Introduction to 'Process Analytical Technology' (PAT) for the Sampling Community

By Gary E. Richie[1]

DOI: 10.62178/sst.004.002

ABSTRACT

The evolution of Process Analytical Technology (PAT) from its roots in Process Analytical Chemistry (PAC) to its current integration with the Theory of Sampling (TOS) marks a pivotal transformation in pharmaceutical manufacturing. This article traces the historical, technical, and regulatory milestones that have shaped PAT into a comprehensive framework for ensuring product quality through real-time process understanding and control. It is also significant, and highly beneficial for society, that the modern PAT concept/framework has been found to be applicable in many other industry sectors.

At the heart of this transformation lies the recognition that sampling is not merely a preparatory step but is in fact a critical determinant of analytical reliability. The incorporation of TOS principles, particularly the concepts of material heterogeneity, lot dimensionality, Total Sampling Error (TSE) and sampling process supremacy with regard to representativity, has elevated the role of sampling from being viewed as a procedural necessity to a scientifically governed critical discipline. The key to its adoption by regulators and industry is the implementation of control strategies for materials, sampling equipment (hardware and software), methods, training, and lifecycle management. When combined with chemometric modeling and multivariate calibration, TOS and PAT becomes powerful enablers of Quality by Design (QbD), continuous manufacturing (CM), and lifecycle management.

Regulatory guidance from the Food and Drug Administration (FDA), International Council for Harmonisation (ICH), and international standardization bodies now reflect this integrated perspective, emphasizing the need for representative sampling, robust analytical procedures, and risk-based process validation. The convergence of PAC, PAT, and TOS provides a unique, powerful *unified strategy* for minimizing uncertainty, maximizing process efficiency, and ensuring regulatory compliance across the pharmaceutical product lifecycle.

As the involved industries move forward, the continued refinement of sampling strategies, sensor technologies, and data analytics will continue to be essential. The future of PAT lies not only in further technological innovation but in the disciplined application of scientific principles that *bridge* the gap between relevant, optimized measurement (sampling-*and*-measurement) and meaning—between critical data and optimal decision-making. This article also presents a first, hopefully inspiring, scope of potential further PAT impact(s) in industry sectors that already rely on TOS.

1. Introduction

This article introduces the concept of Process Analytical Technology (PAT), with underpinnings from analytical chemistry, signal processing, process engineering, along with developments from computer science, mathematical, statistical and multivariate sciences, and considerations for necessary reference samples needed for calibrations.

In 1989, Harald Martens and Tormod Næs published a pioneering book *Multivariate Calibration*. Although the book's primary focus was on what we today refer to as chemometric data analysis and modeling — aka "soft modeling", a subject that will be discussed briefly later in article, the authors also comment on an aspect of data modeling that is useful for here, with regard to connecting and relating process characterization with first principles of acquiring relevant calibration and validation samples.

1 Equipment Validation Engineer, Actalent

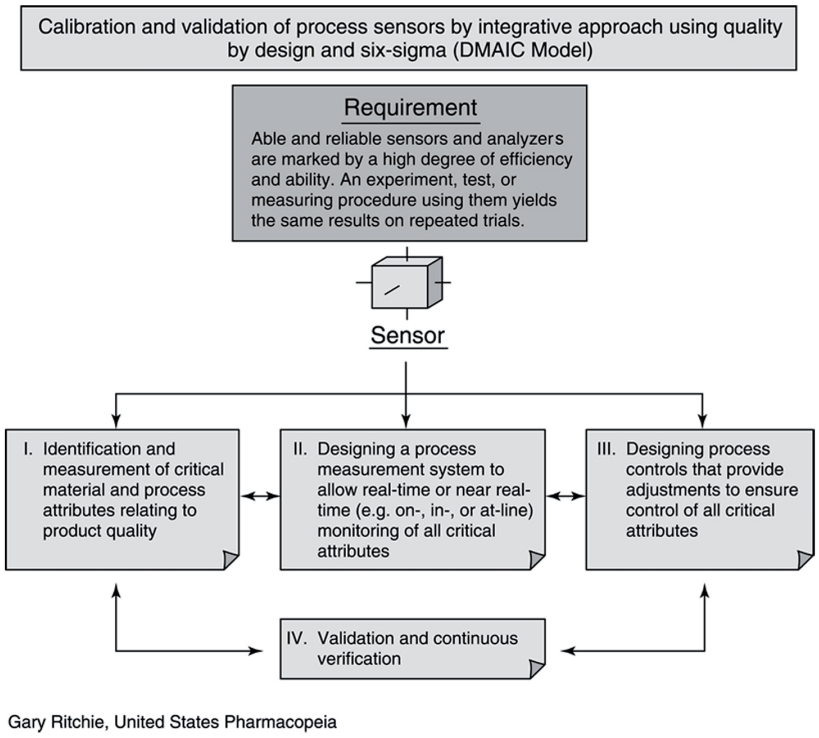


Figure 1: Integration of calibrations and validation, DoE and QbD considerations of process sensors.

They emphasized that there are critical conditions necessary for converting sensor data into reliable analytical results leading to process knowledge. To quote Martens and Næs (1989):

The same holds true for process sensors. It will be shown that, through transduction and transmission of signal into impulse, or through calculation into a determined value, the flow of process data into process information, which then may be transformed into process knowledge, can be turned into useful process understanding only if:

a) *the knowledge behind the sensor systems intended application can be shown to be valid*
b) *the quality of the data is accurately and precisely verifiable when that information is needed*
c) *is based on appropriate measuring reference systems*

A further condition that is necessary is that the system must be adjustable (preferably in real or near-real time).

The requirement that process sensors (Figure 1) must be able and reliable, and give consistent and repeatable results is a cornerstone of PAT that has evolved out of an integrative approach from QbD, DoE, and quality management system principles (Dziki et al., 2004).

Thirty-six years later, it is now obvious that another important factor can also be shown to be critical for the uncertainty of the analytical result obtained from the raw data of a process, that being the *process sampling* procedure itself (Esbensen, 2025). It is fair to say that Martens, Næs and many others who pioneered the use of process analysis focused mainly on what came *after* the sampling step; trying to understand how the raw data (spectral) properties impact modelling characteristics (linear, non-linear) and prediction behaviors. However, with the realization that must now be regarded as very firmly established, the following must be considered with respect to sampling in process analysis (DS-3077, 2024; Esbensen, 2000; Esbensen, 2025):

1. There are significant error effects (leading to uncertainty contribution) originating from inadequate (non-representative) sampling of heterogeneous materials (the domain of TOS).
2. The objective is to achieve representative process sampling (the domain of TOS).
3. Ensure valid analytical data quality assurance (the domain of PAT).

