CHAPTER 5

CLEANING VALIDATION USING HPLC FOR ANALYSIS

5.1 INTRODUCTION

This chapter reports the validation of the HPLC method developed for the detection of selected detergents (Ekon D concentrate® and LaboClean FT concentrate®) for glassware cleaning validation purposes for a pharmaceutical contract testing laboratory. Results of the validation will be reported and discussed in this chapter.

Validation is simply the act of confirming that a method performance is sufficient for the intended purpose. A compelling reason for validation is that it is a regulatory requirement. Cleaning validation, as with validation of other processes there may be more than one way of validating the process. In the end, the test of any validation process is whether scientific data shows that the system consistently does as expected and produces a result that consistently meets predetermined specifications (FDA, 2010).

Validation of cleaning methods requires limit tests and quantitative analysis. Both the analytical method and the sampling method should be challenged to ensure whether contaminants can be recovered from the cleaning surface and to what level. Linearity, accuracy, precision, range, specificity, limit of detection (LOD), limit of quantitation (LOQ), ruggedness and robustness are the validation parameters that will be addressed as stated in regulatory guidelines for cleaning validation purposes; details of the validation parameters were discussed in chapter 2, section 2.5.

5.2 VALIDATION

Three sets of data will be reported in this chapter. The first data set reports the method validation results generated by the method developing analyst in the laboratory of study. The second data set reports a method transfer conducted by an inexperienced student in a different research laboratory using a Shimadzu® UFLC instrument. The

third report is a validation conducted on the Shimadzu® UFLC by an inexperienced analyst. The second and third data sets will be reported as part of ruggedness data.

5.2.1 Scope

To validate the developed HPLC method for the detection of selected detergents (Ekon D concentrate® and LaboClean FT. concentrate®), used for the cleaning of glassware in a pharmaceutical contract testing laboratory.

5.2.2 Chromatographic conditions

Table 5.1 shows the chromatographic conditions used for validating the developed HPLC method for the detection of selected detergents.

Table 5.1 HPLC method validation chromatographic conditions

Analytical Instrument	Agilent® 1100 series DAD isocratic system using Chemstation software®
Mobile phase	Mobile phase: Acetonitrile: buffer (25:75), with buffer containing 0.02 M hexanesulphonic acid sodium salt, with pH adjusted to 3.0 with phosphoric acid. Filtered and degassed.
Column	µBondapak C_{18} (10 µm) (300 x 3.9 mm) column at ambient temperature
DAD detector	205 nm & 220 nm
Injection volume	25 µl
Solvent	Milli-Q water
Flow rate	1.0 ml per minute

5.2.3 Standard preparation

Table 5.2 shows the preparation of variable standard concentrations of Ekon D concentrate® used for the validation process.

Table 5.2 Standard preparation of Ekon D concentrate®

Ekon D Concentrate®	
Reference standard	Weigh 1 ml of Ekon D concentrate® to a 100 ml volumetric flask. Add and make up to volume with solvent and mix well.
Recovery standard	Weigh 1 ml of Ekon D concentrate® to a 100 ml volumetric flask. Add and make up to volume with solvent and mix well.
20% v/v standard	Dilute 4 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
50% v/v standard	Dilute 10 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
75% v/v standard	Dilute 15 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
112.5% v/v standard	Prepare this standard after the preparation of the 150% v/v standard. Dilute 15 ml of the 150% standard solution to a 2 0ml volumetric flask and make up to volume with the solvent and mix well.
150% v/v standard	Weigh 3 ml of Ekon D concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.

Table 5.3 shows the preparation of variable standard concentrations of LaboClean FT concentrate® used for the validation process.

Table 5.3 Standard preparation of LaboClean FT concentrate®

LaboClean FT Concentrate®	
Reference standard	Weigh 3 ml of LaboClean ft concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.
Recovery standard	Weigh 3 ml of LaboClean FT concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.
20% v/v standard	Dilute 4 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
50% v/v standard	Dilute 10 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
75% v/v standard	Dilute 15 ml of the reference standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
150% v/v standard	Prepare this standard after the preparation of the 200% v/v standard. Dilute 15 ml of the 200% standard solution to a 20 ml volumetric flask and make up to volume with the solvent and mix well.
200% v/v standard	Weigh 6 ml of LaboClean FT concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.

Tables 5.4, 5.5 and 5.6 report the validation summary of the data generated in the laboratory of study by the HPLC method developing analyst.

Table 5.4 Ekon D concentrate® peak 1 area response summary report obtained for the validation of the developed HPLC method, conducted by the analyst developing the HPLC method

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
5057.5	49.9	1.21	1.12				1.16	0.061	5.22
7586.3	74.9	2.10	1.93				2.02	0.122	6.03
10115.0	99.9	2.74	2.82	2.55	2.76	2.77	2.73	0.102	3.73
11383.8	112.5	2.97	2.95				2.96	0.019	0.630
15177.0	149.9	3.75	3.82				3.79	0.051	1.35

Control Standard								
Theoretical concentration (µg/ml)				**10120.0**	**Calculated concentration (µg/ml)**		10110.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**	
Control 1	2.75	10698.9	2.70	0.072	2.66	**103.8**	285.5	
Control 2	2.64	10303.3						

SUMMARY OUTPUT		**SYTEM SUITABILITY CONDITIONS**		**LOD**	**LOQ**
Regression Statistics		**Response factor 1**	N/A	1568.6	5228.7
Multiple R	0.993	**Response factor 2**	N/A		
R Square	0.986	**USP tailing**	1.52		
Adjusted R Square	0.982	**Theoretical plate count**	8406.0		
Standard Error	0.134	**Capacity**	1.11		
Observations	5	**Resolution**	N/A		

