CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF THE STUDY LABORATORY

The pharmaceutical industry is complex, and can be divided into three principal tasks: production, distribution, and research and development (R&D) (González & Gascón, 2004). This industry is highly regulated as it accounts for the health of millions of human lives and plays a critical role in the ascent of market economies of many countries (Cardinal & Hatfield, 2000). Regulatory authorities of the pharmaceutical industry ensure uniformity of rules and regulations in different countries to facilitate structured unbiased scientific compliance and business opportunities. With regards to compliance and business opportunities, commercialization of pharmaceutical products is subject to thorough scrutiny by pharmaceutical contract testing laboratories.

There are different types or categories of pharmaceutical laboratories namely; first party, second party and third party laboratories of which the latter will be the study focus. The laboratory of study is categorised as a third party industrial pharmaceutical laboratory based on its activities in the pharmaceutical industry as recognised by international and/or local regulatory authorities.

The laboratory of study is mainly a pharmaceutical contract testing laboratory, operating within the School of Pharmacy in the Health Sciences Faculty, of the North-West University, Potchefstroom Campus. This laboratory performs non-clinical testing on nearly every type of pharmaceutical, cosmetic and veterinary product/dosage forms and on the associated active and non-active starting materials, on a contract basis, for quality assessment purposes. The work performed in the laboratory of study serves South Africa and foreign pharmaceutical, veterinary and cosmetic companies, as well as other organisations and agencies such as the World Health Organisation (WHO), Clinton Foundation and South African Medicines Control Council.

With approximately forty years in operation the laboratory of study has since become the largest and most experienced university affiliated quality assessment facility in South Africa. This facility has been used by a number of small and large companies to acquire data necessary to register pharmaceutical products in South Africa. Several currently marketed products in South Africa also have their origin in this unique research and development facility.

The scope of operation and testing performed in this facility includes techniques such as the ultra-pressure liquid chromatography (UPLC), high pressure liquid chromatography (HPLC), gas chromatography (GC) and ultraviolet spectrometry (UV). The facility also has instruments pertaining to the physical analysis of pharmaceuticals i.e. differential scanning calorimetry (DSC) and infrared red (IR) to name but a few. A wide spectrum of other chemical and physical tests applied in pharmaceutical analysis is also carried out in this facility.

One of the major policies governing the laboratory of study, is the assurance that all aspects of the work (testing/service/administration) performed within the facility shall be done in accordance with relevant nationally and internationally recognised quality guidelines, such as the South African guide to GMP, GLP, ISO/IEC 17025:2005, and FDA guidelines, to assure a professional work environment in which study integrity or validity, customer satisfaction, public health and safety, and environmental protection are a priority.

This facility is intended to generate data that is correct and consistent every time, the first time, in order to ensure the integrity and validity of test results generated. Trustworthy and timely results are of essence to the operation of this facility and hence every aspect in the generation of quality reliable data must not be compromised.

2.2 CHROMATOGRAPHY

Quantitative or qualitative analyses result in data that must somehow be interpreted and reported to make meaningful decisions. Chromatographic techniques present some of the most reliable and leading sources of generating analytical data that must be acquired, interpreted, quantified, compared, reported and finally archived (Ahuja, 2005).

High pressure liquid chromatography (HPLC), sometimes called high performance liquid chromatography is a chromatographic technique widely used in the pharmaceutical industry. HPLC can be used in analytical developments to quantify the active pharmaceutical ingredient (API) and to evaluate impurity and degradation product profiles of drug products (Rasmussen *et al.,* 2005). This chromatographic technique can additionally be used to determine content uniformity of dosage forms, monitor dissolution profiles, determine antioxidants and microbial preservatives content and to support cleaning validation (Rasmussen *et al.,* 2005). High sensitivity, selectivity, automation characteristics, and the ability to detect compounds with low UV chromophores (Boca *et al.*, 2005), supports HPLC as the chromatographic technique of choice for use in pharmaceutical method development.

