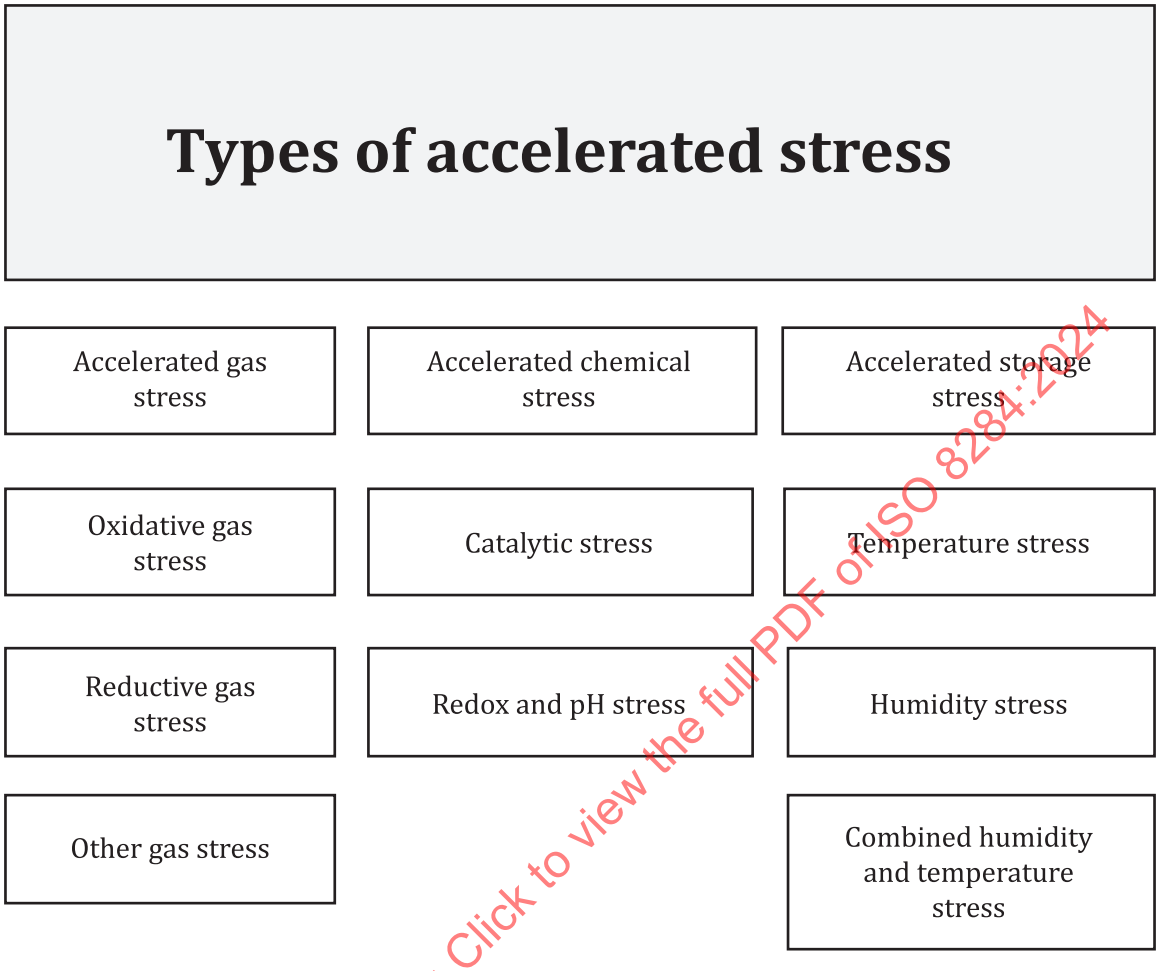


Figure 1 — Types of accelerated stress

4.2.2 Accelerated gas stress simulation

Typical gas stress can be generated by:

— oxidative gases;

— reductive gases;

— other types of reactive gases.

Accelerated gas stress simulation should be used to assess the influence of these gases on the stability of finished products (see Figure 1 column 1).

For validation of the proposed test setup, an accuracy criterion is described in Annex A.

4.2.3 Accelerated chemical stress simulation

Typical chemical stress can be generated by:

— catalytic constituents;

— compounds with a specific redox potential;

— acidic or alkaline constituents which modify the pH.

