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SHORT COMMUNICATION article

Comprehensive validation of a GMP-compliant pure steam system for biopharmaceutical manufacturing

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Abstract: Pure steam systems are essential utilities in biopharmaceutical manufacturing, providing sterile steam for cleaning-in-place, sterilization-in-place, and equipment sterilization. However, validation data for pure steam remains limited compared with water systems. This study reports the comprehensive qualification and validation of a GMP-compliant pure steam system, including installation qualification, operational qualification, and performance qualification. Sampling was performed at five representative points of use over one year, twice weekly, to ensure robustness and reproducibility. Analytical testing covered physicochemical parameters (conductivity, total organic carbon, endotoxins), microbiological safety (viable counts, thermophiles, pathogens), and steam quality attributes (non-condensable gases, dryness fraction, superheat). Results consistently met pharmacopeial and regulatory requirements: conductivity < 1.0 µS/cm, total organic carbon < 100 ppb, endotoxins undetectable, and negligible microbial counts. Steam quality testing confirmed non-condensable gases < 2.0%, dryness fraction > 0.95, and controlled superheat. These outcomes align with European Pharmacopoeia, United States Pharmacopeia, EU GMP Annex 1, FDA guidance, and ISPE recommendations, demonstrating the reliability of pure steam systems for sterile manufacturing. The novelty of this work lies in presenting complete validation data for pure steam as an independent utility, contrasting with the limited case reports that address steam only within water-system validation. Additionally, the study highlights the importance of continuous monitoring and lifecycle management to maintain validated states, ensuring long-term compliance and operational robustness. By providing detailed evidence of system performance, this study supports industry efforts to strengthen quality assurance frameworks and to establish pure steam as a validated, reliable, and indispensable utility in biopharmaceutical production.

Introduction

Pure steam is a critical utility in biopharmaceutical manufacturing, serving as an indispensable medium for sterilization of equipment, piping, and process vessels, as well as for cleaning-in-place (CIP) and sterilization-in-place (SIP) operations [1]. Unlike utility-grade steam, pure steam is generated from water for injection (WFI) or highly purified water, ensuring the absence of chemical additives, corrosion inhibitors, and contaminants that could compromise product quality [2]. Its role in maintaining aseptic conditions makes it a cornerstone of sterile

manufacturing environments. Beyond its established applications, pure steam is essential in the production of WFI itself, safeguarding the integrity of distillation and condensation processes. In fermentation facilities, it enables reliable sterilization of fermenters, ensuring microbial control and product consistency. It also supports aseptic sampling procedures, reducing contamination risks during critical in-process controls, and serves as the standard medium for sterilization of laboratory and production materials in autoclaves, reinforcing its versatility across the biopharmaceutical value chain. Regulatory frameworks highlight the importance of pure steam quality. The EU GMP Annex 1 emphasizes stringent control of sterilization processes, including steam quality attributes such as dryness fraction, non-condensable gases, and superheat [3, 13]. FDA guidance on aseptic processing underscores the need for validated steam systems to ensure consistent sterilization efficacy [4]. The ISPE Good Practice Guide provides methodologies for commissioning and qualification of pharmaceutical water and steam systems, reinforcing the necessity of structured validation approaches [1, 5]. Validation of pure steam systems encompasses installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). Expected outcomes include compliance with pharmacopeial requirements for condensate quality (conductivity, total organic carbon, endotoxins), microbiological safety (absence of thermophiles and pathogens), and steam quality parameters (dryness fraction ≥ 0.95, non-condensable gases ≤ 3.0%, controlled superheat) [6, 7]. Equally important is to maintain the validated state, achieved through continuous monitoring, preventive maintenance, and periodic requalification, ensuring long-term compliance and operational robustness [7].

Materials and methods

System description: A GMP-compliant pure steam generation and distribution system was installed in a sterile manufacturing facility. The system was designed to produce pure steam from purified water, ensuring compliance with pharmacopeial and regulatory requirements [1, 3]. The distribution loop included multiple points of use strategically located to support SIP and CIP operations. The system configuration, comprising the sequential arrangement of the pure steam generator, the distribution line, and ancillary equipment utilizing this service, is depicted in **Figure 1**.

