CHAPTER 3

EQUIPMENT, MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter discusses the experimental equipment, materials and methods used to conduct this study. Results will be presented and discussed in chapter 4.

3.2 GLASSWARE CLEANING PROCEDURES

Two glassware cleaning procedures are currently used in the laboratory of study. A brief explanation of the procedures will be given in the following paragraphs.

3.2.1 Automated glassware cleaning procedure

An automated glass-washer is employed in the cleaning of volumetric glassware. The G 7783 CD Mielabor glass-washer is a front-loading automatic machine for the efficient washing, neutralising and rinsing of laboratory glassware (Miele Co. Ltd. RSA). A single wash cycle programme has five steps, namely; pre-wash, wash/ disinfecting, interim rinse, final rinse and drying.

This instrument is operated on normal tap water supply. The instrument also has an "AD" (aqua destillata) programme with final rinses using purified water. The electronic control unit offers a choice of temperature programmes of up to 90°C for cleaning and final rinsing phases of up to 70°C. This machine is fitted with a drying unit and water softener as standard. The detergent LaboClean Ft® used in this machine is specially supplied by Miele Co. Ltd. RSA. The glass-washer has the capacity to load only thirty seven volumetric flasks, for a single sixty minutes cycle wash. These thirty seven volumetric flasks should comprise of eight 5 ml to 25 ml volumetric flasks, twenty three 50 ml to 100 ml volumetric flasks and six 200 ml to 1000 ml volumetric flasks. On completion of the automatic programme sequence, laboratory glassware is expected to be clean to the standard required for analysis. Drying of glassware can be achieved

using the Miele glass-washer program, use of a designated oven, or by hanging the glassware on the designated drying rack.

3.2.1.1 Cleaning procedure

- Programme C with T1 temperature of 50°C and T2 temperature of 70°C is used.
- The volumetric flasks should be emptied before being loaded into the machine.
- Care should be taken not to allow any acid or chlorine solution to spill in the washing cabinet
- Stoppers and labels should be removed from the containers before loading them into the machine.

3.2.1.2 Possible drawbacks of the current in-house automated glassware cleaning procedure

Possible shortcomings were identified on the use of the current in-house automatic glassware cleaning procedure.

1. Instructions for the use of the washing programme are not explained in the standard operating procedure (SOP).
2. Generally it is good practice to rinse glassware with tap water or with a suitable organic solvent followed by water after use, before commencing with the general cleaning procedure.

   On a day to day scenario of a pharmaceutical contract testing laboratory, it is difficult to closely monitor exposure of glassware to acidic or chlorinated chemicals or to monitor the severity of exposure.

The convenience of an automated glassware washer does not always suffice for the washing of all the laboratory glassware due to its limited capacity, shape incompatibility of some glassware containers and high demand for clean glassware on a day to day basis. Such cases result in manual glassware washing. Figure 3.1 shows a schematic summary of the automated glassware cleaning procedure.

