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Reflection paper on microbiological aspects of herbal medicinal products and traditional herbal medicinal products

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7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 Facsimile +44 (0)20 7418 8416
E-mail info@ema.europa.eu Website www.ema.europa.eu

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1. Introduction

Directive 2001/83/EC as amended and Directive 2001/82/EC as amended provide definitions for herbal substances, herbal preparations, and herbal medicinal products (HMPs)[1]. The basic legislation applies to both HMPs for human and veterinary use[2]. An additional simplified registration procedure has been established for traditional herbal medicinal products (THMPs) for human use under Directive 2004/24/EC. The principles of this reflection paper apply equally to such THMPs.

According to these definitions a herbal medicinal product is any medicinal product, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations.

THMPs may also contain vitamins and minerals, provided that the action of the vitamins and minerals is ancillary to that of the active herbal ingredient(s).

HMPs have a number of characteristics that differentiate them from medicinal products containing chemically defined active substances. Specific guidelines have therefore been established for HMPs which cover particular aspects that general guidelines do not. Herbal substances and herbal preparations are complex mixtures of natural constituents and, potentially, also contaminants, with a natural variability. Being of natural origin herbal substances generally have a higher microbial content compared to chemical drug substances. In this reflection paper consideration is given as to how the microbial contamination of herbal substances, herbal preparations, and HMPs can be limited by preventative measures and by applying decontamination processes. The need for testing and regulatory documentation is also discussed.

Sterile dosage forms and methods of sterilisation are not covered by this paper.

1 The term “herbal substance” should be considered as equivalent to the term “herbal drug” as defined in the European Pharmacopoeia, and the term “herbal preparation” should be considered as equivalent to the term “herbal drug preparation” as defined in the European Pharmacopoeia.

2 Directive 2001/83/EC as amended and Directive 2001/82/EC as amended.

2. Discussion

The active ingredients of HMPs are herbal substances and/or herbal preparations derived from herbal substances. Being of natural origin, the active ingredients in HMPs tend to have higher microbial contamination (bioburden) than chemically defined active substances and the microbial population present may differ qualitatively and quantitatively. Therefore, particular attention should be paid to the microbiological quality of HMPs and specific guidance should be provided. The Ph. Eur. recognises the need to allow wider acceptance criteria for the microbial quality HMPs depending on the nature of the product and method of preparation e.g. herbal teas.

Herbal substances/preparations may be contaminated with numerous species of bacteria and fungi (yeasts and moulds). Viruses are not usually considered to be a concern with herbal substances/preparations. The content of live bacteria, fungi and their spores should be determined and limited in herbal substances/preparations and HMPs.

Pathogenic micro-organisms

Some bacterial species are pathogenic (e.g. *Salmonella* spp.*, Shigella* spp., some pathovars of *Escherichia coli; Listeria monocytogenes* and some clostridial species) and therefore pose a risk of inducing infectious diseases or other unwanted effects in patients taking the HMP. Such micro-organisms should not be present in the HMP.

Spores

Endospores are bacterial spores formed by certain Gram-positive bacteria e.g. *Bacillus* and *Clostridium* species. Spores are formed when bacteria are exposed to unfavourable environmental conditions (heat, drought, irradiation or depletion of nutrients). Generally, a higher number of spores are found in dry herbal substances compared to fresh herbal substances, especially when inappropriate drying procedures are used. Bacterial spores are highly resistant to various environments (desiccation, freezing, dry heating, vapour, elevated pressure, UV radiation and various chemicals including extraction solvents such as ethanol). Bacterial spores have the potential to be reactivated into the vegetative state as bacteria when favourable environmental conditions are present again. Nutrients and elevated temperatures are used during the incubation phase of testing of total aerobic microbial count (TAMC; Ph. Eur. 2.6.12, 2.6.13, 2.6.31) of a product and thus spores of certain bacterial species (mostly aerobic from *Bacillus* spp.) are detected together with the bacteria by these quantitative *in vitro* methods.

Fungi, and particularly moulds, also produce spores (conidia). However, they are generally not as resistant as bacterial spores to unfavourable environmental conditions.

Physicochemical characteristics

From a quality point of view, some micro-organisms can alter the physicochemical characteristics of the product which may lead to detrimental changes to the product's quality. Constituents of the plant material may be metabolised by the micro-organism, leading to chemically changed substances. It is undesirable to have chemical degradation of constituents of the plant material, (especially constituents with known therapeutic activity), active markers and chemical preservatives (added to e.g. a liquid aqueous extract or liquid dosage form). Any potential reduction or change in the therapeutic activity of the product must be evaluated.

Micro-organisms may also lead to sensory changes (appearance, smell, or taste) and to changes in pH of the HMP, due to metabolic substances formed by the micro-organism. If the pH changes significantly in a HMP containing a chemically ionisable preservative and the efficacy of that preservative is pH dependent (e.g. benzoic acid and sorbic acid), then the efficacy of the preservative may be diminished. Such risks should be considered.

Mycotoxins

During mycelial growth on substrates, some moulds produce mycotoxins. These substances are secondary metabolites with lipophilic (e.g. aflatoxins and ochratoxin A) or hydrophilic (e.g. fumonisins) properties. Mycotoxins can be formed during plant growth (cultivation or wild growth) or during storage of the herbal substance/preparation or HMP.

