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HOLISTIC VALIDATION OF INTEGRATED PHARMACEUTICAL WATER SYSTEMS: FROM PRETREATMENT TO PURE STEAM AND PURIFIED WATER

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ABSTRACT

Pharmaceutical water systems are fundamental to product quality and patient safety, requiring rigorous validation across pretreatment, purified water, and pure steam subsystems. This study presents a holistic validation approach encompassing activated carbon filtration (ACF), reverse osmosis (RO), purified water (PW), pure steam, and condensate recovery, with extended determinations across multiple production cycles. Pretreatment with ACF and RO consistently removed chlorine (<0.1 mg/L), maintained hardness below 5 mg/L, and achieved conductivity values of 6–7 µS/cm with microbial counts <100 CFU/mL. These results confirm the protective role of ACF in safeguarding RO membranes and the reliability of RO as the cornerstone of pharmaceutical water purification. Pure steam (PS) met Water for Injection (WFI)-equivalent quality, with conductivity ≤1.0 µS/cm, endotoxin levels <0.05 EU/mL, TOC ≤185 ppb, and microbiological counts ≤3 CFU/100 mL. Purified water remained within pharmacopeial specifications throughout validation, with TOC and microbial monitoring reinforcing reproducibility under routine operating conditions. Condensate recovery consistently met PW specifications (conductivity ≤1.3 µS/cm, TOC ≤150 ppb, microbial counts <2 CFU/mL), demonstrating both compliance and sustainability benefits. Importantly, validation data confirmed stability across production cycles, highlighting condensate recovery as a robust and efficient component of the water purification framework. Overall, the integrated validation confirmed that pharmaceutical

water systems not only comply with USP and Ph. Eur. monographs but also demonstrate reproducibility across extended operation. The inclusion of condensate recovery as a validated source of PW represents a novel contribution, enhancing sustainability while maintaining GMP compliance. This holistic validation strengthens the evidence base for reliable and sustainable pharmaceutical water systems.

KEYWORDS: Pharmaceutical water systems, Pure steam validation, Purified water quality, Condensate recovery, Good Manufacturing Practices.

INTRODUCTION

Pharmaceutical water systems are essential utilities in drug manufacturing, directly influencing product quality, patient safety, and regulatory compliance. Current Good Manufacturing Practices (GMP) emphasize not only the validation of individual subsystems but also the holistic assessment of integrated water treatment processes.[1] Yet, most published studies remain focused on isolated components such as purified water (PW) or water for injection, leaving a notable gap in the literature regarding full-cycle system validation. Pretreatment stages are critical to downstream reliability. Activated carbon filtration (ACF) is widely applied to remove chlorine, chloramine, and organic impurities, thereby protecting reverse osmosis (RO) membranes from oxidative damage and ensuring consistent performance.[2] Building upon this, reverse osmosis is considered the cornerstone of pharmaceutical water purification, providing a robust barrier against dissolved salts, microorganisms, and endotoxins.[3] RO system failures may jeopardize entire production batches, underscoring their criticality within GMP frameworks.

Extending beyond purification, pure steam generation represents a vital utility for sterilization and clean-in-place operations. Pure steam must meet stringent quality attributes equivalent to WFI, with conductivity and endotoxin levels tightly controlled to comply with international pharmacopeias and GMP requirements.[4] When recovered under controlled conditions, pure steam condensate can be utilized as purified water, providing an efficient, GMP-compliant pathway for producing high-quality utility water.[5]

This integrated approach—from activated carbon pretreatment through reverse osmosis, pure steam generation, and condensate recovery—highlights the importance of validating water systems as complete entities. To date, no peer-reviewed studies have reported consolidated validation data across the entire pharmaceutical water cycle. By presenting such data within a

unified IQ/OQ/PQ framework, this work fills a critical gap in the literature and offers a replicable model for pharmaceutical engineering practice, aligning with WHO, EMA, and FDA guidelines on pharmaceutical water use.[6] By presenting consolidated validation data across the entire cycle, this work addresses a critical gap in the literature and provides a novel contribution to pharmaceutical engineering practice.