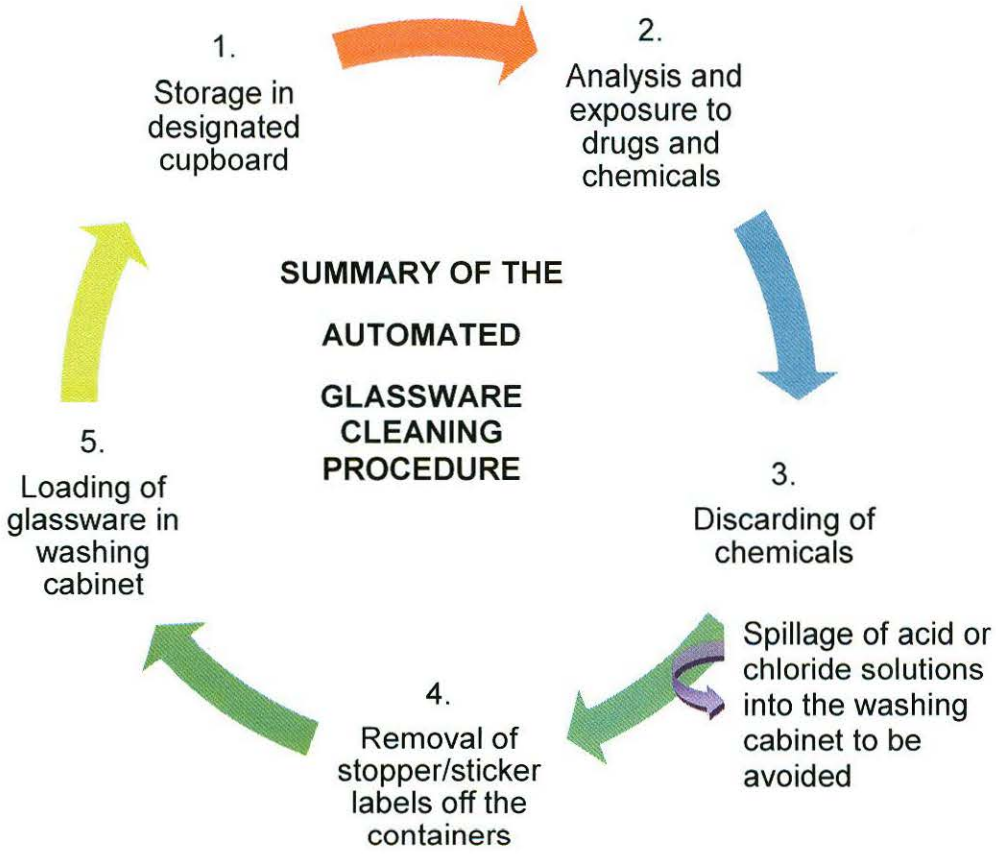

Figure 3.1 Schematic representation summary of the automated glassware cleaning procedure.

3.2.2 Manual glassware cleaning procedure

This section briefly outlines the current in-house manual glassware cleaning procedure.

3.2.2.1 Detergents and non-corrosive cleaning agents

Only approved detergents suitable for laboratory cleaning purposes are used. Ekon D concentrate® supplied by Merck (Pty) Ltd. RSA, is generally used for the manual cleaning of glassware. Another non-corrosive cleaning agent that is available, though casually used in the laboratory of study is Contrad concentrate® (catalogue code 56022), manufactured under licence from Decon laboratories by Merck (Pty) Ltd. RSA.

The SOP requires the use of these detergents according to the supplier/manufacturer instructions. Figure 3.2 shows a schematic summary of the manual glassware cleaning procedure.