With the foundational concept of the Total Sampling Error (TSE), analytical results can now be considered complete with respect to uncertainty arising from both what comes *before* analysis (Esbensen, 2020; Esbensen, 2025) and from what comes *after* sampling, the traditional Total Analytical Error (TAE).

Just as the practitioners of process analytics in pharmaceutical manufacturing now have guidance from TOS principles in evaluating analytical results obtained from Process Analytical Technology (PAT) (Romanach, 2025), the analysts too could greatly benefit by investing an effort to understand that beside analytical uncertainty, there are other error sources that contribute to the overall uncertainty of an analytical result. Today, these sources are well characterized and understood throughout the history of the development of process analysis and other disciplines.

Presented below is a condensed version of the historical developments of PAT. The rest of this article will explore key highlights from process analysis' early beginnings, to the pivotal launch of Process Analytical Chemistry (PAC), initially funded by the National Science Foundation (NSF) in the USA, later implementation in pharmaceutical manufacturing from the FDA (Food and Drug Administration, 2004b), a core component of their 21st century Current Good Manufacturing Practice (CGMP) Initiative, and on through to the current era of ICH guidance development and adoption to the integration of TOS for Continuous Manufacturing (CM).

2. Background

Process analysis has its beginnings from its development in industrial engineering and analytical chemistry (Baugmann, 2005; Center for Process Analytical Chemistry, 1984a, 1984b; Callis et al., 1987). Baughman (2005) provides a historical perspective of PAC (Process Analytical Chemistry) from its early introduction in oil (crude) processing and in petrochemical (refined) manufacturing, to the development and application of instrumentation for the near real and real-time measurement and analysis of manufacturing processes. Later works by Callis, Illman, and Kowalski, associated with the establishment of the Center of Process Analytical Chemistry (CPAC) at the University of Washington, Seattle, and Layloff, Hussain, Afnan, and Watts, for ushering in Process Analytical Technology (PAT) initiative at the Food and Drug Administration (FDA), and many others, provides a firm footing for future progress of process analytical chemistry and technologies.

For the purposes of this article, interest in Process Analytical Chemistry & Technology (PCA&T) is primarily focused on the quality of the analytical result and on the critical role of all three domains: TOS, analysis and chemometrics (multivariate data analysis and – modeling).

Traditionally, an analytical result has been thought of consisting of two parts, a value (the determined concentration of the analyte) and an uncertainty component arising from the measurement itself.

TRADITIONAL ANALYTICAL RESULT

$$\text{Analytical Result} = \text{Value} + \underbrace{\text{Uncertainty } (MU_{ANAL})}_{\text{MEASUREMENT UNCERTAINTY } (MU_{ANAL})}$$

The measurement is typically a single end point measurement, but in the case of process streams, could also reflect a continuous measurement. The Measurement Uncertainty (MUANAL) reflects an error to the measured value stemming exclusively from the analytical method, the Total Analytical Error (TAE). The numerical value is the true, or accepted value of a quantity.

Following the comprehensive understanding presented by the Theory of Sampling (TOS) (Danish Standards, 2024; Esbensen, 2020; Esbensen, 2025), it is necessary to *augment* this understanding, by including the Total Sampling Error (TSE). Thus, the total, effective Measurement Uncertainty (MU_{TOTAL}) associated with a process analytical result is today understood as comprised by two components, one originating with sampling (TSE), the other associated with the analytical method (TAE). These two errors manifest themselves as additive MU components:

REVISED ANALYTICAL RESULT

$$\text{Analytical Result} = \text{Value} + \underbrace{MU_{SAMP} + MU_{ANAL}}_{\text{SAMPLING + MEASUREMENT}}$$

3. Process Analytical Chemistry (PAC)

PAC is described as originating from five alternative analytical interactions with a process, which is often envisaged as a material stream in a pipeline: Measurement setups can be *off-line*, *at-line*, *on-line*, *in-line*, or can be *non-invasive* analysis. These concepts stem from having the focus on the way (mode) of a PAT probe (sensor) is *interacting* with the process stream in order to obtain a measurement.

Table 1: Process Sampling Modes.

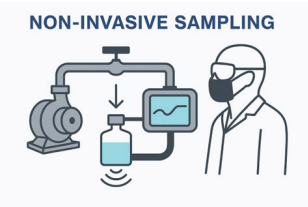
Analysis Mode	Sampling Mode	Example
Off-Line	Manual removal of the sample and transport to the measuring instrument.	
At-Line	Manual removal of the sample and transport to the measuring instrument, installed in close proximity to the process line.	
On-Line	An automated sampling system is used to extract the sample, condition it, and present it to an analytical instrument for measurement.	
In-Line	Chemical analysis is done in situ, i.e. directly inside the process line, using a probe that is chemically sensitive.	
Non-invasive	Analysis based on a probe that does not physically interact with the sample.	

Table 1 shows the PAC main applications described by their principal sampling modes. See also Figure 2 on page 72.

It is important to realize that during the timeframe in which these developments in PAC were happening (1980s), Pierre Gy's 1998 book *Sampling for Analytical Purposes* had not yet been published. By then, TOS had gained recognition and use by many industry sectors in which understanding and managing sampling uncertainty resulted in huge financial savings (e.g., in mining, minerals processing, cement, pharmaceutical industry sectors).

Key to the success of TOS at any scale, especially in the analytical laboratory, and subsequently at manufacturing scales, was a comprehensive understanding of how sampling variation arising from *both* the compositional property of materials, the unit sampled (the sample mass), and the spatial distributional material heterogeneity, determines the quality of the analytical result. Gy revolutionized both theory and practice in realizing that the correct understanding of sampling variability needs to take into account all heterogeneity manifestations as well as the appropriate sampling procedure, a scope emphasized in DS-3077 (2024) and by Esbensen (2020, 2025).

2 Contrary to many current understandings, it is not possible to ascertain the representativity status of an individual extracted 'sample' by any characterization of the sample itself. With TOS, a representative sample is defined as the result of a representative sampling process. It is the sampling process only that can be designed, implemented and verified as representative; this is a key tenet of TOS (Danish Standards, 2024; Esbensen 2020; Esbensen 2025).

An important factor determining the possibility of securing a *representative* sample is also related to the lot geometry and size. Accordingly focus must also be on *lot dimensionality*. TOS classifies lots into four geometrical categories, i.e., lot dimensionalities: zero (0)-D, one (1)-D, two (2)-D and three (3)-D lots. This provides the basis for considering the geometry and the scale of material lots to be sampled, which also determines the scale of the sampling process (the sampling tools). (Danish Standards, 2024; Esbensen, 2020; Esbensen 2025; Romanach, 2015) describes the framework of the theory of Sampling (TOS) in more detail and gives many foundational references for the interested reader.

