Development and Validation of An HPLC Assay for Simethicone in Pharmaceutical Formulations

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Development and Validation of An HPLC Assay for Simethicone in Pharmaceutical Formulations

A thesis submitted in fulfillment of the requirements for the degree of Master of Pharmaceutical Sciences at the University of Sydney

By

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PREFACE

The work described in this thesis was carried out in the Department of Pharmacy, The University of Sydney. The material presented is original and has not previously been submitted to this or any other university for a degree. Full acknowledgment has been made where the work of others has been used or cited.

ACKNOWLEDGEMENTS

I sincerely thank my supervisor, Associate Professor Douglas E. Moore for his patience, understanding, consistent encouragement and fantastic support.

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Finally, I thank for my parents for their love, understanding and encouragement during the study.

ABSTRACT

A high performance liquid chromatography (HPLC) assay has been developed and validated for the antifoam agent Simethicone (polydimethylsiloxane + silicon dioxide) as used in antacid formulations. The method relies on a reversed-phase gradient HPLC system with the key component being the use of an evaporative light scattering detector (ELSD). The ELSD is a universal detector with particular application to eluants which have no UV chromophore. Separation of Simethicone was achieved using a reversed-phase column (C_8) and elution with an acetonitrile- chloroform gradient. A low molecular weight polydimethylsiloxane (MW 1800) can be resolved completely into its oligomers by the appropriate solvent gradient, whereas a higher molecular weight polydimethylsiloxane (MW 27,000) is resolved to a limited extent only. The Simethicone content in a commercial antacid emulsion formulation was quantitatively determined, and the validated method used to assay samples of the formulation in stability trials.

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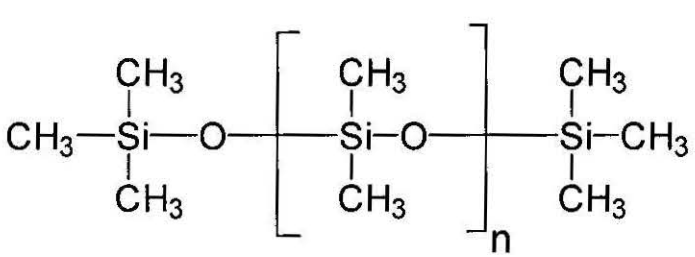
Chapter 1: Introduction

1.1 Polydimethylsiloxane

A polymer is a large molecule built up by the repetition of a simple chemical unit, or monomer. In some cases the repetition is linear, much as a chain built up from its links, but in other cases, the chains are branched or interconnected to form three-dimensional networks (Urbanski et al, 1977). Commercial polysiloxanes, commonly referred to as silicone oils, are the product of hydrolytic polycondensation of the monomers, which are low molecular weight organosilicon compounds polydimethylsiloxane (PDMS) is an example of the polysiloxanes, having the Chemical Abstract name of α-(trimethylsilyl)-ω-methylpoly[oxy(dimethylsilylene] and CA registry Number [8050-81-5]. The main trivial name for PDMS is dimethicone, although the European and British Pharmacopoeias refer to it as Dimeticone (USP, 2000). There are also a number of more or less proper synonyms and commercial names used for this polymer, such as Dimethylsiloxane, Methyl-polysiloxane, Polymethylsiloxane, Permethylpolysiloxane and Antifoam A or MS, (BP, 2000). In this thesis, the name polydimethylsiloxane and acronym PDMS will be used. The chemical structure of PDMS is:



where n is the degree of polymerization or chain length, normally having a value between 150 and 400. PDMS is synthesized by hydrolysis and polycondensation of dichlorodimethylsilane with chlorotrimethylsilane. Any individual preparation of PDMS is a mixture of polymeric molecules, i.e., it contains a distribution of chain lengths centered about the average degree of polymerization. Various different preparations are therefore available, depending upon the average chain length. The characterizing physical property of each silicone oil is its viscosity. The different dimethicones are therefore distinguished by a number after the name indicating the declared viscosity which, in terms of kinematics viscosity, is between 20 and 1300 $mm^2 s^{-1}$ (BP, 2000). PDMS is a clear colorless liquid, insoluble in water, but miscible with many organic solvents. It has a number of applications in food processing, cosmetics and pharmaceuticals as an antifoaming and lubricating agent, resulting from its water repellent and surface tension lowering effects (Luck, 1997).