The most important mycotoxins are highly toxic and carcinogenic aflatoxins. Aflatoxin B1 is considered to be the most toxic mycotoxin.

In principle, aflatoxins are only formed by specific fungal species, which favour certain plants, plant parts and growing conditions. For example, formation of aflatoxins may be initiated only after exposure of the plant to unfavourable environmental conditions (e.g. drought or flooding). The geographical origin may have a marked impact on the extent of aflatoxin formation. Aflatoxin forming moulds prefer elevated temperatures and humid conditions, so herbal substances originating from plants grown in (sub)tropical climates may show significantly higher levels of aflatoxins than those grown in cooler, drier climates. Formation of aflatoxins is also dependent on the pH of the material.

The main producer organisms for aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*. Generally all plant parts are at risk of contamination by aflatoxins. However seeds, fruits, roots, and rhizomes present a greater risk as they contain the best combination of nutrients for growth of the fungi. Furthermore, as *A. flavus* and *A. parasiticus* are soil borne this presents an added risk for roots and rhizomes. The presence of water is essential for both growth of micro-organisms and formation of aflatoxins; therefore the content of water is a critical parameter and testing of loss on drying or water content is crucial for dried herbal substances, preparations and HMPs.

Some plant materials (e.g. liquorice root) may be contaminated by ochratoxin A. This toxin is produced by *Aspergillus ochraceus*, *Penicillium verrucosum* and some other species of *Aspergillus* and *Penicillium*. Ochratoxin A is nephrotoxic and carcinogenic.

Aflatoxins and ochratoxin A are heat stable and soluble in hydro-alcoholic solvents. There is therefore a potential risk of carry-over of aflatoxins and ochratoxin A from the herbal substance to the herbal preparation or HMP which could lead to the presence of higher concentrations of aflatoxins in the herbal preparation or HMP. This risk should be fully evaluated by validation of the extraction process of a herbal preparation.

2.1. Minimizing microbial contamination by prevention

Microbial contamination originates from primary and secondary contamination. Primary contamination is the naturally occurring microbial flora of the plant to be harvested. Secondary contamination is caused by handling of the plant material (human intervention, equipment, buildings, air ventilation systems, and contamination during transportation). Minimising contamination with micro-organisms and microbial toxins should be ensured ideally by monitoring and limiting both primary and secondary contamination, i.e. by prevention rather than by use of decontamination methods.

The herbal substance should be manufactured in compliance with good agricultural and collection practice (GACP) and, from the starting material onwards, the herbal preparation should be

manufactured in compliance with good manufacturing practice (GMP). Some herbal substances/herbal preparations (e.g. certain essential oils) exhibit a certain degree of inherent antimicrobial activity. This should not be used to justify a lack of compliance with GACP and GMP.

2.1.1. Herbal substances

For cultivated plants, the growing conditions should be chosen in order to avoid unnecessary microbial contamination. If manure is used as a fertiliser, the manure should be carefully composted before use. In view of the fact that many micro-organisms are host specific human faeces must not be used as fertiliser and direct use of sewage must also be avoided.

Where justified, fungicides can be used during cultivation of the plant in order to reduce fungal growth. For both cultivated and wild plants, the time of harvest should be chosen so that the presence of external water on the plants is limited, i.e. by avoiding harvesting during or immediately after rainfall or heavy morning/evening dew. Growing the plants in green houses provides some opportunity to control airborne and animal contamination which may help to reduce microbial contamination.

After harvest, unless frozen, herbal substances intended for fresh use, should be processed immediately. If the herbal substance is to be dried before use, the drying process (method and time) should be described. Drying should be as fast and uniform as possible, as this step is the most critical for the growth of moulds and bacteria and formation of mycotoxins. Insufficient drying which leads to increased levels of microbial contamination should not be resolved primarily by applying decontamination methods to the product.

Any use of cleaning (dusting off or washing), cutting, freezing and storage of the herbal substance may have a positive or negative impact on the final level of microbial contamination. If the herbal substance is cleaned by washing with water, the quality of the water should be considered as a possible risk for microbial contamination.

The packaging material and storage conditions for the herbal substance should be chosen in order to prevent microbial growth and secondary contamination. Storage at low temperatures may lead to formation of condensed water, which may pose a contamination risk.

2.1.2. Herbal preparations

The principles of fast, efficient and homogenous processing during manufacture for the herbal substance should also be applied to herbal preparations. Relevant steps and in-process controls include extraction temperatures and times, in particular for aqueous extractions, vacuum evaporation of extracts, distillation of essential oils and holding times. The manufacturing method should be validated and appropriate IPCs should be set. Expressed juices and herbal extracts prepared with water or with low concentrations of alcohol are at particular risk of microbial contamination. The addition of preservatives to extracts and expressed juices may be considered as an option. The choice and concentration of the preservative should be fully justified, in accordance with current guidelines, which should include evidence of preservative efficacy.

In addition to microbial contamination arising from the herbal substance itself, microbial contamination arising from water, organic extraction solvents, excipients for standardisation or technological purposes should also be considered.

The packaging material and storage conditions for the herbal preparation should be chosen in order to prevent microbial growth and secondary contamination.

2.1.3. Herbal medicinal products

The principles for addressing microbial contamination in herbal substances and herbal preparations also apply to manufacture, transportation and storage of the HMP.