MATERIALS AND METHODS

Qualification Procedures (DQ, IQ, OQ, PQ)

The qualification of critical systems was conducted in four sequential stages: Design Qualification (DQ)**,** where the system design was assessed for compliance with regulatory requirements and user specifications; Installation Qualification (IQ)**,** which verified that all components were installed and calibrated according to approved documentation; Operational Qualification (OQ)**,** aimed at demonstrating consistent system performance under routine and stress conditions through functional testing, alarm verification, failure simulations, challenge tests, and recovery checks; and Performance Qualification (PQ)**,** which confirmed that the system, when operated under actual production conditions, consistently delivered results meeting predefined acceptance criteria. This comprehensive qualification procedure was performed to ensure reliability, regulatory compliance, and adherence to GMP**,** thereby confirming that the system was properly designed, correctly installed, robust in operation, and capable of sustaining performance in real-world use, safeguarding both data integrity and product quality.

The pharmaceutical water system subjected to validation was designed in accordance with international regulatory requirements, including the United States Pharmacopeia (USP), the European Medicines Agency (EMA), and the World Health Organization (WHO) guidelines for pharmaceutical-grade water. The system comprises a pretreatment stage and two critical production units.

The pretreatment stage integrates activated carbon filters (ACF) and a reverse osmosis (RO) unit, which collectively remove organic contaminants, residual chlorine, and dissolved ionic species, thereby producing demineralized water suitable for pharmaceutical applications. This pretreated water is subsequently directed to two downstream systems: a pure steam generator, intended to supply sterile steam for manufacturing processes, and a purified water system by condensation, which ensures continuous production of purified water meeting pharmacopeial specifications for conductivity, total organic carbon (TOC), endotoxin levels, and

microbiological quality. The configuration of the system, including the sequential arrangement of pretreatment and production units, is illustrated in Figure 1.

Activated Carbon Filtration (ACF)

The activated carbon stage was validated for its ability to remove free chlorine and to control microbial growth. Parameters monitored: free chlorine, TOC, and microbiological counts. Analytical method: free chlorine was determined using the DPD (N, N-diethyl-p-phenylenediamine) colorimetric method, in accordance with Standard Methods for Water Examination.[7] Acceptance criteria: microbial counts maintained at < 500 CFU/mL, consistent with potable water specifications.[8] This pretreatment step was essential to protect downstream reverse osmosis membranes from oxidative damage.

Reverse Osmosis (RO)

The RO subsystem was validated under "Demi water" conditions. Parameters monitored: conductivity, TOC, and microbiological counts. Acceptance criteria: conductivity ≤ 10 µS/cm and microbial counts < 100 CFU/mL.[2] The upstream ACF stage ensured chlorine removal, preventing membrane degradation and maintaining RO rejection efficiency.[9]

Regulatory Framework

All validation activities were conducted in alignment with current GMP guidelines (WHO, EMA, FDA) and ISPE Baseline Guide recommendations [10]. Sampling points were strategically located at subsystem outlets and at the final distribution loop. Analytical methods followed pharmacopeial standards (USP <643>, <645>, Ph. Eur. monographs).

The assays were conducted by the laboratories of the Quality Control Department (Free Chlorine Residual Test, Total hardness, total aerobic mesophilic count, presence of pathogens, conductivity, and presence of nitrates) and the Process Control Department (TOC and LAL). The alert, action, and specification limits for each of these quality attributes are shown in Table 1. The sampling points are described in Table 2.

Table 1: Tests and specification, alert, and action limits for each quality attribute evaluated in Water system.

Assay	Specification	Alert limit	Action Limit
Free Chlorine Residual Test	<0.1 mg\L	-	-
Total Hardness	< 5 mg\L	-	-
Conductivity	Current USP	-	-
TOC	≤ 500 ppb	200 ppb	300 ppb
Endotoxin (LAL)	≤ 0,25 UE/mL	0,1 UE/mL	0,2 UE/mL
Microbiology (PS)	≤ 10ufc/100 mL	3 ufc/100 mL	6 ufc/100 mL
Microbiology (PW)	≤ 100 ufc/mL	20ufc/mL	50 ufc/mL
Presence of pathogens	Absence/200 mL	-	-
Nitrate	< 0,2 mg/L	-	0,2 mg/L

Table 2: Code and location of the sampling points of the system during Performance Qualification the whole water system.