1.2 Simethicone and Simethicone Emulsion

Simethicone is defined by the United States Pharmacopoeia as a mixture of fully methylated linear silicone polymers containing repeating units of the formula (-$(CH_3)_2SiO$-), stabilized with trimethylsiloxyl end-blocking units of formula (-$(CH_3)_2SiO$-), and finely divided silicon dioxide. In other words, it is PDMS with particulate SiO_2 added to enhance the defoaming properties of the silicone. The degree of polymerization n has an average value such that the corresponding nominal viscosity is in a discrete range between 20 and 30,000 centistokes (USP24/NF19, 2000).

Simethicone is a milky-white paste with essentially no characteristic odor, it is a viscous, oil-like liquid with a density range of 0.965 to 0.970, and the viscosity at 25°C is about 60,000 centistokes (cs). It is immiscible with water and alcohol, but miscible with chloroform and ether. It has no UV chromophore and contains many individual oligomers

with different chain length. The molecular weight of the PDMS in Simethicone is approximately 15,000 to 27,000 (Merck, 1996). Simethicone Emulsion is a water-dispersible form of Simethicone with the addition of suitable emulsifiers, preservatives, and water. It may contain suitable viscosity-increasing agents (USP24/NF19, 2000). Simethicone 30% emulsion is a preparation for pharmaceutical use and contains between 27.80 to 31.60 percent of polydimethylsiloxane.

The BP does not contain a monograph of Simethicone, but its dimeticone monograph defines dimeticone as a polydimethylsiloxane obtained by hydrolysis and polycondensation of dichlorodimethylsiloxane and chlorotrimethylsilane. Different grades exist, distinguished by a number indicating the nominal viscosity placed after the name. Their degree of polymerization (n = 20 to 400) is such that their kinematic viscosities are nominally between 20 cs and 1300 cs. Each is a clear, colorless liquid of designated viscosity, insoluble in water, very slightly soluble to practically insoluble in ethanol, but miscible with ethyl acetate, methyl ethyl ketone and toluene (BP, 2000).

1.3 Applications of Simethicone

1.3.1 General uses

Simethicone is a common ingredient in cosmetics and hair-care products, where it serves as a spreading agent and moisture retainer, adding lustre and sheen. It is claimed to allow cleaning and conditioning in one step (Rushton et al, 1994), overcoming problems with anionic surfactants and cationic conditioners that result in charge interaction and complexing of the ingredients (Heidenkumer and Kampik, 1991).

In food processing, Simethicone is used as a defoaming agent (Gooch, 1993). Its effect on foaming systems containing synthetic gastric juice and a surface active substances (anionic surfactant, cationic surfactant, soap solution) was quantified by measuring the

surface tension, foam stability and initial foam density (Bergeron et al, 1997). The effect of Simethicone was the result of combination of two actions: the drainage of liquid from foam films and the rupture of relatively thick liquid films. The mechanism of these actions may be described as liquid drainage followed by bridging of the liquid film by PDMS droplets, helped by hydrophobic silica particles also present in the antifoam agent, leading to the rupture of the film surface and air escape (Brecevic et al, 1994).

1.3.2 Pharmaceutical uses

The principal application of Simethicone in a pharmaceutical sense is for the alleviation of intestinal gas, a cause of significant discomfort to many people (Ali et al, 1998). Simethicone is therefore a common additive to antacid formulations. Its efficacy was assessed by comparing the effect of a Simethicone-containing antacid gel with a simple antacid gel in a double blind trial in 45 patients with reflux oesophagitis. The results suggested that a Simethicone–containing antacid is of value in the treatment of symptomatic gastro-oesophageal reflux. Intestinal gas is also the most common cause of technically unsatisfactory abdominal ultrasound scans. In an attempt to improve the visualization of abdominal organs, many investigators use antifoaming agents or laxatives (Olivie and Atkinson, 1996). Examples of antacid–containing preparations designed to provide relief from indigestion, upset stomach, heartburn and wind pain, include liquid and tablet formulations. A typical liquid formulation (Mylanta Antacid Liquid-manufactured by Pfizer-Warner-Lambert Consumer Pty Ltd) contains magnesium hydroxide (400 mg/10 mL), aluminium hydroxide (400 mg/10 mL) and Simethicone (40 mg/10 mL) with the necessary preservatives and flavors. A tablet formulation of Mylanta is constituted as follows: aluminum hydroxide-dried (equivalent to 50% aluminum oxide, anhydrous, 200 mg/tablet), magnesium hydroxide (200 mg/tablet) and Simethicone (20 mg/tablet) with preservatives and flavours.