Data acquired from HPLC analysis is presented in a form of chromatograms. A chromatogram is an output of the signal produced by sample components transported to the detector by the mobile phase versus time (Agilent Technologies, 2004). Retention times provide the qualitative aspect of the chromatogram and retention time of a compound will be the same under identical chromatographic conditions while the chromatographic peak height or peak area is related to the quantity of the analyte (Agilent Technologies, 2004). Literature by Agilent Technologies (2004) says to determine the actual amount of the analyte, the area or height is compared against standards of known concentration. Chromatograms offer an integrated presentation of the instrument, method and performance of the chromatographic conditions used for analysis. Chromatograms offer a visual source of data that can be used as evidence of reference for making meaningful decisions.

2.3 HPLC METHOD DEVELOPMENT

Logic and simplicity lay the foundation of method development. Information on the compound of interest and the objective of the method to be developed form the basis of early phase method development. Method development can be time consuming and costly, hence a comprehensive literature search may be worth investing in.

Method development can be greatly facilitated by the use of chromatographic software programs. DryLab®, ChromSword®, EluEx®, and LabExpert® are computer assisted chromatography software packages that have been developed to make method development easier. Usage of these software packages range from using retention data from scouting runs for subsequent retention and resolution prediction via simulation to using structure fragments and dipole-dipole interactions to predict retention behaviour (Rasmussen *et al.,* 2005). These software packages are efficient however very expensive. For this study, the trial and error approach was used for method development. Using this approach requires the method objectives to be clearly defined, theoretically sound and practically applicable for its intended purpose to save costs and time.

Guidelines for HPLC method development are available in literature. The basic principles underlying HPLC method development are the same however the objectives of the method shape the end product of the development. In this study guideline from Richardson & Erni (2005) and Thompson & LoBrutto (2007) will be used.

Table 2.1 summarises some of the basic chemical properties to consider about the analyte used to guide HPLC method development as highlighted in literature.

Table 2.1: Properties of the analyte to guide method development

Properties of the analyte	Application
Solubility	Assist in the choice of a suitable mobile phase
	Guides sample preparation and choice of suitable solvent
UV properties	Determination of optimum wavelength absorption
	Determination of the UV- cut off of the solvent
pKa values	Determining if compound is acid or a base
	Suitable pH to obtain ion suppression or ion pairing
Stability	To stipulate time constraints or special sample manipulation procedures
	Choice of a suitable sample solvent

2.4 PROCEDURE FOR DEVELOPING AN HPLC METHOD

This section reviews details of the procedure followed to develop the HPLC method for glassware cleaning validation purposes. One of the objectives for developing the HPLC method for this study was to enable the detection of detergents used in the cleaning of laboratory glassware. The method development was initiated by means of a thorough literature search.

2.4.1 Analytes/compound of interest

Three detergents that are used in the laboratory of study were investigated. Two of these detergents namely; Ekon D concentrate uniTEK® (frequently used) and Contrad concentrate® (occasionally used) are used for the manual/hand washing of all washable laboratory equipment, including glassware and washable instrument components.
Ekon D concentrate uniTEK® is described by the manufacturer on the container label as a cleaning agent for highly contaminated glass and plastic ware, containing ionic and anionic surfactants whereas Contrad concentrate® is described as strongly alkaline in its undiluted form.

The third detergent LaboClean FT (neodisher®) is used for the automated cleaning of laboratory glassware. This detergent contains potassium hydroxide and/or sodium hydroxide and sodium hypochlorite solution (1-5% active chlorine) as described on the container label.

Detergent formulations are kept confidential due to competition amongst manufacturers (Resto *et al.*, 2007). However, literature reveals that surfactants and builders are two main ingredients in detergent formulations. These components must possess improved washing power and capacity for sequestering all the hardness in water. Components must be biodegradable and non-polluting (Yu *et al.*, 2008).

Surfactants

Surfactant is an abbreviation for surface active agent, meaning active at a surface (Yu *et al.*, 2008). Surfactants are characterised by having a molecule which carries a hydrophobic (water repelling) hydrocarbon tail and a hydrophilic (water-attracting) head (Thomssen & McCutchenon, 1949).
Based on the polar head group, surfactants used in detergent formulations can be classified into four groups: anionics, nonionics, cationics, and zwitterionics (Yu *et al.*, 2008). Some detergents however contain a mixture of different types of surfactants to strengthen their performance capability and to remain mild to the skin.