Process Step	Code	Location
Pretreatment	VL-PM001	FCA A Inlet
	VL-PM002	FCA A Outlet
	VL-PM003	FCA B Inlet
	VL-PM004	FCA B Outlet
	VL-PM005	RO Outlet
Pure Steam Generator	VL-PM007	GVP Sampling Point
Purified Water	AN-PM008	Condenser Outlet
	AN-PM009	Pump Outlet
	AN-PM010	Loop Return
	AN-PU001	Sink
	AN-PU002	Solution Preparation
	AN-PU003	Synthesis 2

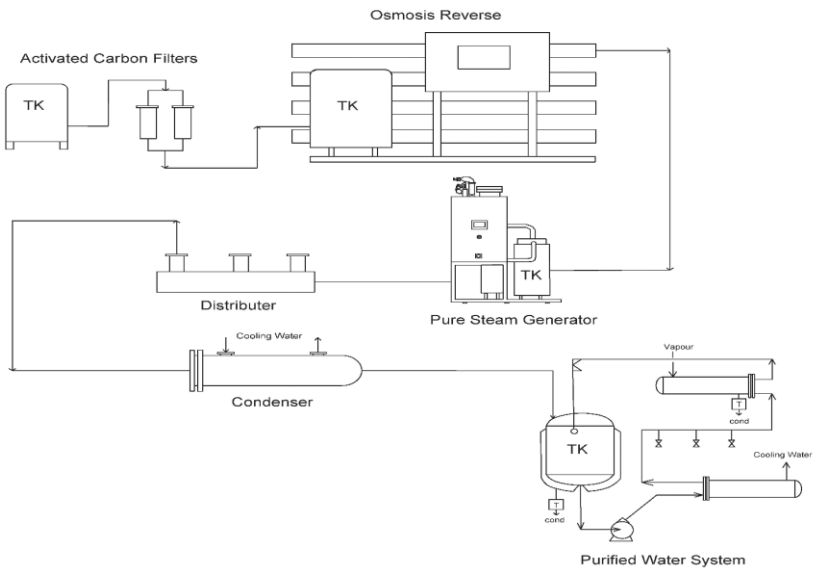

Figure 1: Illustration of the pretreatment, production and distribution system for Pure steam Generator and purified water system.

RESULTS AND DISCUSSION

Pretreatment: Activated Carbon Filtration (ACF) and Reverse Osmosis (RO)

Activated carbon filtration consistently demonstrated high efficacy in removing free chlorine, organic matter, and controlling microbial growth. Across 79 determinations, chlorine concentrations remained undetectable (<0.05 mg/L, DPD method), in full compliance with APHA recommendations.[7] The graphical data confirm that values were stable and consistently below the regulatory limit, underscoring the reliability of ACF in protecting downstream processes. Microbiological counts remained below the potable water threshold of 500 CFU/mL,[8] while total hardness was maintained under 5 mg/L, in accordance with manufacturer specifications (Figure 2). The tight clustering of hardness values across determinations highlights reproducibility in mineral removal, further validating the robustness of pretreatment. Reverse osmosis (RO) consistently achieved conductivity values of 6–7 µS/cm, well below the acceptance limit of 10 µS/cm, with microbial counts <100 CFU/mL.[2] These findings corroborate previous reports identifying RO as the cornerstone of pharmaceutical water purification.[9] The upstream integration of ACF proved critical, as chlorine breakthrough is known to compromise RO rejection efficiency and reduce membrane lifespan.[10] The graphical data reinforce this synergy: ACF safeguarded RO membranes by eliminating oxidative stressors, while RO provided the necessary conductivity and microbiological control for pharmaceutical-grade water.