Microbial contamination of excipients used to produce the chosen dosage form should be controlled and monitored.

The limits for microbiological purity of the finished product will depend on the dosage form and administration route, cf. section 2.3.

2.2. Methods for reduction of microbial contamination

As described in the sections above, microbiological quality of HMPs is the result of the quality of the materials used and the manufacturing process. According to GMP criteria, good quality cannot be controlled at the end of the process but should be built-in and should include the quality of the starting material.

Minimisation of microbial content of herbal materials during cultivation, harvesting, storage and processing is essential because the possibility of reducing the microbial bioburden in herbal materials by means of post-processing treatments is very limited. This is due to the fact that herbal materials are prone to deterioration by many of the treatments available; but, in addition, the potential for harmful residues to remain needs to be addressed fully.

This issue is highlighted in the Ph. Eur. monograph “Herbal drugs”, which under the section on production states: “if a decontamination treatment has been used, it is necessary to demonstrate that the constituents of the plant are not affected and that no harmful residues remain.”

Despite its effectiveness in bioburden reduction (including endospores) the use of ethylene oxide for the decontamination of herbal substances has been prohibited in the European Union since 31 December 1989 by Directive 89/365/EEC due to the formation of toxic by-products, such as ethylene chlorohydrin and ethylene glycol.

2.2.1. Justification for applying a decontamination process

Complete elimination of micro-organisms from a given herbal substance, preparation or HMP, by sterilisation methods, is not necessary, provided that pathogenic micro-organisms are excluded, microbial contamination is limited to an acceptable level and any microbial growth can be controlled during storage until the end of shelf-life.

Information on microbiological quality of a product should be provided to justify the need for the decontamination treatment and to establish a procedure to reduce microbial contamination. A risk assessment should be performed based on the microbial population and the initial level of contamination taking account of the recommended acceptance criteria for non-sterile pharmaceutical products: total aerobic microbial count (TAMC) and total combined yeasts/moulds count (TYMC), as defined in the Ph. Eur.

The use of a decontamination process should be selected and fully justified on the basis of the type and composition of the herbal material, its intended use and route of administration. Important considerations are the initial microbial bioburden and the desired maximum final microbial contamination level and should take account of the subsequent steps in the manufacturing process and factors likely to influence microbial growth such as the water activity and the proposed shelf-life and storage conditions.

A decontamination treatment should not be used simply as a precautionary measure and decontamination treatments should not be used where the herbal substances/preparations/HMPs have microbial contents unfit for human or animal consumption. The presence of pathogenic bacteria must be avoided or these bacteria must be completely killed or removed. Micro-organisms capable of producing toxins, such as *Clostridium botulinum* or fungi, are harmless provided conditions prevent their growth; however once the toxins are produced they are very difficult to eliminate. Therefore the possible presence of microbial metabolites needs to be carefully considered since the majority of microbial decontamination methods lead to reduction of viable microorganisms (TAMC and TYMC) but do not reduce the levels of mycotoxins or endotoxins. Furthermore, only some decontamination methods reduce the number of spores.

The quality of a decontaminated herbal substance/preparation/HMP can be greatly influenced by storage and shipping conditions due to the growth of bacteria surviving the process and chemical reactions such as oxidation and biochemical modifications of the chemical constituents of the herbal material.

If a decontamination method is used, it should be demonstrated that the chemical profile of the product has not been affected by the process. If any change in the chemical profile occurs this should be addressed and fully justified. The impact on safety and efficacy aspects of the herbal substance/preparation/HMP should be considered and degradation products should be qualified toxicologically, as appropriate.

2.2.2. Choice of decontamination method

A number of different methods are available which may be used to reduce microbial contamination of the herbal substance, the herbal preparation or during manufacture of the finished product. Where used, they should be performed as early as possible in order to maintain microbial quality at an appropriate level throughout the entire manufacturing process and to minimise further microbial growth during and after manufacture of the product.

Any treatment should be chosen to be as gentle as possible in order to avoid unwanted changes (chemical and physical) in the quality of the product. The choice of method and establishment of process parameters (times, temperatures, pressures, concentrations, dose etc.) should be based on development and validation data.

The extraction process itself

In many cases, the manufacturing process itself may provide a degree of microbial decontamination to a certain extent. For example, extraction of the raw material with an alcoholic solution may represent a microbial-reducing method. However, only higher alcohol concentrations (60 to 80%) have marked decontamination effects because, at lower concentrations of alcohol, the presence of water potentially facilitates the growth of the micro-organisms.

No obvious differences in microbial decontamination have been shown between the use of ethanol and methanol. Vegetative cells, particularly those of Gram-negative species, are very sensitive to heat and alcoholic solutions. The residual microbial contamination from such extraction processes is represented mainly by bacterial endospores, which are resistant to e.g. ethanol. Hydroalcoholic extraction with heating usually yields products with relatively low TAMC ($<10^4$ CFU/ml).

Production of a dry extract normally involves cautious evaporation of the organic solvent in a vacuum-evaporator. In most cases, the resulting aqueous soft extract is mixed with suitable excipients and then further evaporated to dryness using suitable equipment (e.g. spray drier or belt drier): the total

microbial level may be increased after alcohol evaporation, as the aqueous soft extract is an ideal medium for microbial growth.