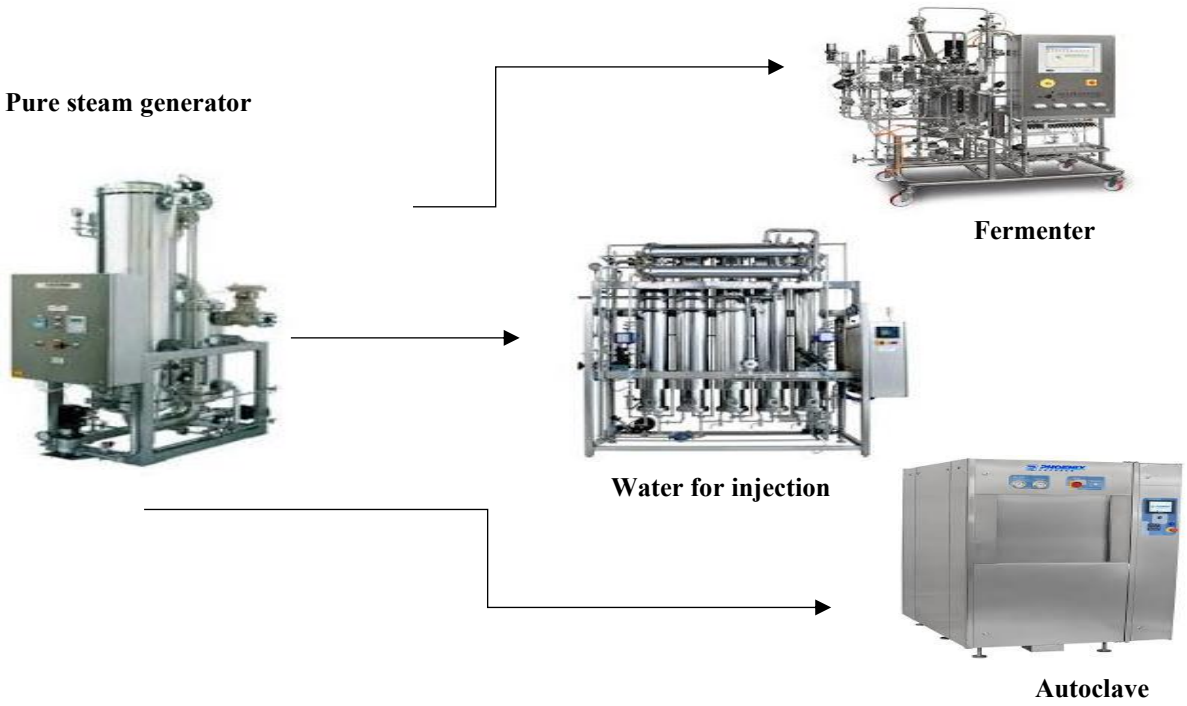

Figure 1: Design of a pure steam system

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Qualification protocols

- *Installation qualification:* Verification of installation, materials of construction, and documentation, including piping and instrumentation diagrams (P & IDs), certificates of materials, and calibration records [1].
- *Operational qualification:* Assessment of operational parameters such as pressure stability, temperature control, and condensate quality. Functional testing included alarms, control systems, and steam-generation capacity [4].
- *Performance qualification:* Long-term evaluation under routine production conditions, with repeated sampling to confirm reproducibility and compliance [7]. PQ was conducted over one year, with sampling performed twice per week, to ensure sufficient data to demonstrate system reliability and reproducibility under actual manufacturing conditions.

***Sampling strategy**:* Multiple points of use were selected across the distribution system, including distal ends, high-use points, and representative locations in the whole system. This approach ensured that validation data reflected typical and challenging operating scenarios**.** Sampling was performed during qualification and subsequently as part of routine monitoring to maintain the validated state [7]. Samples were analyzed for physicochemical parameters (conductivity, total organic carbon, endotoxins), microbiological safety (viable counts, thermophiles, pathogens), and steam quality attributes (non-condensable gases, dryness fraction, superheat), following pharmacopeial and regulatory guidelines.