Taken together, the combined pretreatment strategy of ACF and RO not only fulfilled regulatory and manufacturer specifications but also demonstrated consistency and reproducibility across multiple determinations. This stability reflects not merely compliance with GMP but the establishment of a validated, controlled process. The results emphasize the importance of sequential pretreatment, where ACF ensures membrane protection and RO guarantees water quality. This synergy underscores the robustness of the purification process and its critical role in holistic validation of pharmaceutical water systems.

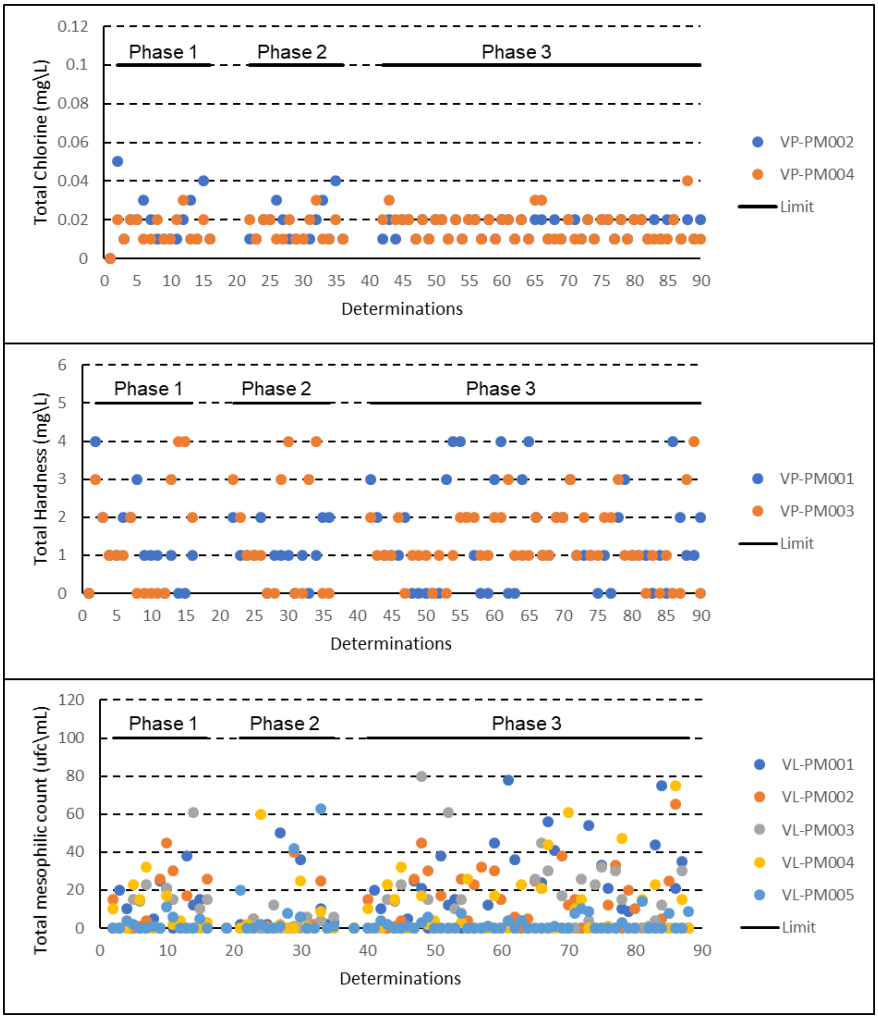

Figure 2: Results of free chlorine content, total hardness and microbiology in the pretreatment system.