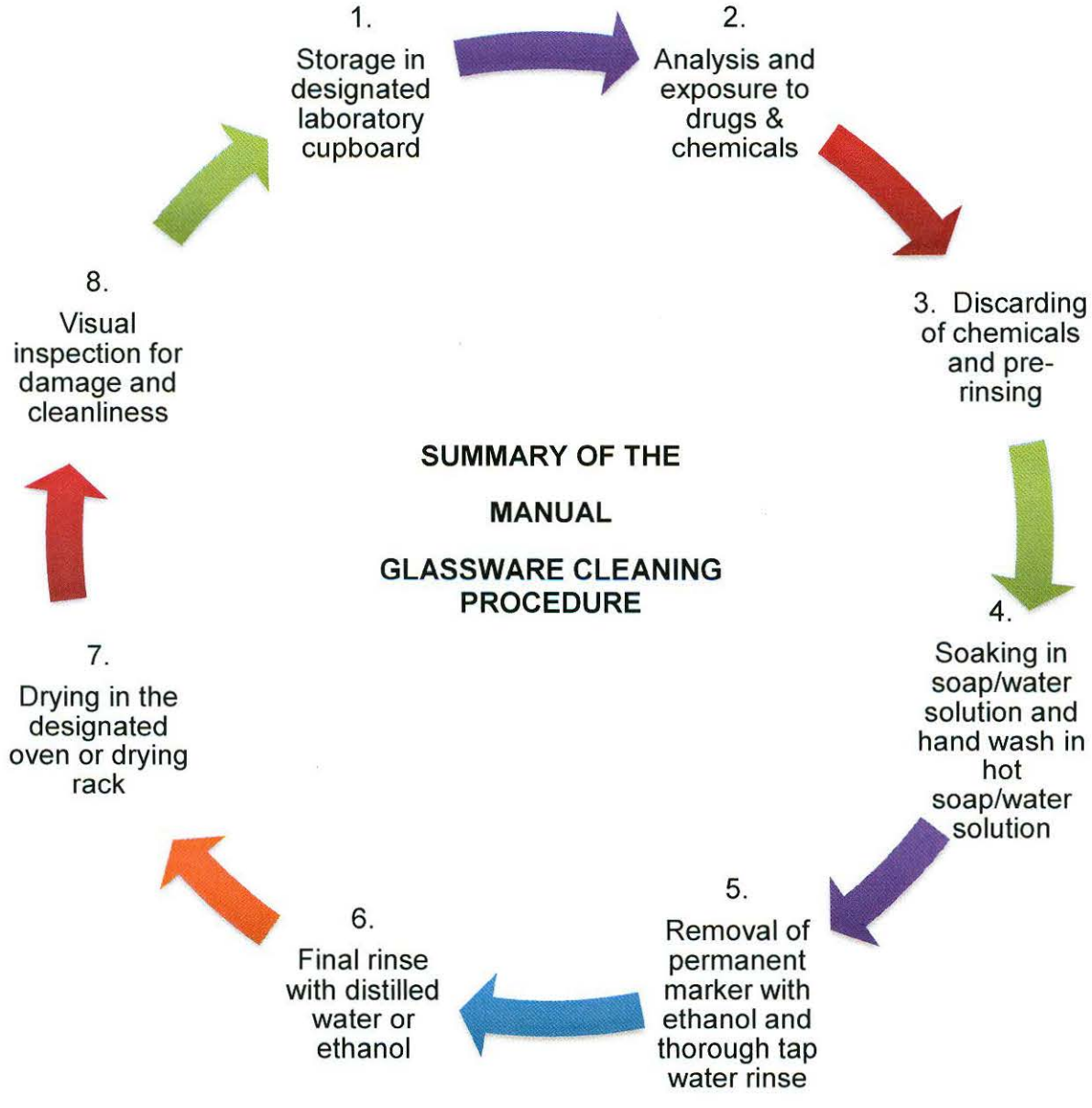

Figure 3.2 Schematic representation summary of the manual glassware cleaning procedure.

3.2.2.2 Pre-rinsing/ prewashing of glassware

Generally it is good practice to rinse glassware with tap water or with a suitable organic solvent followed by water after use, before commencing with the general cleaning procedure with the detergent solution.

3.2.2.3 General manual cleaning/ washing of glassware

After appropriately pre-rinsing glassware with water, the glassware is soaked in a suitably prepared soap solution with time frame dependent on the severity of the dirty glassware. After soaking, the glassware is washed manually using hot water and low residue detergents.

The washing solution may be replaced with a fresh one on regular intervals. Ethanol is used to remove permanent pen marking on the outside of the glassware. Glassware is rinsed with successive amounts of tap water after a thorough wash until no signs of soap or samples are left. Finally the glassware is rinsed with ethanol or purified/distilled water.

3.2.2.4 Drying glassware

Washed glassware is dried in the designated drying oven with temperature set between 50-60°C or it is hanged on a drying rack for only a period necessary for the glassware to dry.

3.2.2.5 Visual inspection

Dried glassware is then subject to visual inspection for damage and cleanliness before storage in the designated storage cupboards.

3.2.2.6 Possible drawbacks of the current in-house manual glassware cleaning procedure

Possible shortcomings were identified on the use of the current in-house manual glassware cleaning procedure. Each shortcoming is briefly explained.

