Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2015; 6(2); 54-72

ISSN 0975 9506

Research Article

Assessment of Purified Water Quality in Pharmaceutical Facility Using Six Sigma Tools

Eissa M.E.*, Seif M. , Fares M.

HIKMA Pharma pharmaceutical company, P.O. Box 1913, 2nd Industrial Zone, Plot no.1, 6th of October City, Cairo, Egypt.

Available Online: 16th April, 2015

ABSTRACT

The performance of water treatment plant for production of purified water is critical aspect in drug pharmaceutical manufacturing especially for semisolid and liquid products; where water represents the major bulk component. The water station investigated; herein, thermally and chemically sanitize city waterand is primarily made out of three major compartments: pretreatment unit, Reverse Osmosis-Electrodeinonizer (RO-EDI) compartment and distribution system. In order to assess the quality of different water treatment steps, water samples were taken from each processing stage in the water station and analyzed microbiologically using standard procedure. Conductivity and total organic carbon (TOC) measurements were assessed in samples of the final output waterat two points viz: after the tank of purified waterand at the return point of the distribution loop. Monitoring was conducted along a two years period of data gathering and was transformed logarithmically to approximate normality. Results were interpreted and analyzed using statistical software package and *Six Sigma* tools to identify the area of risk, processes that need improvement and control chart structuring for each stage. RO-EDI phase wasfound to be the most critical part, showing the greatest impact on the total performance of water station and need improvement. This partition contributed to 87% of the total defect based on*Cumulative Pareto* with rolled throughput yield (RTY) of 0.88. RO-EDI is sensitive to microbial burden and needs continuous monitoring and preventive measures especially during maintenance and shutdown intervals by using proper thermal sanitization at relatively higher rate than the other compartments.

Keywords: Purified water; Reverse Osmosis; Electrodeionizer; Conductivity; Total Organic Carbon; Six Sigma; Control Chart; Cumulative Pareto; Rolled Throughput Yield; Sanitization.

INTRODUCTION

Purified water for pharmaceutical manufacturing is a crucial component in several processes including bulk manufacturing of products.According to European Agency for the Evaluation of Medicinal Products[1], the use of purified water in no-sterile medicinal products includes nubeliser solutions, oral, cutaneous, nasal, ear, rectal and vaginal preparations in addition to isolation and purification of active pharmaceutical ingredients (APIs) during their manufacturing. Machine washing, initial and final rinse including Clean-In-Place (CIP) and cleaning and sanitization of clean area are conducted using water as vehicle.In addition, water is the most widely used substance, raw material or starting material in the production, processing and formulation of pharmaceutical productssuch as granulation, tablet coating and as a component in the formulation prior to non-sterile lyophilisation. Water sources and treated water should be monitored regularly for chemical and microbiological contamination. Thus, the performance of water purification, storage and distribution systems should be monitored on regular bases. Records of the monitoring results, trend analysis and any actions taken should be maintained[2].

Practically, purified water is used as an excipient in the production of official preparations and in pharmaceutical applications; therefore, it must meet the requirements for ionic and organic chemical purity and must be protected from microbial proliferation[3].It is prepared using Drinking Water as feed water and is purified using unit operations that include deionization,distillation,ion exchange,reverse osmosis, filtration or other suitable procedures, which necessitates the validationof Purified Water (PW) systems. Production, storageand circulation of water under ambient conditions in PW systems result probablyin the establishment of tenacious biofilms of microorganisms, which can be the source of undesirable levels of viable microorganisms in the effluent water. Therefore, these systems require frequent sanitization and microbiological monitoring in order to ensure the production of water of appropriate microbiological quality at the points of use[4].

Implementation of statistical and analytical tools to monitor the pharmaceutical water processing plants is very important to control them and make any improvements. Six Sigma is one of the systematic development and combination proven tools and methods that are applied for improving processes. By applying such a tool, emphasis is placed on the consistent orientation to customer

*Author for Correspondence

requirements and a concept of quality that integrates the "benefit" for the stakeholders. Interestingly, Six Sigma is applicable in every industry and service branch and is broadly accepted on capital and labor markets. Six Sigma shows that the demands to enhance quality and simultaneously reduce costsmust not contradicteach other[5].

The aim of the current study is to develop a system for continuous monitoring and improvement of water treatment plant by applying Six Sigma and other statistical tools using statistical software package. Fulfillment of such aim would ensure better control on the purified water quality used in pharmaceutical products manufacturing and minimize the risk of microbiological excursions as a part of a quality control program.

MATERIAL AND METHODS

The investigated system

The system for water purification is intended to remove organic substances, ions and some microorganisms. The station for production of purified water (PW)under investigation;herein, is thermally sanitized with hot water up to 80°C and composed of the following stages (based on the actual and documented design of the manufacturer):

Pretreatment Unit (PRT)

*Feed inlet valve**: for passage of the incoming raw city water.

Sodium hypochlorite (NaOCl) dosing station: for the control of Chlorine level in the city water.

Backwashable Multimedia filters (MMF):in stainless steel housing with 3 different layers of mineral sands.

*Double filtration (80/50 µm) cartridges unit**.

*Cationic double softener unit**: working alternatively and each is supplied with a dosing station of Brine for automatic regeneration of the resin.The unit is followed by hardness analyzer.

Sodium metabisulphite dosing unit (SMB): for the neutralization of free Chlorine.

Stainless steel Softened water tank 2000L: for the storage of water from softener, second pass RO and EDI.

Reverse Osmosis-Electrodeionizer (RO-EDI) unit

*Plate heat exchanger**: for temperature control and raising temperature for sanitization.

Sodium hydroxide dosing station (NaOH): pH control of water before entering to RO.

*Double filtration (25/5 µm) cartridges unit**.

*SMB dosing unit**: to ensure the freedom of inlet water to RO unit from Chlorine.

*First and second pass RO unit**: both in stainless steel pressure vessel.

*EDI unit**: combines electrodialysis and mixed bed ion exchange system in one module.

*Ultraviolet disinfection unit (UV)**: composed of 6 UV lamps with indicator for working hours and intensity.

III-PW storage and distribution system

*PW storage tank 6000L**.

Double tube sheet heat exchanger.

PW cold loop: with 10 use points (UPs).*

The Investigated Parameters

Conductivity at 25°C and total organic carbon (TOC) were measured at both PW storage tank and after the return of the loop online using temperature compensated transmitter and TOC analyzer, respectively. Microbiological water analysis was performed using conventional method described in an earlier study [6] and the sampling points are denoted by asterisks (*) in the previously described water station. The study period was conducted for two years from June 2012 to June 2014.

Data Analysis

Drinking water (potable water) has a requirement for a bioburden level of not more than (NMT) 500 CFU/mL [7]. As for PW, the compendia recommend an action limit of NMT 100 CFU/mL [3]. Gathered results were Log_{10} transformed as described by other researchers [8] for further statistical processing. A comparison was performed between raw and transformed data to measure the improvement in data normalization. Statistical analysis was conducted using GraphPad Prism® v6.01 for Windows and control charts and other Six Sigma tools were performed using Minitab® v17.1.0 while other calculations were carried on using Microsoft Office Excel 2007. It should be noted that only the pattern of data above the control limit (CL) is of interest in the current study since the acceptance criteria of results should not exceed certain values but there are no lower limits.An overall capability index (Cpm), a measures of potential process capability (Cpk) and a measure of overall process capability (Ppk) benchmark value of 1.33was used as a reference value in many industries. Points that are highlighted in red in control charts generated by Minitab® indicate out of control conditions that could be interpreted as follows:

Abnormal freak due to extraneous causes: Single point more than 3σ from the center line.

A shift in the process mean: 6 to 9 points in a row on the same side of the center line.

Trend either improving or deteriorating due to operators'change in skills, maintenance or machine wearing: 6 points in a row, all increasing or all decreasing.

*Homogeneous subgroups, dissimilar stratification or reciprocating factors affecting the system:*14 points in one row, oscillating up and down.

5-and 6- *Early warning of potential process drift*: 2 out of 3 or 4 out of 5 points more than 2σ and 1σ respectively from the center line.

RESULTS

Results of the processes monitoring at all stages of water treatment are presented graphically in figures from (1) to (8) showing Individual-Moving Range (I-MR) control charts, a run chart to look for evidence of patterns in the last 25 observations. In addition to a probability plot (PP or QQ) to verify thedegree of fitness of data to the chosen distribution, capability histogram and capability plot to visually compare the distribution of data from water treatment processes to the specification spread. It also includes the capability statistics to assess the capability of eachof the processing stages quantitatively.Outliers are identified graphically by Boxplot by labeling the observations that are at least 1.5 times the interquartile

range (Q3 – Q1) from the edge of the box as shown in Figure (9) for raw income water, before 25 µm filter, after SMB injection, second pass RO and EDI. Sample weight as fraction of the total samples in relation to time is indicated in Figure (10) showing that in about half the period of the study more than 80% of data were centered for city water then declined gradually in PRT unit till reaching about 50% for the other preceding starting from heat exchanger point.Furthermore, Figure (11) is showing graphical presentation of different processing stages of water in relation with time and the general trend during 2 years of the study. Cumulative Pareto Chart presented in Figure (12) demonstrates that 87% of the microbiological defect was a resultof RO-EDI compartment contamination with EDI unit contributing alone for 37% of the excursions while 2% only resulted from the loop and distribution system but PRT accounted for 11% of the defects.On the other hand, process performance metrics shown in Table (1) confirmed the results obtained from cumulative Pareto chart in terms of defects and throughputs. One-Way ANOVA analysis in Table (2) showed that microbial count was raised significantly after 50 µm filter till reaching highest counts in RO-EDI compartment which declined significantly after UV unit.The relatedness of processing stages microbiologically is illustrated in Table (3) showing an interesting strong correlation between the seasonal temperature variation and bioburden of storage tank. Similarly, the same observation was recorded for pooled softner output with heat exchanger and before 25 µm filter. The process of data transformation had improved normalization of the results. This finding is illustrated in Tables (4), (5) and (6).