Extraction with boiling water reduces the TAMC and TYMC as shown by several studies on the effects of the use of boiling water to prepare herbal teas. Experiments with artificial contamination by non-sporulating (*E. coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Enterobacter cloaceae*) and spore-forming microbial species (*Bacillus cereus*) demonstrated that the non-sporulating bacteria were fully eliminated while the sporulating organisms survived extraction with boiling water almost completely. However, as water is ideal for the growth of micro-organisms, the storage period should be less than 24 h at the temperature of a refrigerator (2-8°C) to prevent microbial growth.

Extraction with supercritical carbon dioxide reduces the TAMC and TYMC by combining the effect of the solvent with the effect of high pressure which both reduce the level the micro-organisms.

Distillation of essential oils usually leads to very low microbial contamination because of the process itself (high temperature and phase change) and, additionally, due to the often intrinsic antimicrobial properties of the essential oils.

Treatment with ethanol

Data are available on the bactericidal effects of ethanol as a function of concentration and contact time. Ethanol is bactericidal in aqueous mixtures at concentrations between 60-95% V/V but is ineffective against bacterial spores.

In view of the fact that extraction with ethanol helps to reduce the microbial contamination, repeated treatments with ethanol followed by evaporation may be performed to minimise the microbial content. However, use of ethanol may cause chemical changes in the composition of extracts and such changes should be evaluated.

Heat treatment: dry or steam

In order to minimise microbial contamination, short heat treatment (ultra high heat (UHT)) or pasteurisation may be performed before drying, if necessary.

However, such treatments are not usually suitable for extracts with high contents of resinous substances, highly viscous extracts (dry residue more than 50%) or extracts with thermolabile or volatile constituents.

The use of heating as a microbial decontamination method is based on the assumption that the death of micro-organisms is log-linear with time. However, the use of this method may be limited by the highest temperature that can be used, particularly when thermolabile and volatile constituents are present in the herbal material.

Drying at high temperatures for a few minutes, such as in tumble dryers used for industrial production, generally reduces the microbial bioburden. Drying at lower temperatures in static dryers for a longer time, may have a lesser impact on some chemical constituents, but does not sufficiently reduce the viable count as much as in tumble dryers and has no effect on spores. The spores of Gram-positive bacteria are highly heat resistant and temperatures required to kill them may induce physicochemical, chemical and sensory changes to the product.

Water vapour treatment at 65°C may destroy certain undesirable micro-organisms (e.g. Salmonellae, *E. coli* and *Pseudomonas aeruginosa*). However, residual moisture should be removed and carefully controlled after the treatment in order to avoid subsequent microbial growth.

Fumigation

Fumigation of herbal substances to control pests and plant diseases may also reduce microbial contamination. It is recommended that the use of fumigant products is limited as far as possible and should only be used when a genuine need is identified. Fumigation should be carried out at the earliest possible stage and the choice of fumigant, concentration and conditions of use (temperature, humidity, exposure time) should be carefully assessed to minimise residues in the herbal material. Potential carry-over of residues to the herbal preparation and HMP should be addressed fully and controls applied where necessary. Aspects of fumigation of herbal substances are discussed in the *Reflection paper on the use of fumigants* (EMEA/HMPC/125562/2006) and in the *Questions & answers on quality of herbal medicinal products/traditional herbal medicinal products* (EMA/HMPC/41500/2010, as revised).

Irradiation

Irradiation is restricted or not permitted in a number of European Member States and, when allowed, it should only be used when there is a reasonable need and no other methods can be applied.

Irradiation should be carried out under specified conditions and the safety of irradiated products should be fully evaluated.

Three different types of ionising radiation are used; gamma rays, X rays and electrons.

The effectiveness of the treatment is dependent on several factors including the composition of the substrate, the number and types of micro-organisms and the dose applied. The lethal dose of radiation varies depending on the type of radiation and the type of micro-organisms. In general, vegetative forms of bacteria are more sensitive to ionizing radiation than fungi are. The number of spores may also be reduced by X ray and gamma irradiation.

Freeze drying

Freeze-drying is reported to decrease microbial contamination, but there is little information on the effect of this technique. Moreover, the sensitivity of micro-organisms may differ considerably to this method and conditions capable of reducing microbial contamination should be evaluated. On the other hand, the use of cryo-protectants as part of the process may allow for the survival of micro-organisms and their subsequent recovery and proliferation after reconstitution. This should be addressed during method validation.

High pressure processing

High pressure processing (HPP), also known as high hydrostatic pressure processing (HHPP) and ultrahigh pressure (UHP), is a method of processing, where the material is subjected to elevated pressures, up to 1000 MPa (145,000 psi), applied with a pressure-transmitting medium (water or other liquids as appropriate). The process inactivates/kills micro-organisms, reduces the need for preservatives and eliminates post-process contamination, while retaining organoleptic properties such as freshness, flavour and colour of the plant material. Moreover HPP can also be used to inactivate (or to activate) enzymes.

Although the mechanism of inactivation by HPP is not well understood, it is considered that the compression process induces cell membrane rupture and macromolecular transformations, e.g. protein denaturation.

HPP can be applied to solids, liquids and to packaged products as high pressure acts instantaneously and uniformly without a gradient of effectiveness from surface to centre, regardless of shape, size, and composition. Pressure, temperature and exposure time can be adjusted for optimal results, and the process may be carried out at ambient, cooling or freezing temperatures, with exposure times ranging from a millisecond to over 20 minutes.

