PAT in Biotechnology Manufacture

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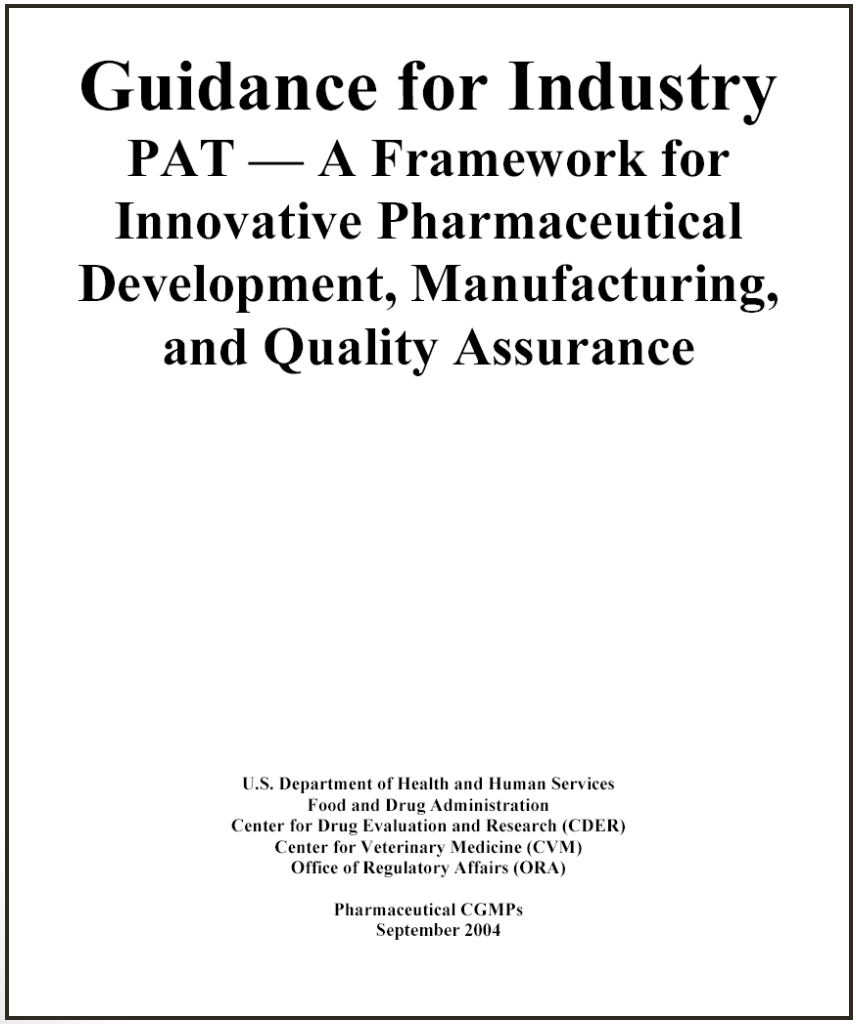
Views presented are those of the speaker & not necessarily official FDA policy

PAT Guidance



- Released September 29, 2004
- Scientific principles and tools
  - Process Understanding
  - PAT Tools
  - Risk-Based Approach
  - Integrated Approach
- Regulatory Strategy accommodating *innovation*
  - Training
  - Lab research
- www.fda.gov/cder/gmp
- Can this be applied to biotech?

The Essence of PAT

Product quality is monitored and controlled during the manufacturing process.

Process decisions are based on assessments of material attributes.