DISCUSSION

Wateristhemostwidelyusedingredientinpharmaceuticalma nufacturingandthebasic component required for equipment and system cleaning. However, controlling its microbial quality is difficult being obtained from either municipal or non-municipal water systems, which represents the major exogenous source of microbial contamination of pharmaceutical waters. It is estimated that there are70 different types of bacteria in waste water [9]. Interestingly, several different types of microbes cancross watertreatment barriers and are found in pharmaceutical waters [10]. It is therefore not surprising that proper control and monitoring of income water as raw material are crucial to ensure acceptable quality of the produced water.

The observed low values of performance indices below the reference acceptable value (1.33) are indicative that microbiological qualities of water at different stages are in a state of "out-of-control". By reviewing the microbiological defenses of the water station, it was found that at the PRT partition the first protecting mechanism was not adequate since the antimicrobial activity of Chlorine was set at about 600 mV measured by oxidation reduction potential (ORP) sensor while it is recommended to be 800 mV by the manufacturer. Moreover,an earlier study [11] demonstrated that the microbial survival declined significantly when ORP increased from below 620 mV to more than 665 mV. For instance *Listeria monocytogenes* survival time significantly reduced by more than 10 times, *Salmonella* spp. dramatically by more than 15 times and interestingly more than 5760 times for thermo-tolerant coliform. In addition RO membrane that was faced with low quality water provides good media for microbial colonization and possible biofilm (known as membrane fouling) formation as warned by the manufacturer.

The second defense system against microbial contaminationis the UV lamp control after EDI unit in RO-EDI compartment. Although the 6 UV lamps worked and maintained appropriately during the period of study, yet it masked the hidden defect as it disinfected water terminally before its passage to the distribution and storage system.

Points of weakness in water station must be considered as they could contribute to failure in water processing. Perforated heat exchangers can also lead to direct contamination of the water system. The FDA technical guide, Heat Exchangers to Avoid Contamination, discusses the design and potential problems associated with heatexchangers[12]. The guide references two main methods for preventing water contamination by leakage: (a) provide gauges to constantly monitor pressure differentials in order to ensure that the higher pressure is always on the clean fluid side and (b) utilize the double-tube sheet type heat exchanger. Also,as a preventive measure, the FDA recommends that heat exchangers must not be drained of the cooling water when not in use to prevent pin holes formation in the tubing after they are drained as a result of corrosion of the stainless steel tubes in the presence of moisture and air. As stated by WHO [2], ambient-temperature systems such as ion exchange, RO and ultrafiltrationare especially susceptible to microbiological contamination, particularly when equipment is static during periods of no or low demand for water. It is essential to consider the mechanisms for microbiological control and sanitization.Special care should be taken to control microbiological contamination of sand filters, carbon beds and water softeners. Once microorganisms have infected a system, the contamination can rapidly form biofilms and spread throughout the system. Thus unacceptable level of PRT unit may be viewed as the source of the low efficacy of RO-EDI partition in the current case Techniques for controlling contamination such as back-flushing, chemical and/or thermal sanitization and frequent regeneration should be considered as appropriate.

The term "Normal distribution approach" has been describedin The PDA Technical Report [13] as a method that calculates the alert level as the mean plus twice the standard deviation (2SD), and the action level as the mean plus three times the standard deviation (3SD) of a population of data points. This method suits a population with high microbial counts best.In the current study, thiswas applicable for purified water where relatively high counts are expected. However, microbial population is not normally distributed [14] so the logarithm transformation to the base 10 for microbial count improved the normalization process [15]. In such case, alert and action levels could be calculated from the control charts provided

that continual trending of data are ensured, updated values of the levels could be calculated. An earlier study [14]demonstrated that microbial count increased in summer months in PW station. This was in agreement with the finding in the current study which showed that microbial count in the pooled purified water in storage tank is strongly correlated with seasonal temperature variation. Most other significant correlations are normally expected since they were found in the related sequential processing stages.

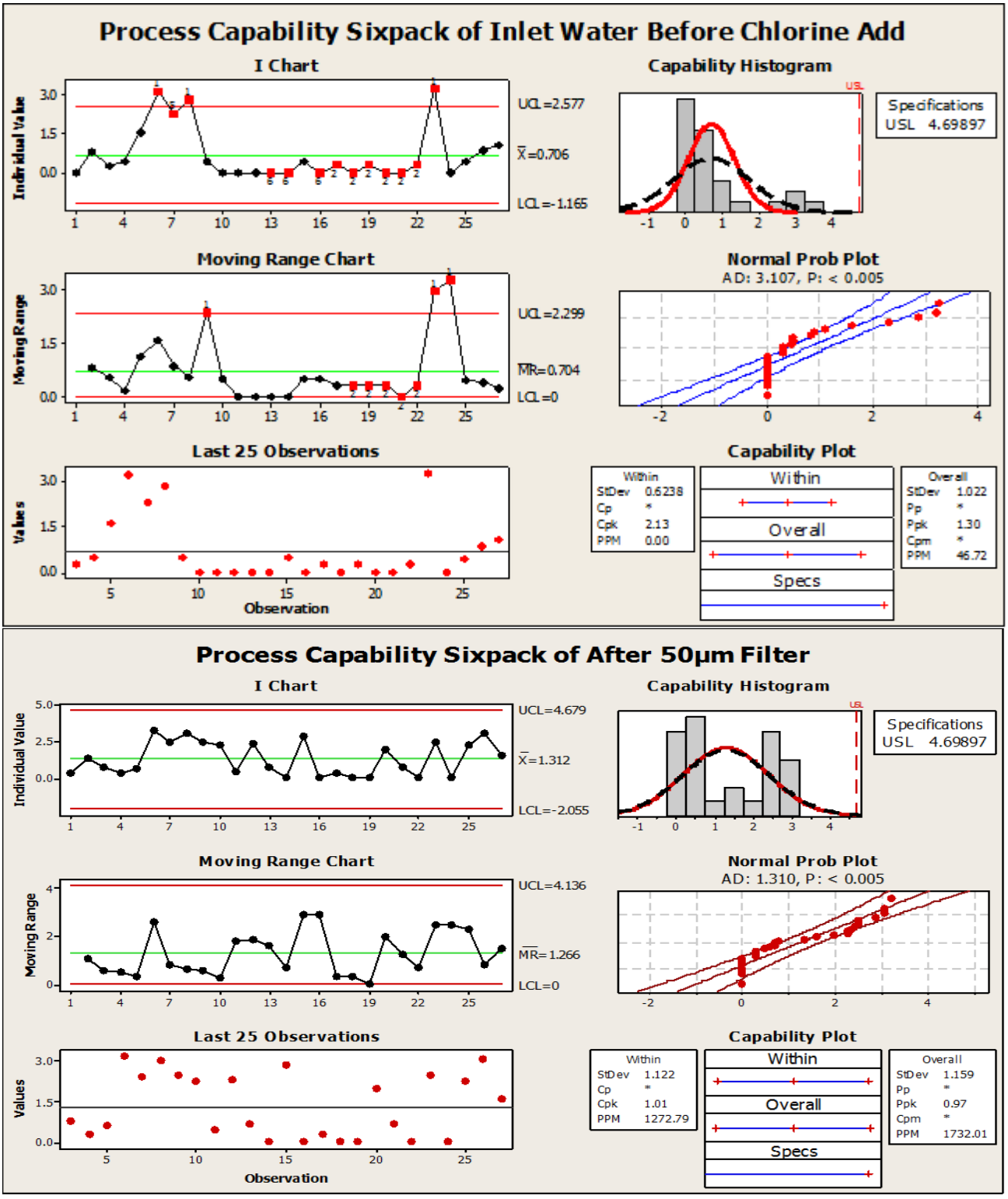

Figure 1: Capability sixpack using quality tools in Minitab® v17.1.0for Log_{10} transformed microbiological count of both city water before NaOCl injection and after 50 µm filter cartridge in the PRT unit to approximate the normal distribution