Pharmaceutical Water Quality: Pure Steam and Purified Water

Pure steam consistently met pharmacopeial requirements equivalent to Water for Injection quality, with conductivity values ≤1.0 µS/cm, endotoxin levels <0.05 EU/mL, and non-condensable gases at 2.8% v/v. Total Organic Carbon (TOC) measurements across the three validation phases reached a maximum of 185 ppb, remaining below the alert limit for this quality attribute. Likewise, microbiological counts did not exceed the alert threshold of 3 CFU/100 mL. These findings confirm full compliance with USP and Ph. Eur. Monographs.[5,6] Purified water also demonstrated consistent quality, with parameters remaining within pharmacopeial specifications throughout validation. The integration of TOC monitoring and microbiological control provided additional assurance of system reliability and product safety. Comparable studies have reported similar steam quality; however, few have documented validation data across multiple production cycles. The inclusion of

longitudinal validation results in this work therefore represents a novel contribution, strengthening the evidence base for the robustness of pharmaceutical water systems (Table 3). Overall, the results highlight that both pure steam and purified water systems not only comply with international pharmacopeial standards but also demonstrate reproducibility across extended operation. This reinforces their critical role in ensuring product quality and patient safety within GMP.

Table 3: Results point VL-PM007 of pure steam generator.

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
	TOC (ppb)	15	15	5.3	35.6	179	-	-	500
	LAL (EU\mL	15	15	0.063	0.063	0.063	-	-	0.25
	Microbiology (10cfu/100mL)	15	15	0	0	2	-	-	10
2	Conductivity (µS/cm)	15	15	-	-	-	-	-	USP
	Nitrate (mg/L)	15	15	-	-	-	-	-	≤ 0.2
	TOC (ppb)	15	15	6.4	42.3	168	200	300	500
	LAL (EU\mL	15	15	0.063	0.063	0.063	0.1	0.2	0.25
	Microbiology (10cfu/100mL)	15	15	0	0	1	3	6	10
3	Conductivity (µS/cm)	104	104	-	-	-	-	-	USP
	Nitrate (mg/L)	104	104	-	-	-	-	-	≤ 0.2
	TOC (ppb)	104	104	4.8	38.7	185	200	300	500
	LAL (EU\mL	104	104	0.063	0.063	0.063	0.1	0.2	0.25
	Microbiology (10cfu/100mL)	104	104	0	0	2	3	6	10

Pharmaceutical Water Quality: Pure Steam

The validation of pure steam confirmed full compliance with pharmacopeial requirements equivalent to Water for Injection (WFI) quality. Conductivity values consistently remained ≤1.0 µS/cm, endotoxin levels were <0.05 EU/mL, and non-condensable gases measured 2.8% v/v. Total Organic Carbon (TOC) values across the three validation phases reached a maximum of 185 ppb, remaining below the alert limit of 200 ppb. Microbiological counts did not exceed 3 CFU/100 mL, well below the specification limit of 10 CFU/100 mL. These findings, supported by longitudinal data (Table 1), confirm reproducibility and robust compliance with USP and Ph. Eur. Monographs.[5,6] Comparable studies have emphasized that pure steam must consistently meet WFI-equivalent standards to ensure sterility assurance in pharmaceutical manufacturing. ISPE guidance highlights that steam quality directly impacts sterilization efficacy and equipment integrity, particularly in autoclaves and clean-in-place systems.[12] Our results align with these observations, demonstrating that the integration of TOC and microbiological monitoring provides additional assurance of system reliability.

Moreover, while previous publications have described validation strategies for purified water systems,[11] few have documented extended validation data across multiple production cycles. These results confirm the absence of contamination at the points of use and sampling with Gram-negative bacteria, as previously reported by Martínez et. al., 2004.[13] The inclusion of longitudinal validation phases in this work therefore represents a novel contribution, strengthening the evidence base for the robustness of pharmaceutical water systems.

Purified Water and Condensate Recovery

Purified water also demonstrated consistent quality, with all parameters remaining within pharmacopeial specifications throughout validation. The integration of TOC monitoring and microbiological control provided additional assurance of system reliability and product safety. This is consistent with literature emphasizing that validation must demonstrate reproducibility under routine operating conditions, not only during initial qualification.[14] By documenting performance across extended operation, our study provides evidence that purified water systems can maintain compliance and reliability over time, reinforcing their critical role in GMP frameworks. Condensate recovered from pure steam consistently met Purified Water (PW) specifications, with conductivity values ≤1.3 µS/cm, TOC ≤150 ppb, and microbial counts <2 CFU/mL. These results demonstrate that condensate recovery, when controlled under GMP conditions, can serve as a reliable and compliant source of PW. Importantly, validation data confirm that condensate recovery maintains stability across production cycles, reinforcing its robustness as part of the water purification framework.