Regulatory framework: Validation activities were carried out in compliance with current GMP guidelines issued by the World Health Organization (WHO), European Medicines Agency (EMA), and U.S. Food and Drug Administration (FDA)**,** as well as the ISPE Baseline Guide recommendations [1]. Compliance was demonstrated through documented qualification protocols (IQ, OQ, PQ), deviation control procedures, and risk assessments, ensuring traceability and reproducibility of results. Sampling points were strategically located at subsystem outlets and at the final distribution loop to capture both representative and worst-case conditions. Analytical procedures adhered to pharmacopeial standards, including USP <643> (total organic carbon), USP <645> (conductivity), USP <85> (bacterial endotoxins test), and relevant microbiological chapters (e.g., USP <61> and <62> for microbial enumeration and specified microorganisms), as well as Ph. Eur. monographs. This comprehensive framework ensured that physicochemical and microbiological attributes of pure steam were consistently evaluated against internationally recognized acceptance criteria. The assays were performed by the laboratories of the quality control department (free chlorine residual test, total hardness, total aerobic mesophilic count, pathogen detection, conductivity, nitrate determination) and the Process Control Department (total organic carbon, LAL), as part of the joint analytical work conducted on the water samples [8, 9]. The alert and action limits, which were specifically established during Phase 1 of the validation process, together with the specification limits for each quality attribute, are presented in **Table 1**. The sampling points are detailed in **Table 2**.

Table 1: Tests and specifications, alert, and action limits for quality attributes evaluated in purified water and pure steam

Assay	Specification	Alert limit	Action limit
Conductivity	Current USP	-	-
Nitrate	< 0,2 mg/L	-	0,2 mg/L
Total organic carbon	≤ 500 ppb	200 ppb	300 ppb
Endotoxin (LAL)	≤ 0,25 UE/mL	0,1 UE/mL	0,2 UE/mL
Microbiology (PS)	≤ 10cfu/100 mL	3 cfu/100 mL	6 cfu/100 mL
Microbiology (PW)	≤ 100 cfu/mL	20ufc/mL	50 ufc/mL
Presence of pathogens	Absence/200 mL	-	-
Thermophiles	Present	-	-
Non-condensable gases (%)	≤ 3.0%	-	-
Dryness fraction	≥ 0.95	-	-
Superheat (°C above sat.)	≤ 25 °C	-	-

Table 2: Code and location of the sampling points of the system during performance qualification of the pure steam system

Quality	Code	Location
Purified water	VP-PM004	Tank GVP Sampling Point
Pure steam	VP-PM001	PSG sampling point
	VP-PU020	Autoclave sampling point
	Vap-Trap	Fermenter sampling point
	AI-PM001	WFI1 condenser
	AY-PM001	WFI2 condenser

Results and discussion

Extensive validation testing was performed at five sampling points across the pure steam distribution system. The analytical data, summarized in **Tables 3** and **4** and illustrated in **Figures 2 - 4**, consistently demonstrated compliance with pharmacopeial and GMP requirements, thereby confirming the robustness of the system under operational conditions. At sampling point VP PM004 (feed tank of the pure steam generator), purified water quality was verified. This location is critical because purified water serves as the raw material for pure steam generation; its compliance with quality attributes directly determines the reliability of the steam produced. All parameters met the established specifications and acceptance limits defined for system validation. Notably, nitrate concentrations consistently remained below the specification limit (< 0.2 mg/L), confirming the absence of nitrates and demonstrating compliance with European Pharmacopoeia criteria for this test [6]. Total organic carbon levels remained consistently below 102 ppb throughout the performance qualification period. Counts of mesophilic aerobic microorganisms were maintained below 20 cfu/mL, and no pathogenic microorganisms were detected at any stage, as presented in **Table 3**. These results confirm the microbiological integrity of the system under routine operating conditions.

Table 3: Results point VP-PM004 feed tank pure steam generator (purified water quality)

Phase	Assay	Samples	In control	minimum	median	maximum	Alert Limit	Action Limit	Specification Limit
1	Conductivity (µS/cm)	15	15	-	-	-	-	-	USP
	Nitrate (mg/L)	15	15	-	-	-	-	-	≤ 0.2
	Total organic carbon (ppb)	15	15	2.3	15.1	99	-	-	500
	Microbiology (cfu/mL)	15	15	0	0	20	-	-	100
2	Conductivity (µS/cm)	15	15	-	-	-	-	-	USP
	Nitrate (mg/L)	15	15	-	-	-	-	-	≤ 0.2
	Total organic carbon (ppb)	15	15	3.5	10.9	102	200	300	500
	Microbiology (cfu/mL)	15	15	0	0	10	20	50	100
3	Conductivity (µS/cm)	104	104	-	-	-	-	-	USP
	Nitrate (mg/L)	104	104	-	-	-	-	-	≤ 0.2
	Total organic carbon (ppb)	104	104	5.9	12.6	89	200	300	500
	Microbiology (cfu/mL)	104	104	0	0	20	20	50	100

Although nitrate testing is typically associated with purified water, it was performed at key points of the system, including pure steam, to ensure feed water quality control. Any deviation in nitrate levels could compromise steam integrity, underscoring the need for purified water to consistently meet pharmacopeial standards. The selection of VP PM004 was risk-based, as it represents the entry point of water into the steam generator, where deviations would directly affect downstream steam quality. This rationale is consistent with recent literature highlighting the impact of total organic carbon, endotoxins, and nitrates in purified water on pure steam robustness, thereby supporting the findings presented here [8, 9].

