Gloria Marnini, Annalisa Scali, Luisa Torchio
Euticals S.p.A

PROCESS DEVELOPMENT AND APPLICATION OF ICH Q11 TO SEMISYNTHESIS API PROCESSES

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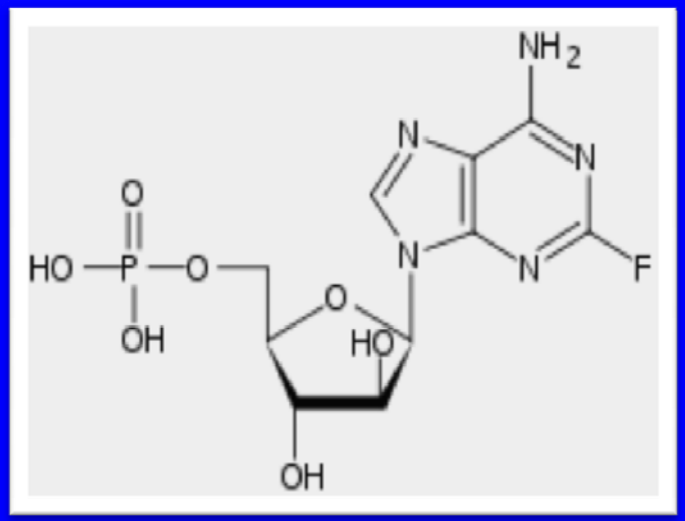
Process description

- The object of the presentation is to describe the synthetic pathway used for the preparation of the Active Pharmaceutical Ingredient FLUDARABINE PHOSPHATE, obtained through enzimatic transglycosilation reaction from the precursor ARA U.
- The potential application of ICH Q11 principles to the process will be then discussed.

Process description

Fludarabine is an Active Ingredient having purine analog structure and anticancer properties.

It is obtained as phosphate.



Formula C10H13FN5O7P

Molar Mass 365.212 g/mol

CAS # 75607 – 67 – 9

ATC # L01BB05

Process description

Many literature references are available for the preparation of this class of compounds by chemical synthesis.

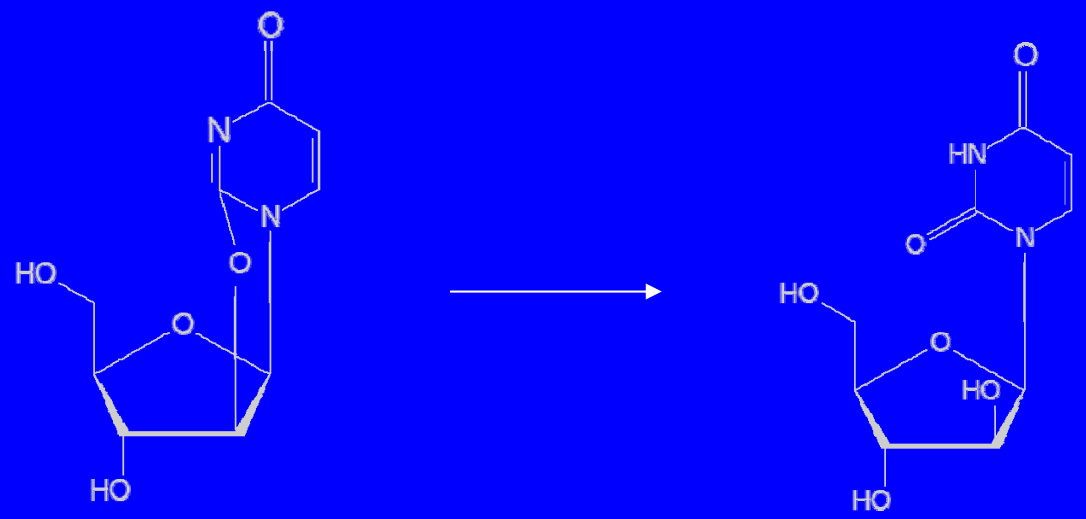
The alternative way, by enzimatic process, assures to obtain the same compound with faster reaction, better stereoselectivity and less reaction steps.

The enzymes used in this transglycosilation process are UP (Uridine phosphorilase) and PNP (Purine Nucleoside Phosphorilase).