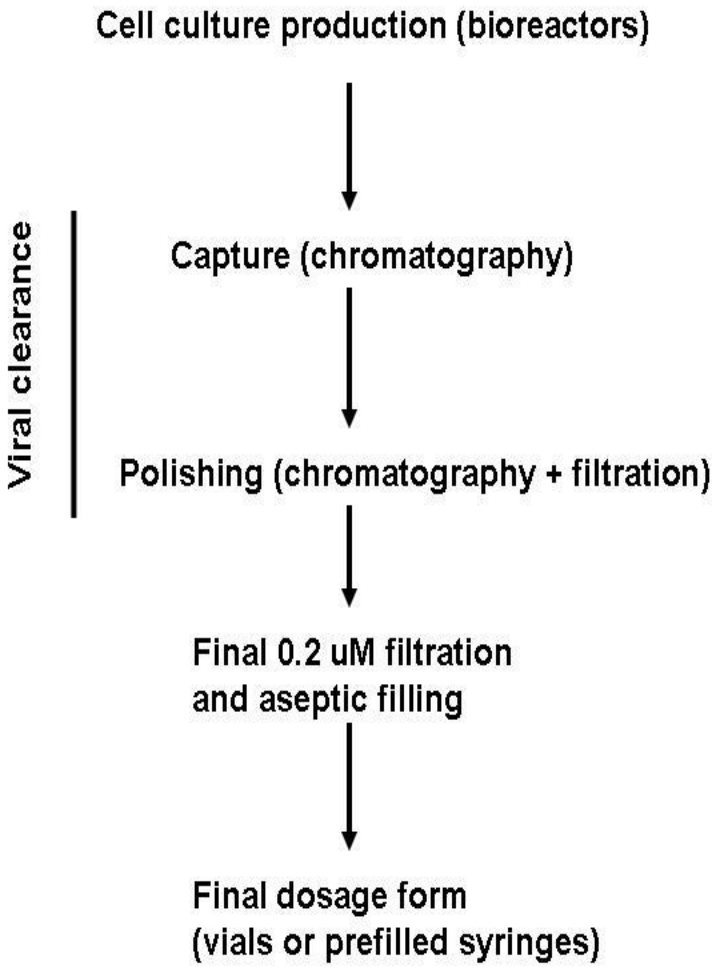
- Forward-feed of incoming material
- In-process monitoring & control

- Critical product attributes measured/assessed either
  - Instantaneously (on-line, in-line, at-line) or
  - Before decision point (near at-line)
    - With as large a window as feasible

Potential Critical Quality Attributes (CQA's) for Biopharms

- Potency/strength
- Post-translational modifications
- Isoelectric point
- Aggregation
- Size
- Sterility
- Adventitious agents
- Impurities (e.g., DNA, Host Cell Proteins)
- Formulation components

Major Stages in Bioprocessing



Each stage has one or more unit operations (e.g. bioreactors, columns, etc.)

In biotech, PAT can be applied on a unit operation basis

Biotech Unit Operations are composed of sequential steps

Cell culture

- Bioreactor prep
- Media fill
- Inoculate
- Feed
- Harvest

CHROMATOGRAPHY

- Equilibrate the column
- Load the column
- Wash away unbound material
- Elute the bound material

Transition from one step to the next

Decision points

- Points in a process at which transition decisions are made.

Decision criteria

- The information that triggers a transition.

- **Note:** In PAT, *Decision criteria assessment doesn't need to be instant, but must close enough to decision point to influence outcome*

Decision points - Examples

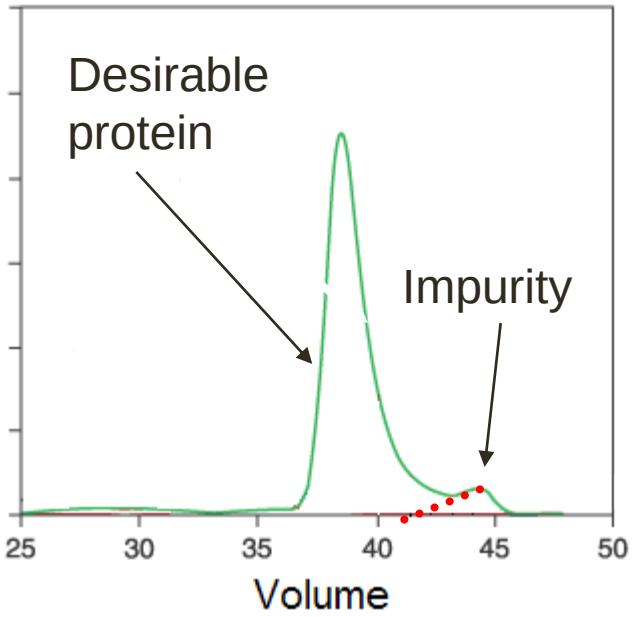
- When to feed the bioreactor
- When to harvest the bioreactor
- When to stop equilibrating a column
- When to start/stop collecting column eluate
- When to stop diafiltration
- When to stop mixing a protein solution
- When to stop lyophilization