Applying TOS principles to analytical chemistry and later, process analytical chemistry, is one of the core focus areas for the International Pierre Gy Sampling Association (IPGSA): https://www.intsamp.org/

Having forged its beginning from very practical objectives (major industrial and trading sectors, (Esbensen et al., 2019; Esbensen, 2020; Esbensen 2025), but also from a fundamental need to scientifically define sample (vs. specimen), analytical aliquot, measurement, and analytical result, TOS fundamentally changed how sampling is viewed in key analytical domains (e.g., in *vibrational spectroscopy*, visible, NIR, RAMAN) because of the introduction of the critical concepts of sampling representativity[2], material heterogeneity, and lot dimensionality. Considered together, PAC and TOS provide a complete theoretical basis for controlling the effective total analytical result uncertainty by carefully considering and controlling the sampling process as responsible for delivering a defensible representative analytical aliquot to the domain of analysis. It is fair to say today that TOS is the agent responsible for a renewed focus on the fact that the aliquot is the only miniscule portion of the original lot that is actually analyzed. Since aliquots typically only make up from 1:103 to 1:109 of the original lot volume/mass, the role of a representative sampling process spanning six orders of magnitude cannot be undervalued!

The generously available TOS literature since 2000, concluding with the iconic compendium "Economic Arguments for Representative Sampling" (Esbensen, 2021) explored the many ways leading to a sampling bias and the resulting uncertainty that will arise in practice, mainly as related to PAT.

4. Process Analytical Technology (PAT)

Process sampling is differentiated from static sampling by virtue of the sample, or the sampler, is *moving*; one has to sample a *dynamic* material system (a heterogeneous material system at that). TOS states: "The movement involved is relative: either the matter streams, or flows, past the sampler/sampling equipment, or the sampler "walks up and down" along the extended dimension of the lot" (the latter obviously dependent upon competent involvement of appropriate engineering). From the point of view of TOS, both modes are considered equal in contributing to the TSE. Most importantly though: from a PAC or PAT perspective, the goal of sampling has not changed from static to dynamic sampling - minimization the of TSE before TAE.

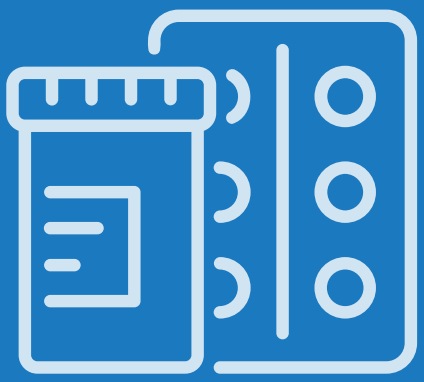
PAT, one leg of the FDA's monumental '21st century initiative for pharmaceutical quality' (see Fact box and USDA, 2004), was started in response to the FDAs concerns over low efficiency and poor quality in pharmaceutical manufacturing. The FDA devised and implemented a framework approach to encourage innovation and continuous improvement. Key achievements include new guidance documents, a quality system approach to inspections, and a shift towards process understanding and control.

The FDA stated (2004): "*The goal of PAT is to enhance understanding and control the manufacturing process, which is consistent with our current FDAs drug quality system: quality cannot be tested into products; it should be built-in or should be by design.*" This is in agreement with PAC's focus on the control of the quality of the analytical result and with the TOS control of the TSE. TOS helps achieve control over process sampling by designing valid (correct, unbiased, representative) sampling procedures (very often automated), that are qualified and continuously verified, to yield representative sensor measurement results. Figure 1 shows an archetype implementation of a PAT sensor (probe) with which to *interact* with a moving material stream. PAT sensors/probes are very nearly always multi-spectral (vibrational spectroscopy), demanding powerful *chemometric multivariate calibration and validation* (Martens & Næs, 1989; Esbensen, 2020; Esbensen, 2025). Note how all PAT sensor/probe installments must comply with TOS' demand to cover a *complete slice* of the moving material stream.

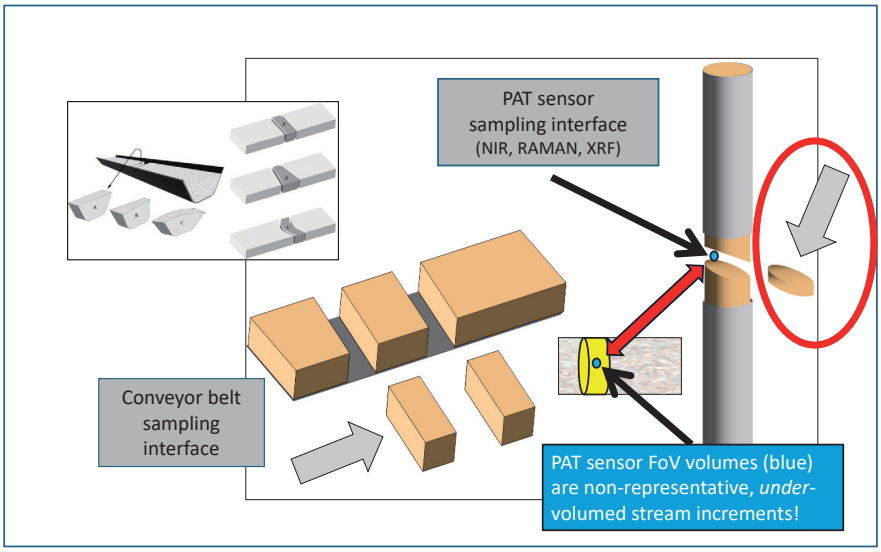
The field-of-view (FOV) of PAT sensors/probes (blue circles in Fig. 2) obviously do not cover a complete slice of the contemporary stream. This is unfortunately a massively neglected obligation in very many current 'PAT solutions', as documented in (Esbensen, 2025).

FACTBOX - 21st Century Initiative for Pharmaceutical Quality

- The FDA's initiative began in 2002 in an attempt to modernize pharmaceutical manufacturing regulation by integrating a science-based, risk-based, and quality systems approach across the product lifecycle. FDA developed a wide Quality Systems Framework and issued draft guidance for industry.
- Piloted inspection site prioritization model. Integrated risk into CMC review
- Issued PAT guidance. Promoted continuous improvement and innovation
- Collaborated with ICH, (VICH), Pharmaceutical Inspection Co-operation Scheme (PIC/S) Supported Q8, Q9, Q10 initiatives
- Updated compliance policy guide. Planned revision of 1987 guideline
- Launched pilot program. Drafted formal guidance
- Issued final guidance. Initiated rulemaking process
- Created Pharmaceutical Inspectorate. Enhanced training and certification
- Draft guidance issued. Enabled post-approval changes without prior FDA review
- Formed cross-functional team. Supported ASTM E55 standards



(CMC) - Chemistry Manufacturing and Control
(VICH) - International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
(PIC/S) - Pharmaceutical Inspection Co-operation Scheme



Credit: KHE Consulting; used with permission.