Accelerated chemical stress simulation should be used to assess the influence of these compounds on the stability of finished products (see Figure 1 column 2).

4.2.4 Accelerated storage stress simulation

Typical storage stress can be generated by storage under:

— defined temperature storage conditions;

— defined humidity storage conditions;

— defined combined temperature and humidity storage conditions.

Accelerated storage stress simulation should be used to assess the influence of storage conditions on the stability of finished products (see Figure 1 column 3).

5 Application methods for accelerated gas stress simulations

5.1 General

On a critical examination, with regard to a certain long-term relevance, six candidates are identified. These, in addition to external supply, are also subject to certain de novo conditions that can arise during storage as a result of degradation reactions in the product.

In order to make core statements about the lability of the marker compound in the shortest possible time in an accelerated stress procedure, novel experimental set-ups are designed from common consumables in the laboratory, which can then be disposed simply and without risk after use as single-use approaches.

The system is designed in such a way that the relevant gaseous reactants from chemical precursors are only produced de novo at the time of simulation. This also eliminates the problem of having to maintain and use corresponding compressed gas cylinders with partly toxic contents to a greater extent. Due to their small quantities, the critical gases can be discharged safely for the employees via normal exhaust ventilation.

Three basic chemical types are identified as possible stability-relevant reactions:

— oxidation reactions;

— reduction reactions;

— further reactions.

5.2 Stability-relevant reactions

5.2.1 General

Based on the different types of gas stress (see 4.2.2), the typical gaseous reactants described in 5.2.2 to 5.2.4 shall be applied for accelerated gas stress simulation.

5.2.2 Oxidation with gaseous reactants

In order to detect oxidative degradation and rearrangement processes at an early stage, a standardized amount of test sample is exposed to a standardized amount of oxidizing agent in a standardized period of time in the respective test arrangements and thus also the associated marker compound.

The following three gases are identified as potentially effective candidates:

— oxygen (O_2);

— sulfur dioxide (SO_2);

— nitrogen oxides (NO_x).

By suitable chemical reactions it is possible to produce equimolar amounts in a simplified and standardized way. In this special case 500 ml of these gases are produced at normal pressure and passed through the test sample including the marker compound.

5.2.3 Reduction with gaseous reactants

In addition to the experiments described in 5.2.2, corresponding reactions with reducing agents can also be simulated under identical conditions in the same experimental set-ups.

These are:

— carbon monoxide (CO);

— hydrogen (H_2).

5.2.4 Other gaseous reactants

In some cases, natural carbon dioxide (CO_2) in the ambient air and as a left-over group during degenerative processes can be responsible for changes and thus loss of stability of marker compounds and products. In order to exclude this risk, CO_2 is also included in the test. The experimental setup described in 5.2.2 shall be used.

5.3 Reagents

— zinc (laboratory grade);

— calcium carbonate (laboratory grade);

— copper (laboratory grade);

— sodium sulfite (laboratory grade);

— manganese (IV) oxide (laboratory grade);

— sulfuric acid 96 % (laboratory grade);

— formic acid 100 % (laboratory grade);

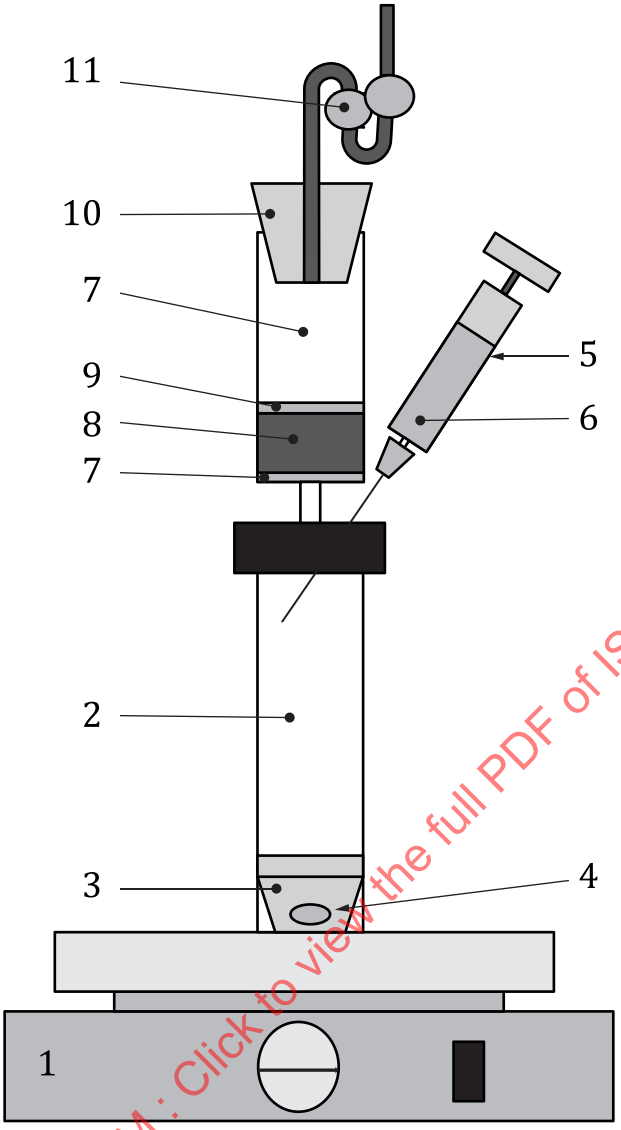
— hydrogen peroxide 15 % (laboratory grade);