Decision Criteria – Column Example

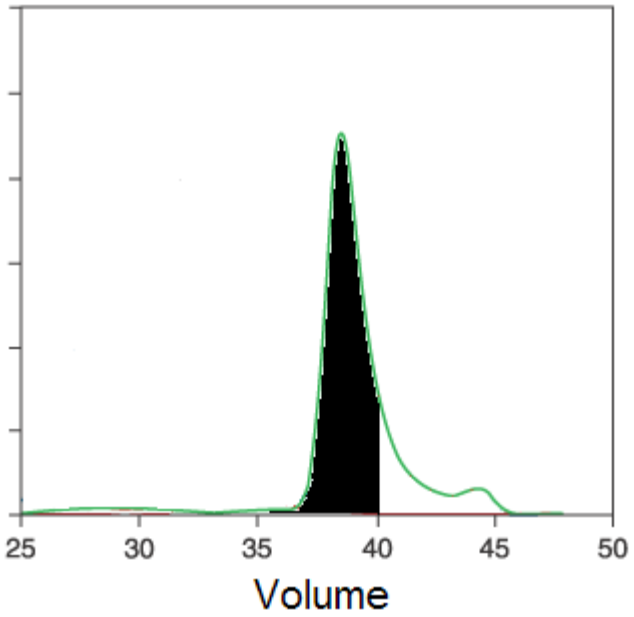
Elution of bound material from a column

- Elute with 40 Liters of buffer
- Elute with 2 column volumes
- Elute until A280 drops to a value of X
- Elute until slope of A280 trace decreases to a value of Y
- Elute until an unwanted component elutes

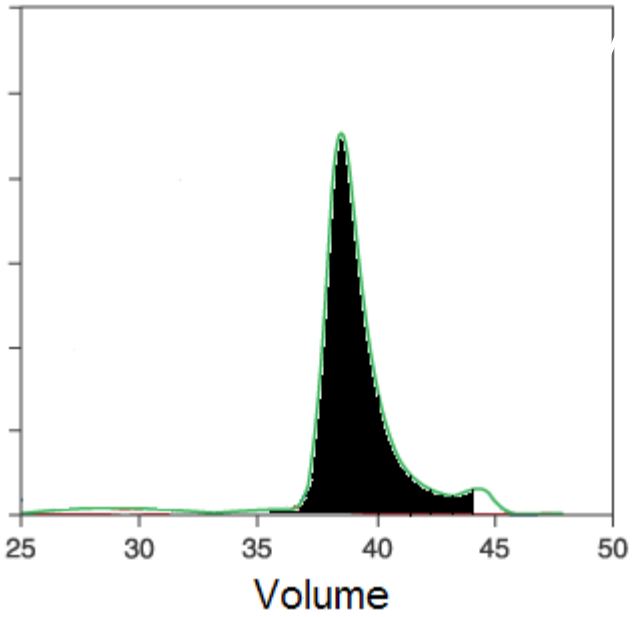
Decision Criteria Example: eluting a protein from a column



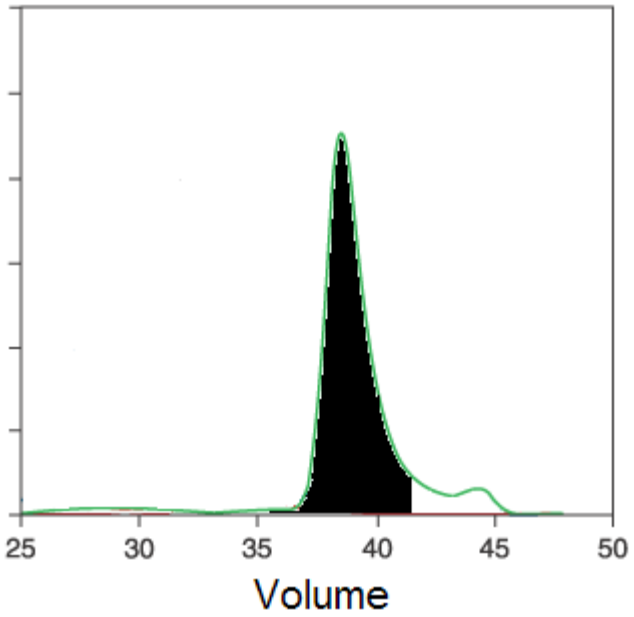
Decision Criteria – 40 LITER CUT: Yield loss