Figure 2: Principally identical PAT sampling demands to samples extracted from conveyor belts or pipeline moving material streams.

5. PAT – Historical Development

Table 2 shows a timeline of the history of development of the FDA PAT initiative. Since its inception, PAT has seen a slow but steady growth and has had a significant impact also in many other industry sectors than pharmaceutical manufacturing. Several pivotal moments from the development of PAT have resulted in some of its successes today in 2025, as we are now entering a continuous manufacturing (CM) phase of PAT (more of which below). PAT (incorporating TOS) serves as a crucial enabler for the successful implementation and improvements in CM.

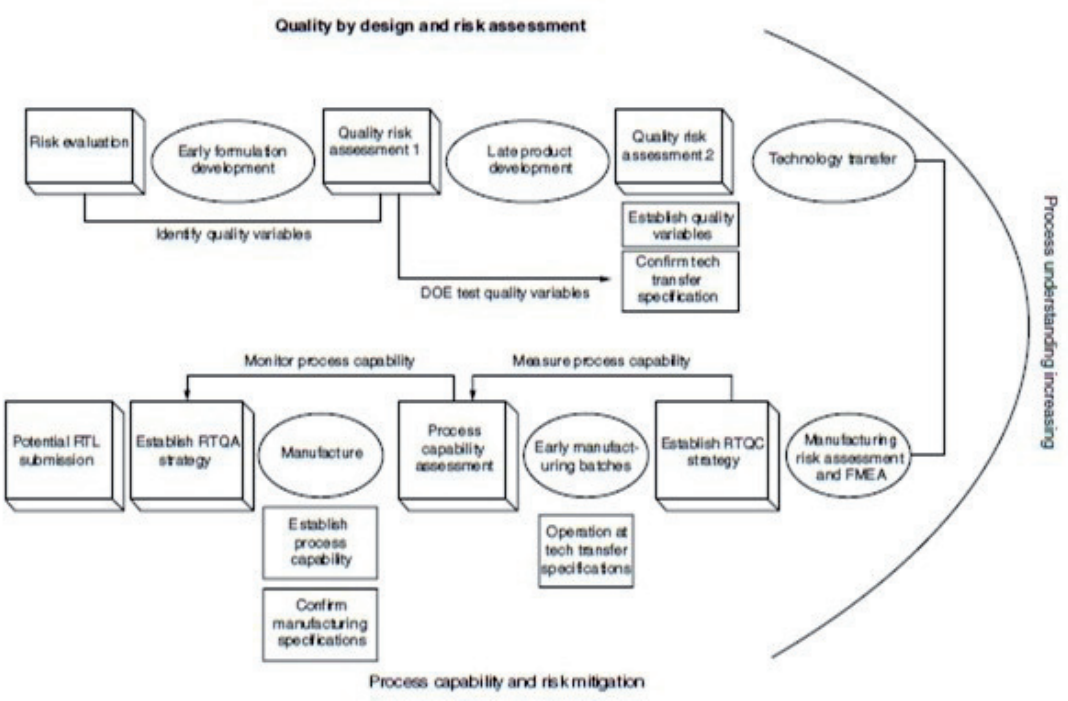
The next successful step for the development of PAT for pharmaceutical manufacturing was introducing the concept of Quality by Design (QbD).

6. Quality by Design (QbD)

Developed from the ICH, the guidance provides current thinking by the FDA on Quality by Design (QbD) as a systematic approach to pharmaceutical development. QbD emphasizes building quality into a product from the start by focusing on product and process understanding, risk management, and continuous improvement throughout the product lifecycle. This approach helps ensure product quality, enhance manufacturability, and potentially leads to greater regulatory flexibility (FDA 2009a, 2012a, 2023b). Figure 3 shows a mind map of PAT elements needed for understanding and managing process risk through designing, validating, and monitoring process variation.

Table 2: PAT Development Timeline.

1993	Early concepts of Process Analytical Technology (PAT) introduced by Tom Layoff et al. at AOAC conference.
2000	Initial proposal for PAT initiative met with limited support within FDA.
2001	**July**: Introduction of PAT topic to FDA Advisory Committee for Pharmaceutical Science. **November 16**: Key presentations at FDA Science Board set the stage for initiative: • GK Raju analyzes low efficiency in pharmaceutical manufacturing. • Doug Dean and Francis Britain highlight "don't use and don't tell" culture at Pfizer. • Ray Scherzer emphasizes cultural and historical barriers to quality by design.
2002	**January**: Establishment of PAT subcommittee with industry and academia representation. **June**: PAT initiative formally launched. Goals: improve manufacturing efficiency, reduce costs, address FDA resource challenges.
2003	September 11th and rising drug affordability concerns add urgency to initiative. Shift towards CGMPs initiative addressing broader quality systems.
2004	**July**: Finalization of PAT Guidance for Innovative Manufacturing and Product Development Quality Assurance. **September**: Major announcement with over 18 guidance documents issued (including PAT Guidance, Aseptic Manufacturing Guidance, Quality System Approach to CGMP Inspections). Start of paradigm shift within FDA: • Reduced turf issues between CMC review and CGMP inspection. • Scientific assessment prioritized over procedural compliance. • Process validation redefined with focus on understanding and control. • International collaboration through (ICH) Q8 (Risk Assessment & Risk Management), Q9 (Quality), and potential Q10 (Continuous Improvement & Change Control).
Ongoing & Future	Five major pharma companies led the way in adopting PAT principles and submitting innovative proposals. • Continuous improvement in efficiency and reduced regulatory burden expected for compliant companies. • Principles of systems approach and quality by design applied to other initiatives like Critical Path. • International collaboration and harmonization efforts continue. • The timeline focuses on early key milestones and highlights. Further details and specific dates for individual actions can be found in official FDA records and publications. The initiative is ongoing and continues to evolve into CM.



Credit: R.S. Chisholm; used with permission.

Figure 3: PAT Mind Map.