1. The SOP requires the use of detergents according to the supplier/manufacturer instructions. Ekon D concentrate® is widely used for the manual glassware cleaning in the laboratory of study. The supplier describes this Ekon D concentrate® as a phosphate free cleaning agent for highly contaminated laboratory glass and plastic ware. This detergent however, does not have specific supplier/manufacturer user instructions.
2. The current procedure does not specify the in-house glassware soaking/washing solution concentration and the exact intervals the washing solution should be replaced.
3. Ethanol is a widely used organic solvent for laboratory housekeeping purposes however; it does not suffice for the removal of permanent markers on the glassware.

3.3 MANUFACTURES AND/OR SUPPLIERS

Hexanesulfonic acid sodium salt used was of analytical grade purchased from Merck (Pty) Ltd. RSA. The solvents used (methanol, acetonitrile, phosphoric acid and ethanol) were of HPLC and analytical grade also purchased from Merck (Pty) Ltd. RSA. The Milli-Q water purification system, a Millipore product was purchased from Microsep, RSA.

3.4 PHYSICAL PROPERTIES

Physical properties are properties that can be observed and measured without changing the composition of a substance (Kotz & Treichel, 2003). Colour, state of matter, solubility and density were the four physical properties that were examined for the three detergents. Each physical property observation is briefly mentioned in the next paragraph.

3.4.1 Colour, solubility & state of matter observations

- Ekon D concentrate uniTEK® is a homogenous thick clear liquid. The detergent is highly soluble in water and highly foams when mixed with water.

- Contrad concentrate® is a non-homogenous watery clear liquid. The detergent is highly soluble in water and highly foams when mixed with water.
- LaboClean FT (neodisher®) is a homogenous yellowish watery liquid concentrate. This concentrate is highly soluble in water and slightly foams when mixed with water.

3.4.2 Density analysis

Density is mass per unit volume of a substance (Ansel, 2010). It is usually expressed as grams per cubic centimetre (g/cc) or grams per millilitre (g/ml). Specific gravity is a ratio, expressed decimally of the weight of a substance to the weight of an equal volume of a substance chosen as a standard, both substances at the same temperature or the temperature of each being known (Ansel, 2010). Water is used as the standard for the specific gravities of liquids and can be calculated by dividing the weight of a given substance by the weight of an equal volume of water.

The three detergents were subjected to density analysis. This quantitative property was measured using the Anton Paar DMA 38 density meter. The DMA 38 is an oscillating U-tube density meter measuring sample density values accurately to 0.001 g/cm^3 in a temperature range of 15 to 40°C (Anton Paar GmbH, Graz, Austria). Sample viscosity, surface tension and colour have no influence on the measuring results.

Before analyses could be carried out, the measuring cell was cleaned and then conditioned by passing adequate amount of the sample through it. The sample was filled into the measuring cell using a plastic syringe. The sample was filled into the measuring cell by slowly and continuously pressing the plunger of the syringe, thereby avoiding tiny invisible bubbles which might influence the measuring results. When the measuring cell was full with sample a reading was taken. The measured results are automatically converted into concentration, specific gravity or other density related units. Results are presented and discussed in chapter 4, section 4.1.

3.5 UV-SPECTROPHOTOMETRIC ANALYSIS

Spectroscopic analyses are quantitative. These type of analyses are based on the relationship between the amount of light absorbed and the amount of the absorbing substance. The degree of absorbance of light is proportional to the concentration of the absorbing substance according to Beer's law as discussed in chapter 2, section 2.4.6.
A spectrophotometer consists of two instruments, a spectrometer for producing light of any wavelength and a photometer for measuring the intensity of light. The instruments are arranged so that the liquid in the cuvette can be place between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer (Caprette, 2005).