The sensitivity of micro-organisms to HPP is variable and influenced by several factors, therefore the processing conditions (holding time of the pressure, temperature of pressure processing, composition of the medium) have to be carefully selected for the individual herbal material to be treated. Conditions and specifications should be validated. Any impact of pH modifications applied during the HPP process on the composition of the product should be evaluated.

To improve the efficacy HPP can be combined with heat and/or low pH as well as pressure cycling treatments for inactivation and control of outgrowth of spores. Ultrasound, alternative currents, high-voltage electric pulses and antimicrobial agents may also be used.

HPP may only damage the microbial cells, thus the sub-lethally injured cells may recover and multiply when they find suitable conditions during subsequent processing and storage. This phenomenon may lead to an over-estimation of microbial reduction because the counts determined immediately after HPP will be lower than those reached after the recovery of injured cells.

Instant controlled pressure drop

In recent years a new technology, Instant controlled pressure – drop (DIC for Détente Instantanée Contrôlée) has been developed (and patented) as a decontamination process, particularly for heat-sensitive solids and powders. It is based on short time heating of the material and an instantaneous pressure drop towards vacuum, which causes an abrupt cooling by evaporation of part of the water of the treated material. The micro-organism cells (both spores and vegetative forms) explode as a consequence of a thermo-mechanical effect. Heating of the material may be achieved by saturated or superheated steam injection (STEAM-DIC) or compressed air but other media can be used such as carbon dioxide, when a dissolution effect is expected to be achieved (e.g. extraction of non-volatile constituents). The higher the amount of the steam or gas injected and the shorter the pressure drop time, the more efficient the mechanical effect is. When the process is repeated several times it is possible to lower the heating temperature to achieve the desired microbial contamination reduction, thus preserving thermolabile constituents (Multi-cycle DIC).

A possible negative impact of this method is the loss of volatile constituents through auto-vaporisation. However, this feature offers a potential for use of DIC technology in the extraction of essential oils from aromatic plants.

Treatment with alkaline or acidic substances

Treatment with alkaline or acidic chemical substances is known to reduce microbial contamination, including spores. However, such treatments are not usually applicable to herbal substances or herbal preparations, as alkaline and acidic compounds may lead to significant chemical alterations of the constituents of the herbal substance/preparation. Residues of any toxic substance applied should also be avoided.

Preservation

Addition of a preservative is not considered to be a decontamination method. However, addition of preservatives to prevent microbial growth on storage and to cover the entire shelf-life should be

considered when the unpreserved product supports microbial growth. Preservatives must not be used to replace GACP and GMP or to disguise products with initial high levels of microbial contamination.

New, alternative methods

The list of methods outlined above is not exhaustive and other methods may be applied. Manufacturers and regulatory agencies have a responsibility to ensure appropriate microbiological quality of herbal substances/preparations/HMPs and where necessary, appropriate decontamination methods could be employed to reduce microbial contamination.

2.2.3. Herbal substances

Methods for reducing microbial contamination of herbal substances are not only dependent on the above mentioned specific factors, but also on the subsequent use of the herbal substance. When the herbal substance is intended for further processing it might be sufficient to dry or freeze the plant material to prevent microbial growth and spoilage until further processing takes place.

Fumigation may be appropriate for herbal substances but it should be limited when other approaches are possible and should be applied at the earliest possible stage, taking into consideration all relevant aspects, precautions and prohibitions.

The use of steam is, in general, not advisable to reduce the microbial contamination of plant material, unless, following the process, the material is dried immediately as any residual water may affect the subsequent processing stages.

Irradiation should be limited to exceptional circumstances, when no other method is feasible. Attention should be paid to herbal substances imported from Third Countries, which may have been irradiated but this is not declared or adequately documented. A suitable test to detect possible irradiation should be established for herbal substances at risk.

HPP can be used for eliminating bacteria of concern and to ensure microbiological safety. This process is used in the food area for fruit juices and fruit and is suitable for the treatment of herbal substances. Heat sensitive products with a high acid content are particularly good candidates for the application of this technology.

2.2.4. Herbal preparations

The extraction process itself may contribute to microbial contamination reduction notably when high concentrations of ethanol are used. However it should be noted that extraction with cold water may result in large increases in microbial levels such as in case of maceration.

Fumigation is not a suitable treatment for herbal preparations and irradiation of herbal preparations is not advisable.

Preservatives may be added to herbal preparations in order to prevent microbial growth but not to lower microbial contamination.

Heat treatments (e.g. UHT on soft extracts) or HPP may be suitable; specific conditions have to be selected and validated to allow assessment of the impact on the composition of the preparation. Possible changes should be investigated and justified.

2.2.5. Herbal medicinal products

Microbial quality of HMPs is determined by the quality of starting materials, hygiene conditions and the manufacturing process. Therefore, following application of GACP and GMP criteria the need for microbial decontamination of the finished product should be minimal.

The Ph. Eur. recognises the need to allow wider acceptance criteria for the microbial quality HMPs depending on the nature of the product and method of preparation, as discussed below.

In the specific case of herbal teas for example, relatively high TAMC and TYMC are accepted taking account of the method of preparation with boiling water (brewing). However, consideration should be given to the fact that herbal teas inappropriately prepared, using only hot instead of boiling water, may result in preparations with inadequate microbial quality.