— nitric acid fuming 100 % (laboratory grade);

— hydrochloric acid 32 % (laboratory grade).

5.4 Apparatus

The apparatus for accelerated gas tests is described in Figure 2, Table 1 and Table 2.



Key

1 magnetic stirrer
2 centrifuge tube with seal screw cap
3 reagent 1
4 stir fish
5 syringe with hollow needle
6 reagent 2
7 SPE cartridge with base frit
8 test material
9 cover frit
10 rubber stopper
11 fermenting tube filled with water

Figure 2 — Stress simulation setup

5.5 General stress simulation setup

Table 1 — Components of the reaction apparatus and their functions

Components	Function
Centrifuge tube with plug seal screw cap 50 ml	Reaction vessel for gas generation
Chemicals in centrifuge tube with plug seal screw cap and syringe	Reagents
Cover with central hole	Coupling for SPE cartridge
SPE cartridge with base-frit	Receptacle for the test material
Test substrate 2 g	Test sample/test material
Cover frit	Holds the test sample and prevents blowing out
Syringe + hollow needle	Controlled and regulated addition of reagent 2
Magnetic stirrer with mi-ni-stirring bar	For homogenizing the gas generation reaction
Rubber plug with bore	Tight connection piece to the fermenting tube
Fermenting tube with water filling	Control of gas generation and closure against ambient atmosphere

5.6 General implementation

— Add the appropriate quantity (see Tables 4 to 9) of reactant I to the centrifuge tube with plug seal screw cap.

— Carefully move a stir-bar with the magnetic stirrer.

— Screw the cover to the tube.

— Fit the SPE cartridge filled with the corresponding test material.

— Insert injection needle for reactant 2 (see Tables 4 to 9).

— Place rubber stopper with fermenting tube gas-tight.

— Pour water into the fermenting tube as an indicator and closure against the ambient atmosphere.

— Take up reactant 2 in a suitable disposable syringe and add it in doses.

Table 2 — Reaction process

Parameter	Specification
Test sample	Place 2 g test material in loose powder form between two frits
Gas quantity	500 ml of reaction gas are produced in each case
Application time	Adding reagent 2 within 10 min each (slowly dosed)
Reaction time	Allow to react for approximately 20 min after completion of addition Total time = 30 min
Ventilation time	Approximately 60 min without rubber stopper and fermenting tube

WARNING — To carry out the individual experiments, positioning in a suitable laboratory fume cupboard is recommended in order to meet personal protection requirements for the persons carrying out the experiments.

It is appropriate to prepare all test set ups and then to carry out one complete approach (30 min each, one after the other). After completion, the simple test set-up can be stopped, the test sample taken and the remaining disposables collected for disposal. Then the following approach can be carried out.

The experiments produce manageable but partly toxic and mainly reactive gases, parallel performance is not recommended (e.g. hydrogen and oxygen, as well as nitrous gases and others).

The "stressed" test patterns obtained shall then be analysed and evaluated regarding the entire spectrum of constituents on the one hand and the stability of the projected marker compound on the other (see Table 3).