The three detergents (Ekon D®, Contrad® and LaboClean Ft. concentrate®) were subjected to spectroscopic analysis. A Shimadzu UV-2450 PC single monochromator system was employed in the basic determination of the three detergent's maximum wavelength of absorption. This system has a detection wavelength guaranteed for performance in the range 190 to 900 nm.
It also has wavelength repeatability of +/- 0.1 nm and wavelength accuracy of +/- 0.3 nm (Shimadzu, 2011). Concentrations 0.2 mg/ml, 2.0 mg/ml and 20.0 mg/ml of the detergents, were separately prepared and scanned on the spectrophotometer. Milli-Q water was used as a solvent and a blank. The analytes were scanned in the range of 190 nm to 300 nm on a 1 cm cuvette, starting with the most concentrated to the least. The spectra was then recorded and analysed. Results are reported and discussed in chapter 4, section 4.2.

3.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS

HPLC analyses formed a major component of the experimental work conducted for this study. Experiments conducted for HPLC method development are explained below.

3.6.1 Instrument used for method development

The Agilent 1100 series HPLC system was used in this study. The Agilent 1100 is a series of modern component stackable HPLC systems. Key features of Agilent 1100

series include an online vacuum degasser, which allows reduced operating costs and ensures high instrument performance. The series is optimized for improved sensitivity and allows for easy front access to exchange flow cells and lamps. The temperature management system offer optimum baseline stability. Agilent 1100 series diode-array detector ensure the highest light output from 190 to 950 nm, for the lowest detection limits over the entire wavelength range (GMI, 2011). Latest models come standard with an automatic sampler and injector. The systems were controlled using the Agilent ChemStation software.

3.6.2 HPLC method chromatographic conditions

In chapter 2, the steps followed for the HPLC method development were explained in detail. In this section the established parameters and protocols will be mentioned. Table 3.1 is the summary of chromatographic conditions established for the HPLC. Table 3.2 is the system suitability conditions proposed for the HPLC method.

Table 3.1 Chromatographic conditions for the HPLC

HPLC Chromatographic conditions	
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

Table 3.2 System suitability conditions

System suitability conditions	
Preliminary set up	Prepare the mobile phase and set up the equipment as specified in the standard procedure. Employ a run time of 20 minutes.
System suitability	Inject the standard solution six times and calculate the average of the peak area results. The recovery solution is injected twice.
	Perform a system suitability test on the six standard injections, calculating the parameters according to the formulae as specified in the USP.
Acceptance criteria	The relative standard deviation of the peak areas due to the active for the six replicate injections will be determined according to USP guidelines.

3.6.3 Preparation of standard solutions

The three detergents Ekon D®, Contrad® and LaboClean ft® concentrate were used as references standards. For each detergent, a reference standard and a recovery standard both with the same concentration were prepared. The areas of the identified peaks obtained from preliminary HPLC analysis of the reference standard and the recovery standard were used to determine the limit of detection and limit of quantitation of the detergents. Following preliminary HPLC analysis for the identification of active peaks for each detergent, a reference standard, recovery standard, LOD and LOQ standard and a hundred and fifty percent concentration standards, relative to the predetermined reference standard was also prepared. This was done in order to construct a regression line as specified in the USP guidelines.

Table 3.3, 3.4 and 3.5 are a summary of the standard preparations of the Ekon D®, LaboClean ft® and Contrad® concentrate respectively.

Table 3.3 Standard preparation of Ekon D concentrate®

Ekon D Concentrate®	
Reference standard	Accurately weigh 1.0 ml of Ekon D concentrate® to a 100 ml volumetric flask. Add and make up to volume with solvent and mix well.
Recovery standard	Accurately weigh 1.0 ml of Ekon D concentrate® to a 100ml volumetric flask. Add and make up to volume with solvent and mix well.
LOD & LOQ	From the reference standard prepare 20% solution and a 70% solution in solvent, and label LOD and LOQ respectively.
150% standard	Accurately weigh 3.0 ml of Ekon D concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.