Process description

- The process is divided in different steps:

  The first step is the synthesis of Ara U (Uracil Arabinoside) starting from beta-D-O2,2'-Cyclouridine, by chemical reaction.

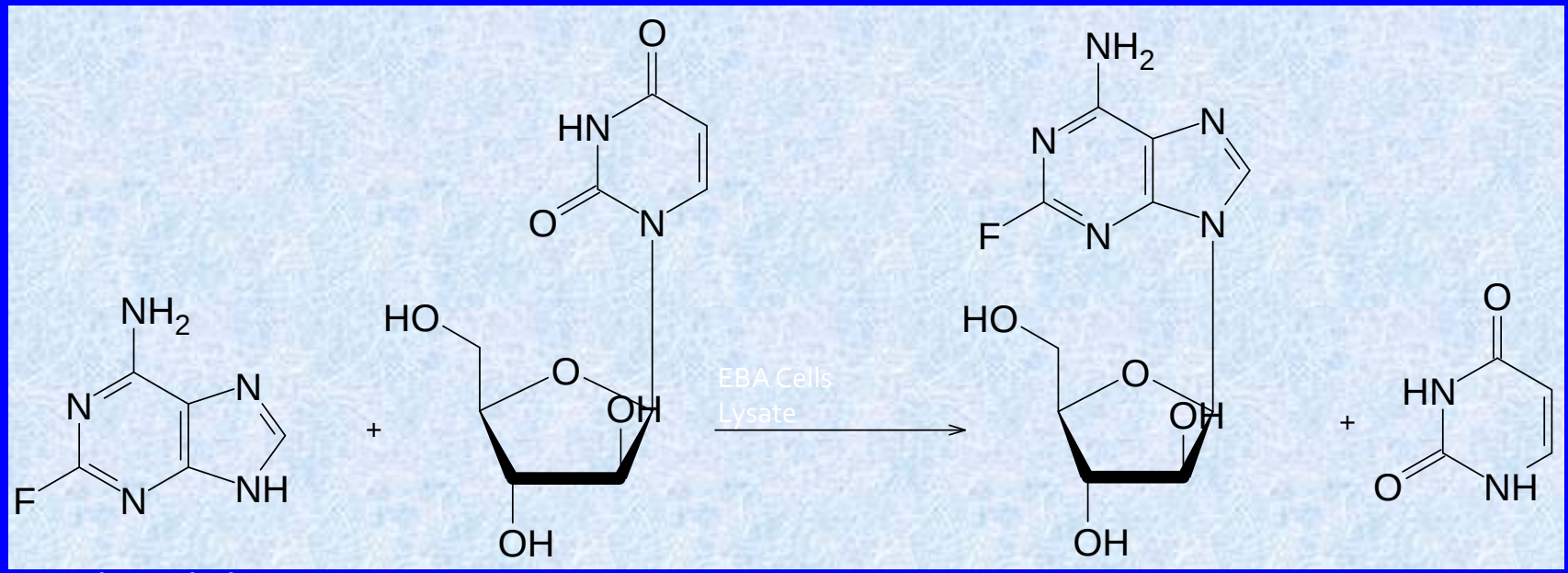


β-D-O2,2'-Cyclouridine
M.W: 226.18 g/mol

Uracil Arabinoside
M.W: 244.20 g/mol

Process description

- In the second step, ARA U is reacted with 2-Fluoroadenine in presence of EBA cells lysate as transglycosilation promoter, to obtain Crude Fludarabine and Uracile.