5.7 Analysis

Table 3 — Analytical evaluation

Parameter	Specification
Test sample	Remove the sample from the cartridge after the reaction time
Sample amount	Weigh the corresponding sample quantity
Sample preparation	According to requirements for analytical testing of the respective marker compound
Analysis	With an appropriate and valid method e.g. HPLC for quantitative determination or TLC

The effects of different gas applications on the test samples shall be analysed qualitatively and quantitatively by HPLC and TLC analysis.

The analytical methods used as well as the sample preparation should be identical to those used for quality control prior to product release.

NOTE Due to the increased salt content in the preparations, higher signals in the hydrophilic chromatogram range of HPLC analysis can be expected (salt peak).

5.8 Oxidative gas stress simulation

5.8.1 Oxygen as gas stress factor

The production of oxygen shall be realized as follows:

$$2\,H_2O_2 \xrightarrow{MnO_2} 2\,H_2O + O_2$$

For the production of 500 ml O_2, the reagents and amounts are specified in Table 4.

Table 4 — Production of oxygen

Reagent	Specification	Quantity
Reagent I	Manganese (IV) oxide	100 mg
Reagent II	Hydrogen peroxide 15 %	9 ml

5.8.2 Sulfur dioxide as gas stress factor

The production of sulfur dioxide shall be realized as follows:

$$Na_2SO_3 + 2\,HCl \rightarrow 2\,NaCl + H_2O + SO_2$$

For the production of 500 ml SO_2, the reagents and amounts are specified in Table 5.

Table 5 — Production of sulfur dioxide

Reagent	Specification	Quantity
Reagent I	Sodium sulfite	2,8 g
Reagent II	Hydrochloric acid 32 %	4,3 ml

5.8.3 Nitrogen oxide as gas stress factor

The production of nitrogen oxide shall be realized as follows:

$$3\,Cu + 8\,HNO_3 \rightarrow 3\,Cu(NO_3)_2 + 2\,NO + 4\,H_2O$$

For the production of 500 ml NO_x, the reagents and amounts are specified in Table 6.

Table 6 — Production of nitrogen oxide

Reagent	Specification	Quantity
Reagent I	Copper	2,1 g
Reagent II	Nitric acid (fuming) 100 %	3,7 ml

5.9 Reductive gas stress simulation

5.9.1 Carbon monoxide as gas stress factor

The production of carbon monoxide shall be realized as follows:

$$HCOOH + H_2SO_4 \rightarrow H_2O + CO + H_2SO_4$$

For the production of 500 ml CO, the reagents and amounts are specified in Table 7.

Table 7 — Production of carbon monoxide

Reagent	Specification	Quantity
Reagent I	Formic acid	0,85 g
Reagent II	Sulfuric acid 96 %	1,3 ml

5.9.2 Hydrogen as gas stress factor

The production of hydrogen shall be realized as follows:

$$Zn + 2\,HCl \rightarrow H_2 + ZnCl_2$$

For the production of 500 ml H_2, the reagents and amounts are specified in Table 8.

Table 8 — Production of hydrogen

Reagent	Specification	Quantity
Reagent I	Zinc powder	1,5 g
Reagent II	Hydrochloric acid 32 %	4,3 ml

5.10 Other gas stress simulation by use of carbon dioxide as gas stress factor

The production of hydrogen shall be realized as follows:

$$CaCO_3 + 2\,HCl \rightarrow CO_2 + CaCl_2 + H_2O$$

For the production of 500 ml CO_2, the reagents and amounts are specified in Table 9.

Table 9 — Production of carbon dioxide

Reagent	Specification	Quantity
Reagent I	Calcium carbonate	2,2 g
Reagent II	Hydrochloric acid 32 %	4,3 ml

6 Application methods for accelerated chemical stress simulations

6.1 General

Multiple influencing variables for changes exist in the composition of a drug preparation, which can be divided into “internal” and “external factors”. The external influences come from reactants such as atmospheric oxygen, carbon dioxide, humidity, light and heat, as well as catalytic heavy metal ions or enzymes. The internal, i.e. preparation-related, factors such as excipients, type of solution, H^+ and OH^- ions or buffer substances also influence the behaviour of the ingredients. These parameters can interact to produce various effects within the preparation. The most common chemical changes can be caused by:

— hydrolysis;

— oxidative decomposition;

— racemization;

— decarboxylation;

— substitution reactions;

— polymerization reactions.