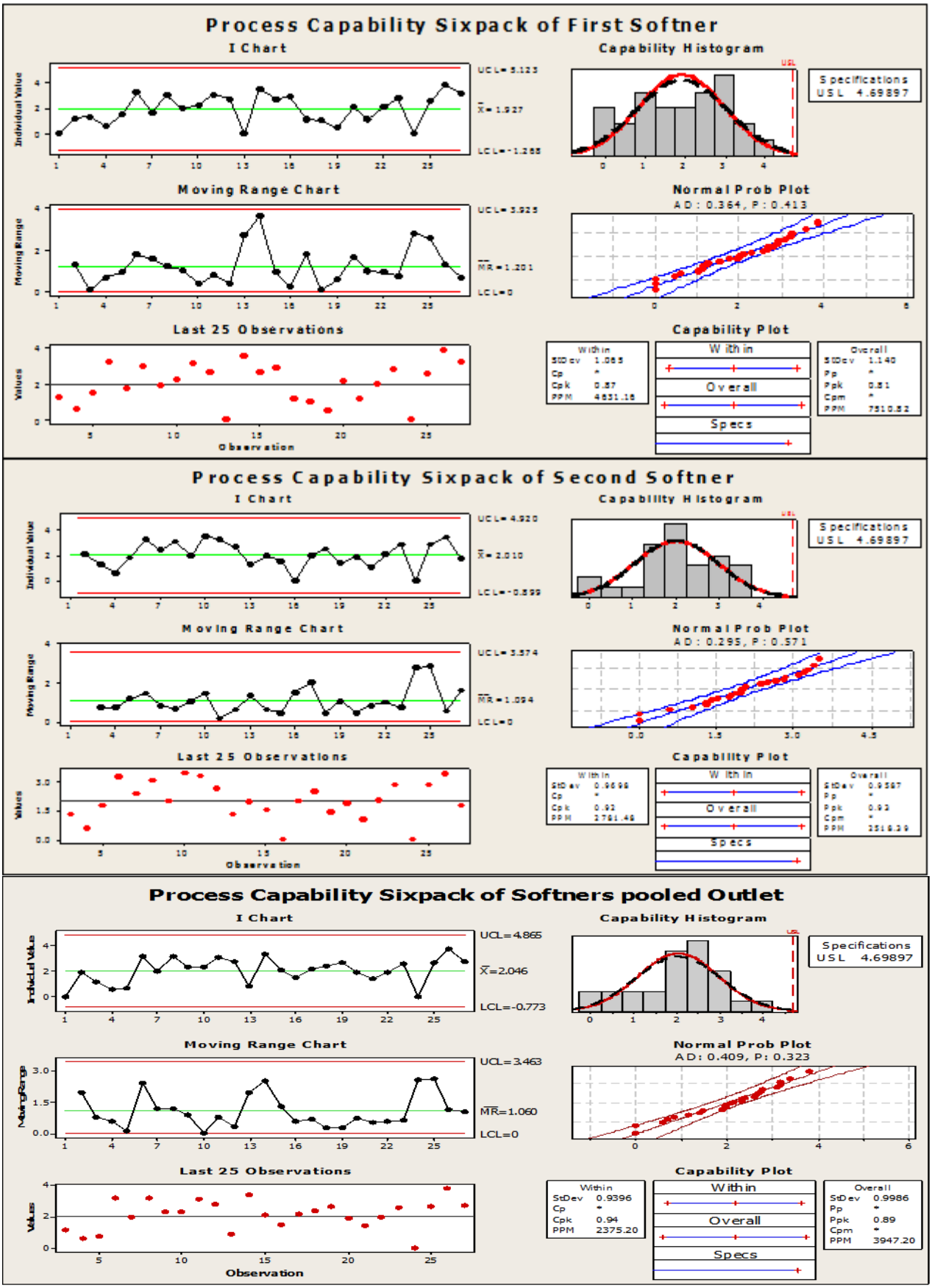

Figure 2: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed microbiological count of the first and second softeners and the pooled softener output water in the PRT unit to approximate the normal distribution.

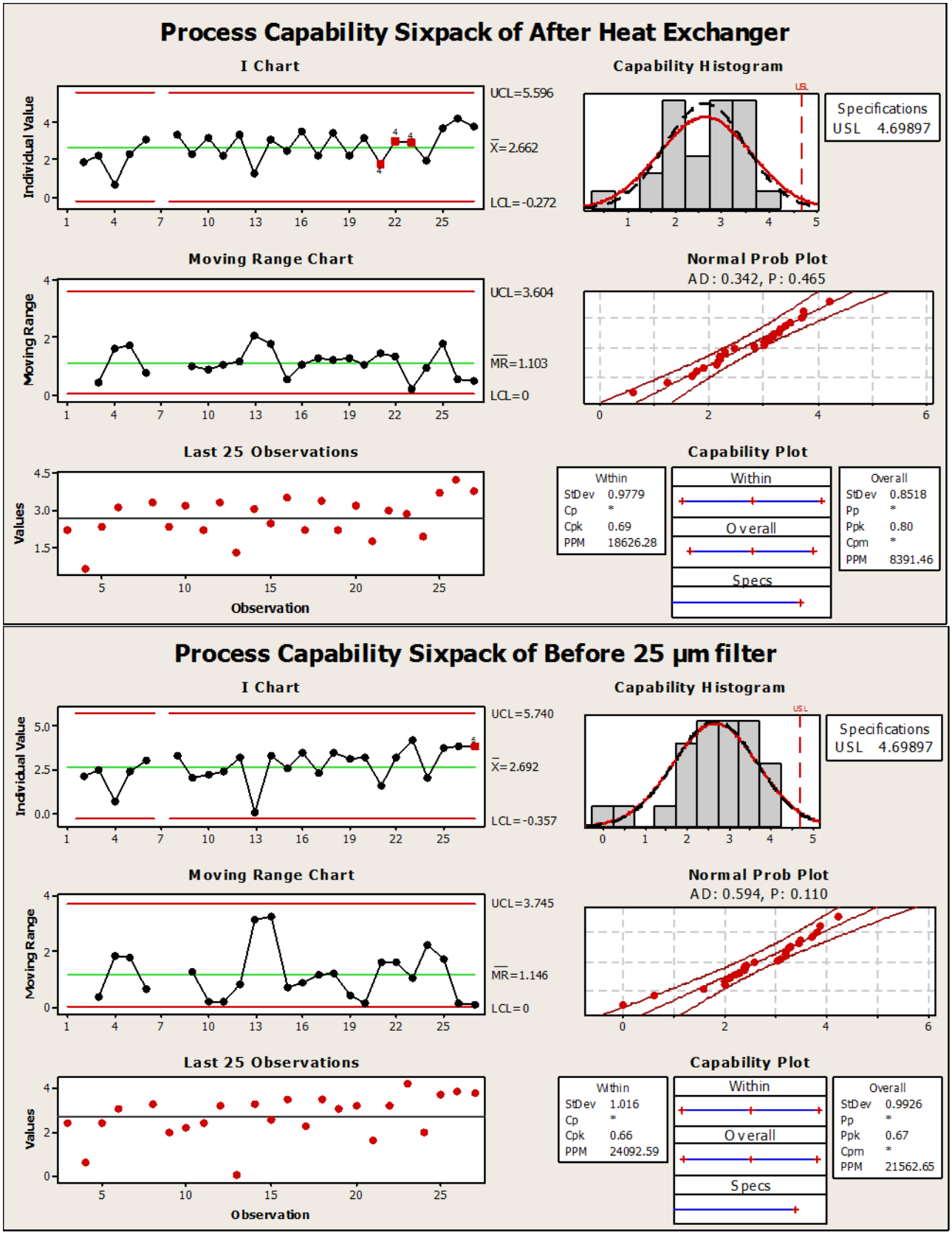

Figure 3: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed microbiological count of after heat exchanger point and before 25µm cartridge filter in the RO-EDI partition to approximate the normal distribution. N.B. I-MR charts of the points after heat exchanger and before 25 µm filter were interrupted due to missing one point within regular schedule.

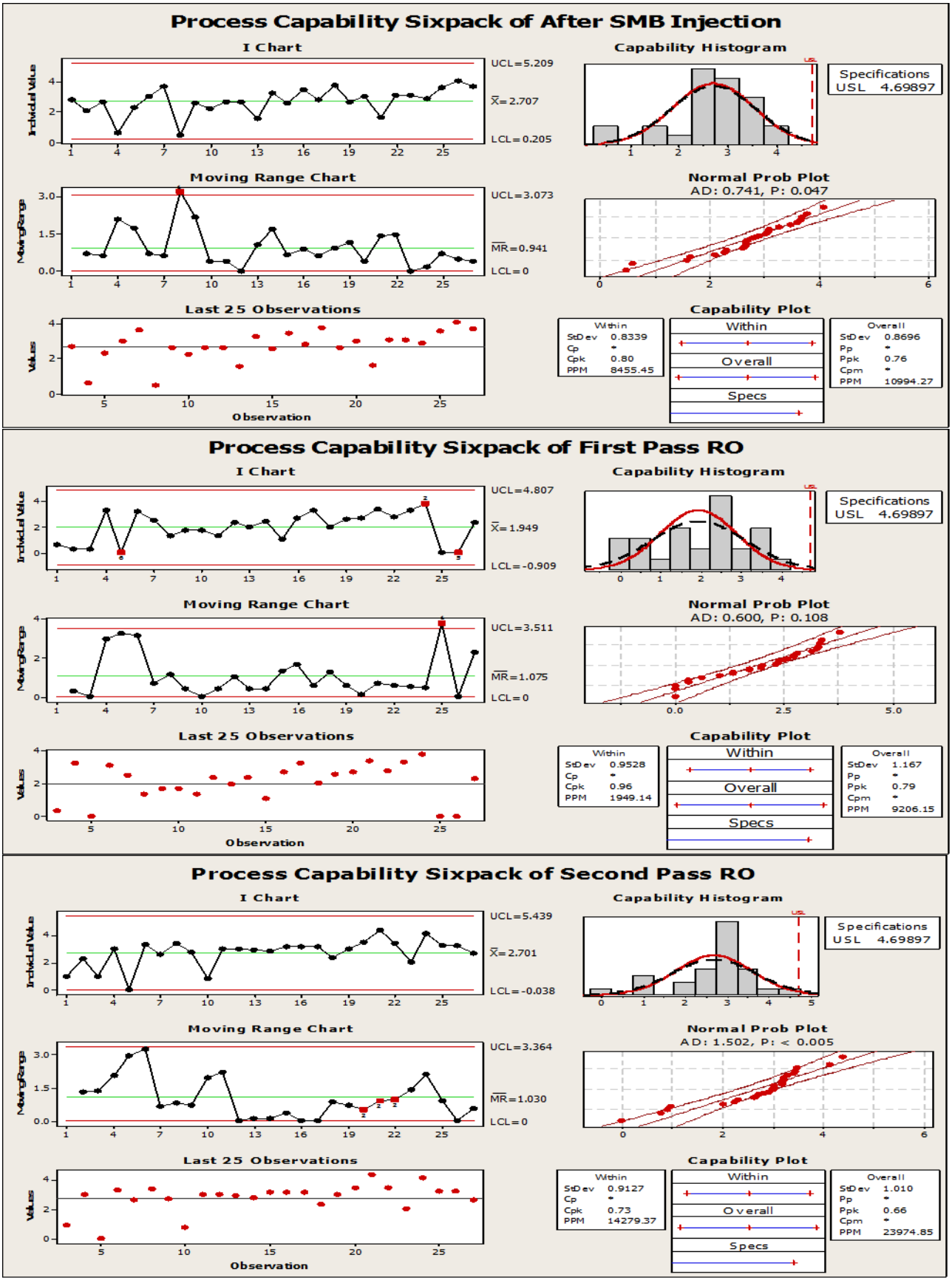

Figure 4: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed microbiological count of point after SMB injection and the first and second pass ROs units in the RO-EDI partition to approximate the normal distribution.