2-Fluoroadenine
MW: 153.12 g/mol

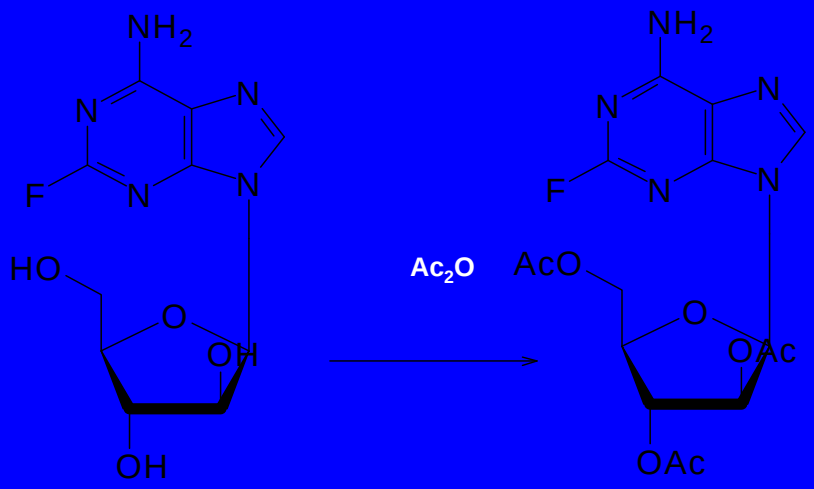
Uracil Arabinoside
MW: 244.20 g/mol

Crude Fludarabine
MW : 285.2 g/mol

Uracile
MW:112,08g/mol

Process description

- In the third step, Crude Fludarabine is then suspended in Acetic anhydride to obtain the triacetylated derivative

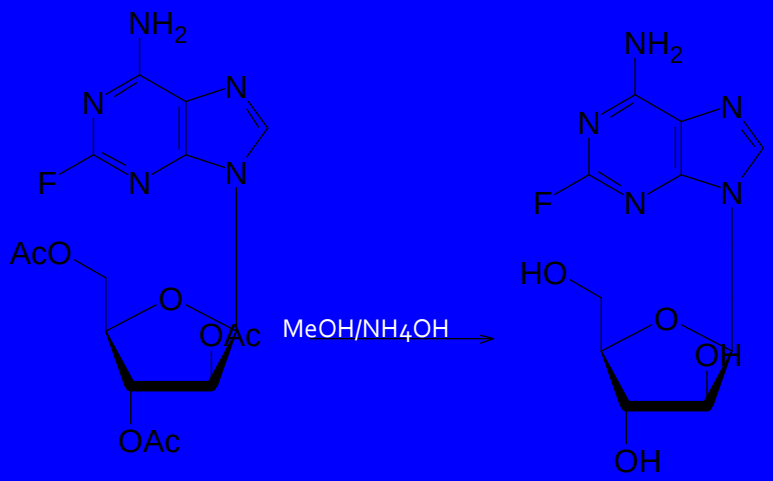


Crude Fludarabine
MW :285.2 g/mol

Triacetylated derivative

Process description

- In the fourth step the triacetylated derivative is treated with methanol and Ammonia solution to give pure Fludarabine.