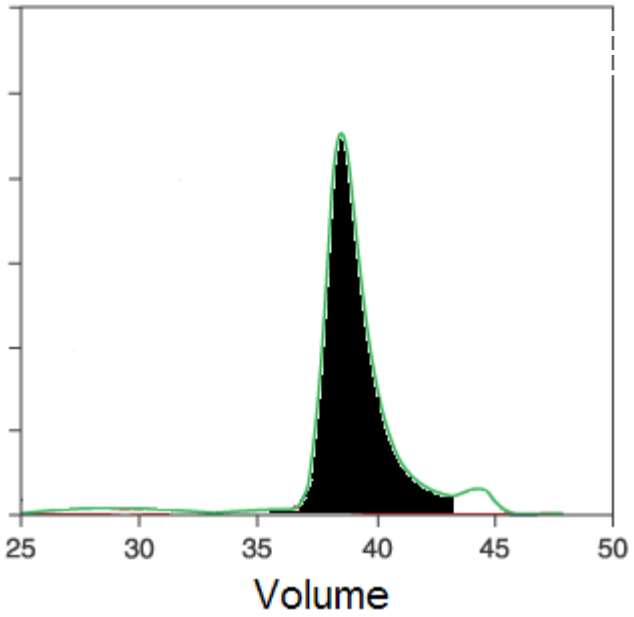
Decision Criteria – 2 Col. Vol. Cut: Impurities



Decision Criteria – A280 Target Cut: Better, but still yield loss

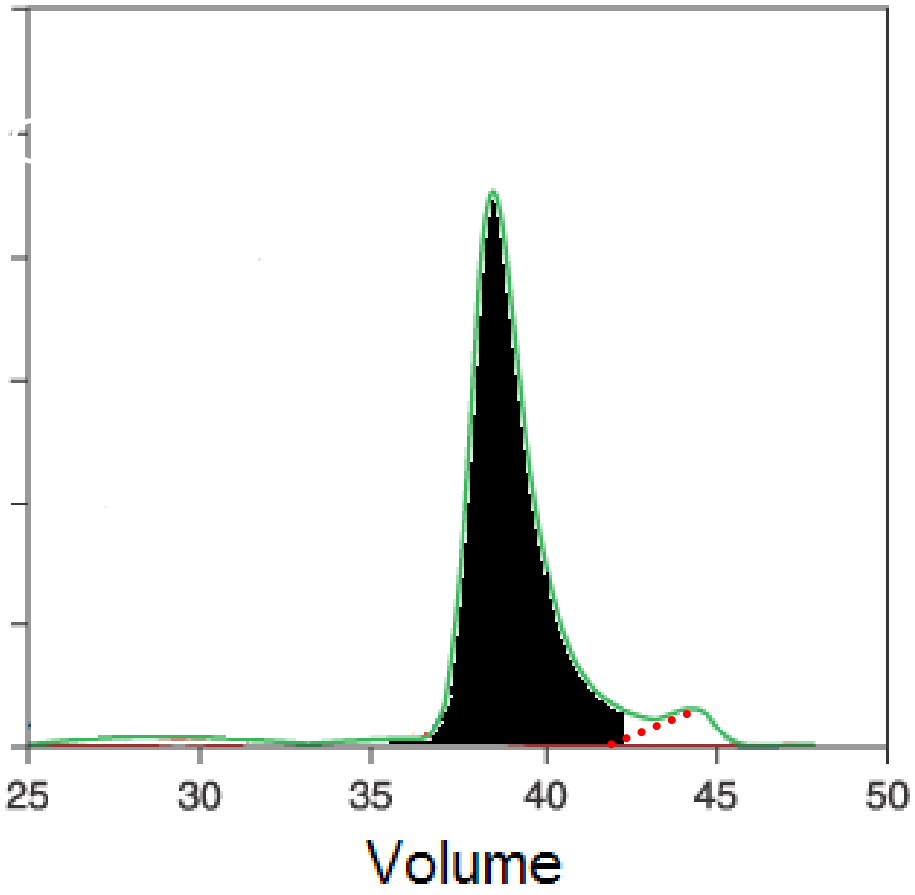


Decision Criteria – A280 Slope Cut: Better, but still has impurities



Decision Criteria – Component Cut:

Best balance *if* impurity can be monitored in-line (or near-at-line) to allow active control



Aggregates in theory can be measured/detected via in-line capable methods like CD, light scattering, FTIR, A_{410}, other techniques

(Brorson and Phillips, BioProcess Intl Nov. 2005)

Potential Controls in Literature

- Cell culture- various critical parameters (non-CQA) are already monitored and controlled on-line (pH, Temp, etc.)
  - Potential for at-line sampling + rapid analysis
- Diafiltration/ Ultrafiltration- UV, pH and/or conductivity
- Proteolytic & Conjugation reactions- process dependent
- Solution mixing- UV, pH and/or conductivity
- Lyophilization- NIR spectroscopy, Manometric temperature measurement (MTM)
- Fill volume- NMR

The biotech world presents a unique set of challenges:

- Production by finicky and highly complex cell-based biological systems
  - highly sensitive to external conditions;
- In-process intermediates can be complex mixtures
  - desired protein may be a fraction of the bulk liquid;
- Worrisome, low level impurities (e.g., viruses) still a concern
  - even when present at levels undetectable by even the most sensitive in-line/on-line/at-line technologies.
  - Removal validation for now

- In contrast, some significant challenges for small molecule drugs may not apply to biotech;
  - blending of aqueous protein solutions

Common objection for PAT in bioprocessing-

"This is great for small molecule drugs, but real-time monitoring not always applicable in biotech"

Reality: Some CQA's not presently amenable to instantaneous on, in, at-line monitoring (e.g. complex biochemical attributes, low level impurities, virus)

However:

- Some obvious examples for simple unit ops exist-
  - solution mixing,
  - End point decisions for diafiltration

Near-at-line monitoring (sampling + rapid analysis) technological improvements are rapid

- Sampling and/or testing column effluents.
- Automated sampling of cell culture.

State of PAT in bioprocessing?

- Surveyed literature for examples of PAT in bioprocessing
  - Read et al. Biotech & Bioeng 2010
- PAT is defined in three main ways: Process control based on real-time, direct measurement of
  - Type #1: product (or raw material) critical quality attributes (CQA)
  - #2: parameters that directly correlate with a CQA
  - #3: parameters that confirm that a unit operation/piece of equipment continues to be fit for purpose
- Very few examples of true PAT (type #1) in bioprocessing, at that time (2010)

Process control and monitoring of product CQAs: 2010 Examples (Type 1)

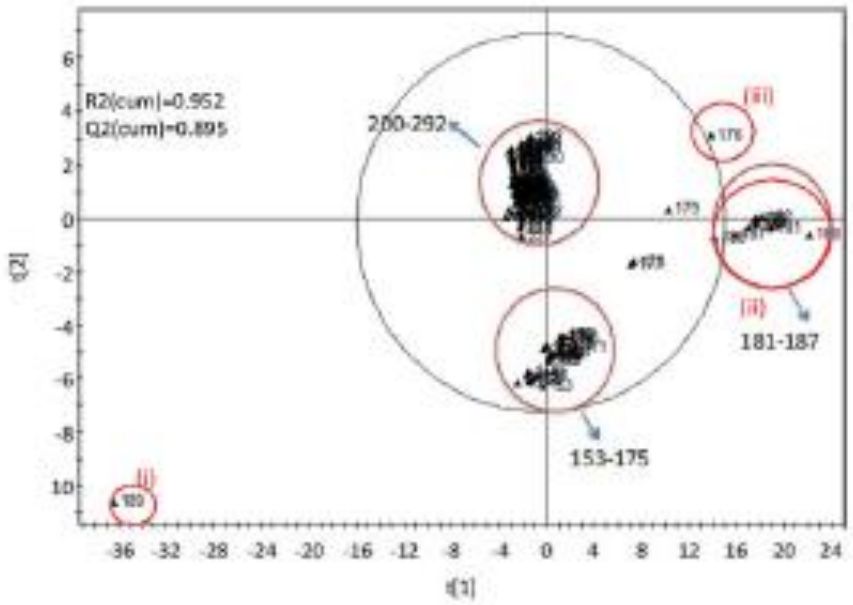
Sensor	measurement principle	Application	Stage	Reference(s)
Surface Plasmon Resonance	Refractive index change	Assess product concentration and affinity	U	Jacquemart et al., 2008
High Performance Liquid Chromatography	Physicochemical properties	Assess product concentration and structure	U C, D	Larson et al., 2002 Rathore et al., 2009
Capillary Electrophoresis	Physicochemical properties	Assess product concentration and structure	D	Klyushnichenko and Kula, 2005