Builders

Builders are often used in conjunction with surfactants in detergent formulations (Yu *et al.*, 2008). Builders remove calcium and magnesium ions existing in hard water by forming soluble complex compounds and keep calcium and magnesium salts from forming precipitates (Eby & Tatum, 1989). This action in turn greatly increases the efficiency of surfactants in hard water and improves its performance in soft water. In addition to softening the water these builders should also satisfy a large number of requirements including buffer capacity, effects on fish and aquatic animals, environmental and economic considerations (Yu *et al.*, 2008).

Though limited information was specified by the manufacturers with regards to the chemical composition of the detergents, a few useful pointers were taken:

- The presence of cationic, non-ionic and zwitterionic surfactants
- Alkaline pH, and
- Solubility in water.

2.4.2 Selection guide to HPLC chromatographic mode

Figure 2.1 shows a flow chart representation of the selection of HPLC mode based on sample properties of solubility and polarity as depicted in LabHouse (Pty) Ltd, (1999).

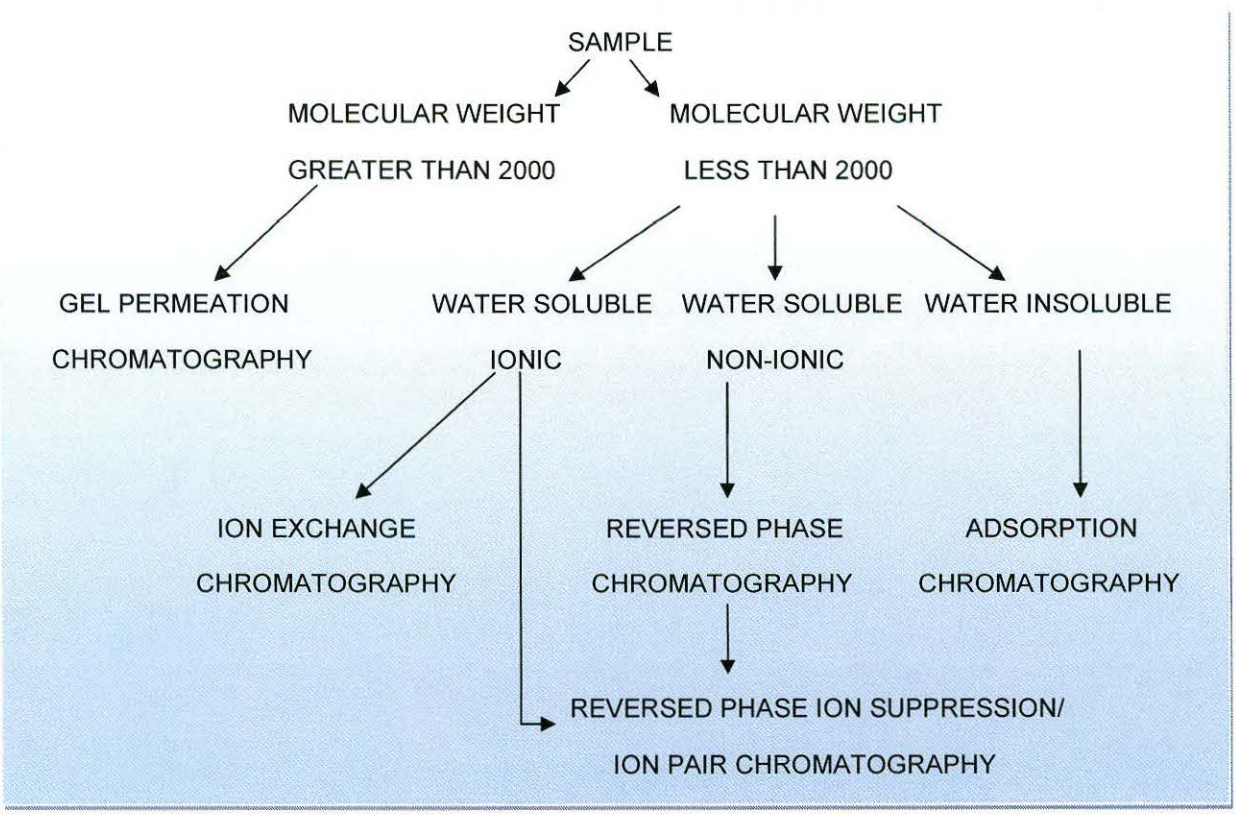

Figure 2.1: Schematic representation to selecting HPLC chromatographic mode.