HMPs sensitive to heat (e.g. emulsions and suspensions) may be treated with HPP without affecting their physico-chemical properties.

Irradiation of HMPs is not advisable.

Addition of preservatives should be minimised, but may be considered for medicinal products, which could potentially support the growth of micro-organisms, if unpreserved, and when packaged in multidose containers. Antimicrobial preservative effectiveness should be demonstrated according to Ph. Eur. 5.1.3, during development, scale-up, at the end of shelf-life and in-use of the product (e.g., in stability testing), and chemical testing of preservative content (ID and assay) is the attribute normally included in the specification.

2.3. Testing of the herbal substance, herbal preparation, and herbal medicinal product

Microbiological contamination is evaluated by the microbial count. Microbial count is determined by a microbiological plate-count technique with enumeration of colony forming units (CFU) per ml or g of herbal material.

Microbial counts: Analytical methods

Usually the assessment of microbiological quality of herbal substance, preparation and HMP is performed in accordance with the reference methods given in three general chapters of the Ph. Eur. i.e. 2.6.12 “Microbiological examination of non-sterile products: Microbial enumeration tests”, 2.6.13 “Microbiological examination of non-sterile products: Test for specified micro-organisms” and 2.6.31 “Microbiological examination of herbal medicinal products for oral use and extracts used in their preparation”.

The tests described in Ph. Eur. 2.6.12 allow quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic conditions. Ph. Eur. 2.6.31 describes tests for the specified micro-organisms *E. coli*, bile-tolerant gram-negative bacteria and *Salmonella*. Specified micro-organisms listed in Ph. Eur. 2.6.13 include the same micro-organisms as in 2.6.31, with the addition of *P. aeruginosa*, *S. aureus*, *Clostridia*, and *Candida albicans*.

As conventional microbiological methods are slow (results are not available before an incubation period of 5-14 days), an additional chapter has been published in the Ph. Eur. for information in order to facilitate the use of alternative methods (5.1.6. “Alternative methods for control of microbiological quality”): some of these methods have shown potential for real-time or near-real-time results with the possibility of earlier corrective action. For each method, the basic principle is stated and the benefits and disadvantages of the method are then discussed. Chapter 5.1.6 may be used in the process of

choosing a microbiological method as a supplement or as an alternative to conventional microbiological approaches and to give guidance on the process of validating the chosen method.

Microbial counts: Acceptance criteria

Microbial limit testing is seen as an attribute of both GMP, and quality assurance.

Chapter 5.1.8 “Microbiological quality of Herbal medicinal products for oral use and Extracts used in their preparation” of the Ph. Eur. provides general acceptance criteria for a non exhaustive list of specified micro-organisms and maximum acceptable counts (expressed as TAMC and TYMC). However testing for other micro-organisms may be necessary or less-stringent criteria may be applied on the basis of a risk-assessment which takes into due consideration the nature of the starting materials, the qualitative and quantitative characterisation of the microbial contamination, the manufacturing process and the intended use of the HMP or extract.

Finished HMPs are grouped into three categories A, B and C, taking into account the manufacturing method, the intended use and, in the case of herbal teas, the method of preparation by the patient.

Extracts for oral use should fulfil the acceptance criteria for category C when it is demonstrated that the method of processing would not reduce the level of micro-organisms sufficiently to reach the criteria of category B.

More-stringent acceptance criteria may be required for extracts that are to be incorporated into pharmaceutical preparations to be administered by other routes of administration as reported in Ph. Eur. chapter 5.1.4 “Microbiological quality of non-sterile pharmaceutical preparations”. This chapter includes special Ph. Eur. provisions for other dosage forms containing raw materials of natural origin (e.g. herbal) for which antimicrobial pre-treatment is not feasible and for which TAMC of the raw material exceeding 10^3 CFU/g or CFU/ml may be accepted.

The absence of specific bacteria of concern should be tested (e.g. *S. aureus, E. coli, Salmonella enterica subsp, P. aeruginosa*). The source of the herbal material should be taken into account when considering the inclusion of other possible pathogens (e.g. *Shigella*, *Campylobacter* and *Listeria* species) in addition to those specified in the Ph. Eur.

Acceptance criteria for herbal substances and herbal preparations other than extracts are not currently given in Ph. Eur. Limits for TAMC, TYMC and specified micro-organisms should be established on a case-by-case basis.

Further indications on interpretation and risk-assessment as well as guidance on the parameters to be taken into account in setting these limits by the applicant are given in the document “Questions & Answers on quality of herbal medicinal products/traditional herbal medicinal products” (EMA/HMPC/41500/2010 current revision) and in the “Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products” (EMA/CPMP/QWP/2820/00 Rev. 2).

Mycotoxins

The potential for mycotoxin contamination should be fully evaluated, even when microbial decontamination treatments have been carried out. For aflatoxins, the Ph. Eur. has included a method 2.8.18 for determination of Aflatoxin B1 in herbal substances and sets limits for herbal substances, unless otherwise indicated in the monograph, at NMT 2 µg/kg. The Ph. Eur. method of analysis 2.8.18 states that the Competent Authority may also require compliance with a limit for the sum of aflatoxins (B1, B2, G1 and G2) of NMT 4 µg/kg.

For ochratoxin A, the procedure is described in Ph. Eur. 2.8.22 and acceptance criteria are given in specific monographs.