Two key practices that must be integrated with QbD elements from product development practices are risk management and an appropriate quality management system:

1. There must be a QbD-aligned *sampling plan* detailing the method, procedure, quantity, frequency, and location of sampling at various stages of the process, including raw material, in-process, and finished product analysis, according to data integrity and good documentation practices (FDA 2018). TOS is a critical success factor in this endeavor.
2. Special attention needs to be paid when sampling heterogeneous materials, where variations in composition or properties necessitate well-designed, flexible *sampling strategies* to always ensure representativeness. The statistical discipline Design of Experiment (DOE) helps understand the relationship between input variables (e.g., process settings) and output parameters (e.g., product quality). DoE is an important component of QbD. It provides meaningful data with which to validate that a process consistently produces a product meeting its specifications e.g., via Critical Quality Attribute (CQA) monitoring, which is crucial for FDA compliance (Adelberg, 2024).

7. Process Validation

Process validation is the first necessary step for the advancement of PAT in drug and product manufacturing. Process validation (Adelberg, 2024) is defined as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product. Process validations occur in three stages:

Stage 1 – Process Design: The commercial manufacturing process is *defined* during this stage based on knowledge gained through *R&D, pilot studies and scale-up activities*.

Stage 2 – Process Qualification: During this stage, the process design is evaluated to determine if the process is capable of *reproducible commercial manufacturing*.

Stage 3 – Continued Process Verification: *Ongoing assurance (QC/QA/QM)* is to be gained during routine production that the process remains in a *state of control*.

Incorporating process sampling methods and technologies into drug and product remanufacturing processes, according to the TOS, will ensure that the variability of the sampling method, and the representativity of the resulting samples are controlled, qualified, and continuously verified.

ICH is an organization involving regulators and the pharmaceutical industry worldwide. Its purpose is to improve efficiency of new drug development and registration processes, promote public health, prevent duplication of clinical trials in humans and minimize the use of animal testing without compromising safety and effectiveness (Adelberg, 2024). The following topics introduce the ICH guidelines for drug substance manufacturing, drug product lifecycle, Continuous Manufacturing of Drug Substances and Drug Products, and analytical method development.

8. Development and Manufacture of Drug Substances

The primary sampling principles of the TOS are founded on the concept of heterogeneity, a.o., defined by the concept of lot dimensionality. This approach can be shown to be related to the core principles discussed in the development and manufacture of drug substances (FDA, 2012b), these being risk management, quality assurance and process control. Several properties of pharmaceutical starting materials may be desirable and considered in order to provide strict control over the Critical Quality Attributes (CQA) of the final drug substance - especially impurities, but also moisture, particle size, residual solvents and heavy metals for instance. However, it is indeed remarkable how much evidence can be found today in peer-reviewed publications in which TOS postulates that only one criterion is necessary to ensure that the starting materials are correctly selected and justified based on the requirements for representative sampling (Table 3).

Sampling representativity is the general criterion to be considered for materials' characterization (e.g., physical, chemical, biological, and microbiological properties) and subsequent processing (sub-sampling) must also be carried out by TOS-complying Sample Unit Operations (SUO) (Esbensen and Wagner, 2015).

Recall that sampling of materials involved in manufacturing of drug substances are typically performed by one of five PAC sampling modes (Table 1). Measurement characteristics for each mode may vary and depend on whether or not samples are taken directly from a process (and are thus 'consumed') or material properties are measured indirectly (non-invasively and non-destructively). Because of these demands proper selection and justification of drug manufacturing starting materials can be considered by design (i.e., critical process parameters (CPPs) and critical quality attributes (CQAs). This means that risk management, quality assurance and process control strategy can be properly addressed based on a sampling lifecycle strategy framework 'from starting materials to final drug substance'.

9. Bias testing – brief interlude

Within pharma there has been a longstanding focus on bias testing, as an integral part of Quality Control and Quality Assurance (QC/QA). However, in the last 5-10 years it has gradually become clear that this is a severely limited approach as concerns representativity. Bias testing does not guarantee representativity across time, material batches, or process changes. And anyway, bias testing is a *post hoc* approach that positively *invites* many batch rejections (unwanted). Much better to adopt TOS' approach of designing, implementing and verifying unbiased sampling processes (Danish Standards, 2024; Esbensen, 2020; Esbensen, 2025; Romanach, 2025).

Table 3: Trying to Ensure Representativity Through Bias Testing.

Purpose of Bias Testing	Validate that a sampling system yields representative samples compared to a reference method.
Reference Methods	Commonly used: Stopped Belt Sampling, Manual Grab Sampling, or Composite Reference Sampling.
Limitations	Bias tests reflect only the conditions during testing. They do not guarantee representativity across time, material batches, or process changes.
Correct Sampling First	Eliminate Incorrect Sampling Errors (ISE) before optimizing for precision, i.e., minimizing Correct Sampling Errors (CSE), e.g., increasing the operative number of increments.
Regulatory Implication	A fully TOS-compliant sampling plus analysis system will be able to reduce or eliminate the need for repeated bias testing, supporting long-term compliance and audit readiness.

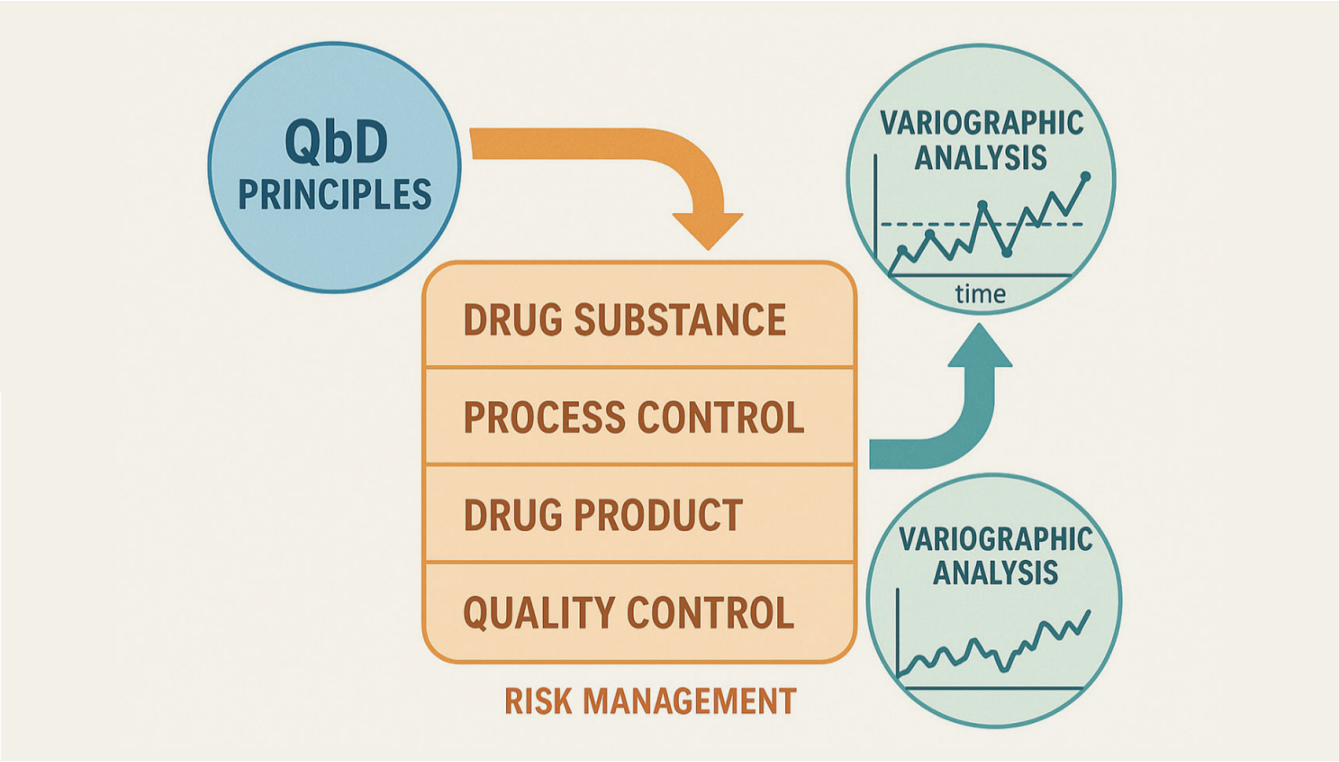
10. Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management Guidance for Industry