Table 5.5 Ekon D concentrate® peak 2 area response summary report obtained for the validation of the developed HPLC method, conducted by the analyst developing the HPLC method

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
5057.5	50.0	1.72	1.85				1.78	0.090	5.05
7586.3	75.0	2.54	2.54				2.54	0.000	0.005
10115.0	99.9	3.38	3.35	3.33	3.10	3.21	3.28	0.115	3.52
11383.8	112.5	3.48	3.65				3.57	0.118	3.31
15177.0	149.9	4.96	5.45				5.21	0.341	6.55

Control Standard							
Theoretical concentration (µg/ml)			**10120.0**	**Calculated concentration (µg/ml)**		10110.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	3.25	9796.4	3.11	0.211	6.82	**92.4**	285.1
Control 2	2.95	8896.2					

SUMMARY OUTPUT		SYTEM SUITABILITY CONDITIONS		LOD	LOQ
Regression Statistics		**Response factor 1**	N/A	1577.9	5259.8
Multiple R	0.993	**Response factor 2**	N/A		
R Square	0.986	**USP tailing**	1.34		
Adjusted R Square	0.981	**Theoretical plate count**	9109.0		
Standard Error	0.175	**Capacity**	2.24		
Observations	5	**Resolution**	9.88		

Table 5.6 LaboClean FT concentrate® peak area response summary report obtained for the validation of the developed HPLC method, conducted by the analyst developing the HPLC method

Theoretical 100% concentration (µg/ml)				19575.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
9792.5	50.0	215.2	221.7				218.4	4.56	2.09
14688.7	75.0	352.2	355.9				354.0	2.67	0.753
19585.0	100.1	489.5	493.1	495.5	497.1	498.1	494.6	3.44	0.696
29377.5	150.1	750.3	750.8				750.6	0.327	0.044
39170.0	200.1	999.0	999.9				999.4	0.596	0.060

Control Standard							
Theoretical concentration (µg/ml)			19575.0	**Calculated concentration (µg/ml)**		19500.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	497.6	20044.2	497.8	0.339	0.070	**102.4**	201.7
Control 2	498.1	20062.3					

SUMMARY OUTPUT		SYTEM SUITABILITY CONDITIONS		LOD	LOQ
Regression Statistics		**Response factor 1**	N/A	917.7	3059.1
Multiple R	0.999	**Response factor 2**	N/A		
R Square	0.999	**USP tailing**	1.15		
Adjusted R Square	0.999	**Theoretical plate count**	11559.0		
Standard Error	8.13	**Capacity**	0.526		
Observations	5	**Resolution**	N/A		

5.2.4 Results and discussion

Table 5.4 and 5.5 is validation summary reports of Ekon D concentrate® peak 1 and peak 2 respectively. Table 5.6 is the validation summary report of LaboClean FT concentrate®. Validation parameter results of Table 5.4, 5.5 and 5.6 are discussed in the following section.

5.2.4.1 Validation test procedure and acceptance criteria

Linearity, accuracy, precision, range, specificity, LOD, LOQ, robustness and ruggedness are validation requirements that will be discussed for the developed HPLC method based on the data generated. The reported results were calculated using the in-house validated Microsoft® Excel® spreadsheet.

a. Linearity and range

A minimum of five concentration ranges were investigated and a plot of the detector response versus the detergent's concentration was plotted.

Figure 5.1 and 5.2 is a linear regression plots of Ekon D concentrate® peak 1 and peak 2. The response of the detergent's concentration ranging from 5000 µg/ml to 15 000 µg/ml were plotted and the regression analysis were calculated using a validated Microsoft® Excel® spreadsheet. The calculated residual sum of squares for linearity evaluation of Ekon D concentrate® peak 1 and peak 2 is 0.986. This R^2 value meets the acceptance criteria of $R^2 \geq 0.98$ specified by Lister (2005) for cleaning validation purposes. The R^2 value obtained for Ekon D concentrate® peak 1 and peak 2 confirms the direct proportionality of the detergents concentration and the instruments detector response.

Figure 5.3 is a linear regression plot of LaboClean FT concentrate® peak. The response of the detergent's concentration ranging from 10 000 µg/ml to 40 000 µg/ml were plotted and regression analysis were calculated using a validated Microsoft® Excel® spreadsheet. The calculated R^2 value of LaboClean FT concentrate® peak 1 is 0.999.