Simethicone is regarded as an active component whose purpose is to act as an antifoam agent to disperse any accumulated gas that often causes indigestion and stomach pains.

When taken orally it causes small bubbles of gas in the gastrointestinal tract to coalesce, thereby aiding their dispersion. Simethicone is taken in doses of 20 - 40 mg three or four times daily, as required.

1.4 Degradation of Polymers

The current project is concerned with the use of polymeric material in pharmaceutical formulations. The product development process must include stability trials for shelf-life determination of the product. Thus, it is important to have an understanding of the nature of possible breakdown processes that may affect PDMS.

Organic polymers when first introduced were thought to be relatively indestructible. In fact many are regarded now as constituting an environmental problem because of their resistance to biodegradation. Thus a very considerable research effort is directed toward understanding the degradation of polymers (Leung et al, 1989). Additionally, the use of polymeric materials in increasingly demanding applications in recent years has also contributed to the demand for knowledge of polymer stability (Grassie, 1978). Polymer behavior and performance, particularly the durability of the polymeric material during manufacture and in service are generally the concern of industry laboratories (Jellinek, 1983).

A study of the effect of temperature on the process of polymerization has shown that it is in some case reversible, so that above a so-called "ceiling" temperature, reversal of reaction may occur to give greater amounts of the original monomer rather than polymer. On the other hand, degradation of polymers leads to lower molecular weight products which are quite different from the starting monomer (Zhu, 1997).

Polymers can be broken down by a variety of environmental agents. The term "polymer degradation" is used to denote changes in physical properties of the polymer caused by

chemical reactions involving bond scission in the backbone of the macromolecule. In linear polymers, these chemical reactions lead to a reduction in molecular weight, i.e., to a diminution of chain length or degree of polymerization (West and Hench, 1994).

Thermal degradation refers to the situation where the polymer, at elevated temperatures, undergoes chemical changes without the simultaneous involvement of another agent. The chemical change occurring during thermal treatment of polymers can be characterized by the following phenomena:

- Chemical bonds in the main chains and/or in the side chains are ruptured, as evidenced by a diminution of the molecular weight of the polymer, and the evolution of low molecular weight gaseous products, respectively.
- Intramolecular reactions such as cyclization and elimination may occur.
- In the case of linear polymers, intermolecular cross-linking can occur indicated by an augmentation of the molecular weight (Fig 1.1).

Figure 1.1 Depiction of cross-linking during Thermal Degradation

All of those processes are believed to occur via the adventitious formation of free radicals in the polymer molecule as a result of thermal activation. Degradation due to photochemical or high-energy radiation follows similar initiation steps.

Mechanical degradation of polymers is observed when structural changes occur due to various kinds of mechanical operations (milling, shear stress, tensile forces, compression, ultrasonic radiation, etc). It is relevant to a manufacturing process involving polymeric components, where the modifying (filling, pumping) stages may cause polymer degradation. In all these cases chemical bonds in the polymer chain can be ruptured (Basedow and Ebert, 1977).

Types of mechanical degradation in solution include:

- Ultrasonic degradation.
- Stress-induced chemical degradation.
- Freezing and thawing degradation.

In its broader sense, the mechanical degradation of a polymer covers fracture phenomena, as well as chemical changes induced by mechanical stress (Grassie, 1956).

Polymer degradation in solution

A stress-induced reaction can occur if the polymer solution is subjected to high-speed stirring, shaking, turbulent flow or ultrasonic treatment. In systematic studies, stress-induced reactions are frequently induced by forcing a polymer solution to flow through a narrow capillary tube or by treating the polymer solution in a rotational viscometer, or a high-speed homogenizer (Kendrick et al, 1989). If polymer in solution is subjected to the influence of ultrasonic waves, then, generally, main-chain degradation occurs. With the aid of ultrasonic generators, high molecular weight polymer samples can be converted to low molecular weight material (Gressbach and Lehmann, 1999).

The mechanism of ultrasonic degradation is explained as follows (Basedow and Ebert, 1977). When a liquid is exposed to ultrasonic waves of high intensity it expands and gives rise to a negative pressure causing any dissolved gas to form bubbles. The presence of gas

is the driving force for the generation of bond ruptures. Main-chain rupture in macromolecules is thought to be induced by these shock waves, which are assumed to cause a rapid compression with subsequent expansion of the liquid.