6.2 Types of accelerated chemical stress simulations

6.2.1 Catalytic stress simulation

In most cases enzymes and other catalytic compounds like heavy metals are responsible for a wide variety of degradation reactions. The production of pre-materials like extracts reduce typically the amount of intact enzymes so that heavy metals are mostly responsible for degradation.

6.2.2 Redox and pH stress simulation

In addition to the reactions based on catalytic effects much more important degradation result from different pH ranges. The redox potentials in multi-component mixtures like natural products depend on the high variety of secondary constituents originated by growing conditions. There is no possibility for a theoretical approach based on redox potentials and therefore pH stress tests shall be realized.

6.3 Accelerated catalytic stress simulation

6.3.1 General

In addition, a wide variety of interactions can be expected in multi-substance mixtures, such as plant extracts and natural based products. In this context, the points hydrolysis and oxidative decomposition are of greatest importance for the active substances from plant preparations. Esters, lactones, epoxides or acetals are among the structural characteristics of many plant constituents which are at risk of hydrolytic decomposition.

In addition to hydrolysis-sensitive substances, numerous olefins, ethers and amines occur in the herbal ingredient spectrum, which are described as sensitive to oxidative decomposition. Also, light energy, heavy metal ions and elevated temperature shall be mentioned as triggers.

6.3.2 Pre-simulation with a catalytic heavy metal mixture

6.3.2.1 General

Long-term observations with herbal materials show that in most cases only six heavy metals are responsible for degradation processes. These six candidates are implemented in the following methodology.

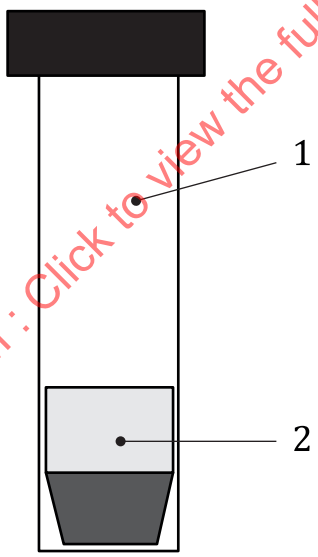
6.3.2.2 Reagents

Table 10 lists the six heavy metals with their concentration in water solutions and their dilution.

Table 10 — Heavy metals as catalysts

No	Heavy metal salts	Molecular weight	Concentration	Dilution for use
1	$PtCl_4$	336,9	33,69 mg/l	1 : 10
2	$PdCl_2$	177,3	17,69 mg/l	1 : 10
3	$NiCl_2$	129,6	12,89 mg/l	1 : 10
4	$CuSO_4$	159,6	16,00 mg/l	1 : 10
5	$ZnCl_2$	136,3	13,65 mg/l	1 : 10
6	$CrCl_3 \cdot 6\ H_2O$	266,5	26,65 mg/l	1 : 10

6.3.2.3 Apparatus



Key

1 centrifuge tube with seal screw cap
2 test material with heavy metal solution

Figure 3 — Apparatus for pre-simulation with a heavy metal mixture

6.3.2.4 Execution

The experimental setup is shown in Figure 3 and Table 11. A two-stage procedure is used to estimate the influence of heavy metals as catalytic variables on active ingredients, markers, extracts and finished products.

The first step is a general preliminary testing for the stability influencing effect of heavy metals.

With this general pre-test, the nonspecific influence on the test sample shall be quantitatively recorded. A so-called “heavy metal catalyst mixture” is used, which contains the most important heavy metals in a defined concentration.

In order to achieve catalytic active concentrations, the following water-soluble heavy metal salts shall be prepared in an aqueous solution to form a mixture.

The selected concentration here is 10 µM each.

Table 11 — Description of the method

Components	Function
Centrifuge tube with plug seal screw cap 50 ml	Reaction vessel
Test substrate 2 g	Test material
Heavy metal solution 6 ml 10 µM (6 ml × 1 ml each)	Aqueous catalyst cocktail for suspending the test substrate
Addition of ampicillin and kanamycin	Preservation against microorganisms
Storage dark at room temperature or in refrigerator at 4 °C to 8 °C	Exclusion of light influence
Reaction period	3 months

NOTE 1 In contrast to sterile synthetic active substances, the use of water-based experimental approaches (in this case heavy metal salt solutions) leads to new problems with natural substances (raw materials, extracts but also finished products for oral absorption). Since such substances and products are generally not sterile, minor to massive microbial contamination often occur in such aqueous preparations, especially in the physiological range (neutral pH value). Experience from decades of laboratory work shows that under certain circumstances storage at room temperature can lead to completely false results after only a few days, as spreading micro-organisms with their specific metabolism make the entire analysis unusable by up, down and conversion reactions.