The dual benefit of condensate recovery is noteworthy: not only does it provide water of pharmacopeial quality, but it also enhances sustainability by reducing overall water consumption in pharmaceutical operations. Previous publications have primarily focused on conventional PW systems alone.[11] In contrast, the integration of condensate recovery into a holistic validation strategy, as presented here, represents a novel contribution. By documenting performance across multiple validation phases and linking outcomes to GMP compliance, this study expands the evidence base for condensate recovery as a viable and efficient component of pharmaceutical water systems (Figures 2–4). No pathogenic microorganisms were detected in any of the samples analyzed.

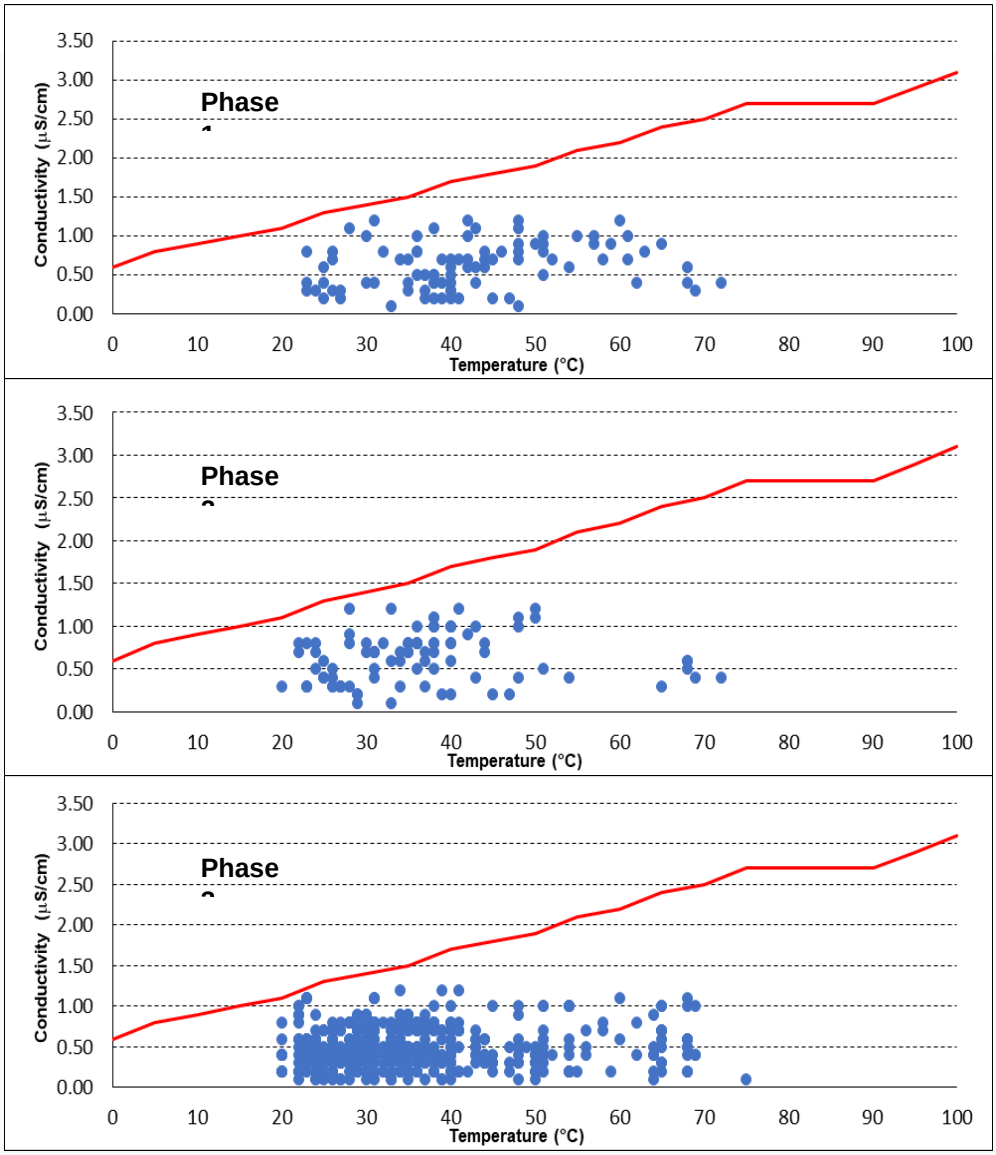

Figure 3: Results of the conductivity of the points of the purified water system during the three validation stages with the relationship between temperature and conductivity without temperature compensation.

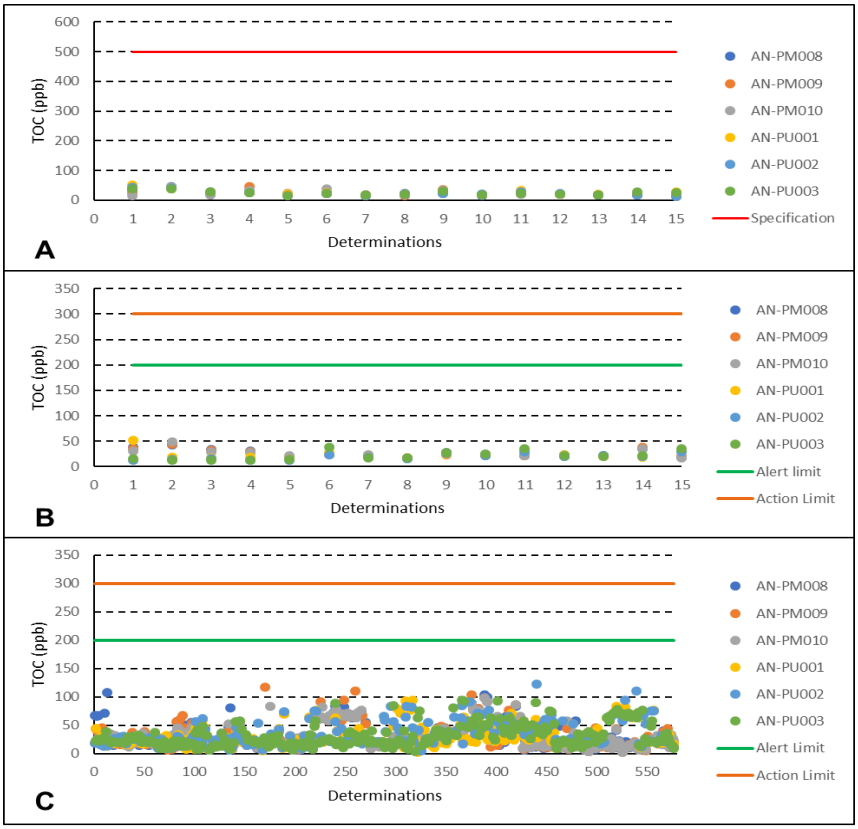

Figure 4: TOC results during the three validation stages. Phase 1(A), phase 2 (B) and phase 3 (C).