Following a sampling management lifecycle strategy designed to control the variability of analytical results by counteracting the adverse effects from material composition and spatial distribution heterogeneity throughout a manufacturing process (i.e., drug product and substance development, registration, and launch), this guidance addresses the commercial phase of the product lifecycle, particularly post approval changes through specially developed tools for managing and communicating control and control of changes over the process (FDA, 2021). More importantly, this strategy is one that links and harmonizes manufacturing process control regulations across three global regions: European Union (EU), United States (FDA), and Japan (MHLW/PMDA). This provides a way for the set of requirements for procedures and documentation elaborated on in the TOS standard (Danish Standards, 2024), to be easily and readily applied seamlessly to the drug substance and product manufacturing domain. For instance, sampling and measurement error management can be used for supporting the qualification of sampling hardware and software. Validation can be performed on process and sampling/measurement methods (modes). This data provides the desired knowledge to understand the CPP necessary to manage process variability resulting from sampling uncertainty.

This tool provides meaningful diagnosis as to root causes, and can be used to scientifically communicate, implement and assess changes so that the final quality of analytical results from pharmaceutical product lifecycle framework, risk management, and manufacturing processes remain in a state of control. Figure 4 shows how QbD principles guide process understanding and design space, how risk management identifies and mitigates critical quality risks, and how variographic analysis (the most recent innovation in pharma from the sampling domain (TOS), see a parallel article in this issue of SST) monitors spatial and temporal variability in drug substance and product manufacturing. Each element flows into the next, supporting a robust control strategy.

11. Continuous Manufacturing of Drug Substances and Drug Products Guidance for Industry

Movilla-Meza et al. (2025) reviewed several CM studies where QbD principles, PAT tools, and variographic analysis were assessed for improving PAT development, implementation, and quality control. They concluded and showed that it is possible to differentiate process variability from sampling and analytical errors.



Credit: Gary Richie; used with permission.

Figure 4: Sampling / Measurement Lifecycle: TOS Integrated with PAC/T Principles: A. QbD Principles B. Risk Management C. Variographic Analysis.

This becomes the central thesis and justification for designing a sampling strategy in the widest scope possible (sampling plan, validation plan, quality plan, lifecycle management, and a communication plan) for filing for approval and post approval changes to a pharmaceutical process and design space (FDA, 2023a). This guidance can use and rely on the TOS framework for adhering to a scientific, risk-based approach for addressing the sampling mode, placement, heterogeneity, dimensionality, and frequency. Movilla-Meza, et al. (2025) conclude:

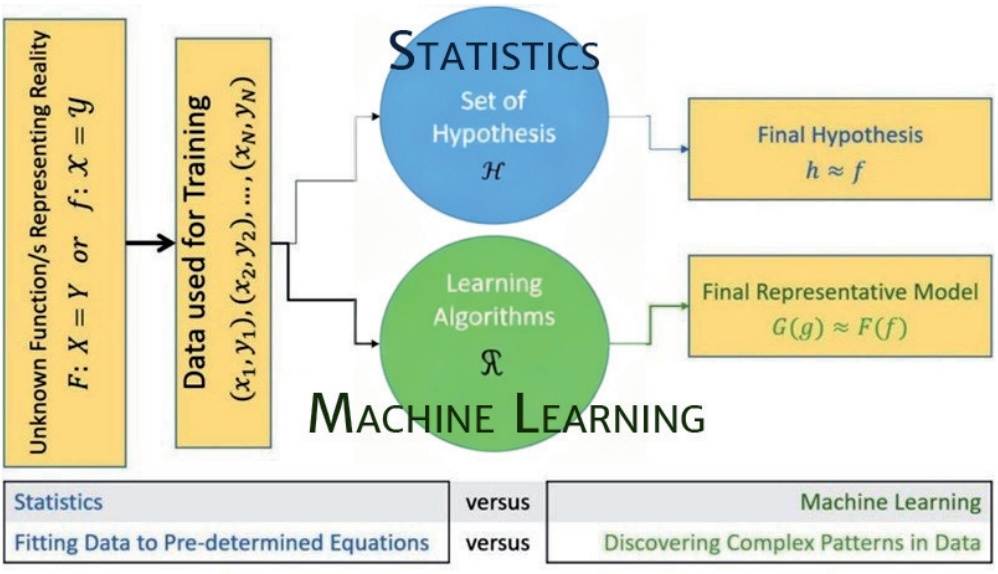
TOS has guided the sampling of CM processes. The repeatability study and variographic analysis have provided a greater understanding of the sources of error in PAT methods used in CM. Future studies are needed to better understand the cyclical behavior that is observed in variograms. PAT has progressed over the last 20 years and found a role in maintaining the state of control in CM processes.

12. Analytical Procedure Development

In the first ever published book on chemometrics by Sharif et al. (1986), the authors introduce the reader to sampling in the first chapter. A review of this chapter and its references show that the state of the art at the time this was written was rooted exclusively in classical statistical theory. While ample consideration is given to representativity of sampling from a traditional statistical population, it does not address how ill-reflected sampling for material composition will cause significant bias and other sampling error variability.