Table 3.4 Standard preparation of LaboClean FT concentrate®

LaboClean FT Concentrate®	
Reference standard	Accurately weigh 3.0 ml of LaboClean ft concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.
Recovery standard	Accurately weigh 3.0 ml of LaboClean ft concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.
LOD & LOQ	From the reference standard prepare 20% solution and a 70% solution in solvent, and label LOD and LOQ respectively.
150% v/v standard	Accurately weigh 4.5 ml of LaboClean ft concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent and mix well.

Table 3.5 Standard preparation of Contrad concentrate®

Contrad concentrate®	
Reference standard	Accurately weigh 1.0 ml of Contrad concentrate® to a 100 ml volumetric flask. Add and make up to volume with solvent and mix well.
Recovery standard	Accurately weigh 1.0 ml of Contrad concentrate® to a 100 ml volumetric flask. Add and make up to volume with solvent and mix well.
LOD & LOQ	From the reference standard prepare 20% solution and a 70% solution in solvent, and label LOD and LOQ respectively.
150% v/v standard	Accurately weigh 3.0 ml of Contrad concentrate® to a 200 ml volumetric flask. Add and make up to volume with solvent.

3.6.4 Preparation of sample solutions

Two sampling procedures were used in the preparation of glassware analytes. Each procedure is explained in the next paragraph.

a. Rinse sampling

Rinse sampling involves using a liquid to cover the surface to be sampled (LeBlanc, 2008). This procedure allows sampling of residues in all surfaces of the equipment, even on the most difficult to clean surfaces. Sampling with this procedure gives a snapshot of the overall contamination of the equipment (LeBlanc, 2008). A variety of rinsing solvents can be used, catering for the water insoluble contaminants.

Procedure: To an empty volumetric flask pipette 10 ml solvent (Milli-Q water), cap the flask and hand shake vigorously for about a minute. Allow flask to stand on the bench for a few minutes. Filter through a 0.45 µm membrane filter into an HPLC vial and inject.

b. Swab sampling

Swabbing involves the use of a cotton swab moistened with a suitable solvent and then drawn over a defined area using a systematic, multi-pass technique always moving from clean to dirty areas to avoid contamination (McLaughlin & Zisman, 2005). The residue is then extracted or desorbed from the swab head into the suitable solvent for subsequent analysis (LeBlanc, 2008).

Procedure: Wet a swab in solvent and rub the swab against the inside of the glassware, in all areas possible to reach with the swab.

To an HPLC vial add 1.0 ml of solvent and dip a used swab. Leave the swab in the solvent filled vial for a minute. Remove the swab from the vial, by slowly pulling the swab while twisting and rubbing it against the vial walls, to remove the excess solvent from the swab bud, cap the vial and inject.

Cleaned laboratory volumetric flasks varying from 20 ml to 250 ml were randomly used as samples. Glassware washed with the automatic laboratory glass-washer and manually washed glassware was subjected to the sampling procedures.
Six cleaned volumetric flasks varying in sizes were randomly selected for sampling every day for a period of a week. A clear distinction was established between glassware washed with the automatic laboratory glass-washer and manually washed glassware.

A standard addition and recovery procedure was also employed to prove that the cleaning procedure works and that the glassware is indeed clean after being hand washed or automatically washed with the glassware washer. The analyte (drug) is applied to the surface as a solution, allowed to dry, and then either swabbed or rinsed off to prove that if it is there it will be removed by either technique. This procedure also helps in choosing the best sampling procedure that can be used to investigate the effectiveness of the glassware cleaning procedures. Thoroughly clean and rinsed laboratory glassware is also sampled but serve as control.

3.6.5 Conclusion

The purpose of the HPLC analysis of cleaning samples is to prove with data that the equipment is indeed clean. The HPLC chromatographic finish is reproducible and can be used to prove that the samples analysed by the HPLC quantitatively remove any residues of the analyte left behind or incompletely removed by the cleaning procedure. The outcomes of the established method, chromatographic conditions and sampling procedures will be reported and discussed in chapter 4.