In the case of simulation series in an aqueous environment, without appropriate preservation over a longer period of time, it cannot be assumed that the stability is specifically dependent on targeted stress factors, but rather that microbial digestion takes place.

NOTE 2 Catalytic concentrations of heavy metal salts do not necessarily have antimicrobial properties such as those described for silver, for example.

In addition, the use of antibiotics is therefore necessary in order to eliminate the potential of microbial contamination by multiplication of the microorganisms already present in the samples to be tested. Consequently, it shall be taken into account that an additional comparison between test samples after short-term aqueous treatment and subsequent drying with an untreated test material after processing and extraction with methanol is necessary in order to assess the effect of antibiotics on the overall analysis.

6.3.2.5 Analysis

The test preparations are stored over the specified reaction time under the ambient conditions (refrigerator or room temperature in the dark).

The samples should then be freeze-dried to produce a system that is as dry as possible and can be quantitatively extracted with 100 % methanol in the same way as the other test approaches, without initiating a shift in the spectrum of constituents (see Table 12).

Table 12 — Sample preparation

Designation	Execution
Test sample after stress simulation	Freeze-drying or drying via vacuum centrifugation concentration or drying under reduced pressure
Extraction	Adding the appropriate amount of methanol (typically 20 ml)
Test extract	After filtration

The effects of heavy metal catalysts on the test samples in aqueous suspension shall be analysed qualitatively and quantitatively by HPLC and TLC analysis.

The analytical methods used as well as the sample preparation should be identical to those used for quality control prior to product release.

NOTE Due to the increased salt content in the preparations, higher signals in the hydrophilic chromatogram range of HPLC analysis can be expected (salt peak).

6.3.2.6 Results and consequences

If there are no relevant changes regarding the marker and/or the fingerprint, the experiment can be terminated with the heavy metal mixture.

If, on the other hand, there are relevant changes with regard to the marker and/or fingerprint, each individual heavy metal shall be used separately and analysed after the reaction time.

6.3.3 Identification of a specific stability-influencing catalytic reaction

6.3.3.1 Reagents and apparatus

See 6.3.2.2 and 6.3.2.3.

6.3.3.2 Execution

Analogous to the execution in 6.3.2.4 and Table 13, the test samples are treated with the individual heavy metal solutions. Thus the heavy metal mixture is replaced by single element solutions, whereby everything else remains the same.

Table 13 — Description of the method

Components	Function
Centrifuge tube with plug seal screw cap 50 ml	Reaction vessel
Test substrate 2 g	Test material
Heavy metal solution 6 ml 10 µM/l	Aqueous single catalyst solution for suspending the test substrate
Addition of ampicillin and kanamycin	Preservation against microorganisms
Storage dark at room temperature or in refrigerator at 4 °C to 8 °C	Exclusion of light influence
Reaction period	3 months

6.3.3.3 Analysis

The effects of individual heavy metal catalysts on the test samples in aqueous suspension shall be qualitatively and quantitatively analysed by HPLC and TLC analysis.

The analytical methods used as well as the sample preparation should be identical to those used for quality control prior to product release.

NOTE Due to the increased salt content in the preparations, higher signals in the hydrophilic chromatogram range of HPLC analysis can be expected (salt peak).

6.3.3.4 Accuracy criterion for accelerated catalytic stress simulation (validation)

The accuracy of this test method can be realized by measurement of the osmolality of the heavy metal solutions.

6.4 Accelerated redox and pH stress simulation

6.4.1 General

In order to estimate the influence of different pH values as a degenerative influencing factor on active substances, extracts, marker compounds and finished products, a new experiment is established.

To generate reliable data, a special test with a focus on this problem shall be implemented and realized (see Figure 4).