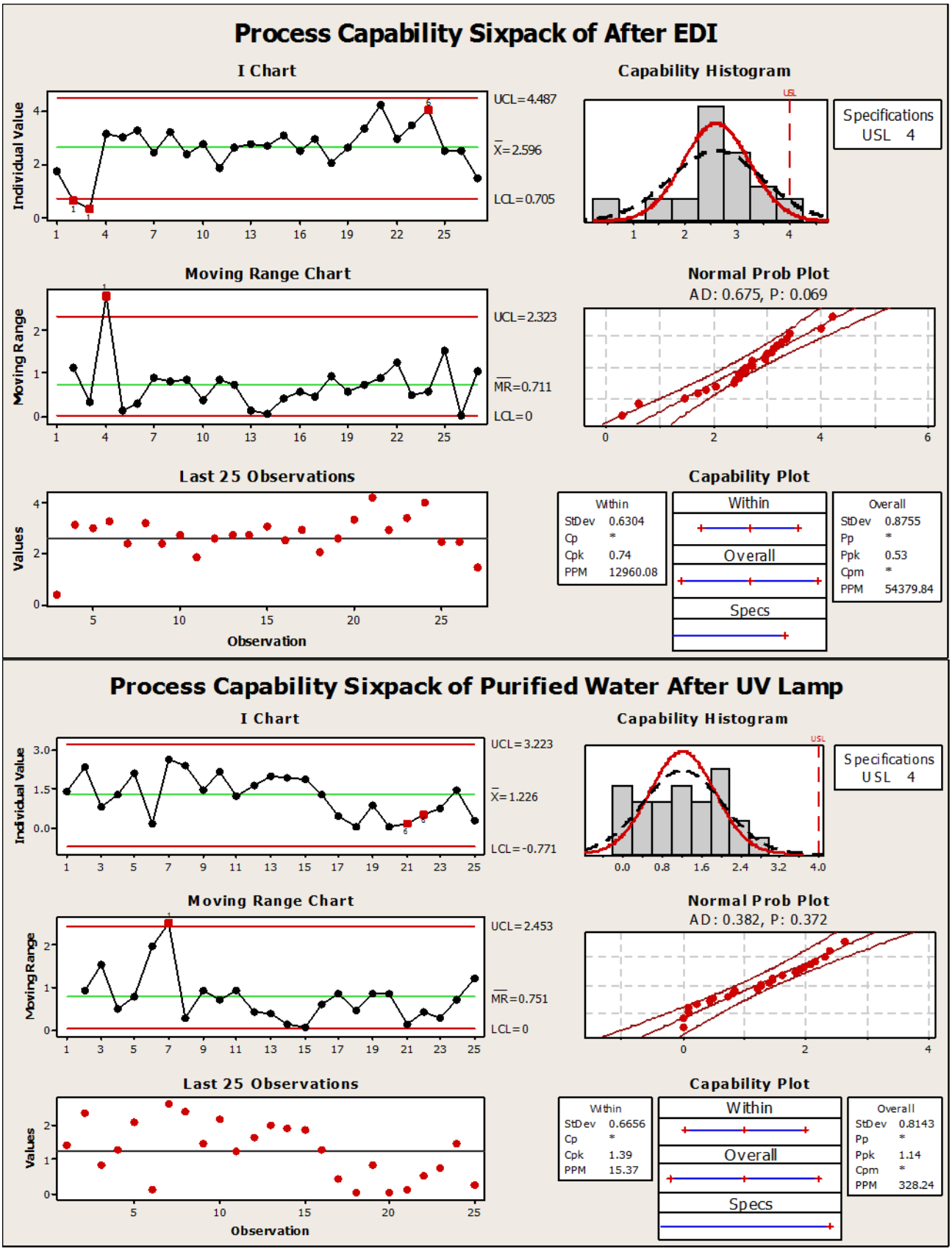

Figure 5: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed microbiological count of point next to EDI unit and after UV station in the RO-EDI partition to approximate the normal distribution

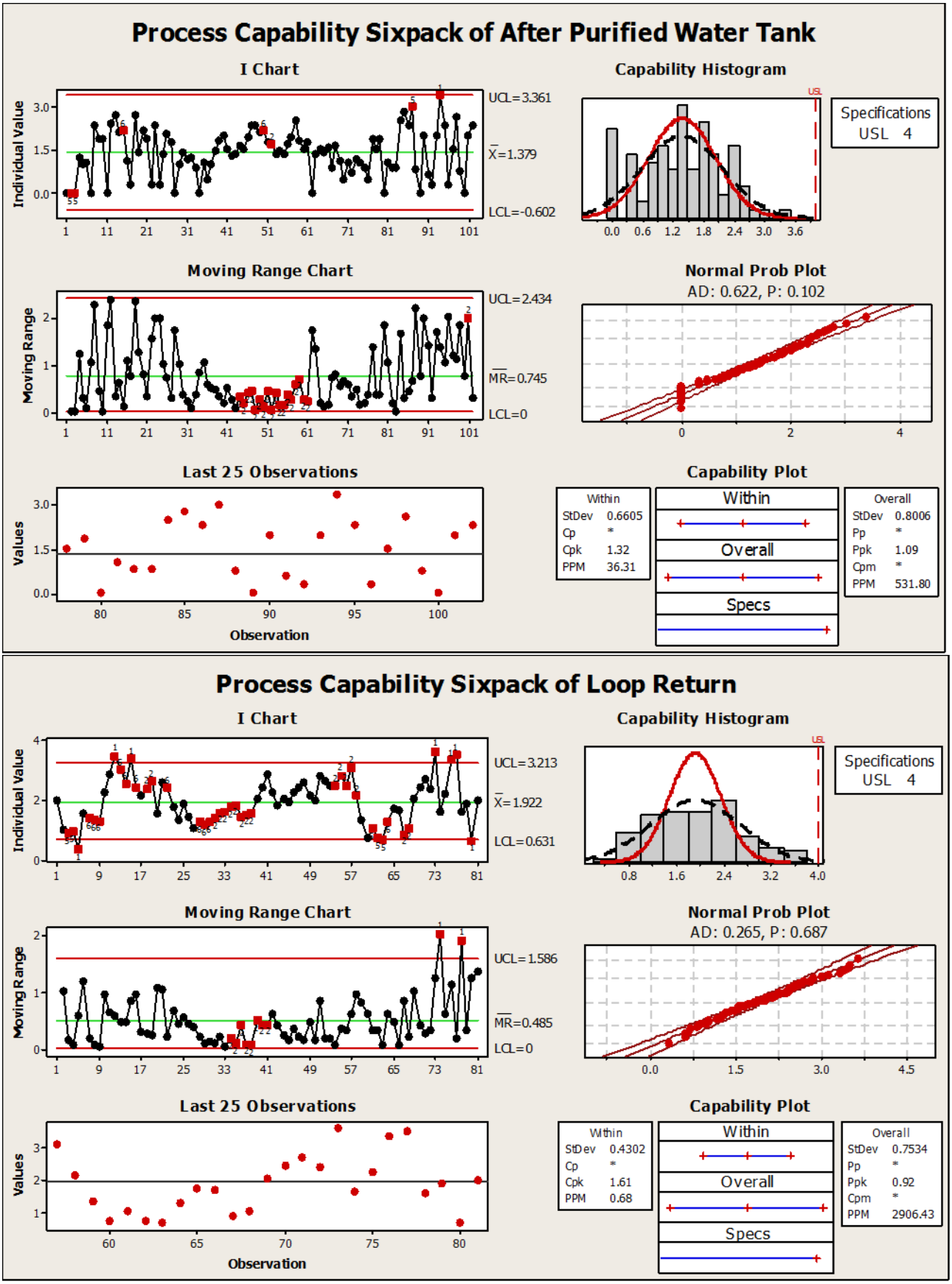

Figure 6: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed microbiological count of point next purified water tank and after the return of the loop in the PW storage and distribution loop system to approximate the normal distribution.