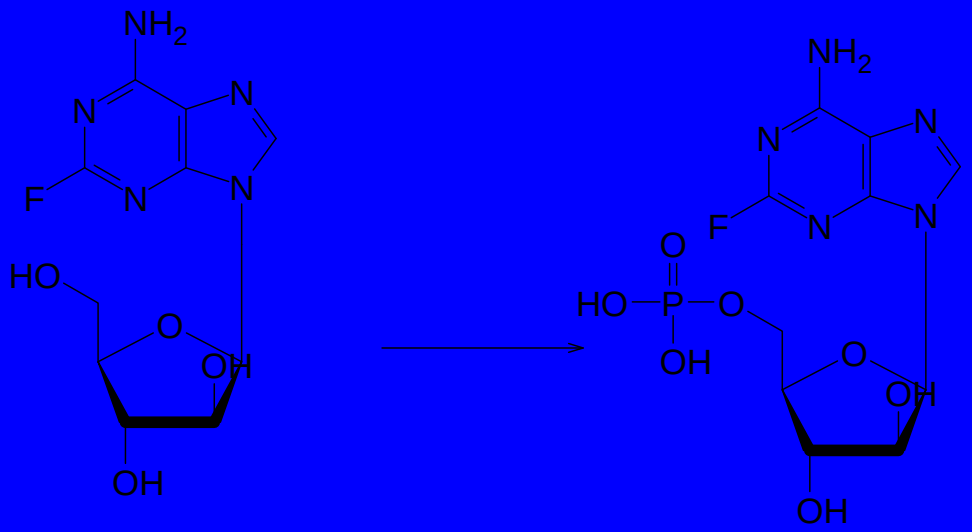


Triacetylated derivative

Pure Fludarabine
MW: 285.2 g/mol

Process description

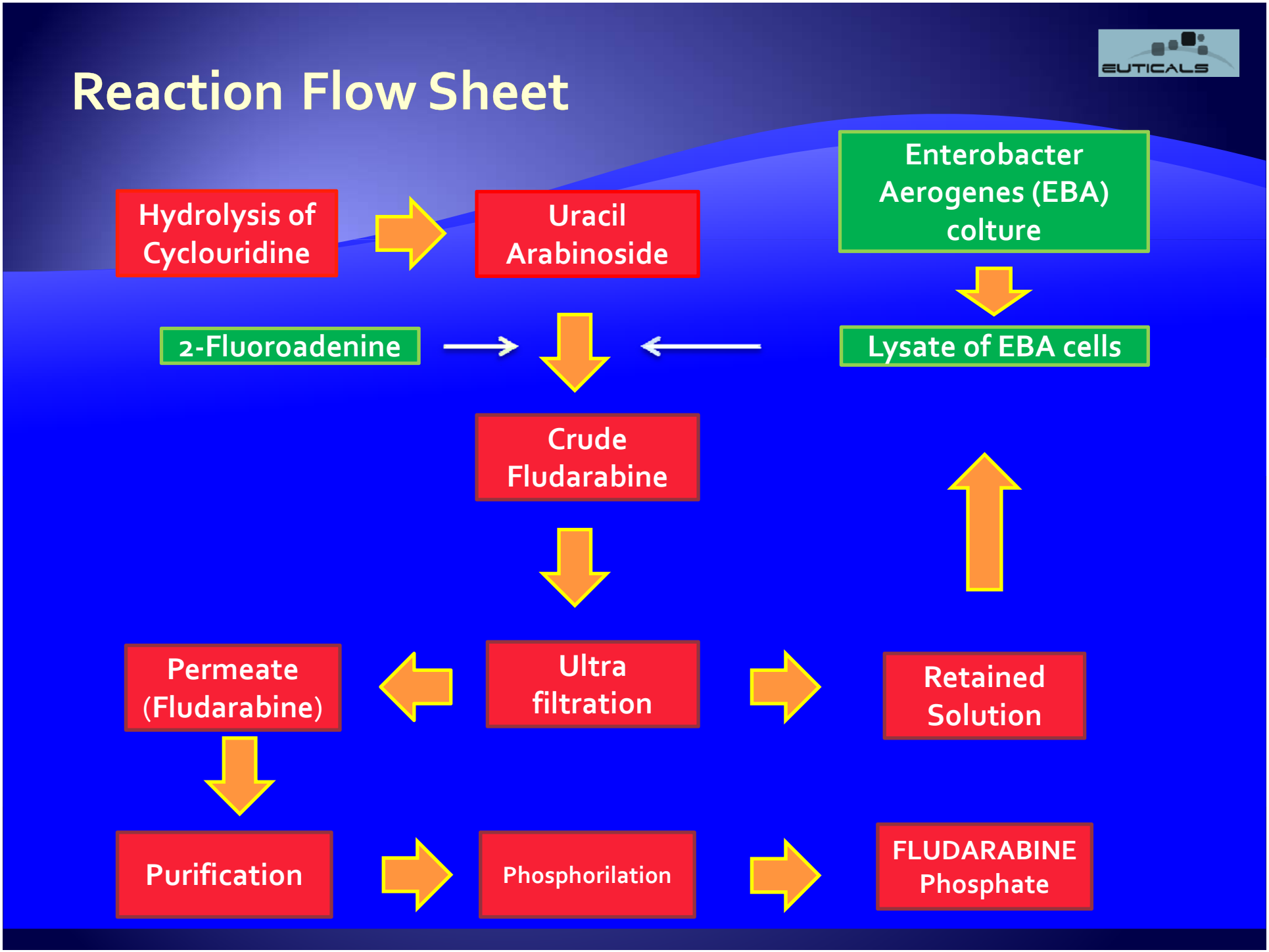
- The Pure Fludarabine after a phosphorylation is isolated as Fludarabine Phosphate, final API.