According to figure 2.1 sample molecular weight guides the first level of chromatography mode selection. Each chromatographic mode will be briefly discussed.

- Gel permeation or gel exclusion chromatography is categorised under size exclusion chromatography, where separation occurs because of mass or size constraints (Agilent Technologies, 2004). Gel permeation is when size exclusion is performed with an organic phase (Agilent Technologies, 2004). This technique separates analytes based on their sizes in solution and can handle high molecular weight samples (Wong & Datla, 2005). Gel permeation is mainly associated with polymer analysis and separation can only occur between components if the molecular weight differs by at least 10% (Agilent Technologies, 2004). The stationary phase is prepared from porous silica (Boyer, 2002), with the order of elution of the compounds from the column being determined by the degree to which they can permeate the pores of the packing material (Thompson & LoBrutto, 2007).

For samples with a molecular weight less than 2000 g/mol; several chromatographic techniques can be employed.

- Normal phase (adsorption) chromatography is the use of a solvent/column system where the stationary phase is more polar than the mobile phase (Agilent Technologies, 2004). Retention occurs through polar interactions, such as hydrogen bonding and dipole interactions, between the solute and the stationary phase (Thompson & LoBrutto, 2007); the sample needs to displace the mobile phase from the surface of the stationary phase for retention to occur (Agilent Technologies, 2004). Silica (adsorption), cyano (bonded phases), diol, and amino stationary phases are commonly used for the normal phase chromatography (Thompson & LoBrutto, 2007). This technique is used for the separation of structural isomers and compounds that are not readily soluble in organic/water mobile phases (Agilent Technologies, 2004).

- Ion exchange chromatography is used exclusively for the separation of

compounds that can be ions in solution; neutral compounds are not separated by this technique (Agilent Technologies, 2004). Resolution is based on the strength of the ionic interaction of ionisable compounds with ionic functional group on the stationary phase (Thompson & LoBrutto, 2007). The stationary phase is made of a cationic or anionic species covalently bound to an inert support like a polymer (Agilent Technologies, 2004).

The mobile phase is an aqueous solution of buffer which may contain counter ions and organic modifiers (Thompson & LoBrutto, 2007) whose ionic strength is adjusted to effect resolution (Agilent Technologies, 2004). At low ionic strength, the counter ion will compete with the analyte for the charged sites, whereas as the mobile phase ionic strength increases, less analyte retention occurs. Ion chromatography can be applied for the quantitation of inorganic impurities, drug substance counter ions, degradation products and ionic synthetic impurities (Thompson & LoBrutto, 2007).

- Reversed phase chromatography is the preferred HPLC mode in the pharmaceutical industry deriving its popularity from its mobile-phase compatibility with polar drug substances, higher efficiencies associated with this mode, shorter re-equilibration times, and the ability to run gradient methods covering a large range in polarity (Thompson & LoBrutto, 2007). In reversed phase chromatography, the stationary phase is less polar than the mobile phase and the main mechanism of retention is partitioning (Agilent Technologies, 2004). If the sample is more soluble in the stationary phase and is in effect squeezed out of the mobile phase by the polar water content, then retention occurs, whereas if the sample is more soluble in the mobile phase, then it will be less retained and elute quickly (Agilent Technologies, 2004). Commonly utilized stationary phase for this technique are C_8 (Octyl silyl), C_{18} (Octadecyl silyl), octadecylsilane (ODS), phenyl, and cyano. Reversed phase chromatography can be used for the separation of non-polar compounds, polar compounds, weak acids and bases, strong acids and bases (with ion-pair reagents), proteins, peptides, and homologs (Agilent Technologies, 2004).

The presence of ionic and anionic surfactants, alkalinity and solubility in water were three properties acquired in literature about the analytes/compound of interest. Based on figure 2.1 and the information acquired from literature about the analytes/compound of interest, the best suited HPLC chromatographic mode for this study is reversed phase chromatography.

2.4.3 Selection of the mobile phase

The mobile phase controls HPLC separation. While the HPLC stationary phase provides retention and influences the separation mechanism, it is the mobile phase which controls the overall separation (Ornaf & Dong, 2005). Finding a set of mobile phase conditions that can provide adequate separation of the analyte peak(s) from other components in the sample is key to a successful HPLC method development (Ornaf & Dong, 2005).