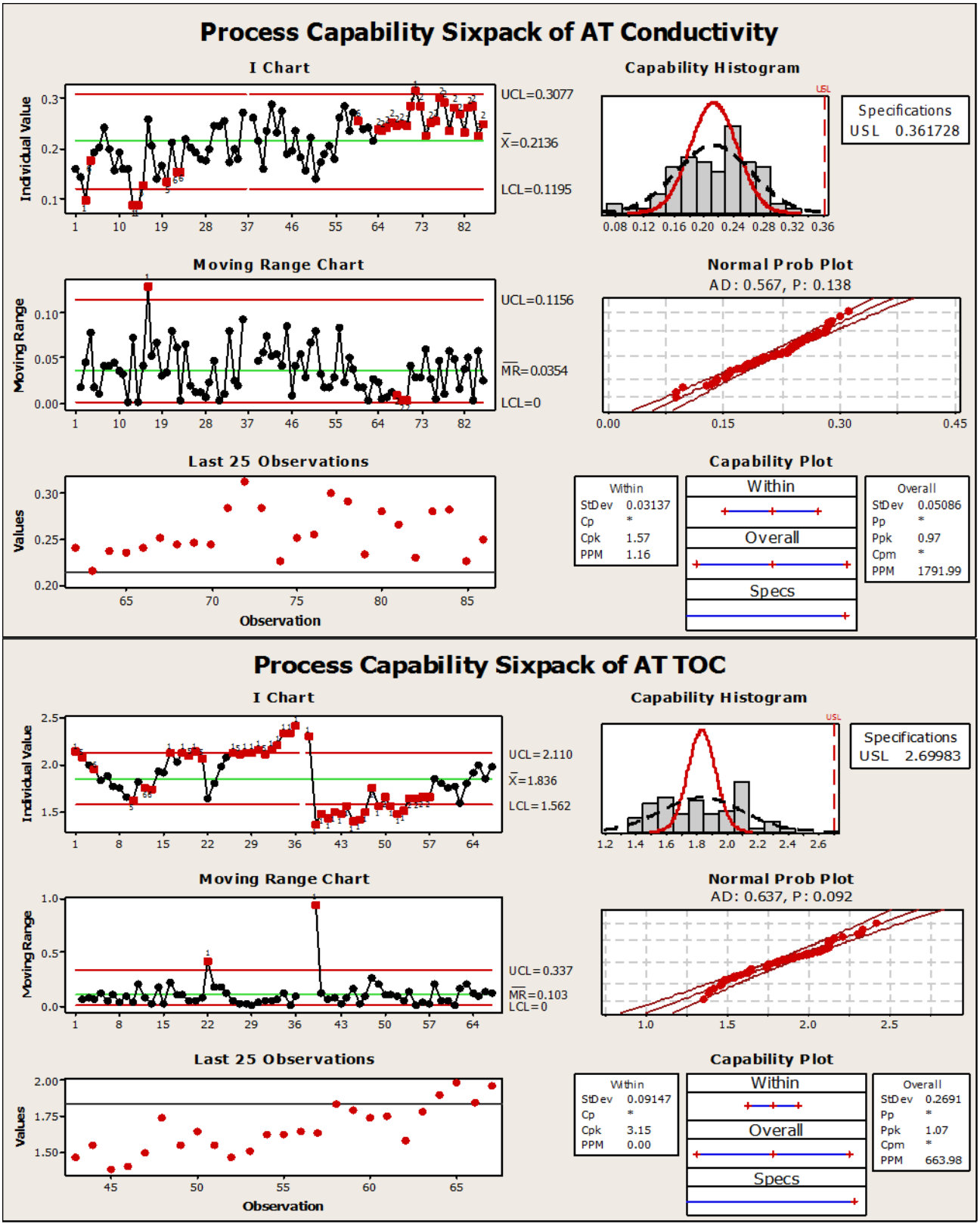

Figure 7: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed data of conductivity at 25°C and TOC of after purified water storage tank (AT) point in the PW storage and distribution loop system to approximate the normal distribution. N.B. I-MR charts of conductivity and TOC of AT point were interrupted due to missing one point within regular schedule.

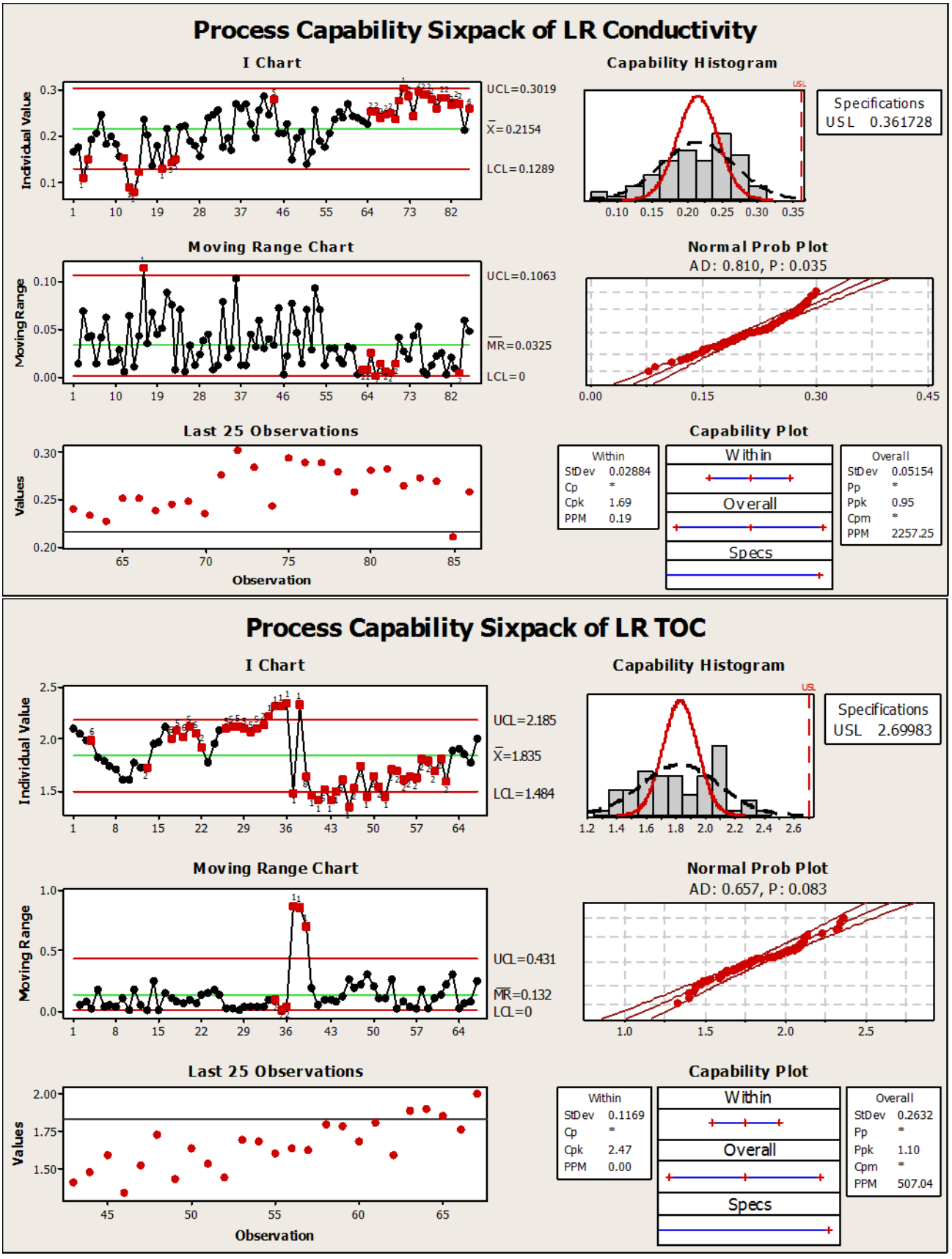

Figure 8: Capability sixpack using quality tools in Minitab® v17.1.0 for Log_{10} transformed data of conductivity at 25°C and TOC of the loop return (LR) point in the PW storage and distribution loop system to approximate the normal distribution.

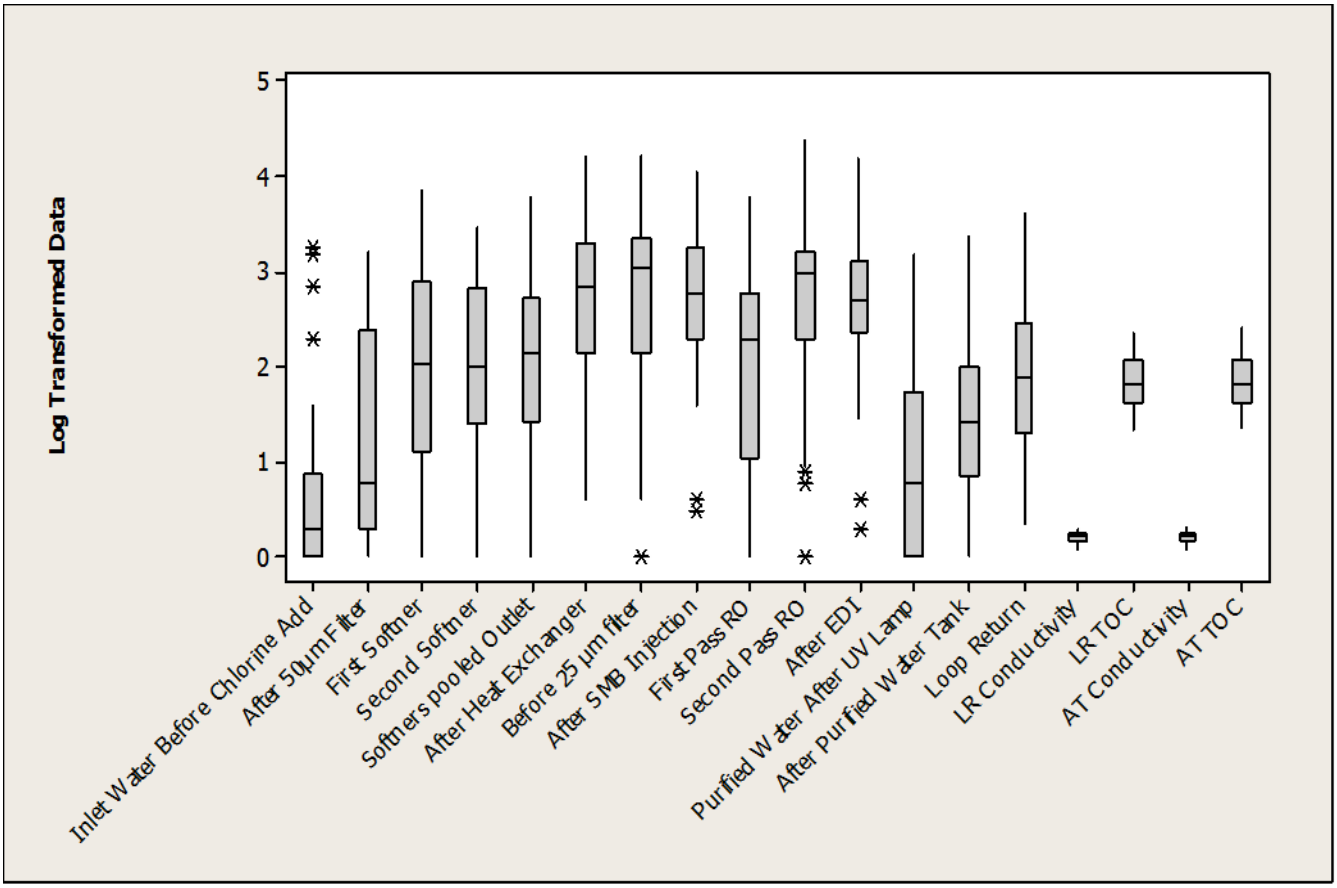

Figure 9:Box-and-Whisker diagram with asterisks (*) representing outlier values, spacing between the different parts of the box indicates the degree of dispersion and skewness in the data and whiskers indicating variability outside the upper and lower quartiles. (Graph was generated using Minitab® v17.1.0)