Chemical Degradation

Changes in polymers may also occur due to conditions other than mechanical forces (e.g. oxidation, irradiation, other chemical reactions, etc.) thereby altering their properties. Chemical degradation of a polymer containing a heteroatom linkage in the main chain may occur by the attack of solvolytic or oxidative reagents due to main-chain scission, thus causing changes in the physical properties. The solvolysis reactions involve the breaking of C-X bonds, where X designates a hetero atom, such as O, N, P, S, Si or halogen, under the influence of the solvent, YZ such as as water, alcohol (R-OH), etc, as indicated by Fig 1.2.

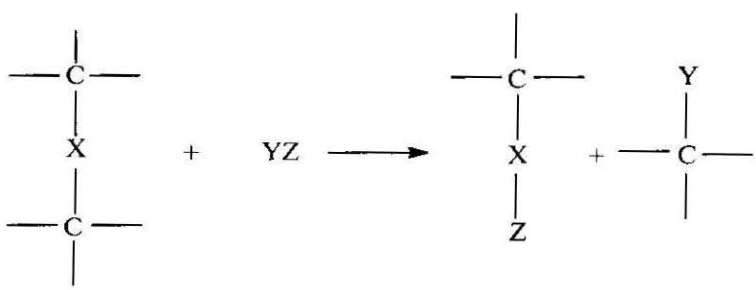


Figure 1.2 General equation for the solvolysis reaction of a polymer containing a hetero atom X by solvent YZ

Oxidative degradation of a polymer proceeds by a free radical chain mechanism consisting of three important steps: initiation, propagation, and termination. The initiation step may be effected by thermal or photochemical action, or by the participation of metal ion catalysis or peroxidic substances, while oxygen participates in the propagation step

(Conley, 1970). The term biodegradation in relation to a polymer refers to the degradation and assimilation of organic polymers and compounds by living organisms, principally microorganisms such as fungi, bacteria and actinomycetes. The term can be enlarged to include attack by all forms of living things (Economy and Mason, 1970).

Degradation of Silicone polymers

Silicone polymers (such as polydimethylsiloxane) generally are viscous liquids at room temperature, depending on the molecular weight. Their maximum use temperature is designated in the region 150-200°C, mainly because of the reduction in viscosity with increasing temperature (Conley, 1970). Degradation of polydimethylsiloxane has been demonstrated following heating at 300°C. Volatile products have been detected by gas chromatography as shown in Figure 1.3. The products D_3, D_4, etc, have been identified as the cyclic trimer, tetramer, etc, up to heptadecamer D_{17} (Grassie and Scott, 1985).