Generally the mobile phase for reversed phase chromatography, consist of mixtures of water or aqueous buffer with various water-miscible organic solvents. The stronger the organic mixed with water, the faster sample components will elute from the column. A weaker mobile phase improves the separation and analytes are retained longer on the column (Agilent Technologies, 2004).

A mobile phase with a pH range from 2.5-3 may serve as good starting point for most pharmaceutical applications. Low pH suppresses the ionisation of acidic solutes and the silanols sites of the stationary phase resulting in their high retention (Thompson & LoBrutto, 2007). Basic analytes are however ionised at low pH and might not be retained unless ion-pairing reagents are used (Ornaf & Dong, 2005).

Ion-pairing reagents are added to the mobile phase to provide additional retention and selectivity for the analytes with opposite charge (Ornaf & Dong, 2005). Ion-pairing reagents provide an additional parameter to facilitate resolution of basic analytes from other components in the sample. The alkyl sulfonates are commonly used for the separation of water soluble basic analytes. The alkyl sulfonate binds with basic analytes

to form ion-pairs. This results in neutral ion-pairs that are more hydrophobic than their unassociated protonated amines resulting in higher retention times (Ornaf & Dong, 2005).

In this study, various mobile phases were tested. The mobile phase combinations ranged from the buffer solution containing as little as 0.01 M of hexanesulfonate to a buffer containing 0.02M of hexanesulfonate mixed with different volumes of organic solvents (methanol or acetonitrile). With the trial and error approach, a mobile phase containing 25% acetonitrile and 75% 0.02 M hexanesulfonate buffer adjusted to pH 3.0 with phosphoric acid gave acceptable retention and resolution for peaks of the analytes.

2.4.4 Choosing a column

Column selection is difficult due to the wide variety of reversed phase columns available. C_{18} columns have longer alkyl chain length as compared to C_8 columns. C_{18} columns give better retention when compared to C_8 columns operated under the same conditions (i.e. mobile phase) (Synder *et al.*, 1988). C_{18} columns are more conductive to ion pairing due to the free silanols that are sterically more protected from secondary retention mechanisms because of the longer C_{18} alkyl chain. C_{18} columns give better separation for analytes that differ primarily in carbon content. Efficiency may be greater with C_{18} columns due to faster solute mass transfer.

Column performance is also affected by the particle size of the packing material. Commonly used column packing materials generally include the 10 µm irregular shaped packing and 5 µm spherical packing. Other sizes are available, such as 4 µm and 7 µm packing which closely resemble the 5 µm packing. These values are nominal sizes given for the average diameter of the particles. The smaller and more uniformly packed (i.e. spherical) columns give better resolution and sensitivity (Synder *et al.*, 1988).

Separation is achieved by the use of 15 cm x 0.46 cm, 5µm particle size or 30 cm x 0.39 cm, 10 µm particle size columns. The 15 cm column gives shorter run times and better resolution than their 30 cm counterparts; better resolution is not possible if the

particle size is the same. However, when improved resolution is required for difficult separations, longer columns are employed at the expense of longer run times (Synder *et al.*, 1988). These are typically the 25 cm x 0.46 cm, 5 µm particle size columns. The 10 µm particle size columns are mainly used for preparative work where improved load is required. The 10 µm particle size columns offer the basic column chemistry that can be used for developing a method for analysis of analytes with unknown chemical compositions (Synder *et al.*, 1988). The 10 µm particle size columns can be used as a starting point for method development. Once the method is developed, shorter and more sensitive columns can be employed for method optimization.

2.4.5 Isocratic vs. Gradient analysis

Gradient analyses are suited for complex samples and those containing analytes of diverse polarities. In gradient analysis the strength of the mobile phase is increased with time during sample elution. This type of analysis yield better separation for early eluting peaks and sharper peaks for late eluters (Agilent Technologies, 2004). The disadvantages of gradient analysis are the requirement for more complex instrumentation and difficulties in method transfer (Ornaf & Dong, 2005).

The method developed for this study resulted in isocratic type analysis. Isocratic analysis have an advantage of quick column equilibration, better interaction of analyte, stationary phase and mobile phase and the system remains constant throughout the analysis. Isocratic analysis use simpler HPLC equipment and employs premixed mobile phases. The disadvantages of isocratic analysis are limited peak capacity, and problems with the samples containing analytes of diverse polarities (Ornaf & Dong, 2005). Late eluting peaks may also be difficult to quantify due to excessive band broadening. Long analysis times also characterize isocratic analysis (Agilent Technologies, 2004).