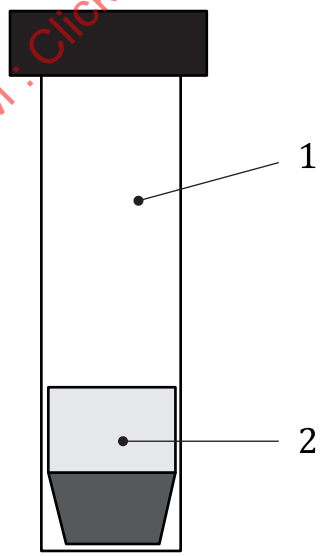
By using suitable buffer solutions, the effect of different pH values and simultaneously the influence of redox systems can be simulated (see Table 15).

6.4.2 Reagents

Table 14 — Buffer reagents

No	Buffer component	Molarity	Concentration
A	KCl	0,2 M	14,91 g/l
B	Potassium hydrogen phthalate	0,1 M	20,42 g/l
C	Potassium dihydrogen phosphate	0,1 M	13,61 g/l
D	Sodium tetraborate × 10 H_2O (Borax)	0,025 M	9,54 g/l
E	Disodium hydrogen phosphate	0,05 M	7,1 g/l
F	HCl 37 %	0,1 M	8,3 ml/l
G	HCl 37 %	0,2 M	16,6 ml/l
H	NaOH	0,1 M	4 g/l
I	NaOH	0,2 M	8 g/l

6.4.3 Apparatus



Key

1 centrifuge tube with seal screw cap

2 test material with buffer solution

Figure 4 — Apparatus for accelerated redox and pH stress simulation

6.4.4 Execution

Table 15 — Description of the method for Redox and pH stress simulation

Components	Function
Centrifuge tube with plug seal screw cap 50 ml	Reaction vessel
Test substrate 2 g	Test material
Buffer redox solution 5 ml	Aqueous buffer as redox system for suspension of the test sample
Addition of ampicillin and kanamycin	Preservation against microorganisms
Storage dark at room temperature or in refrigerator at 4 °C to 8 °C	Exclusion of light influence
Reaction period	3 months

For conceptual simplification, a buffer system successfully propagated by the Handbook of Chemistry and Physics for decades and established worldwide, consisting of only two component solutions without necessary adjustments of the pH values, shall be used.

The necessary solutions for the targeted production of the required buffers can be prepared in advance according to Table 16.

From the solutions (see Table 14), the buffer systems shall then be mixed according to Table 16 without further readjustment. The pH values apply at 25 °C.

Table 16 — Mixing table for buffers

pH	No	Buffer component 1	Amount	No	Buffer comp. 2	Amount
1,0	A	0,2 M KCl	25 ml	G	0,2 M HCl	67,0 ml
2,0	A	0,2 M KCl	25 ml	G	0,2 M HCl	6,5 ml
3,0	B	0,1 M Potassium hydrogen phthalate	50 ml	F	0,1 M HCl	22,3 ml
4,0	B	0,1 M Potassium hydrogen phthalate	50 ml	F	0,1 M HCl	0,1 ml
5,0	B	0,1 M Potassium hydrogen phthalate	50 ml	H	0,1 M NaOH	22,6 ml
6,0	C	0,1 M Potassium dihydrogen phosphate	50 ml	H	0,1 M NaOH	5,6 ml
7,0	C	0,1 M Potassium dihydrogen phosphate	50 ml	H	0,1 M NaOH	29,1 ml
8,0	D	0,025 M Borax	50 ml	F	0,1 M HCl	20,5 ml
9,0	D	0,025 M Borax	50 ml	F	0,1 M HCl	4,6 ml
10,0	D	0,025 M Borax	50 ml	H	0,1 M NaOH	18,3 ml
11,0	E	0,05 M Disodium hydrogen phosphate	50 ml	H	0,1 M NaOH	4,1 ml
12,0	E	0,05 M Disodium hydrogen phosphate	50 ml	H	0,1 M NaOH	26,9 ml
13,0	A	0,2 M KCl	25 ml	I	0,2 M NaOH	66,0 ml

6.4.5 Sample preparation and analysis

The experimental approaches shall be stored analogous to the heavy metal approaches over the given reaction time under the ambient conditions (refrigerator or room temperature in the dark).

The samples shall then after the stress period be freeze-dried to produce a system that is as dry as possible and can be quantitatively extracted with 100 % methanol, analogous to the other test approaches, without initiating a shift in the spectrum of constituents (see Table 17).