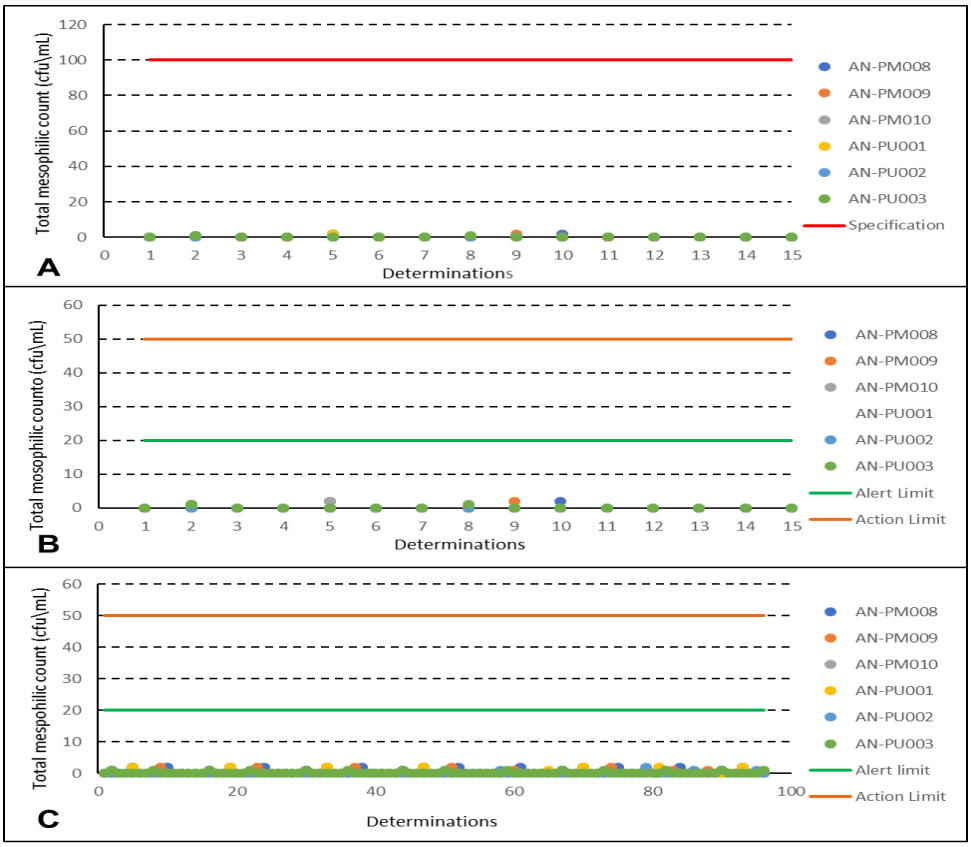

Figure 5: Microbiological results of the purified water system during the three stages of validation. Phase 1(A), phase 2 (B) and phase 3 (C).

Holistic Validation Perspective

The consolidated data confirm that each subsystem not only met its individual acceptance criteria but also contributed to the robustness of the entire cycle. Unlike prior studies that validated PW or WFI systems in isolation,[11,12] this work demonstrates the feasibility and regulatory relevance of validating a complete water system. Such an approach aligns with lifecycle management principles emphasized in current GMP guidelines,[1] offering stronger assurance for regulatory inspections and industrial practice.

CONCLUSIONS

The comprehensive validation of integrated pharmaceutical water systems—from pretreatment to pure steam, purified water, and condensate recovery—demonstrated consistent compliance with international pharmacopeial standards and regulatory expectations. Activated carbon filtration (ACF) and reverse osmosis (RO) provided a robust pretreatment strategy, ensuring protection of downstream processes and reproducibility across multiple determinations. Pure steam consistently met Water for Injection (WFI)-equivalent quality, with stable conductivity, TOC, endotoxin, and microbiological values, confirming its suitability for sterilization and aseptic manufacturing.

Purified water systems likewise maintained quality within pharmacopeial specifications, with longitudinal validation data reinforcing their reliability under routine operating conditions. Importantly, condensate recovery emerged as a validated and sustainable source of Purified Water (PW), meeting all quality attributes while reducing overall water consumption. This dual benefit highlights the potential of condensate recovery to enhance both compliance and environmental stewardship.

By integrating validation data across multiple production cycles, this study contributes novel evidence to the literature, addressing a gap in longitudinal assessments of pharmaceutical water systems. The findings underscore the critical role of holistic validation: rather than treating subsystems in isolation, a unified approach ensures system-wide reliability, regulatory compliance, and patient safety. Ultimately, the robustness and reproducibility demonstrated here reinforce the importance of water systems as a cornerstone of GMP and pharmaceutical quality assurance.

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Conflicts of Interest

The authors declare no conflicts of interest related to this study. The work was conducted independently, and no external funding or commercial sponsorship influenced the design, execution, or reporting of the validation results.

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