2.4.6 Choosing an HPLC detector

Selectivity of a detection system is defined as the ability to select only those compounds of interest in a complex matrix using specific compound properties. A detector is selective if it does not respond to coeluting compounds that could interfere with analyte quantification (Agilent Technologies, 2004). Each detector has performance characteristic, which need to be applied in conjunction with the acceptance criteria which the method applies. A couple of detectors are used in HPLC analysis and each will be briefly described as explained in the Agilent Technologies (2004).

- Fluorescence detection is a specific type of luminescence that is created when certain molecules emit energy previously absorbed during a period of illumination. Luminescence detectors have higher selectivity because not all molecules that absorb light also emit it. The excitation and emission wavelength are characteristic of the individual compound (Agilent Technologies, 2004).

- Electrochemical detection techniques are based on the electrical charge transfer that occurs when electrons are given up by a molecule during oxidation or absorbed by a molecule during reduction. This oxidation or reduction takes place on the surface of a so-called working electrode. The working electrode can be made of material like gold (for sugars, alcohols), platinum (for chlorite, sulfite), silver (for halogens) and combined mercury-gold (in reductive mode for nitrogenous organic compounds) (Agilent Technologies, 2004).

- Refractive index detector is a nonselective or universal detector. It can detect nearly every compound, but not at low levels. The detection principle is based on the comparison of the refractive index of the eluent and sample with a reference solution of the eluent only. Refractive index (RI) detection is based on the difference in RI between the solution in the sample cell and the pure mobile-phase solution in the reference cell. Because the composition of the eluents

must remain fixed throughout the analysis, this detector is not suitable for gradient analysis (Agilent Technologies, 2004).

- Mass spectroscopy (MS) can be used in conjunction with liquid chromatography to identify a wide variety of sample types with great certainty. Complex samples with coeluting compounds can be identified using this technique. Non-volatile, extremely polar, or thermally labile compounds can be successfully separated with LC/MS (Agilent Technologies, 2004).

- UV-Vis detectors are very popular in the pharmaceutical industry. The absorbance of light transmitted through the detector cell is related to the solute concentration according to Beer's Law. Quantification using this detector is possible because of the linear relationship between absorbance and concentration of analyte (Agilent Technologies, 2004).

  Beer's Law: **Log $I_0/I = A = abc$** where;

  I_0 -Radiation intensity before passing through flow cell

  I -Radiation intensity after passing through flow cell

  A - Absorbance

  a - Absorptivity, a proportionality constant characteristic of the absorbing medium

  b – path length of the flow cell

  c – concentration of the absorbing medium (Caprette, 2005).

  A plot of a signal or area versus concentration is an application of Beer's law and allows a calibration table plot for the determination of unknowns. Determination of the concentration of an unknown is valid in the linear region of the calibration curve.

  Diode array detector can provide detection at a single wavelength or simultaneously at multiple wavelengths. The detector has the ability to store

spectra for peak purity analysis, library searching, and creation of extracted signals. The combined tungsten and deuterium lamps emit radiation from 190-850 nm (Agilent Technologies, 2004).

In this study, a UV- Vis spectrophotometer was employed to scan the three detergents to determine their maximum wavelength absorption. The wavelengths determined with the spectrophotometer were then used as the wavelengths of choice in the subsequent HPLC analyses and method validation.

2.5 METHOD VALIDATION

Method validation ensures that an analytical method is accurate, specific, reproducible and robust over a predetermined range within which analysis will be conducted (Shabir, 2003). Validation is meant to demonstrate the suitability and reliability of the developed method for its intended purpose (Thompson & LoBrutto, 2007).

Method validation is a regulatory requirement. Method validation must be performed by regulated laboratories in order to be in compliance with US Food and Drug Administration (FDA) regulations. Guidelines from the FDA, US Pharmacopeia (USP), and International Conference on Harmonization (ICH) provide a framework for performing HPLC method validations (Shabir, 2003). The use of validated methods by a laboratory shows its qualification and competency (Taverniers *et al.*, 2004).