Precise conductivity measurements are essential for maintaining the high purity of pure steam condensate, a critical parameter for ensuring product safety and efficacy. As shown in **Figure 2**, none of the 520 samples analyzed during the performance qualification phases exceeded the specification limit. This outcome was based on the correlation between temperature and conductivity measured without temperature compensation, in accordance with USP <645> requirements [4].

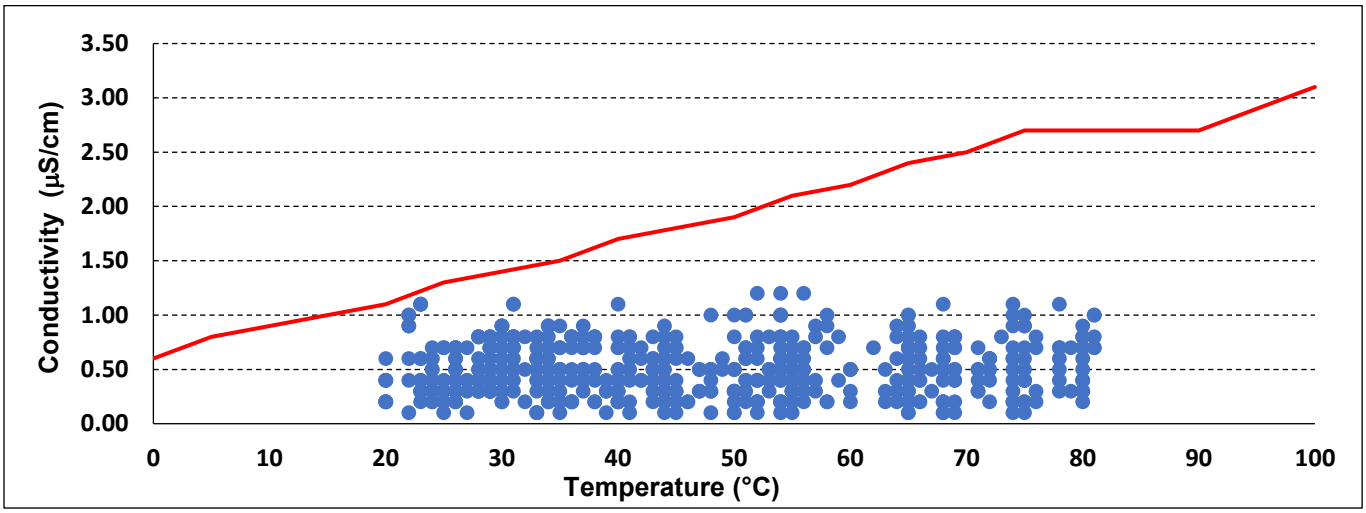

Figure 2: The conductivity of all points during the three validation stages, with the relationship between temperature and conductivity without temperature compensation

Total organic carbon results (**Figure 3**) were consistently below 100 ppb, with median values of 18.9 ppb, well within the USP <643> specification limit of ≤ 500 ppb [4]. Microbiological monitoring (**Figure 4**) revealed negligible viable counts, with no detection of pathogens or thermophiles, thereby ensuring microbiological safety. Counts were consistently ≤ 2 CFU/100 mL, below the action limit, in line with USP <61> and <62> requirements [4]. Sampling was performed twice weekly over one year across five representative and worst-case points of use, ensuring robustness and reproducibility of the dataset. This frequency and duration provided sufficient statistical confidence to demonstrate system reliability under routine and stress conditions. These findings are consistent with recent reports in pharmaceutical engineering literature, which emphasize the importance of long-term trending of conductivity, total organic carbon, and microbiological attributes to confirm lifecycle validation of pure steam systems [8, 9]. The reproducibility of results across multiple points of use reinforces the robustness of the qualification strategy and supports the reliability of pure steam as a validated utility for sterile manufacturing.