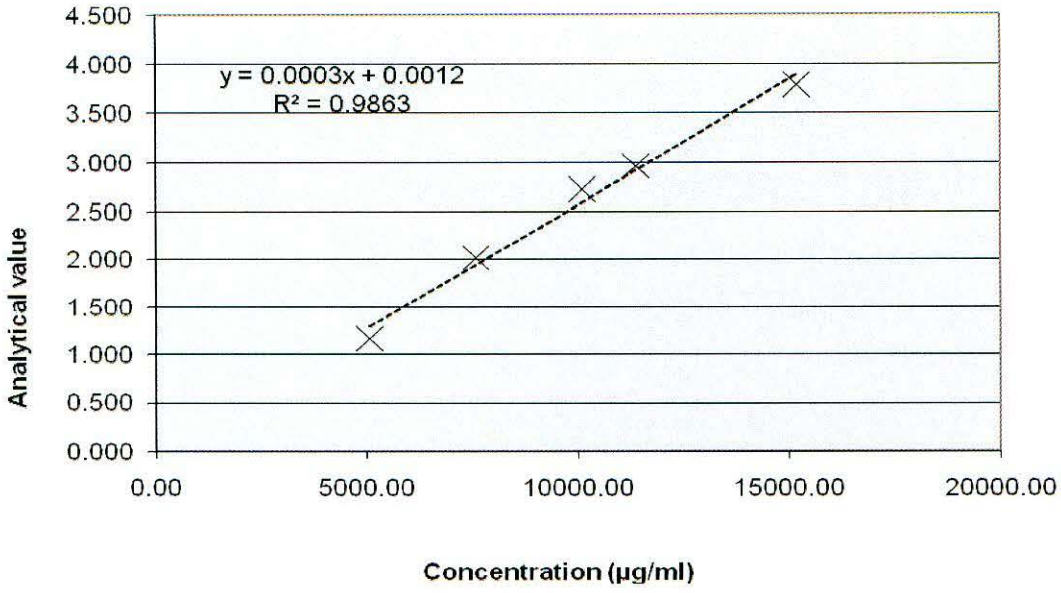

Figure 5.1 Linear plot obtained for Ekon D concentrate® peak 1 for HPLC method validation, conducted by the analyst developing the HPLC method.

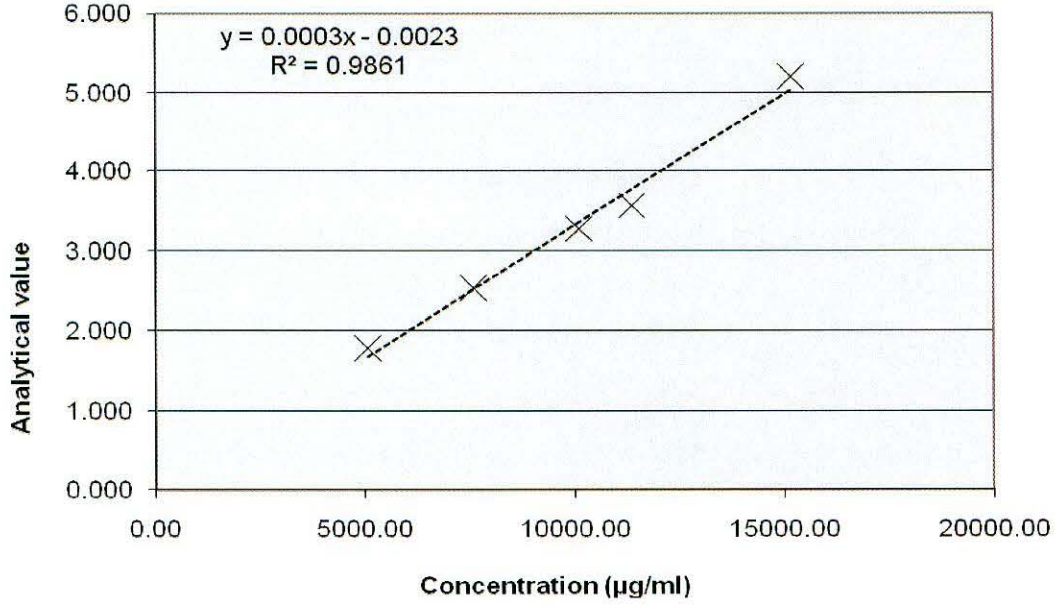

Figure 5.2 Linear plot obtained for Ekon D concentrate® peak 2 for HPLC method validation, conducted by the analyst developing the HPLC method.

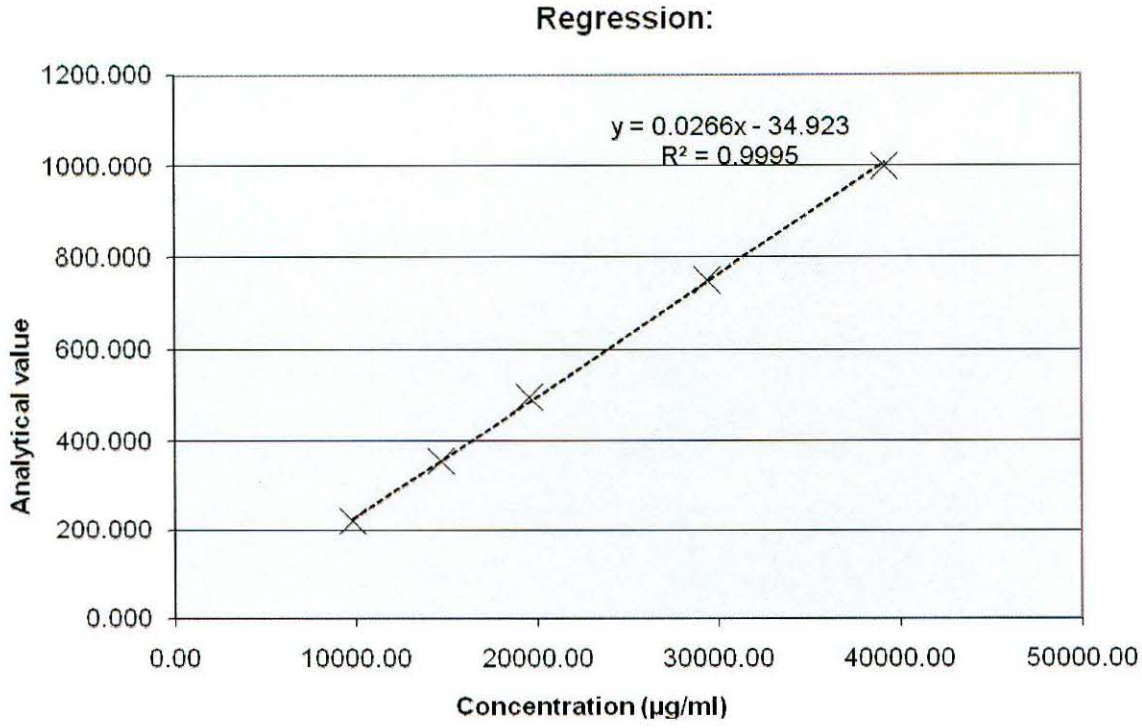

Figure 5.3 Linear plot obtained for the LaboClean FT concentrate® peak for HPLC method validation, conducted by the analyst developing the HPLC method.