Method validation requires a plan, which clearly shows how each step of the analytical test method was validated. The extent of validation and the choice of performance parameters to be evaluated depend on the status and the acceptance criteria of the analytical method (Shabir, 2003; Taverniers *et al.*, 2004). For the purpose of this study, the method developed is a category 2 method as categorized by Ahuja & Dong, (2005), which is intended for the verification of a cleaning protocol.

There are parameters established by the FDA, USP, and ICH, which serve as criteria to establish for different categories of the analytical methods to be validated. These

parameters are simply known as method-performance parameters. A brief summary of these parameters will be mentioned as described by Taverniers *et al.*, (2004) and Shabir, (2003).

The first parameter is *accuracy* and it is defined as the closeness of agreement between a test result and the accepted reference value. *Accuracy* is measured in terms of *precision* and *trueness*.

- *Precision* and bias studies form part of the uncertainty measurement of a method. Precision measures can be further divided into:

    1. Repeatability precision measures s or SD (s_r or SD_r) and RSD (RSD_r);

        Defined as the precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

    2. Intra-laboratory reproducibility precision or 'intermediate precision' measures, SD and RSD; and,

        Defined as the precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory but by different analysts, using the same equipment over an extended period of time.

    3. Inter-laboratory reproducibility precision s or SD (s_r or SD_r) and RSD (RSD_r)

        Defined as the precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

- *Trueness* is expressed in terms of bias or percentages of error. It is the closeness of agreement between the expectation of the test result and an accepted reference value.

Recovery is explained as the fraction of analyte added to the test sample (spiked) prior to analysis, which is measured by the method.

Specificity defines the ability of the method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix.

Limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected, whereas *limit of quantification (LOQ)* is the lowest concentration of an analyte in a sample that can be determined with acceptable precision accuracy under stated operational conditions of the method. LOD and LOQ are used as a measure of the methods *sensitivity*.

Linearity is the ability of the method to obtain test results that are directly proportional to analyte concentration within a given range.

Range is the interval between the upper and lower levels of analyte (exclusive) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

Ruggedness is the intra-laboratory tested behaviour of an analytical process when small changes in the environmental and/or operating conditions are made.

The *robustness* of the method is its ability to remain unaffected by small deliberate variations in method parameters such as pH of buffer on mobile phase, ionic strength, or different columns, which provides an indication of its reliability during normal usage.

For the purpose of cleaning method validation there are additional analytical steps which should be applied in conjunction with the one's mentioned for method validation. These analytical steps, serve as facilitation for the analytical part of the method only and not the cleaning method validation process as a whole. Analytical steps ensure that the results generated in relation to proving the efficiency of a cleaning protocol are scientifically sound. The analytical steps described below will be applied in this study as described by Ahuja & Dong (2005).

Table 2.2 is a summary of chronological steps that will be followed to achieve a complete methodology for the glassware cleaning validation program as described by (Plasz, 2005).

TABLE 2.2 PRACTICAL ANALYTICAL STEPS FOR CLEANING VALIDATION

	ANALYTICAL STEP	PURPOSE
1	Determine cleaning limits	Ensure whether contaminants can be recovered from cleaning surfaces and what level
2	Determine equipment to be tested	In this study: Laboratory glassware
3	Choose the sampling technique (Swabs vs. Rinser)	Both methods should be used for control purposes (Direct and indirect measuring technique)
4	Facilitate the recovery drug and/or detergent of swab	Test the accuracy of the sampling techniques and HPLC method
5	Establish the stability of the swabbing solution	Determine how long the sample preparation are stable
6	Recover drug off surfaces	Determine the sensitivity of the sampling techniques and HPLC method
7	Show that cleaning removes drug	To prove that the cleaning procedure is effective
8	Clean and test	Confirm the HPLC method is effective and sensitive for its intended purpose
9	Document with data the appropriateness of the cleaning procedure	Validate the cleaning method

2.6 CONCLUSION

HPLC is regarded as a leading analytical technique used for the verification of pharmaceutical cleaning validation programs. Its ability to provide linear, sensitive methods for quantitating low levels of residues makes it the technique of choice for investigating the efficacy of the glassware cleaning protocol in a pharmaceutical contract testing laboratory. The chromatographic finish makes HPLC the most reliable part of the cleaning verification process.