Impurities in Drug Substances prepared via fermentation and semi-synthetic processes

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2 October 2015

Overview

- Fermentation
- Definitions and Background
- Expectations
- Importance of Downstream processing
- Enzymes
- Semi-synthetics e.g. Antibiotics

Please note: the scope of this presentation is products covered by Ph. Eur. monographs

Fermentation Impurities – different from the norm

Substances for pharmaceutical use – Ph. Eur General monograph 2034

For impurity control: "The requirements above do not apply to biological and biotechnological products, oligonucleotides, radiopharmaceuticals, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products"

Products of Fermentation – General Monograph 1468

Defined as :

- Active or inactive substances produced by controlled fermentation as indirect gene products.
- Primary or secondary metabolites of micro-organisms such as bacteria, yeasts, fungi and micro-algae, whether or not modified by traditional procedures or recombinant DNA technology. Such metabolites include vitamins, amino acids, antibiotics, alkaloids and polysaccharides.
- They may be obtained by batch or continuous fermentation processes followed by procedures such as extraction, concentration, purification and isolation

It is important to consider what is not in the scope of the monograph

Background

- 1989: Outbreak of eosinophilia-myalgia syndrome (EMS) in the U.S.
  - 1,500 cases of permanent disability and at least thirty-seven deaths.
  - Traced to specific batches of L-tryptophan where genetically engineered bacteria produced the contaminated batches at TRACE levels
- Highlighted need to give regulatory guidance on improving safety of products of fermentation.
- The general monograph was elaborated at a time when analytical methods were not generally available to adequately control the impurity profile of a product of fermentation
  - may be a single well defined substance with impurities
  - **or** a mixture of closely related substances where different components contribute to activity but also where impurities are present.

Examples of Monographs

- **Mitomycin :** Definition 'Substance produced by a strain of Streptomyces caespitotus.'
- **Teicoplanin :** Definition 'Fermentation product'.
- **Arginine :** Definition 'Fermentation product, extract or hydrolysis of protein'.
- **Sodium hyaluronate :** Definition 'it is extracted from cocks' combs or obtained by fermentation from Streptococci, Lancefield Groups A and C'.
  - Production 'When produced by fermentation of gram-positive bacteria.....'

Minimum Expectations

- Marketing history of the source of substance in question
- The characterization of the producer micro-organism
  - Purity of master cell bank and information concerning its deposit (for example culture type collection)
  - for micro-organisms established by recombinant DNA-technology genetic stability of respective plasmid or other introduced rDNA
  - for wildtype micro-organisms, information concerning the origin of the strain should include possible random mutation performed on the WT micro-organism.
  - Maintenance and stability of seed lots
- The fermentation process

Minimum Expectations (Contd)

- Information on fermentation media
  - Components and preparation of the media – are materials of animal/human origin used (e.g. peptone, antifoamers)?
  - If material of animal origin is used and which is susceptible to be concerned by TSE contamination since of ruminant origin, compliance with the Ph. Eur. Monograph Products with risk of transmitting agents of animal spongiform encephalopathies (1483) should be demonstrated. All suppliers should be considered.
  - Sterilisation/heat treatment of the media to prevent contamination
- In-process controls during fermentation and purification
  - As described in Ph. Eur. General monograph 1468
- Downstream processing & purity of the final active substance
  - See later slides

All of the above can aid in the review the potential impurities

Downstream processing

- At the end of fermentation, the producer micro-organism is inactivated or removed.
- As bacterial expression is usually intracellular, the host cell needs to be lysed to release all of the available product
- The culture mixture contains mostly media, the host cell and the desired substance.
- Further processing should reduce residues from the culture media and the resultant culture mixture to acceptable levels
  - E.g. centrifugation, charcoal, ultrafiltration, solvent extraction, crystallisation from organic solutions
- Spoilage with other bacteria could be possible – should be controlled during the fermentation process and Cell Bank Storage

Downstream processing

The basis for evaluation of the efficiency of downstream processing is the statement of the monograph Products of Fermentation (1468):

"It must be demonstrated that the process or processes chosen reduce to a minimum or remove:

— Residues from the producer micro-organism, culture media, substrates and precursors

— unwanted transformation products of substrates and precursors

If necessary, suitable tests are performed either as in-process controls or on the isolated product of fermentation".

The choice of technique for downstream processing can depend on the culture mixture itself (e.g. volume) and the purification stage of the process in question

Downstream processing

Useful techniques

- Ion Exchange Chromatography
- Hydrophobic Interaction Chromatography
- Affinity Chromatography
- Gel filtration/Size exclusion Chromatography
- Microfiltration
- Ultrafiltration
- Nanofiltration

Downstream processing

- Route of administration of the substance can be important to consider
  - E.g. Residual peptides and proteins, from host cell or mutations – especially important if used parenterally
- Purity and impurity e.g. SDS Page, Lowry method
- The absence of nucleic acid (NA) and host cell protein should be demonstrated e.g. Threshold assays, ELISA, PCR.
- Aspects that may need to be considered, depending on specific control rationale:
  - Bacterial Endotoxin or Pyrogen contamination e.g. LAL, Rabbit Test
  - Mycotoxins, Aflatoxins, Sugars etc.