Since mycotoxin contamination is expected to be non-homogenous and contamination may not spread to all parts of the plant, only some parts of a stored herbal material batch may contain mycotoxins (e.g. spot contamination by fungi). This issue must be carefully evaluated and an appropriate sampling regime should be established to determine the risk of mycotoxin contamination[3].

Loss on drying, water content or water activity

Testing for loss on drying, water content or water activity on the herbal substance/preparation is useful for the risk assessment of potential microbial growth. Such testing cannot replace a test on TAMC and TYMC, but it can support a justification for skip testing of the herbal substance/preparation/finished product.

Hygroscopic herbal substances/preparations are more prone to support microbiological growth. Therefore the acceptance criteria for water content should be assessed in the light of the effects of moisture absorption. A loss on drying test may be adequate, however, this may not be reliable for some extracts (e.g. milk thistle) and in such cases the water content determination is preferred (Ph. Eur. 2.5.12).

For essential-oil containing plants a test that is specific for water is required.

The Ph. Eur. describes a test for “Water in essential oils” (2.8.5.), a method “Determination of water by distillation (2.2.13)” which may be used for herbal drugs and a method “Water - semi-micro determination” (2.5.12) useful for the extracts.

Water activity (a_w) is a measure of the energy status of the water in a system and it is one of the most critical factors in determining if and how fast a micro-organism will grow. Since water activity, and not water content, determines the lower limit of available water for microbial growth, the control of a_w is a valuable tool for controlling microbial growth and a test to determine a_w may be useful in predicting the potential for an increase in microbial contamination during storage.

It is generally recognised that in products with a_w below 0.60 moulds and yeasts do not proliferate. The lowest a_w at which the vast majority of bacteria and moulds will grow is about 0.85 and 0.70, respectively, whilst dried herbal materials stored under normal conditions have a lower a_w (usually 0.50-0.60). Halophilic (salt-loving) bacteria will grow at an a_w as low as 0.75, but they pose no known threat to public health. With the exception of *S. aureus*, the minimum a_w level for growth of pathogenic bacteria known to cause food borne infections or intoxications is $\geq$0.93. *S. aureus* can proliferate in products with an a_w as low as 0.86. Production of *S. aureus* enterotoxins may, however, require a higher a_w.

Ethanol

Methods to determine the ethanol content in liquid pharmaceutical preparations such as extracts and tinctures are given in Ph. Eur. chapter 2.9.10 “Ethanol content”. Reduced (or omission of) microbiological testing of the herbal preparation in presence of suitable ethanol concentration must be justified.

[3] Ph. Eur. General Chapter 2.8.20 describes a sampling plan for herbal drugs “Herbal drugs: sampling and sample preparation”

Preservatives

For HMPs needing an antimicrobial preservative, e.g. oral liquids, acceptance criteria for preservative content must be stated, based on the levels necessary to maintain the product's microbiological quality throughout storage and use. The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling microorganisms by using the Ph. Eur chapter 5.1.3. "Efficacy of antimicrobial preservation". A similar approach could be used for preserved herbal preparations.

Release and stability testing for the identification and assay of antimicrobial preservative content should normally be performed. Under certain circumstances, in-process testing may suffice in lieu of release testing. When antimicrobial preservative content testing is performed as an in-process test, the acceptance criteria should remain part of the specification.

Residues of fumigants

The potential for residues of fumigation agents in herbal substances and herbal preparations should be fully evaluated. For HMPs it is not necessary to test residues of fumigants when they are controlled in the herbal substance/preparation.

Where necessary, suitable validated methods should be used to control potential residues and the acceptance criteria should be justified.

Residues of "irradiation"

The potential for residues of irradiation in herbal substances and herbal preparations should be fully evaluated and tested when there is reason, or a concern that irradiation has been performed. Where necessary suitable validated methods should be used to control potential residues and the acceptance criteria should be justified.

Testing frequencies - Release and stability testing

Microbial counts should be determined using pharmacopoeial procedures or other validated procedures and at a sampling frequency and/or time point in the manufacture which is justified by data and experience ("Guideline on quality of herbal medicinal products/traditional herbal medicinal products EMA/CPMP/QWP/2819/00).

Further guidance on routine and reduced microbiological testing as well as testing for mycotoxins and during stability studies is given in the document "Questions & Answers on quality of herbal medicinal products/traditional herbal medicinal products" (EMA/HMPC/41500/2010 current revision) and in "Quality of medicines questions and answers: Part 1 (Active Substance - Starting materials of herbal origin)".

2.3.1. Herbal substances

In general, routine testing is applicable for herbal substances. Limits and acceptance criteria should be established and justified through a risk assessment taking into account the specific microbial contamination, information from validation studies on the capability of subsequent steps of the manufacturing process to decrease the microbial count and the intended use. Possible contamination by mycotoxins should be also considered.

2.3.2. Herbal preparations

Excluding or reducing tests for microbial contamination in herbal preparations such as extracts or tinctures depending on the ethanol content must be justified by scientific evidence. The frequency of testing of herbal preparations should be justified by the applicant e.g. based on the validation of the manufacturing process and of the holding time of the bulk product. Possible contamination by mycotoxins should be also considered.