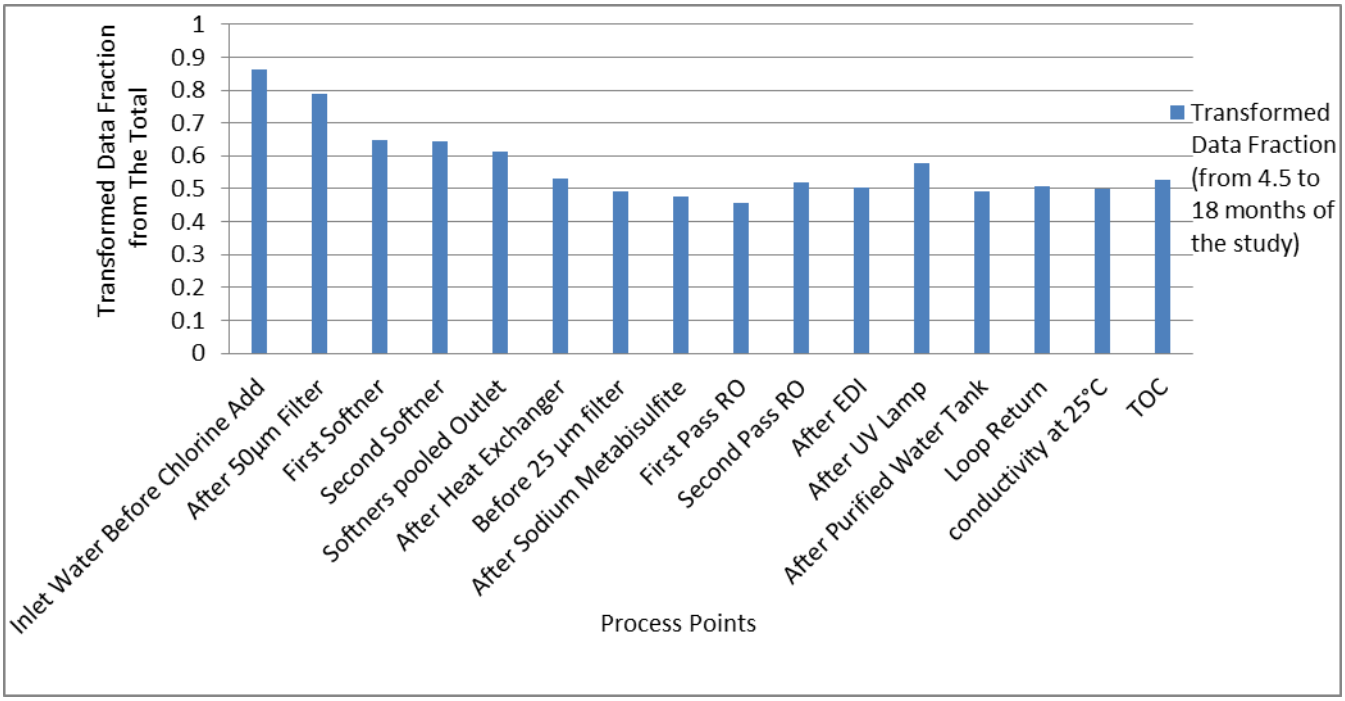

Figure 10: Weight of the fractions of transformed data in about one year period from the total of those gathered during two years period. The period covers time range duration from about 4.5 to 18 months from the study initiation. (Selection of the fraction-time period was based on GraphPad Prism® v6.01 for Windows)

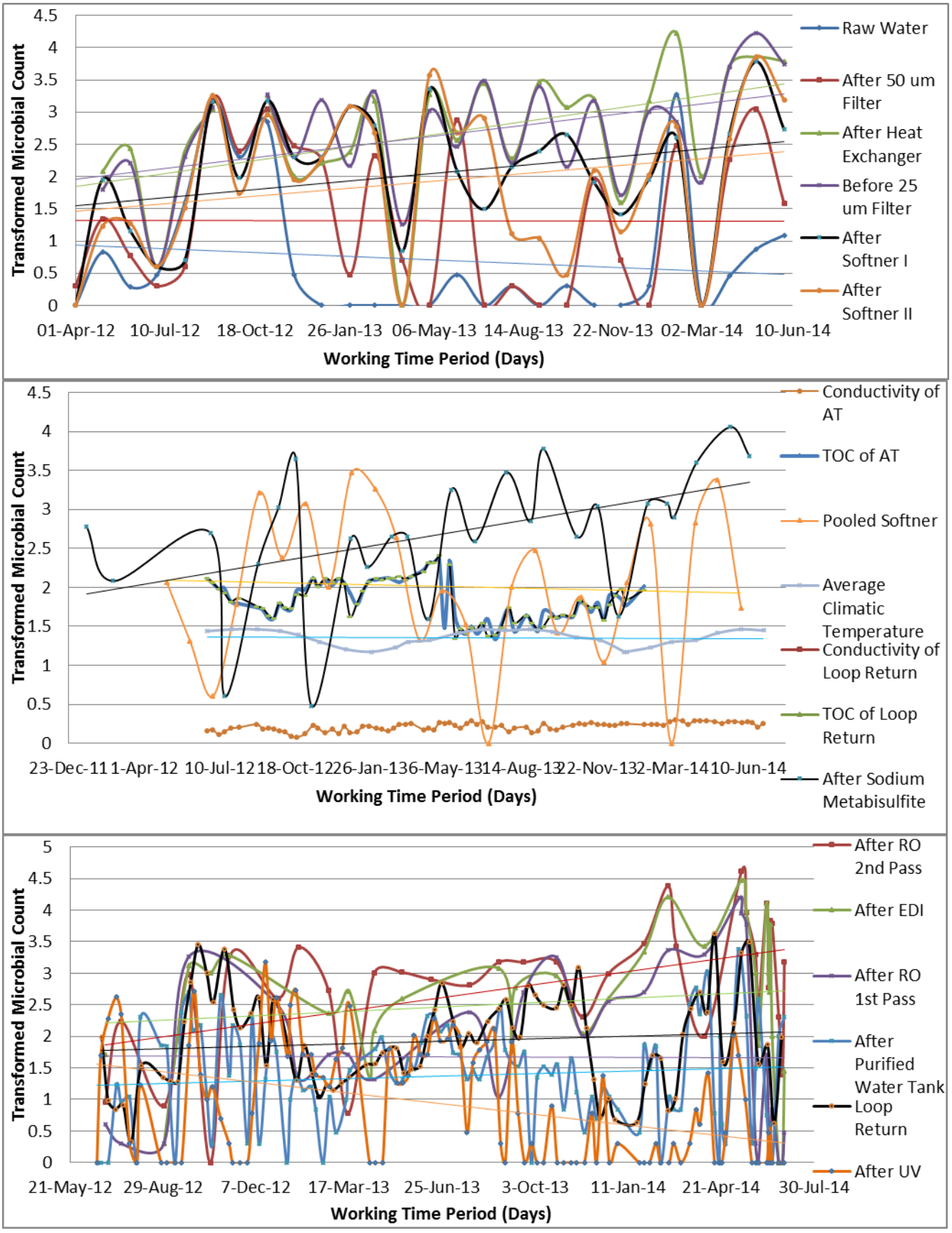

Figure 11: Different parameters and processes measured for water treatment by the facility during two years period showing general trend line for each monitored microbiological parameter (indicated by straight line). (Figures were generated using Microsoft Office Excel 2007)