Table 17 — Sample preparation for Redox and pH stress simulation

Designation	Execution
Test sample after stress simulation	Freeze-drying or drying via vacuum centrifugation concentration or drying under reduced pressure
Extraction	Adding the appropriate amount of methanol (typically 20 ml)
Test extract	After filtration

The effects of different pH values and redox systems on the test samples in aqueous suspension shall be qualitatively and quantitatively analysed by HPLC and TLC analysis.

The analytical methods used as well as the sample preparation should be identical to those used for quality control prior to product release.

NOTE Due to the increased salt content in the preparations, higher signals in the hydrophilic chromatogram range of HPLC analysis can be expected (salt peak).

6.4.6 Accuracy criterion for accelerated Redox pH stress simulation (validation)

The accuracy of this test method can be realized by potentiometric measurement of the pH value of the buffer/redox system.

7 Application methods for accelerated storage stress simulations

7.1 General

In this context, the points hydrolysis and oxidative decomposition are of the greatest importance for the active ingredients of herbal preparations. Esters, lactones, epoxides or acetals are among the structural characteristics of many plant constituents which are at risk of hydrolytic decomposition. For solid preparations such as dry extracts and products, the hydrolysis rate depends on the water content, the temperature, the water adsorption rate and the adsorption isotherm of the respective substances. In the adsorbed water layers, the active ingredient shall first be dissolved before degradation processes take place.

NOTE As part of this document, extensive series of tests are carried out to develop a simple and cost-effective standard procedure that can be implemented quickly in the normal laboratory work, which also does not block valuable resources in GMP storage cabinets and only creates problems in GMP-compliant documentation with regard to allocation to R&D. On closer inspection, therefore, such test series are not carried out simultaneously in GMP rooms and equipment (in this case climate chambers), as this can only result in regulatory problems.

7.2 Types of accelerated storage stress simulation

7.2.1 Temperature stress simulation

Based on the rule of Van't Hoff which shows that an increase of the temperature by 10 °C leads to a doubling or quadrupling of the reaction speed, Arrhenius postulated the so-called Arrhenius equation which can be used for the estimation of the shelf life.

The laboratory tests are realized by the procedure in 7.3.

7.2.2 Humidity stress simulation

Besides the influence of temperature, the relative humidity has the greatest effect on degradation and physical changes. From a defined critical water content, the slope of the degradation kinetics increases linearly. How the degradation ultimately takes place depends on the water adsorption and the resulting surface moisture of the substance particles.

7.2.3 Combined humidity and temperature stress simulation

The testing of the combination of humidity with temperature stress is defined in different national and international regulations.

Details can be found also in Pharmacopoeias.

NOTE The following tests are not mandatory in all countries (see national regulations).

In order to assess the stability of products, the ICH also offers the supplementary possibility of stability tests going beyond the classic "climate zones for stability studies". Proposals for the conditions for such so-called "accelerated stress tests" for "high temperature" and "high humidity" are also listed there.

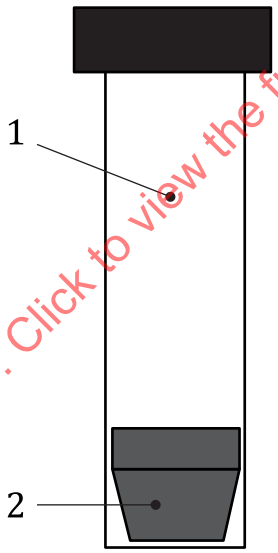
With the aid of the data obtained, estimates of product stability and critical influencing factors can be made in a shorter time. The implementation is described in 7.3, 7.4 and 7.5.

7.3 Accelerated temperature stress tests

7.3.1 Reagents

None.

7.3.2 Apparatus



Key

1 centrifuge tube with seal screw cap

2 test material

Figure 5 — Apparatus for accelerated temperature stress simulation

7.3.3 Execution

Standard PE-tubes (e.g. type falcon 50 ml) (see Figure 5) shall be used to quickly generate reliable and valid measurement data. The test shall be realized according to Table 18.

The ICH Q1A provides for the evaluation of increased temperatures as an influencing factor without simultaneously changing the air humidity. Here three additional temperatures (50 °C, 60 °C and 70 °C), which correspond to the 10 °C intervals proposed in the guideline should be used. The only equipment requirement is access to three ovens with the above temperatures in the R&D environment.