The R^2 value obtained for figure 5.3 meets the acceptance criteria of $R^2 \geq 0.98$ specified by Lister (2005) for cleaning validation purposes. The R^2 value obtained for LaboClean FT concentrate® peak confirms the ability of the developed HPLC method to obtain results that are directly proportional to the analyte concentration over a given range.

b. Limit of detection (LOD) and limit of quantitation (LOQ)

In this report the LOD was determined using a validated Microsoft® Excel® spreadsheet. The LOD for Ekon D concentrate® peak 1 and peak 2 were separately calculated. In Table 5.4 and Table 5.5, LOD for peak 1 and peak 2 gave a concentration just over 1500 µg/ml. The LOD value correlation of Ekon D concentrate® peak 1 and peak 2 also show specificity and accuracy of the developed HPLC method

for the analyte. In Table 5.6 the LOD of LaboClean FT concentrate® gave a concentration of approximately 900 µg/ml.

LOQ of Ekon D concentrate® peak 1 and peak 2 gave a concentration just above 5000 µg/ml. The LOQ of LaboClean FT concentrate® gave a concentration just over 3000 µg/ml.

c. Precision

For the purpose of this study, the accepted relative standard deviation for replicate of six values at ten times the LOQ concentration is 20% (Lister, 2005). RSD results of five replicate values at a concentration approximately 1.5 times the LOQ shown in Tables 5.4, 5.5 and 5.6 for Ekon D concentrate® peak 1and peak 2 and LaboClean FT concentrate® is less that 7%. The two detergents Ekon D concentrate® and LaboClean FT concentrate® pass the specified precision criteria.

d. Robustness

The mobile phase buffer concentration, mobile phase pH, and column dimension, are three parameters that were tempered with to test the robustness of the developed method.

- **Buffer concentration**

The buffer concentration of the mobile phase was adjusted by 50%. 0.01 M of hexanesulphonic acid sodium salt, with pH adjusted to 3.0 with phosphoric acid was used with the same concentration of the organic phase (acetonitrile). Figure 5.4 and 5.5 is representative chromatograms of LaboClean FT concentrate® (19575 µg/ml) and Ekon D concentrate® (10120 µg/ml) after the deliberate adjustment of the mobile phase concentration.

The chromatography, retention time and areas obtained after the deliberate adjustment of the mobile phase buffer were the same as the results obtained with the 0.02M buffer concentration results. The drastic buffer concentration adjustment did not have any

significant effect on the chromatography and hence allows optimisation of the method for future purposes.

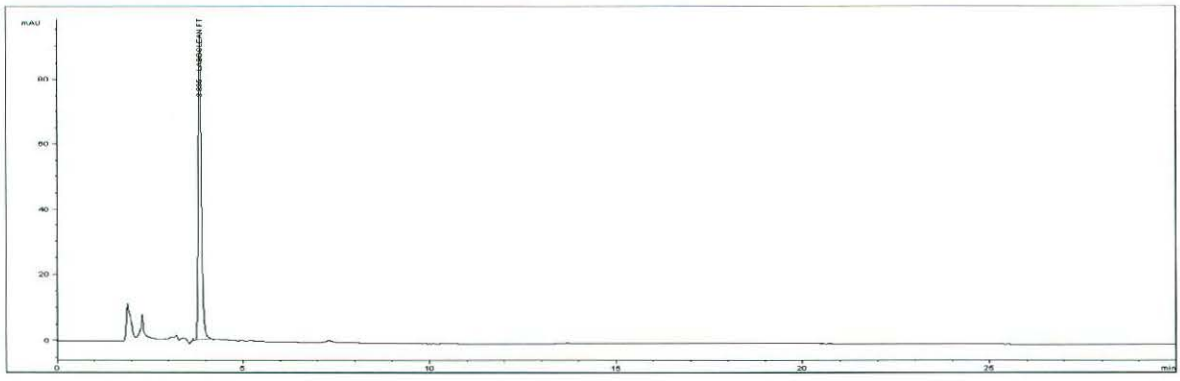

Figure 5.4 Chromatogram obtained for LaboClean FT concentrate® with the mobile phase buffer adjusted by 50%.

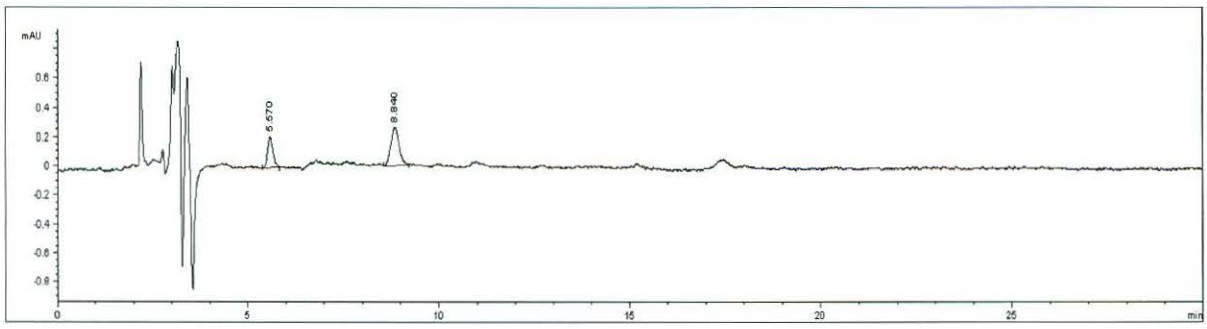

Figure 5.5 Chromatogram obtained for Ekon D concentrate with mobile phase buffer adjusted by 50%.