Purity of the final product

General controls as usual:

An overview of the potential impurities in the final product should be provided and which includes the related substances, residues of the producer micro-organism, substrates, precursors and other media ingredients, toxins, metallic impurities, and how they are removed by subsequent purification steps.

- The suitability of the specific monograph to control the product must also be shown.
- Any additional impurity (not listed in the monograph) should be controlled by a limit that is justified by batch and stability data, and qualified with respect to safety.
- Don't forget solvents (Ph. Eur. general text 5.4 Residual Solvents) and trace elements (EMA guideline on heavy metals/ICH Q3D).

Change control

- Changes can have the potential to impact the impurity profile of the substance especially when simultaneous changes are made.
- If a change has taken place in the micro-organism used for production that causes a significant change in the impurity profile of the product, then the critical steps of the whole production process, particularly the fermentation process have to be revalidated.
- Change in the fermentation medium or procedure (for example, propagation conditions for the master cell culture or seed lots) may induce mutation in the micro-organism. Stability of the producer organism at different propagation steps should be established.
- Changes to Downstream processing are also likely to affect the final impurity profile of a substance

Enzymes

- Biocatalytic transformation : where enzymes are employed to carry out a particular transformation they may be in solution, immobilized or present in a micro-organism.
- E.g. Biocatalytic Transformations of Steroids (hydrocortisone ⟹ prednisolone)
- Possible carryover of Foreign Proteins, DNA, Oligo & Poly Saccharides, Toxins, Endotoxins etc.

- Note: biocatalytic transformations are out of scope of the Ph. Eur. General Monograph, but aspects can be useful to considered nonetheless.

Semi-synthetic Syntheses

- Start with fermentation but are followed with chemical synthetic step(s)
  - *"One or more synthesis steps following fermentation. A synthesis step involves cleavage and formation of covalent bonds." Ref EMA guideline EMA/CHMP/CVMP/QWP/199250/2009 corr*
- Chemical steps can sometimes help remove fermentation derived impurities
- Examples include
  - Amoxicillin Sodium, Cefuroxime Sodium, Flucloxacillin sodium: "Semi-synthetic product derived from a fermentation product"
  - Paclitaxel: "It is isolated from natural sources or produced by fermentation or by a semi-synthetic process"

Impurity limits in Antibiotics

- Guideline on setting specifications for related impurities in antibiotics EMA/CHMP/CVMP/QWP/199250/2009 corr
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129997.pdf
- Thresholds for reporting, identification and qualification of related impurities for antibiotics
- "it is acknowledged that in some cases higher thresholds may be acceptable if necessary and justified taking account of use and exposure of the drug substance/product. This would also include analytical problems"

Impurity Limits in Antibiotics

- Scope of the EMA guideline:
  - Antibacterials that are fermentation products or semi-synthetic substances derived from fermentation products
  - New active substances and for new sources of active substances
    - "It is the Applicant's responsibility to demonstrate that the active substance has already been marketed in the EU when relevant."
- Out of Scope:
  - Active substances used in investigational products/clinical trials
  - Residues from fermentation process

The Thresholds

- Limits should be set for :
  - Each specified identified impurity
  - Each specified unidentified impurity (where relevant)
  - Any unspecified impurity, with an acceptance criterion of not more the identification threshold
  - Total impurities.

- It should be noted that comparison with impurity levels/profiles of active substance sources or products approved in the EU is one of the options for qualifying impurities.

The Thresholds

- There are 6 categories referred to in the guideline:
  - ***Active substances manufactured by semi-synthesis***
  - ***Active substances manufactured by fermentation, single compound***
  - ***Active substances manufactured by fermentation, family of compounds***
  - ***Peptides manufactured by fermentation/semi-synthesis***
  - ***Active substances for veterinary use***
  - ***Special cases for very complex impurity profiles***

- The guideline also elaborates what do for substances with or without Ph. Eur. monographs

The Thresholds

Two Examples:

Active substance manufactured by fermentation, single compound

- Reporting threshold : 0.10 %
- ID & qualification threshold : 0.15 %

Active substance manufactured by fermentation, family of compounds

- Reporting threshold : 0.10 %
- Identification threshold : 0.15 %
- Qualification threshold : 0.50 %/0.2 %
- 0.50 % applies to structurally closely related impurities, 0.2 % applies to any other impurity.

Conclusion

- Consider the General Monograph for Products of Fermentation
- Quality of the producer micro-organism and media constituents
- Control of the fermentation and downstream processes
- Semi-synthetics
- Antibiotic Impurities

Thank you

Any questions?

Useful Ph. Eur. References:

- Products of Fermentation 1468
- Products with risk of transmitting agents of animal spongiform encephalopathies 1483
- 5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products
- 2.6.14. Bacterial endotoxins
- 5.1.10. Guidelines for using the test for bacterial endotoxins