Pure Fludarabine
MW: 285.2 g/mol

Fludarabine Phosphate
MW: 365.212 g/mol

Reaction Flow Sheet



Points to consider

1. CELL COLTURE AND ITS RECYCLING

The enzymatic reaction is performed by means of Enterobacter Aerogens (class II, gram – bacteria) lysate, added to the reaction mass to catalyse the transglicosylation reaction.

The lysate, in fact, contains the UP and PNP enzymes that are able to cut/form the glicosydic bond.

The lysate is obtained by submitting cells to a suitable mechanical treatment, that assures their destruction and makes enzymes available for further steps.

Points to consider

At the end of reaction, the lysate is recovered by ultrafiltration and it can be reused.

Ultrafiltration is performed by using suitable, certified poliethersulfones (PES) membranes, having cut-off below 50,000 D, to assure the proteins removal from filtrate solution.

This kind of filtration assures also the removal of other macroproteins, viruses and bacteria.

During validation, the absence of proteins and genetic material has been demonstrated by PCR (Polymerase Chain Reaction), Biureto (or Gornall) test along with Optical density performed on filtrate sulution, that demonstrated a retention capacity of the membrane > 95%.

Points to consider

- Life of UF membranes

The membranes' life depends on the standard flows that are set at 200-400 L/h. When decline begins, the flow decreases and when it is lower than 100 L/h the membranes are changed. The integrity of these membranes is guarantee performing a diffusion test by measuring the ΔP.

Points to consider

4. Number of cycles of EBA cells lysate

EBA Cells lysate is recycled after ultrafiltration, and it may be reused in the next batch.

The number of cycles is based on the enzimatic activity of the lysate.

Fresh lysate has a substrate conversion rate of about 6 g/L in 18-24 hours of reaction. When the rate decreases to about 2g/L, the lysate is considered exhausted and replaced with fresh one. This decision is taken based on a continuous In-Process Control monitoring.

Points to consider

5. Controls on the final substance

Endotoxins potentially coming from EBA, that is a gram-negative bacterium, are checked in the final substance with a limit of NMT 0.8 EU/mg.

A suitable test for Enterobacteriacee is perfomed on the final substance as well, to assure their complete removal from the final API.

The Q11 enhanced approach

The process described was developed following the so-called «traditional approach» so considering each item of a process as a stand-alone topic (development, validation, control) mainly based on batch history.

The ICH Guideline Q11 is currently a "DRAFT consensus guideline" issued in May 2011 but not yet approved and officially applied. From our prospective as APIs bulk producers, this new guideline will give us the opportunity to find a compromise between the "traditional" guideline Q7 (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients) and the more recent guidelines Q9 (Risk Analyses Management) and Q10 (Pharmaceutical Quality System).

The Q11 guideline, being specifically addressed to API manufacturing, can offer a mediation between the traditional approach and an enhanced one to reach a Quality by Design that could prevent instead of solving the atypical events. Moreover, it could improve the application of this guideline suggesting the right integration with a Quality Risk Assessment.

This guideline suggests to consider an «enhanced approach» based on lifecycle management and continuous process verification as alternative to traditional validation approach based on manufacturing of different batches, data collection and comparison.

For this kind of processes, that join both chemical and biological items, this different approach may be helpful, since it permits to consider the different features as part of an «unicum» that starts from the choice of starting material and continues through the different phases of the process

The choice of starting materials, the choice of meaningful pieces of equipment (membranes in this case), the discussion about potential impurities that may be of chemical and biological origin, the recycling of cell lysate in the next batch may be discussed in a suitable Quality Risk Management document that permits to highlight the real critical phases and set relevant controls in a more effective way.

Moreover, the potential approach based on continuous monitoring rather traditional process validation may be helpful in managing and tracking recycling of lysate.

Another point to consider is the process batch size. These kind of compounds are very active and very expensive, and they are usually manufactured on small scale amounts. The enhanced approach permits to build a scientifically justified model, that is considered predictive and it permits the extrapolation of operating conditions across multiple scales and equipment, if needed by market requirements.

THANK YOU