- **Mobile phase pH**

The pH of the mobile phase 0.02 M buffer at pH 3.0: acetonitrile (75:25) was adjusted to 2.50 with phosphoric acid. Observation of representative chromatograms show that Ekon D concentrate® peaks retention time shifted from 5.57 minutes and 8.84 minutes to 4.95 minutes for peak 1 and 7.94 minutes for peak 2. The retention time of the LaboClean FT concentrate® peak shifted from 3.89 minutes to 3.19 minutes.

Adjusting the mobile phase pH to 2.50 resulted in the reduction of the peak retention times of both detergents. The effect can be employed to shorten the run time of analysis, resulting in saving time and costs of analysis. The chromatographic layout remained acceptable for both detergents, however care must be taken not to shorten the stop time drastically, as this may results in active peaks eluting at the solvent front especially for the LaboClean FT concentrate® peak that elutes at a retention time of approximately 3 minutes. Refer to figure 5.6 and figure 5.7 for representative chromatograms.

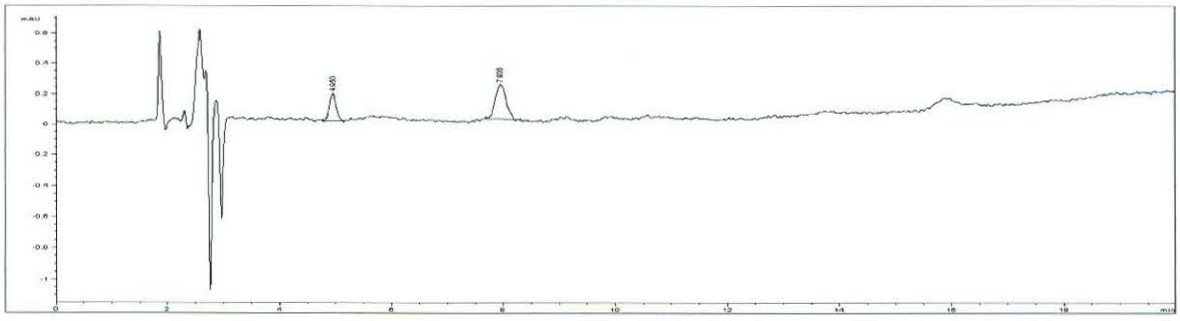

Figure 5.6 Chromatogram obtained for Ekon D concentrate® after adjusting the mobile phase pH to 2.5.

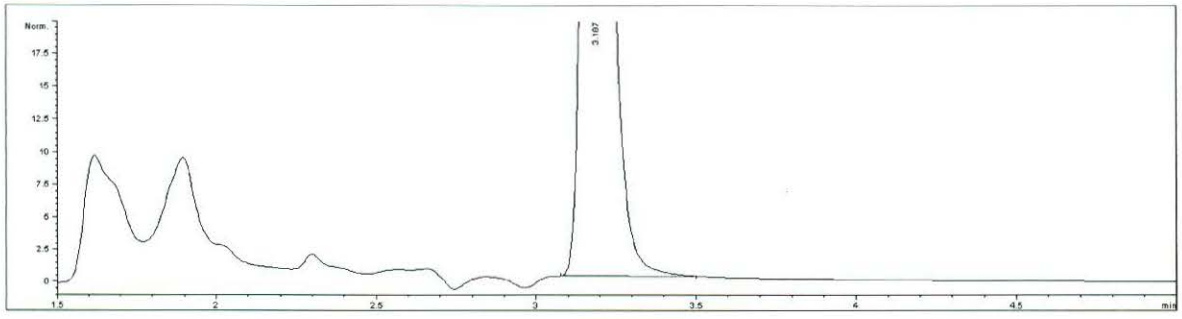

Figure 5.7 Chromatogram obtained for LaboClean FT concentrate® after adjusting the mobile phase pH to 2.5.

- **Column dimensions**

A µBondapak C_{18} 300 x 3.9 mm column with a particle size of 10 µm was used to develop the original method. A Luna C_{18} 250 x 4.6 mm with a particle size of 5 µm was

used in to test the robustness of the method. The Ekon D concentrate® chromatography remained acceptable. Ekon D concentrate® peaks eluted at relatively the same retention time as those achieved with the µBondapak C_{18} 300 x 3.9 mm (10 µm), see figure 5.8.

The LaboClean FT concentrate® chromatography in figure 5.9 showed unacceptable changes. The LaboClean FT concentrate® peak eluted at the solvent front, making it difficult to quantify the active peak.

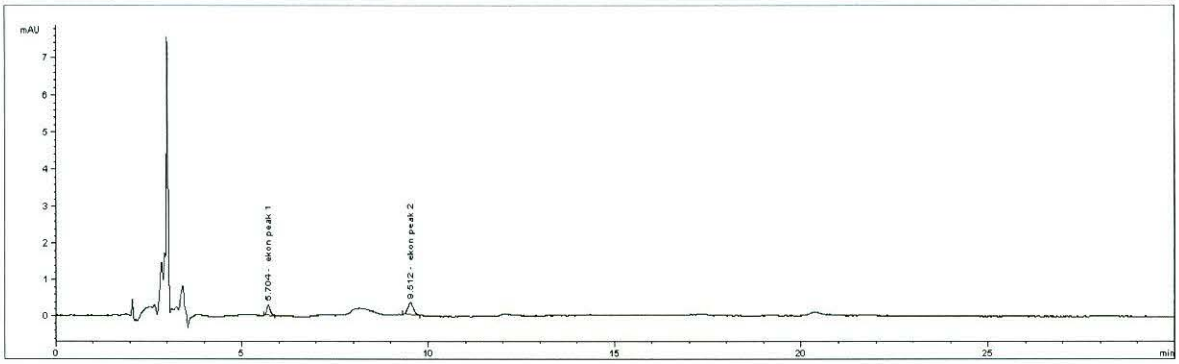

Figure 5.8 Chromatogram obtained for Ekon D concentrate® when using Luna C_{18} 250 x 4.6 mm, (5 µm) column.