2.3.3. Herbal medicinal products

HMPs must be tested for microbiological quality. Skip testing may be applied in circumstances where components are tested before manufacture and validation studies have demonstrated no significant risk of microbial contamination during the manufacturing process. Possible contamination by mycotoxins should be also considered.

Decision tree #8 reported in the "ICH Topic Q 6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances" (CPMP/ICH/367/96) provides additional guidance on the use of microbial limits testing for non-sterile medicinal products.

3. Conclusion

Being of natural origin, herbal substances generally have higher contents of micro-organisms when compared to chemically defined drug substances. This presents particular challenges as the micro-organisms may be carried over to the herbal preparation and herbal medicinal product. In addition, spores and toxic mycotoxins generated by the micro-organisms may also be present and these are more difficult to eliminate, once they are present in the herbal material.

Satisfactory quality of HMPs with respect to microbiological and mycotoxin contamination cannot merely be controlled by final testing; it should be built-in the entire process, from starting material to finished product. Minimizing and testing/monitoring of microbial contamination and mycotoxins in herbal substances, herbal preparations and herbal medicinal products must be based on a case-by-case risk assessment.

A number of critical points need to be considered and taken into account. These include the source of the herbal substance, knowledge about the micro-organisms, the manufacturing processes and any decontamination procedure used, microbiological purity of excipients, the protective capacity of the packaging material chosen, the dosage form, administration route, posology and patient population groups. Most importantly, preventive measures are preferred rather than interventions for decreasing the contamination.

Compliance with GACP and GMP throughout the entire manufacturing process from herbal substance to the finished product is crucial in order to ensure acceptable microbiological quality of the HMP. The HMP should not support microbial growth; drying processes and final contents of water are critical parameters in this respect.

If a decontamination process is to be applied to the herbal material, usually the herbal substance or herbal preparation, the need for such use should be fully justified and the decontamination method should be selected with care. The initial and desired final maximum level of micro-organisms should be taken into account and it should be demonstrated that the decontamination process does not alter the chemical composition of the herbal material or leave residues of toxic components in the product.

The specifications of the herbal substance, herbal preparation and HMP should include tests for TAMC and TYMC and absence of certain specified micro-organisms, unless otherwise justified. Limits and

analytical methods are given in Ph. Eur. for extracts and for HMPs, although alternative validated methods also can be applied. Testing of loss on drying/water content and mycotoxins should also be considered in the risk assessment. However, these parameters cannot replace testing of the microbial contamination itself.

4. Definitions

Acceptance criteria: Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.

Constituents with known therapeutic activity: are chemically defined substances or groups of substances, which are generally accepted to contribute substantially to the therapeutic activity of a herbal substance, a herbal preparation or a herbal medicinal product.

Degradation product: Any impurity resulting from a chemical change in the composition of the active substance brought about during manufacture and/or storage of the active substance/ medicinal product by the effect of, e.g., light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system. Due to the particular nature of herbals, for herbal substances/herbal preparations/herbal medicinal products in general only toxicologically relevant degradation products must be specified.

Extraction solvents: are solvents, which are used for the extraction process.

Herbal medicinal products: any medicinal product, exclusively containing as active substances one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations.

Herbal preparations: are obtained by subjecting herbal substances to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates.

Herbal substances: all mainly whole, fragmented or cut plants, plant parts, algae, fungi, lichen in an unprocessed, usually dried form but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal substances. Herbal substances are precisely defined by the plant part used and the botanical name according to the binomial system (genus, species, variety and author).

Herbal teas: consist exclusively of one or more herbal substance(s) intended for oral aqueous preparations by means of decoction, infusion or maceration. The preparation is prepared immediately before use. Herbal teas are usually supplied in bulk form or in sachets.

Impurity: (1) Any component of the herbal substance, which is not the entity defined as the herbal substance. (2) Any component of the herbal preparation/herbal medicinal product that is not the entity defined as the herbal substance/preparation or an excipient in the herbal preparation/herbal medicinal product.

Markers: are chemically defined constituents or groups of constituents of a herbal substance, a herbal preparation or a herbal medicinal product which are of interest for control purposes independent of whether they have any therapeutic activity. Markers serve to calculate the quantity of herbal substance(s) or herbal preparation(s) in the herbal medicinal product if the marker has been quantitatively determined in the herbal substance or herbal preparation.

There are two categories of markers:

Active markers are constituents or groups of constituents which are generally accepted to contribute to the therapeutic activity.

Analytical markers are constituents or groups of constituents that serve for analytical purposes.

Solvent: An inorganic or an organic liquid used for the preparation of solutions or suspensions in the manufacture of a herbal preparation or the manufacture of a herbal medicinal product.

Specification: A list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a herbal substance/preparation or herbal medicinal product should conform to be considered acceptable for its intended use. "Conformance to specifications" means that the herbal substance/preparation and/or herbal medicinal product, when tested according to the listed analytical procedures, will meet the listed acceptance criteria. Specifications are binding quality standards that are agreed to between the appropriate governmental regulatory agency and the applicant.

Specific test: A test which is considered to be applicable to a particular herbal substance/preparation or a particular herbal medicinal product depending on their specific properties and/or intended use.

TAMC: Total aerobic microbial count.

Traditional herbal medicinal products: are medicinal products for human use, that fulfil the conditions laid down in article 16a(1) of Directive 2001/83/EC, as amended.

TYMC: Total combined yeasts and moulds count.

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