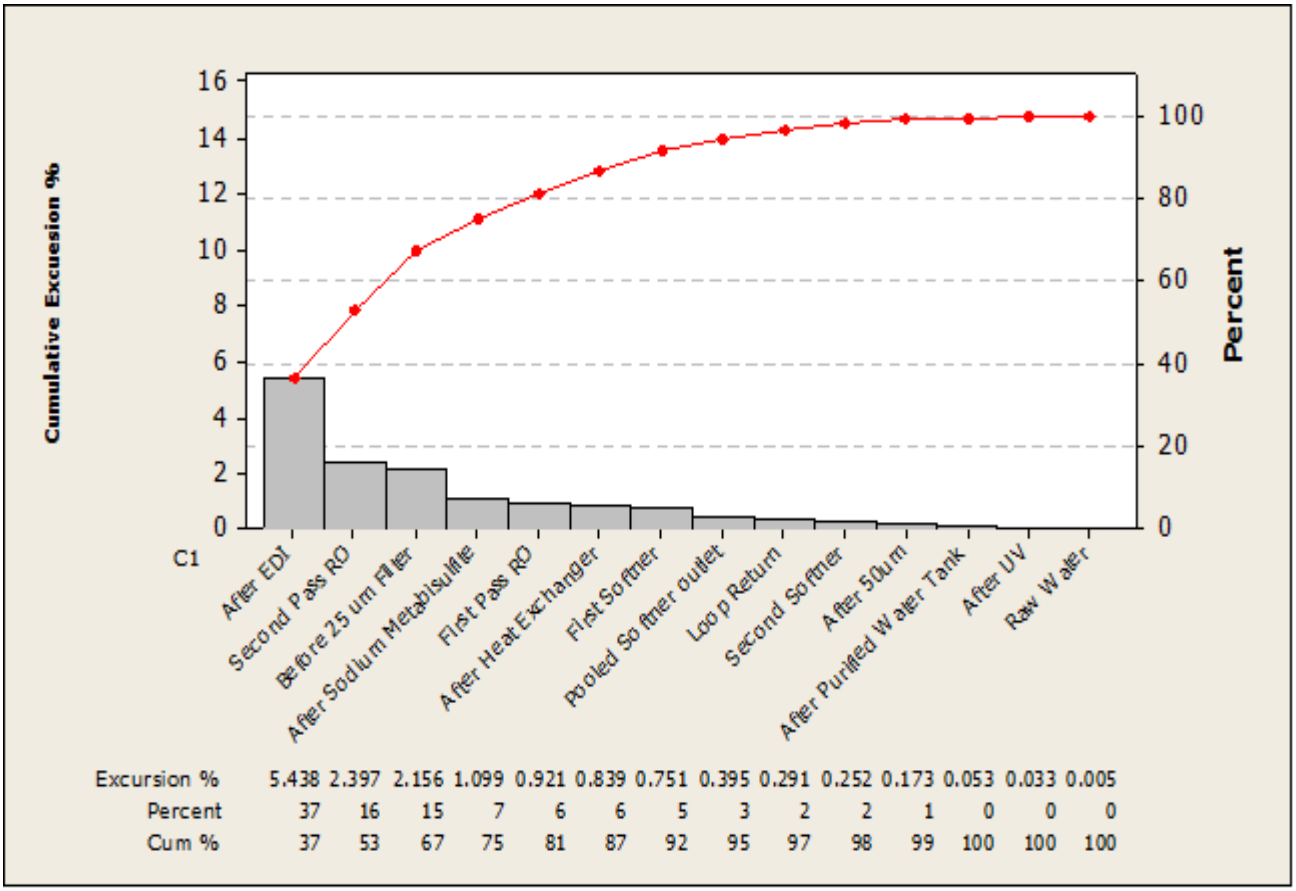

Figure 12: Cumulative Pareto Chart for the microbiological excursion (defect) % of different water treatment stages of the purified water station. (Graph was generated using Minitab® v17.1.0)

Table 1: Process Performance Metrics used in Six Sigma project of the purified water station.(Table and calculations were generated using Microsoft Office Excel 2007)

Water Testing point	PPM	DPU	TPY	RTY	Defect %
Inlet City Water	46.72	0.00004672	0.999953		0.004672
After 50 um Filter	1732.01	0.00173201	0.998269	Pretreatment	0.173201
After First Softener	7510.82	0.00751082	0.992517	Unit	0.751082
After Second Softener	2518.39	0.00251839	0.997485	0.980003	0.251839
Pooled Softener	8391.46	0.00839146	0.991644		0.839146
After Heat Exchanger	3947.2	0.0039472	0.996061		0.39472
Before 25 um Filter	21562.65	0.02156265	0.978668		2.156265
After Sodium Metabisulfite	10994.27	0.01099427	0.989066	(RO + EDI)	1.099427
After First Pass RO	9206.15	0.00920615	0.990836	Unit	0.920615
After Second Pass RO	23974.85	0.02397485	0.97631	0.883033	2.397485
After EDI	54379.84	0.05437984	0.947072		5.437984
After UV Lamp	328.24	0.00032824	0.999672		0.032824
After Purified Water Tank	531.8	0.0005318	0.999468	Loop Unit	0.05318
After Loop Return	2906.43	0.00290643	0.997098	0.996568	0.290643
Total Performance of The Purified Water Station				0.862405	
TOC of Purified Water Tank	663.98	0.00066398	0.999336	Loop Unit	0.066398
TOC of Loop Return	274.07	0.00027407	0.999726	0.999062	0.027407
Conductivity of Purified Water Tank	1791.99	0.00179199	0.99821	Loop Unit	0.179199
Conductivity of Loop Return	2143.17	0.00214317	0.997859	0.996072	0.214317

PPM: Part Per Million. DPU: Defect Per Unit. TPY: Throughput Yield. RTY: Rolled Throughput Yield.

Table 2: One-Way ANOVA with Tukey'spost test at $p<0.05$ showing only statistically different processes in microbiological transformed data.(Data generated by GraphPad Prism® v6.01 for Windows)

Tukey's multiple comparisons test	Mean Diff.	95 CI of diff.
Inlet Water Before Chlorine Add vs. First Softner	-1.2	-2.1 to -0.38
Inlet Water Before Chlorine Add vs. Second Softner	-1.3	-2.2 to -0.45
Inlet Water Before Chlorine Add vs. Softners pooled Outlet	-1.3	-2.2 to -0.49
Inlet Water Before Chlorine Add vs. After Heat Exchanger	-2	-2.8 to -1.1
Inlet Water Before Chlorine Add vs. Before 25 µm filter	-2	-2.8 to -1.1
Inlet Water Before Chlorine Add vs. After SMB Injection	-2	-2.8 to -1.2
Inlet Water Before Chlorine Add vs. First Pass RO	-1.2	-2.1 to -0.40
Inlet Water Before Chlorine Add vs. Second Pass RO	-2	-2.8 to -1.1
Inlet Water Before Chlorine Add vs. After EDI	-1.9	-2.7 to -1.0
Inlet Water Before Chlorine Add vs. After Purified Water Tank	-0.67	-1.3 to -0.0012
Inlet Water Before Chlorine Add vs. Loop Return	-1.2	-1.9 to -0.54
After 50µm Filter vs. After Heat Exchanger	-1.4	-2.2 to -0.49
After 50µm Filter vs. Before 25 µm filter	-1.4	-2.2 to -0.52
After 50µm Filter vs. After SMB Injection	-1.4	-2.2 to -0.55
After 50µm Filter vs. Second Pass RO	-1.4	-2.2 to -0.54
After 50µm Filter vs. After EDI	-1.3	-2.1 to -0.44
First Softner vs. Purified Water After UV Lamp	0.99	0.32 to 1.7
Second Softner vs. Purified Water After UV Lamp	1.1	0.39 to 1.8
Softners pooled Outlet vs. Purified Water After UV Lamp	1.1	0.44 to 1.8
After Heat Exchanger vs. Purified Water After UV Lamp	1.7	1.0 to 2.4
After Heat Exchanger vs. After Purified Water Tank	1.3	0.59 to 2.0
After Heat Exchanger vs. Loop Return	0.75	0.055 to 1.4
Before 25 µm filter vs. Purified Water After UV Lamp	1.8	1.1 to 2.4
Before 25 µm filter vs. After Purified Water Tank	1.3	0.62 to 2.0

Table 3: Correlation Coefficient Matrix (CCM) of transformed microbiological count of different processes for water treatment in pharmaceutical facility using Pearson Correlation at 95 confidence interval.(Data generated by GraphPad Prism® v6.01 for Windows)

CC	TMP												
RWI	-0.08	RWI											
F50	-0.49	0.54	F50										
FST	-0.06	0.30	0.46	FST									
SST	-0.33	0.37	0.52	0.44	SST								
SPO	-0.23	0.24	0.35	0.67	0.70	SPO							
AHE	-0.16	0.17	0.34	0.67	0.44	0.60	AHE						
F25	-0.03	0.19	0.15	0.62	0.30	0.64	0.88	F25					
SMB	0.25	-0.31	-0.20	0.28	0.05	0.30	0.56	0.60	SMB				
FRO	-0.15	-0.12	-0.14	-0.09	-0.20	0.13	-0.09	-0.07	0.02	FRO			
SRO	-0.28	-0.02	0.10	0.12	-0.16	0.30	-0.06	0.01	-0.11	0.71	SRO		
EDI	-0.43	0.25	0.24	0.04	-0.04	0.01	-0.07	-0.13	-0.34	0.54	0.51	EDI	
PWT	0.66	0.02	-0.20	-0.10	-0.34	-0.54	-0.48	-0.46	-0.16	-0.19	-0.19	-0.05	PWT
LRT	-0.29	0.34	0.41	0.16	0.24	0.40	0.55	0.54	0.27	-0.10	0.08	-0.05	-0.53

TMP= Temperature (C°). RWI= Raw Water Inlet (City water before Chlorine addition).F50= After 50 µm Filter.FST and SST= After First and Second Softener respectively.SPO= After Softener Pooled Outlet. AHE= After Heat Exchanger. F25= After 25µm Filter.SMB= After Sodium Metabisulfite. FRO and SRO= First and Second Reverse Osmosis respectively.EDI= After Electrodeionizer. PWT= After Purified Water Tank. LRT= After Loop Return. White cell= Weak or insignificant correlation. Green cell= Perfect correlation. Red cell= Strong correlation. Yellow cell= Moderate correlation.