This difference between varying analytical results (statistical difference) is due to physical properties differences in the heterogeneity of materials. The physical and mathematical nature of analytical results is now superseded by Artificial Intelligence and Machine Learning, and surpassing both the statistical and physical nature of reality, by instead defining the rules leading to steps for finding a solution of a sampling reality for any given material produced by any given process (Figure 5).

From the *Journal of Petroleum Technology*, November 10, 2019, Gy's perspective is simple. A sample's heterogenous nature necessitates counteraction in the sampling process; this is the only way to obtain representativity. This solution is rooted in a thorough understanding of material composition and spatial heterogeneity - not in probability (Pierre Gy is credited with this famous statement: "Sampling—should not be gambling!". Gy powerfully showed that the practice of *grab sampling* is a very poor practice due to its guaranteed lack of representativeness, violating TOS' first governing principle (GP-1), the Fundamental Sampling Principle. This observation goes to the heart of sampling understanding at the time and illustrates the gap that existed in 1986 for sampling as a source of uncertainty also for analytical results. Gy's understanding puts to bed the false notion that sampling error originates purely from statistical properties rather than from inadequate sampling of heterogeneous materials. It would not be



Credit: Mohagheghi, 2019; used with permission.

Figure 5: Statistics versus AI and Machine Learning.

until twelve years later that Gy publishes his seminal *Sampling for Analytical Purposes* (1998), explaining how sampling theory and practice must be considered - not as a statistical matter - but from the basis of proper understanding of material properties based on the principles of physics and empirical evidence, substantiated by physical experimentation.

In today's the guidance for analytical procedure development manual (FDA, 2024), sampling is considered from the following points:

1. The measurement point(s) should be chosen so always to allow representative process sampling, and
2. The sampling interface must remain consistent over the duration of manufacturing and must be robust with regard to the expected processing and environmental variations.

Both these conditions are now aligned with TOS' control strategy for reducing TSE.

The Danish Standard (2024), *Representative Sampling — Horizontal standard* and ICH Q14 (adopted by FDA) answer different but *complementary* questions: TOS ensures that samples are demonstrably representative of the material batch, lot or process, while Q14 ensures the analytical procedure turns samples into reliable measurements across the full product lifecycle. Used together, these guiding documents close the "sampling-to-measurement" loop. These approaches are listed and compared with each other in Table 4.

Table 4: Comparison of the international 'DS 3077:2024' sampling standard with FDA's 'Guidance for Analytical Procedure Development' (Danish Standards, 2024; FDA, 2024)

Dimension	TOS Danish Standard (DS 3077)	ICH Q14 Analytical Procedure Development (FDA-adopted)
Scope	Representative sampling across materials, matrices, and processes	Development, justification, and lifecycle management of analytical procedures
Primary objective	Eliminate sampling bias and control sampling + subsampling + preparation errors to achieve representativeness	Define, justify, and control the method so results are fit for intended use via minimal or enhanced approaches
Core concept	Total Sampling Error taxonomy (correctness vs precision), heterogeneity scales, correct sampling/lot delineation, increment design, composite/pulverization, variography	Analytical Target Profile (ATP); science- and risk-based development; robustness studies; control strategy; Established Conditions (ECs); lifecycle/post-approval change pathways
Risk management	Systematic identification and removal/mitigation of sampling bias sources; design-based error control	Quality risk management per ICH Q9; knowledge- and risk-based identification of critical method parameters and ranges; enhanced approach modeling
Validation link	Focus on sampling correctness and precision; validation pertains to sampling performance and variance components	Paired with ICH Q2(R2) for validation of analytical procedures (accuracy, precision, specificity, etc.)
Deliverables	Sampling plan and SOPs; designed sampling tools and increment protocols; mixing/compositing instructions; documentation of heterogeneity and error control	ATP; development summary (minimal or enhanced); robustness and parameter ranges; method control strategy; ECs; lifecycle and change management dossier
Multivariate/advanced use	Variography, heterogeneity modeling, spatial/temporal sampling designs	Guidance includes multivariate analytical procedures and Real-Time Release Testing considerations
Regulatory status	Horizontal standard widely referenced in industries; not a drug-specific regulatory guideline	International guideline finalized Nov 1, 2023; adopted by FDA Mar 2024; complements ICH Q2(R2)

13. Summary

This chapter traced the historical, technical, and regulatory milestones that shaped PAT into a comprehensive framework for ensuring pharmaceutical product quality through real-time process understanding and control. It was shown how it is highly beneficial for society, that the modern PAT concept/framework has also been found to be applicable in many other industry sectors.

At the heart of this transformation is the recognition that sampling is not merely a preparatory step but is in fact a critical determinant of analytical reliability. The incorporation of TOS principles, particularly the concepts of material heterogeneity, lot dimensionality, Total Sampling Error (TSE) and sampling process supremacy with regard to representativity - has elevated the role of sampling from being viewed as but a procedural necessity to a scientifically governed critical discipline.

The convergence of PAC, PAT, and TOS provides a unique, powerful *unified strategy* for minimizing uncertainty, maximizing process efficiency, and ensuring regulatory compliance across the product lifecycle (pharmaceutical).

As pharmaceutical and other *similar* industries move forward, the continued refinement of sampling strategies, sensor technologies, and data analytics will grow in potential impact. The future of PAT lies not only in further technological innovation but more and more in the disciplined application of scientific principles that *bridge* the gap between [sampling-*and*-measurement] and optimal QC/QA and decision-making. This article presented a hopefully inspiring empowering scope of potential further PAT impact(s) in industry sectors already significantly relying on TOS: process sampling and PAT join forces for the future.

References

Adleberg, J. (2024). International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Overview, U.S. Food and Drug Administration/ HC ICH regional public Meeting, February 22, 2024. https://www.fda.gov/media/177713/download

Baughman, E. (2005). Process Analytical Chemistry: Introduction and Historical Perspective, In Katherine A. Bakeev (Ed.) *Process Analytical Technology Spectroscopic Tools and Implementation Strategies for the Chemical and Pharmaceutical Industries*, 1st ed., Oxford, Blackwell Publishing, pp. 1-12.

Callis, J.B., Illmann, D.L., Kowalski, B.R. (1987). Process Analytical Chemistry, *Analytical Chemistry*, V.59 (9), May., 624a-637a https://pubs.acs.org/doi/pdf/10.1021/ac00136a001?casa_token=XtFSoQwJhFMAAAAA:_rHB40zWPHYvMXxjzsiXVZZIavbOCml-BLnvU-orB6j_pT5dUDZRVja3Fl39DsjwpG3faN_4shjOsAA

Center for Process Analytical Chemistry Receives Planning Grant (1984a). *Analytical Chemistry*, V.56 (1), January. https://pubs.acs.org/doi/pdf/10.1021/ac00265a722?ref=article_openPDF

Center for Process Analytical Chemistry Receives Planning Grant (1984b). *Analytical Chemistry*, V.62 (18), September 12, 965a.