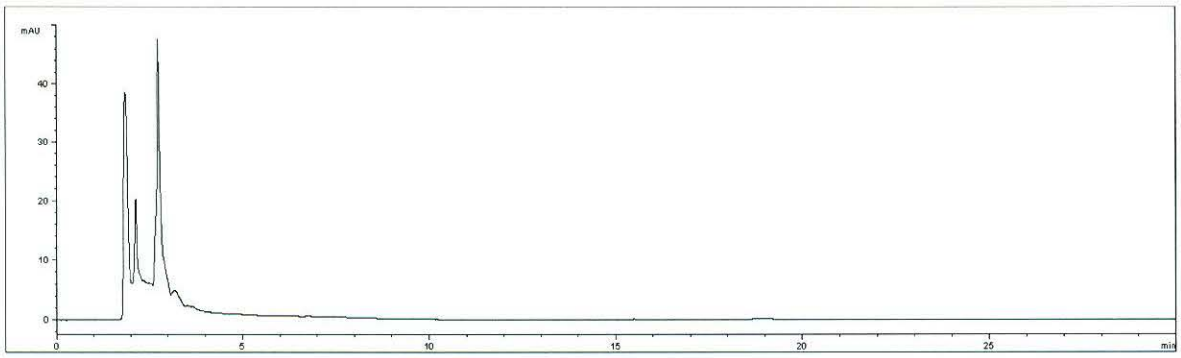

Figure 5.9 Chromatogram obtained for LaboClean FT concentrate® when using Luna C_{18} 250 x 4.6mm, (5 µm) column.

Thorough robustness data can provide the flexibility needed to perform method adjustments if required. Time and costs are a limiting factor in most cases for detailed robustness data, hence it makes sense to perform robustness testing when developing the method to identify critical parameters that can affect method. Section 4.3.3 reported some of the robustness data collected when developing this HPLC method.

e. Ruggedness

Tables 5.7, 5.8 and 5.9 report a summary of the method tranfer conducted on the Shimadzu® UFLC in another research laboratory by a post graduate student.

Tables 5.7 and 5.8 is the method transfer summary reports of Ekon D concentrate® peak 1 and peak 2 respectively. Table 5.9 is the method transfer summary report of LaboClean FT concentrate®. System suitability conditions for the detergent actives are also included in the summary tables.

Tables 5.10 and 5.11 is method validation summary reports of Ekon D concentrate® peak 1 and peak 2 respectively generated on the Shimadzu® UFLC and Table 5.12 is the method validation summary report of LaboClean FT concentrate® data. System suitability conditions for the detergent actives are also included in the summary tables. These results were generated by an inexperienced analyst.

- Table 5.7 and 5.8 presents the method transfer summary reports of Ekon D concentrate® peak 1 and peak 2 respectively whiles Table 5.9 is the method transfer summary report of LaboClean FT concentrate®. The results were generated by an inexperienced post graduate student on a completely different HPLC instrument a Shimadzu® UFLC system. This system presented a completely different set of variables such as tubing, void volume and detector sensitivity to name a few.

Table 5.7 Ekon D concentrate® peak 1 area response summary report obtained for method transfer of the developed HPLC method, conducted by a post graduate student using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
7077.0	69.9	1348.0	1246.0				1297.0	72.1	5.56
10110.0	99.9	2107.0	2074.0	2017.0	2065.0	1982.0	2049.0	49.4	2.41
14500.0	143.3	2685.0	2779.0				2732.0	66.5	2.43

Control Standard							
Theoretical concentration (µg/ml)			10120.0	**Calculated concentration (µg/ml)**		10120.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	2041.0	10120.0	1993.5	67.2	3.37	**100.0**	386.3
Control 2	1946.0	10120.0					

SUMMARY OUTPUT		**SYTEM SUITABILITY CONDITIONS**		**LOD**	**LOQ**
Regression Statistics		**Response factor 1**	N/A	2116.9	7056.6
Multiple R	0.991	**Response factor 2**	N/A		
R Square	0.982	**USP tailing**	1.21		
Adjusted R Square	0.965	**Theoretical plate count**	6728.2		
Standard Error	134.5	**Capacity**	0		
Observations	3	**Resolution**	N/A		

Table 5.8 Ekon D concentrate® peak 2 area response summary report obtained for method transfer of the developed HPLC method, conducted by a post graduate student using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
7077.0	70.0	2802.0	2756.0				2779.0	32.5	1.17
10110.0	100.0	3972.0	4204.0	4137.0	4194.0	4048.0	4111.0	99.4	2.42
14500.0	143.0	5561.0	5450.0				5506.0	78.5	1.43

Control Standard							
Theoretical concentration (µg/ml)			10120.0	**Calculated concentration (µg/ml)**		10120.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	4048.0	10120.0	4041.5	9.19	0.230	**100.0**	273.3
Control 2	4035.0	10120.0					

SUMMARY OUTPUT		**SYTEM SUITABILITY CONDITIONS**		**LOD**	**LOQ**
Regression Statistics		**Response factor 1**	N/A	1459.6	4865.4
Multiple R	0.996	**Response factor 2**	N/A		
R Square	0.992	**USP tailing**	1.33		
Adjusted R Square	0.983	**Theoretical plate count**	7451.7		
Standard Error	176.9	**Capacity**	0		
Observations	3	**Resolution**	N/A		