Table 4: Untransformed microbiological count of water treatment stages of the station: Mean, standard deviation, coefficient of variation, skewness, kurtosis and normality test using three methods. (Data generated by GraphPad Prism® v6.01 for Windows)

	Inlet Water Before Chlorine Addition	After 50µm Filter	After First Softener	After Second Softener	Softeners pooled Outlet	After Heat Exchanger	Before 25 µm filter	After Sodium Metabisulfite	After First Pass RO	After Second Pass RO	After EDI	After UV Lamp	After Purified Water Tank	After Loop Return
Mean	160	228	737	506	605	1823	2150	1686	694	2308	1623	65	103	348
Std. Deviation	460	418	1545	814	1238	3481	3658	2561	1299	4995	3476	178	271	729
D'Agostino& Pearson omnibus normality test														
K2	38	24	44	17	49	47	38	31	41	49	45	158	172	100
P value	< 0.0001	< 0.0001	< 0.0001	0.00022	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Normality test (α=0.05)	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****	****	****	****	****	****
Shapiro-Wilk normality test														
W	0.40	0.61	0.53	0.67	0.52	0.53	0.61	0.66	0.58	0.45	0.47	0.39	0.36	0.50
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Normality test (α=0.05)	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****	****	****	****	****	****
KS normality test														
KS distance	0.33	0.36	0.35	0.32	0.33	0.36	0.35	0.32	0.33	0.36	0.35	0.32	0.33	0.36
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Normality test (α=0.05)	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****	****	****	****	****	****
Coefficient of variation (%)	287.48	183.03	209.53	160.95	204.51	190.97	170.16	151.93	186.99	216.38	214.14	271.76	262.82	209.21
Skewness	3.1	2.2	3.4	1.9	3.7	3.7	3.1	2.6	3.2	3.8	3.5	5.9	6.6	3.4
Kurtosis	9.0	4.4	13	2.8	16	15	11	7.5	12	15	13	43	52	12

Table 5: Transformed microbiological count of water treatment stages of the station: Mean, standard deviation, coefficient of variation, skewness, kurtosis and normality test using three methods. (Data generated by GraphPad Prism® v6.01 for Windows)

	Inlet Water Before Chlorine Addition	After 50µm Filter	After First Softener	After Second Softener	Softeners pooled Outlet	After Heat Exchanger	Before 25 µm filter	After Sodium Metabisulfite	After First Pass RO	After Second Pass RO	After EDI	After UV Lamp	After Purified Water Tank	After Loop Return
Mean	0.71	1.3	1.9	2.0	2.0	2.7	2.7	2.7	1.9	2.7	2.6	0.94	1.4	1.9
Std. Deviation	1.0	1.2	1.1	0.96	1.0	0.85	0.99	0.87	1.2	1.0	0.88	0.92	0.80	0.77
D'Agostino& Pearson omnibus normality test														
K2	13	15	2.7	1.0	1.5	0.99	6.4	7.0	3.4	8.0	5.9	34	2.8	3.6
P value	0.0016	0.0006	0.2637	0.6076	0.4745	0.6090	0.0417	0.0301	0.1832	0.0184	0.0520	< 0.0001	0.2517	0.1690
Normality test (α=0.05)	No	No	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes	No	Yes	Yes
P value summary	**	**	ns	ns	ns	ns	*	*	ns	*	ns	**	ns	ns
Shapiro-Wilk normality test														
W	0.71	0.87	0.96	0.96	0.96	0.97	0.93	0.92	0.93	0.88	0.94	0.86	0.97	0.98
P value	< 0.0001	0.0027	0.2881	0.3448	0.3434	0.6873	0.0763	0.0340	0.0705	0.0039	0.0956	< 0.0001	0.0330	0.1727
Normality test (α=0.05)	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	Yes
P value summary	***	**	ns	ns	ns	ns	ns	*	ns	**	ns	**	*	ns
KS normality test														
KS distance	0.29	0.20	0.12	0.092	0.15	0.14	0.16	0.19	0.13	0.20	0.17	0.21	0.068	0.077
P value	< 0.0001	0.0083	0.2000	0.2000	0.1264	0.2000	0.1081	0.0151	0.2000	0.0060	0.0367	< 0.0001	0.2000	0.1192
Normality test (α=0.05)	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes
P value summary	***	**	ns	ns	ns	ns	ns	*	ns	**	*	**	ns	ns
Coefficient of variation (%)	144.76	88.32	59.13	47.69	48.80	32.00	36.88	32.13	59.86	37.41	33.73	98.61	58.04	40.10
Skewness	1.6	0.28	-0.22	-0.44	-0.52	-0.43	-1.0	-1.0	-0.40	-1.2	-0.86	0.44	-0.084	-0.16
Kurtosis	1.6	-1.6	-1.0	-0.19	-0.31	0.015	1.3	1.3	-1.0	1.3	1.4	-1.2	-0.60	-0.62

Table 6: Untransformed and logarithmically transformed conductivity at 25°C, total organic carbon (TOC) and climatic temperature (°C) data of the water treatment station: Mean, standard deviation, coefficient of variation, skewness, kurtosis and normality test using three methods. (Data generated by GraphPad Prism® v6.01 for Windows)

	Untransformed Data					Transformed Data				
	Loop Return Conductivity	Loop Return TOC	Purified Water Tank Conductivity	Purified Water Tank TOC	Climatic Temperature	Loop Return Conductivity	Loop Return TOC	Purified Water Tank Conductivity	Purified Water Tank TOC	Climatic Temperature
Mean	0.65	81	0.65	82	23	0.22	1.8	0.21	1.8	1.4
Std. Deviation	0.19	50	0.19	52	5.4	0.052	0.26	0.051	0.27	0.11
D'Agostino& Pearson omnibus normality test										
K2	4.3	14	2.3	16	40	4.4	6.4	2.7	6.7	14
P value	0.1146	0.0009	0.3243	0.0003	< 0.0001	0.1115	0.0416	0.2651	0.0354	0.0010
Normality test (α=0.05)	Yes	No	Yes	No	No	Yes	No	Yes	No	No
P value summary	ns	***	ns	***	****	ns	*	ns	*	***
Shapiro-Wilk normality test										
W	0.98	0.88	0.98	0.88	0.94	0.97	0.97	0.98	0.97	0.92
P value	0.1274	< 0.0001	0.4091	< 0.0001	< 0.0001	0.0217	0.0889	0.1191	0.0972	< 0.0001
Normality test (α=0.05)	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No
P value summary	ns	****	ns	****	****	*	ns	ns	ns	****
KS normality test										
KS distance	0.094	0.16	0.085	0.14	0.12	0.11	0.084	0.10	0.090	0.14
P value	0.0588	0.0002	0.1237	0.0028	0.0004	0.0113	0.2000	0.0285	0.1891	< 0.0001
Normality test (α=0.05)	Yes	No	Yes	No	No	No	Yes	No	Yes	No
P value summary	ns	***	ns	**	***	*	ns	*	ns	****
Coefficient of variation (%)	29.16	62.16	29.22	63.79	23.58	23.93	14.35	23.82	14.66	7.71
Skewness	-0.29	1.1	-0.14	1.2	-0.28	-0.52	0.064	-0.39	0.075	-0.56
Kurtosis	-0.67	0.95	-0.59	1.4	-1.2	-0.34	-0.92	-0.35	-0.94	-0.85

Finally, Systems that operate and are maintained at elevated temperatures, in the range of 70–80°C (as in the current case), are generally less susceptible to microbiological contamination than systems that are maintained at lower temperatures. When lower temperatures are required due to the water treatment processes employed or the temperature requirements for the water in use, then special precautions should be taken to prevent the ingress and proliferation of microbiological contaminants [16]. Even though, inappropriate control, maintenance and sanitization can lead to catastrophic excursions in water quality which may lead to sever financial loss and more importantly health hazard risk.

REFERENCES

1. Notes for Guidance on Quality of Water for Pharmaceutical Use, The European Agency for the Evaluation of Medicinal Products (EMEA), London,May 2002.
2. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003394.pdf
3. WHO good manufacturing practices: water for pharmaceutical useAnnex 2revision of WHO Technical Report Series, No. 929, Annex 3, 2005http://apps.who.int/prequal/info_general/documents/TRS970/TRS_970_Annex2.pdf

4. Potdar MA; Pharmaceutical Quality Assurance. 2nd Edition, Nirali .. Press Publication, Delhi, December 2007: 8.2-8.3. 11
5. 2015 USPC Official 12-1-14 - 04-30-15 General Chapters <1231>Water for pharmaceutical purposes.
6. Design for Six Sigma LeanToolset: Implementing Innovations Successfully Christian Staudter‹Jens-Peter Mollenhauer‹RenataMeran‹Olin Roenpage‹Clemens Hugo, Alexis Hamalides,
7. Eissa, M. Studies of Microbial Resistance Against Some Disinfectants, Lambert Academic Publishing, 2014.
8. National Primary Drinking Water Regulations (NPDWR; 2006), United States Environmental Protection Agency.
9. Sutton SV, Proud DW, Rachui S, Brannan DK. Validation of microbial recovery from disinfectants.PDA J Pharm Sci Technol. 2002; 56(5): 255-266.
10. Traeger, H. Pharmaceuticals, The Presence of Bacteria, Endotoxins, and Biofilms in Pharmaceutical Water, Ultrapure Water, March 2003.
11. Clontz, L. Microbial Limit and Bioburden Tests: Validation Approaches and Global Requirements, 2nd Edition, CRC press, 2008.
12. Suslow. TV. Oxidation Reduction Potenial (ORP) for Water Disinfection Monitoring, Control and Documentation Publication 8149. http://anrcatalog.ucdavis.edu/pdf/8149.pdf
13. FDA Technical Guide Heat Exchangers to Avoid Contamination, Department of Health and Education and Welfare Public Health Service, Food and Drug Administration, No. 34, 1979.
14. PDA Technical Report No. 13. Fundamentals of an Environmental Monitoring Program, Parenteral Drug Association, Bethesda, MD. 1990.
15. Sandle, T (2004) An approach for the reporting of microbiological results from water systems. PDA J Pharm SciTechnol 58: 4. 231-237.
16. Sokal, RR, Rohlf, FJ. Biometry: The Principles and Practice of Statistics in Biological Research. 4th edition, W.H. Freeman and Co, New York, 1995.
17. Choudhary, A. WHO: water for Pharmaceutical Use, 2008. http://www.pharmaguideline.com/2010/12/who-water-for-pharmaceutical-use.html