Steam quality parameters provided further confirmation of system performance (**Table 4**). Non-condensable gases averaged 1.7% ± 0.2 (n = 3), consistently below the USP limit of 3.0% [4]. Dryness fraction values ranged from 0.96 to 0.98 (mean 0.97 ± 0.01), exceeding the minimum requirement of 0.95 [1]. Superheat was maintained within 10.0-15.0 °C above saturation (mean 12.0 ± 2.0 °C), preventing premature condensation while avoiding excessive overheating [1]. Endotoxin testing by LAL assay yielded non-detectable levels across all samples (n = 520), underscoring suitability for sterile applications in line with FDA guidance [10]. Collectively, these findings demonstrate that the pure steam system consistently met pharmacopeial and GMP requirements across all sampling points. The dataset presented here is novel, as published validation reports for pure steam systems remain limited compared to water systems. Recent studies have focused primarily on pharmaceutical water validation, with fewer detailed reports on steam quality parameters [8, 9]. By documenting IQ, OQ, PQ, and analytical results across multiple parameters, this study provides a robust reference for pharmaceutical manufacturers, supporting GMP compliance and offering practical guidance for audits and inspections. Beyond

initial qualification, maintaining the validated state is essential. Continuous monitoring of steam quality parameters, trending of analytical data, and preventive maintenance activities-such as inspection of steam traps and calibration of sensors-are critical to ensuring long-term reliability [10, 11]. Periodic requalification, as recommended by ISPE and PDA, reinforces system robustness and regulatory confidence [1, 11].

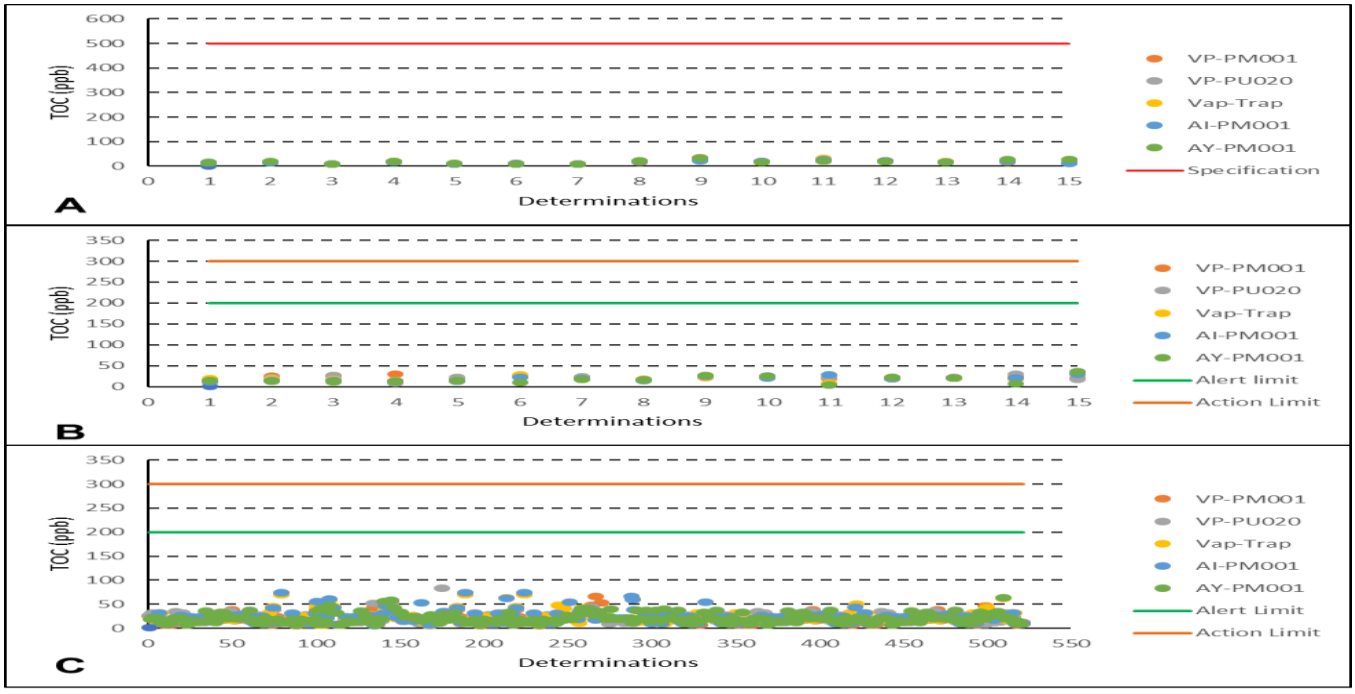

Figure 3: Total organic carbon data during the three validation stages. Phase 1 (A), phase 2 (B), and phase 3 (C)