Table 5.9 LaboClean FT concentrate® peak area response summary report obtained for method transfer of the developed HPLC method, conducted by a post graduate student using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				19575.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
13710.0	70.0	453370.0	455087.0				454228.5	1214.1	0.267
19585.0	100.0	598444.0	624582.0	639238.0	647207.0	650829.0	632060.0	21325.9	3.37
29165.0	149.0	982798.0	989388.0				986093.0	4659.8	0.473

Control Standard							
Theoretical concentration (µg/ml)			19575.0	**Calculated concentration (µg/ml)**		19235.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	603972.0	19235.0	605719.5	2471.3	0.410	**98.3**	1047.7
Control 2	607467.0	19235.0					

SUMMARY OUTPUT		SYTEM SUITABILITY CONDITIONS		LOD	LOQ
Regression Statistics		**Response factor 1**	N/A	1705.3	5684.4
Multiple R	0.999	**Response factor 2**	N/A		
R Square	0.997	**USP tailing**	1.37		
Adjusted R Square	0.995	**Theoretical plate count**	6784.3		
Standard Error	19702.1	**Capacity**	0		
Observations	3	**Resolution**	N/A		

Table 5.10 Ekon D concentrate® peak 1 area response summary report obtained for method validation of the developed HPLC method, conducted by an inexperienced analyst using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
1750.0	17.3	440.0	438.0				439.0	1.41	0.322
6126.0	60.5	1348.0	1353.0				1351.0	3.54	0.262
8751.0	86.5	1877.0	1869.0	1772.0	1740.0	1783.0	1808.0	61.3	3.39
10614.0	104.9	1918.0	1896.0				1907.0	15.6	0.816
14152.0	139.8	2915.0	2836.0				2876.0	55.9	1.94

Control Standard							
Theoretical concentration (µg/ml)			**10120.0**	**Calculated concentration (µg/ml)**		8829.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	1712.0	8469.8	1810.0	138.6	7.66	**88.9**	616.7
Control 2	1908.0	9513.7					

SUMMARY OUTPUT		**SYTEM SUITABILITY CONDITIONS**		**LOD**	**LOQ**
Regression Statistics		**Response factor 1**	N/A	2269.9	7566.4
Multiple R	0.990	**Response factor 2**	N/A		
R Square	0.981	**USP tailing**	1.08		
Adjusted R Square	0.974	**Theoretical plate count**	7635.2		
Standard Error	142.1	**Capacity**	0		
Observations	5	**Resolution**	N/A		

Table 5.11 Ekon D concentrate® peak 2 area response summary report obtained for method validation of the developed HPLC method, conducted by an inexperienced analyst using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				10120.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
1750.0	17.3	375.0	469.0				422.0	66.5	15.8
6126.0	60.5	2158.0	2448.0				2303.0	205.1	8.90
8751.0	86.5	2993.0	2640.0	3007.0	2823.0	2734.0	2839.0	160.3	5.65
10614.0	104.9	3101.0	3315.0				3208.0	151.3	4.72
14152.0	139.8	5357.0	4533.0				4945.0	582.7	11.8

Control Standard							
Theoretical concentration (µg/ml)			**10120.0**	**Calculated concentration (µg/ml)**		8829.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	2907.0	8752.3	2863.5	61.5	2.15	**85.2**	730.1
Control 2	2820.0	8500.2					

SUMMARY OUTPUT		SYTEM SUITABILITY CONDITIONS		LOD	LOQ
Regression Statistics		**Response factor 1**	N/A	2498.6	8328.5
Multiple R	0.988	**Response factor 2**	N/A		
R Square	0.977	**USP tailing**	1.14		
Adjusted R Square	0.969	**Theoretical plate count**	9916.4		
Standard Error	287.4	**Capacity**	0.743		
Observations	5	**Resolution**	N/A		

Table 5.12 LaboClean FT concentrate® peak area response summary report obtained for method validation of the developed HPLC method, conducted by an inexperienced analyst using a Shimadzu® UFLC system

Theoretical 100% concentration (µg/ml)				19575.0					
Analytical values									
Concentration	**% Range**	**Value 1**	**Value 2**	**Value 3**	**Value 4**	**Value 5**	**Average**	**SD**	**% RSD**
3841.6	19.6	37766.0	60479.0				49123.0	16061.0	32.7
13445.6	68.7	262775.0	294857.0				278816.0	22685.0	8.14
19208.0	98.1	465397.0	499252.0	524210.0	541223.0	553572.0	516731.0	35191.0	6.81
43529.3	222.4	1247175.0	1274066.0				1260621.0	19015.0	1.51
58039.0	296.5	1743417.0	1763894.0				1753656.0	14479.0	0.83

Control Standard							
Theoretical concentration (µg/ml)			19575.0	**Calculated concentration (µg/ml)**		19274.0	
Name	**Value**	**Concentration**	**Average**	**SD**	**%RSD**	**% Recovery**	**Uncertainty (x) (µg/ml)**
Control 1	568274.0	21200.0	570672.0	3391.0	0.590	**108.7**	5648.6
Control 2	573070.0	21351.0					

SUMMARY OUTPUT		**SYTEM SUITABILITY CONDITIONS**		**LOD**	**LOQ**
Regression Statistics		**Response factor 1**	N/A	3253.6	10845.5
Multiple R	0.999	**Response factor 2**	N/A		
R Square	0.998	**USP tailing**	1.40		
Adjusted R Square	0.998	**Theoretical plate count**	6673.7		
Standard Error	34417.7	**Capacity**	**0**		
Observations	5	**Resolution**	N/A		

Results show that the method was successfully transferred to another laboratory. The linearity results of the both the detergent peaks fall within specification, where $R^2 \geq 0.98$. The percentage recovery of both detergent peaks was within a range of 95 to 102%.