Danish Standards (2024). DS-3007, *Repræsentativ prøvtagning—Horisontal standard*, Fondan Dansk Standard,Nordhavn, DK.

Dziki, W., Jiang, Z., Radspinner, D.A., Miller, M., Deschenes, R., Newby, P., Moldenhauer, J., Anger, C., Sutton, S., Cundell, T., McCarthy, P., McDaniel, A., Montgomery, S., Denoya, C., Ciurczak E., & Ritchie, G. (2004). The FDA Process Analytical Technology (PAT) initiative - An alternative Pharmaceutical Manufacturing Practice (aPMP). *Pharmacopeial Forum. 30*. 2254-2262. https://www.researchgate.net/publication/289059421_The_FDA_Process_Analytical_Technology_PAT_initiative_-_An_alternative_Pharmaceutical_Manufacturing_Practice_aPMP

Esbensen, K. H. (2020). *Introduction to the Theory of Sampling*, IM Publishing Open, Chichester, UK.

Esbensen, K.H. (Ed.) (2021) Economic arguments for representative sampling in technology, industry, commerce, trade and society, Special Issue, IMP Open, Chichester, UK., *Spectroscopy Europe*, V 33

Esbensen, K. H. (2025). Data Quality: Importance of the 'Before Analysis' Domain [Theory of Sampling (TOS)], *Journal of Chemometrics*, V 39, https//doi.org/1002/cem.70021

Esbensen, K.H. & Wagner, C. (2015). Theory of Sampling (TOS)—fundamental definitions and concepts, *Spectroscopy Europe*, 27(1), 22–25.

Food and Drug Administration (2004, Sept.) Guidance for Industry: PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, & Quality Assurance, U.S. Department of Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Office of Regulatory Affairs, Pharmaceuticals CGMPs, https://www.fda.gov/media/71012/download

Food and Drug Administration (2004a, Sept.). Pharmaceutical CGMPS for the 21st Century-A Risk-Based Approach Final Report, Issued by Dept. of Health and Human Services, U.S. Food and Drug Administration.

Food and Drug Administration (2004b, Oct.). PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance, FDA-2003-D-0032, Issued by: Center for Veterinary Medicine, Office of Regulatory Affairs, Center for Drug Evaluation and Research. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance

Food and Drug Administration (2009a, April) Guidance Document for Pharmaceutical Quality System, FDA-2007-D-0370, Issued by: Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. US Dept. of Health and Services, Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q10-pharmaceutical-quality-system

Food and Drug Administration (2009b, Nov.). Guidance Document Q8 (R2) Pharmaceutical Development, FDA-2005-D-0154, Issued by Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. US Dept. of Health and Services, Food and Drug Administration: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q8r2-pharmaceutical-development

Food and Drug Administration (2011, Jan.). Guidance for Industry Process Validation: General Principles and Practices, U.S. Department of Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Center for Veterinary Medicine, Current Good Manufacturing Practices. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf

Food and Drug Administration (2012a, July). Guidance Document, Q8, Q9 & Q10 Questions and Answers—Appendix: Q &As from Training Sessions (Q8, Q9, Q10 Points to Consider), Issued by U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, & Center for Biologics Evaluation and Research. https://www.fda.gov/media/83904/download

Food and Drug Administration (2012b, Nov.). Guidance Document: Q11 Development and Manufacture of Drug Substances, Issued by U.S. Department of Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). https://www.fda.gov/files/drugs/published/Q11-Development-and-Manufacture-of-Drug-Substances.pdf

Food and Drug Administration (2018, Dec.). Guidance Document: Data Integrity and compliance with Drug CGMP: Questions and Answers, Guidance for Industry, FDA-2018-D-3984, Issued by Center for Veterinary Medicine, center for Biologics Evaluation and research, Center for Drug Evaluation and research-Office regulatory policy. https://www.fda.gov/media/119267/download

Food and Drug Administration (2021, May). Guidance Document, Q12 Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management Guidance for Industry, FDA.2018-D-1609, Issued by Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q12-technical-and-regulatory-considerations-pharmaceutical-product-lifecycle-management-guidance

Food and Drug Administration (2023a, March). Guidance Document: Q13 Continuous Manufacturing of Drug Substances and Drug Products, Guidance for Industry. FDA-2021-D-1047Issued by, Center for Drug Evaluation and Research. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q13-continuous-manufacturing-drug-substances-and-drug-products

Food and Drug Administration (2023b, May): Quality Risk Management: Guidance for Industry, FDA-2022-D-0705, Issued by Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. US Dept. of Health and Services, Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q9r1-quality-risk-management

Food and Drug Administration (2024, March). Guidance Document: Q14 Analytical Procedure Development, FDA-2022-D-1503, Issued by: Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q14-analytical-procedure-development

Gy, P. *Sampling for Analytical Purposes*, John Wiley & Sons, Chicester, UK.

Hassell, C. and Bowman, E. (1998). Process Analytical Chemistry for Spectroscopists, *Applied Spectroscopy*, V. 52 (1).

Martens, H. & Næs, T. (1989). *Multivariate Calibration*, John Wiley & Sons, Chichester, UK.

Mohaghegh, S. D. (2019, Nov.10). Traditional Statistics vs. Artificial Intelligence and Machine Learning, *Journal of Petroleum Technology*, https://jpt.spe.org/traditional-statistics-vs-artificial-intelligence-and-machine-learning

Movilla-Meza, N.A., Patel, D.S., Méndez, R. & Romañach, R.J. (2025). PAT for monitoring the state of control in continuous manufacturing of solid oral dosage forms, *Analytical and Bioanalytical Chemistry*, July 21, 1-13. doi: 10.1007/s00216-025-06012-w.

Ritchie, G. (2007). Calibration and Validation of Process Sensors, Chap.12 in *Handbook of Near-Infrared Analysis Practical Spectroscopy*, Burns, D. Ciurczak, E.(eds.) CRC Press, Taylor and Francis Group, 246-266 https://doi.org/10.1201/9781420007374

Romanach, R. (2025). Process Analytical Technology I—Theory of Sampling in PAT, *Pharmacopeia Forum*, V 51 (3). https://doi.org/10.31003/USPNF_S203344_010101_01

Sharaf, M.A., Illman, D.L., Kowalski, B.R. (1986). *Chemometrics*, New York: John Wiley & Sons.