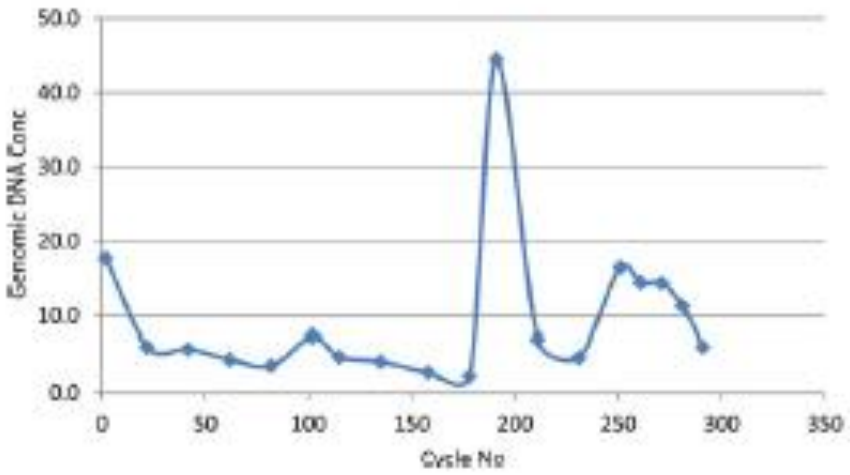
a. Stages of most likely utility. U = Upstream; C = Capture; D = Downstream,

New approaches enabling PAT

- Systems Biology
  - Metabolomics, proteomics, etc. may identify relationships between measurable process variables and cell culture state
  - Examples-
    - Near Infra-Red Spectroscopy established as an input for metabolic flux analysis modeling (Fazenda et al. 2013)
    - Read et al., 2014- Identified rate limiting amino acids by NMR & impacts on glycosylation
- Multivariate data analysis (MVDA)
  - Biotech processes generate huge datasets amenable to MVDA to predict process outcomes
  - Example- MVDA identified bioreactor scale-up issues and causes for batch deviations (Mercier et al. 2013)

MVDA- Chromatography Example





- Protein A cycled 300x with 6M Guanidine cleaning
- Retrospective analysis- original goal was to evaluate viral clearance
- Outliers correlated with repacks and DNA breakthrough
- Lute et al., J Chromatogr A. 2009;1216:3774–3783
- Rathore et al., Biotechnol. Prog., 28: 1308–1314, 2012

New approaches enabling PAT- 2

- Robotics and automation
  - Will enable efficient and consistent sampling of complex process fluids
  - Example- Rapid glycan profiling from cell culture (Doherty et al., 2013)
- Advances in Mass spectroscopy
  - Rapid comprehensive biochemical analysis
- Capacitance probes to measure culture mass
  - On-line measurement of cell biomass and viability

The future: Evolution of PAT in Bioprocessing

- “Type 3 PAT” already routine practice (eg. Back pressure measurement on a column, gas flow meter in bioreactor)
- “Type 2 PAT” enabled by
  - Correlation of measurable process variables with CQA outcomes
  - Multivariate analysis- CQA predictive tools
  - Systems biology
- “Type 1 PAT” gradually surmounting technology barriers
  - Intense and purposeful R & D.
  - Robotics and automation
  - Advances in rapid analytics

Thanks to…

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