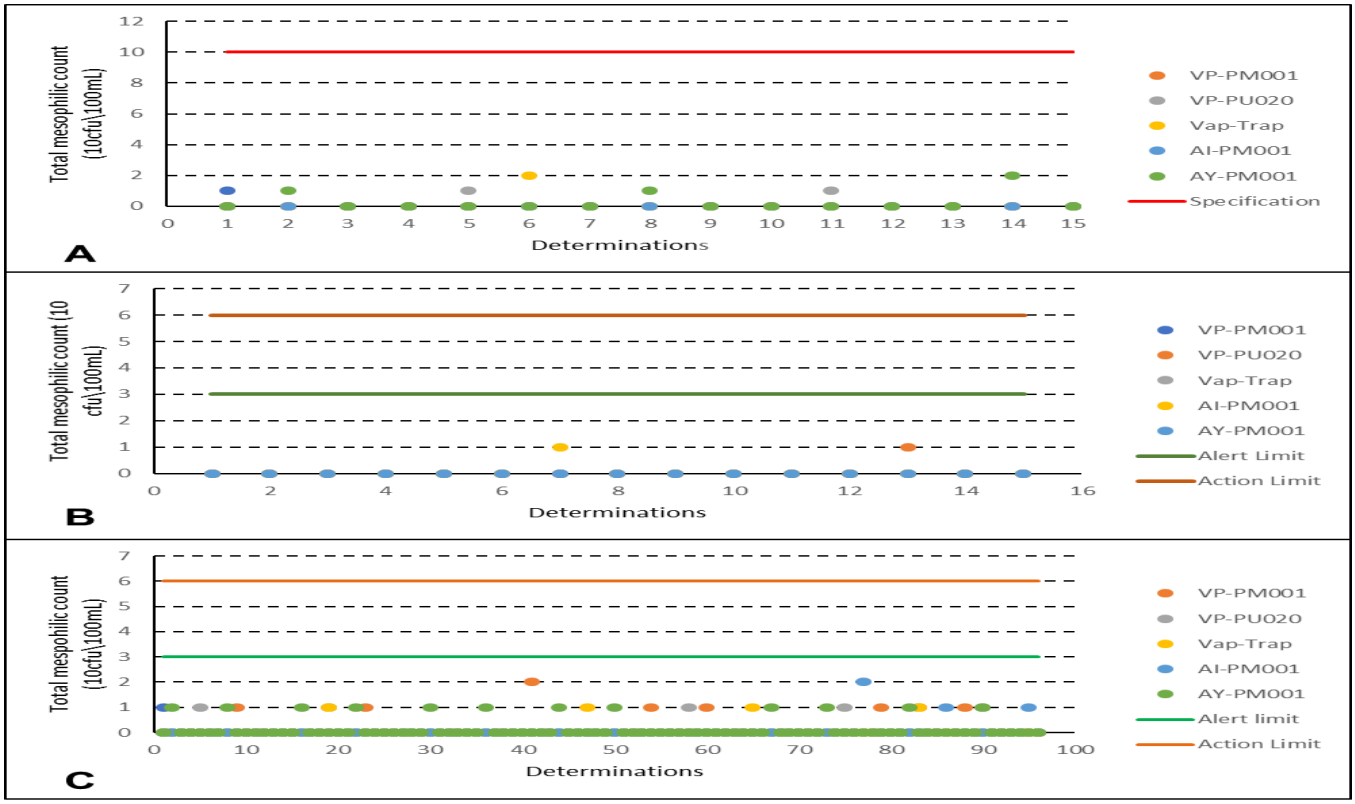

Figure 4: Microbiological data of the pure steam system during the three stages of validation. Phase 1 (A), phase 2 (B), and phase 3 (C)

Table 4: Steam quality parameters

Parameter	Acceptance criteria	Measured values
Non-condensable gases (%)	≤ 3.0%	1.5 - 2.0%
Dryness fraction	≥ 0.95	0.96 - 0.98
Superheat (°C above sat.)	≤ 25.0 °C	10.0 – 15.0 °C