The results show that the method performs well under normal conditions from laboratory to laboratory, instrument to instrument and analyst to analyst.

- Table 5.10 and 5.11 present the method validation summary reports of Ekon D concentrate® peak 1 and peak 2 respectively whiles Table 5.12 is the method validation summary report of LaboClean FT concentrate®. The results were generated by an inexperienced analyst in an attempt to validate the method on the Shimadzu® UFLC system.

  The linearity of the validation attempt for Ekon D concentrate® peak 1 was within specification however linearity for peak 2 was below specification. The percentage recovery of both the Ekon D concentrate® peaks was way below specification. When observing the mass of the detergent weighed by the analyst, it is inevitable that the pipeting technique used to measure the detergent is not mustered by the analyst. Air bubbles in detergents also present a challenge when the detergents are weighed.

  Results for the LaboClean FT concentrate® showed an acceptable linearity fit. The percentage recovery of the active was however above specification. The %RSD on the 20% concentration standard was above specification showing lack of precision in the method.

The ruggedness results obtained in this study show that the method can be successfully transferred from laboratory to laboratory, analyst to analyst and instrument to instrument, however validation of the same method on another instrument is not as easy. The method was found not be rugged enough to be validated on a different instrument, or to be validated by an inexperienced analyst.

f. System suitability

System suitability parameters that will be reported are peak area reproducibility, capacity factor, tailing factor, resolution, and theoretical plate count.

- **Peak area reproducibility**

(%RSD) of replicate injections reported in Tables 5.7, 5.8 and 5.9 was discussed in point c under precision.

- **Capacity factor**

The capacity factor (*k'*) values reported for the validation of the method on the Agilent 1100 system in the laboratory of study (Tables 5.7, 5.8 and 5.9), showed that the active components are retained enough by the column to provide adequate retention. The reported capacity factor values show that analyte gets sufficient opportunity to interact with the stationary phase.

- **Tailing factor**

The peak tailing results reported for the identified active peaks for both detergents in Tables 5.7, 5.8 and 5.9 ranges between 1.1 and 1.5. These results indicate an acceptable interaction of the analyte with the column stationary phase. The results also indicate a good column performance.

- **Resolution**

The resolution reported in Tables 5.7 and 5.8 between the Ekon D concentrate® peak 1 and peak 2 is 9.879 minutes. This resolution value indicates enough separation of the peaks.

- **Theoretical plate count**

The column performance reported for both detergents in Tables 5.7, 5.8 and 5.9 indicate an excellent column performance.

5.3 SUMMARY AND CONCLUSION

The main objective of this study was to validate the developed HPLC method for the detection of detergents and/or API residues in the laboratory of study for glassware cleaning validation purposes.

It was realised that it was a big challenge to develop one HPLC method utilising UV detection alone for detecting API's and detergents. The main challenge was the variety of products (and ultimately numerous API's) that are tested in this particular facility. The logistics to keep used glassware grouped together for a particular API was not possible and made the identification of API's in the development and validation of an analytical method hardly possible. The study was therefore focused on developing a method for detecting detergent traces only.

It is inevitable that the detergents low UV chromophores presented challenges with the precision parameters and system suitability conditions in the attempt to validate the developed HPLC method in another laboratory, by an inexperienced analyst on the Shimadzu® UFLC system. The developed method was however transferable to another laboratory, by an inexperienced student on the Shimadzu® UFLC system under normal conditions.

The validation of the developed method for detecting detergent residues on the Agilent® 1100 systems in the laboratory of study was a success. The developed HPLC method was proved to meet all the performance expectations and acceptance criteria for cleaning validation purposes as stipulated in the guidelines by Plazs (2005). The objective to validate the HPLC method for the detection of detergents for a pharmaceutical contract testing laboratory was met.

5.4 RESEARCH RECOMMENDATIONS

A couple of shortcomings were identified with regards to the developed HPLC method.

- The baseline in some of the chromatograms was unstable. This might have been caused by the HPLC system pressure instability or air bubbles in the HPLC system.

- Carryovers were observed in some of the chromatograms. The cause may have been a short run time employed for the analysis, or unretained compounds which adhered to the stationary phase.
- A solvent associated peak was detected at a retention time almost close to that of the LaboClean FT concentrate®. This solvent associated peak made it difficult for one to identify if there was any LaboClean FT concentrate® detergent residue traces detected from the machine washed glassware.
- A good research can be time and quantity dependant. Sampling of a variety of sizes of the volumetric flasks used on daily basis over a longer period may be worthwhile.
- The operation limit of the developed method was determined to have an LOD of 700 µg/ml for peak 2 of Ekon D Concentrate®. This limit might not be sensitive enough to enable the method to detect contaminants at lower concentration ranges that are dealt with when testing for degradation products of drug and drug related substances in the study laboratory. More sensitive detection techniques like the MS may be a employed to improve the LOD.

It is essential to ensure that the HPLC system used to develop a method is in good working condition to ensure consistency in the data generated. Thorough observation of the chromatograms is essential in the early method development stages to ensure that the run time employed for analyses is enough for all the peaks to elute and that no carryovers are encountered between successive runs. Before commencing with generating data it is worthwhile to trial a variety of solvents over successive runs to avoid elution of ghost peaks. Smaller buffer concentration used to prepare the mobile phase saves costs and the HPLC instrument is protected from exposure to concentrated chemicals. Research conducted over a long period offers an extensive overview of the subject in question. HPLC method optimization is an inevitable issue that arises from the current study findings.