Utilization of pure steam in autoclaves: qualification and validation of sterilization processes. Autoclaves are a cornerstone of sterile manufacturing, ensuring that materials, components, and equipment introduced into controlled environments meet stringent sterility requirements. The use of pure steam eliminates risks associated with chemical additives or contaminants, thereby safeguarding product integrity and patient safety [1]. Regulatory expectations emphasize that autoclave sterilization cycles must consistently achieve a Sterility Assurance Level (SAL) of 10^{-6}, corresponding to a probability of no more than one non-sterile unit in a million sterilized items [12]. EU GMP Annex 1 requires documented evidence of validated sterilization processes, including steam quality attributes and cycle reproducibility [13]. FDA guidance highlights the importance of biological indicator challenges, load pattern mapping, and verification of critical parameters such as temperature distribution and steam penetration [14]. The ISPE good practice guide reinforces a lifecycle approach to validation, integrating risk-based methodologies to account for worst-case scenarios and variability in load configurations [1, 15]. This includes continuous monitoring of steam quality and trending of cycle data to ensure long-term reliability. Validation results demonstrated that the autoclave consistently met critical parameters (**Table 5**). For each condition, three independent tests were conducted, and mean values are presented. Temperature uniformity was maintained within 121-123 °C (mean 121.3 °C ± 0.5 °C, n = 3), while pressure stability remained within ± 0.05 bar of the setpoint. Biological indicator challenges confirmed a ≥ 6 log reduction, achieving the required SAL of 10^{-6}. Steam quality attributes-including dryness fraction (0.96 - 0.98) and non-condensable gases (1.5 - 2.0%) - were within pharmacopeial limits, ensuring effective steam penetration. Condensate testing showed conductivity ≤ 1.0 µS/cm and endotoxins < 0.25 EU/mL, consistent with USP and FDA requirements.

The novelty of this dataset lies in its integration of IQ, OQ, PQ, and analytical results for pure steam autoclaves. While pharmaceutical water validation has been widely studied, detailed reports on autoclave steam quality remain limited [9, 10]. By providing quantitative descriptors from replicated experimental data, this study offers a robust reference for manufacturers, supporting GMP compliance and serving as practical guidance for audits and inspections. Beyond initial qualification, maintaining the validated state is essential. Continuous monitoring of steam quality parameters, trending of analytical data, and preventive maintenance, such as inspection of steam traps and sensor calibration, are critical to ensuring long-term reliability. Periodic requalification, as recommended by ISPE and PDA [1, 9, 10, 15], reinforces system robustness and regulatory confidence.

Table 5: Assessment of critical autoclave parameters against acceptance criteria

Parameters	Measured value	Acceptance criteria	Results
Chamber temp uniformity	121.3 °C ± 0.5	121 °C ± 1.0	Pass
Pressure stability	2.05 bar ± 0.02	2.0 bar ± 0.1	Pass
Steam penetration (BI challenge)	0/20 survivors	SAL 10^{-6} (no BI growth)	Pass
Dryness fraction	0.96	≥ 0.95	Pass
Non-condensable gases	2.50%	≤ 3%	Pass
Superheat	0.3 °C	≤ 1 °C	Pass
Load mapping (worst-case)	All probes within ±1 °C	±2 °C max deviation	Pass
Condensate conductivity	0.8 µS/cm	≤ 1.3 µS/cm	Pass
Endotoxin test	0.063 UE/mL	≤ 0.25 EU/mL	Pass

Conclusion: The validation of the pure steam system demonstrated consistent compliance with pharmacopeial and GMP requirements. Key analytical outcomes confirmed system robustness: Conductivity values ranged between 0.2-1.2 µS/cm (measured without temperature compensation, per USP <645>), total organic carbon median was 18.9 ppb, dryness fraction remained between 0.96-0.98, and endotoxins were non-detectable across all samples. These provide strong evidence of purity, microbiological safety, and steam quality attributes within regulatory limits. The novelty of this study lies in presenting an integrated dataset (IQ, OQ, PQ) and analytical validation of pure steam systems. While pharmaceutical water validation has been extensively documented, published reports on pure steam remain limited. This study addresses that gap by linking qualification protocols with analytical performance data, thereby contributing to regulatory science and supporting GMP compliance.

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