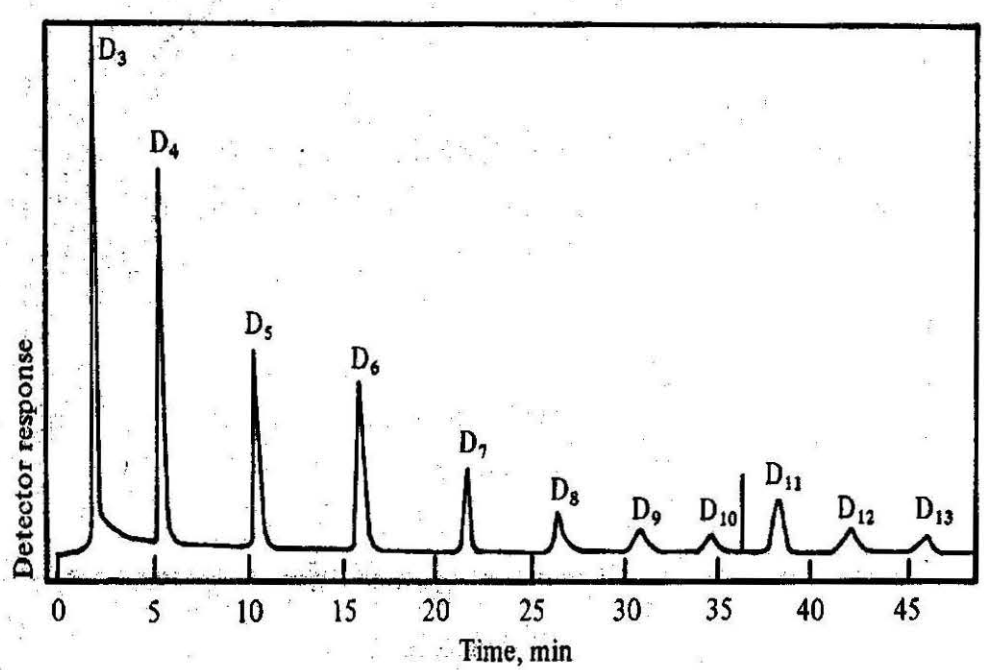


Figure 1.3 Gas chromatography trace of the volatile products from thermal degradation of PDMS (Grassie and Scott, 1985)

While GLC is useful for the detection of volatile products from the polymer degradation process, it gives no information about the residual material. Thermogravimetry (TG) is a technique for the measurement of the change in weight of a material as the temperature of its environment is varied in a controlled manner. TG was used to examine a series of PDMS samples with different average molecular weights and with some variation of the preparation (Husam et al, 1990). In Fig 1.4, curves 1 to 4 are for materials which differ only with respect to their average molecular weight. It can be seen that the threshold temperature at which significant weight loss starts to occur, increases with increase in molecular weight of the polymer. The weight loss can be interpreted as breakdown of the polymer, yielding volatile products of lower molecular weight (Grassie, 1985).

Curve 5 displays the weight loss for sample 2 after end-capping, which means treatment to silanise any free Si-OH groups with trimethylsilyl chloride. The end-capping treatment has imparted greater stability to the polymer. On the other hand, treatment of sample 2 with 5% potassium hydroxide (curve 6) produces a polymer that breaks down at much lower temperature.

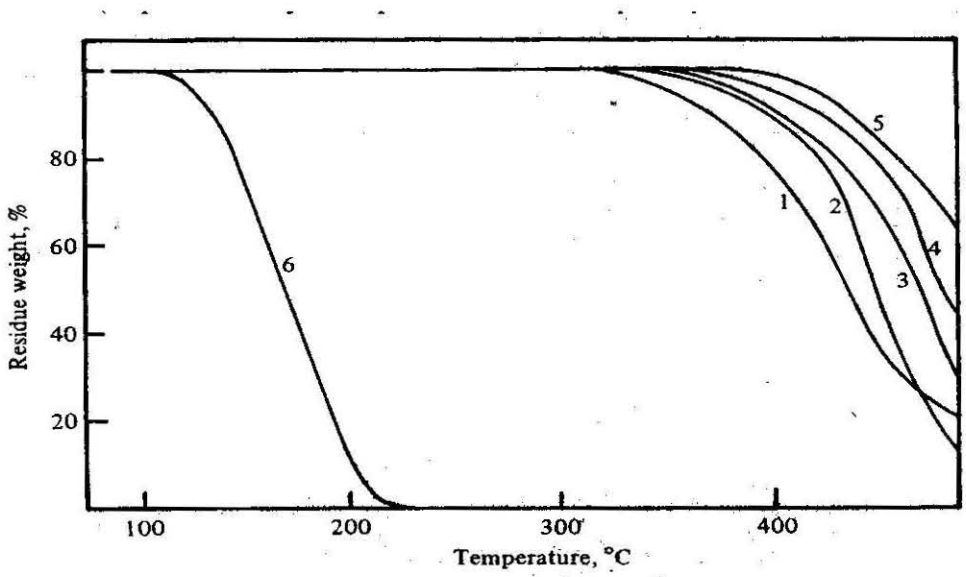


Figure 1.4 Thermogravimetric analysis curves for polydimethylsiloxanes of different number average molecular weights:
1, 94500; 2, 111500; 3, 183000; 4, 258000; 5, 2 end capped; 6, 2 + 5% KOH

Although the designated structure of PDMS does not show the existence of –OH groups, it is found that a very small proportion of the molecules do bear terminal –OH groups. The reason for this is believed to be partial hydrolysis of –Cl substituents in the preparation of the siloxanes (West, 1997).

It is postulated that terminal hydroxyl groups play an important role in initiating the degradation reaction of silicone polymers (Grassie, 1964). The following series of reactions (A – D) in Figures 1.5 - 1.9 are now generally accepted as representing the mechanisms whereby small molecular weight compounds break off from PDMS (John, 1990).

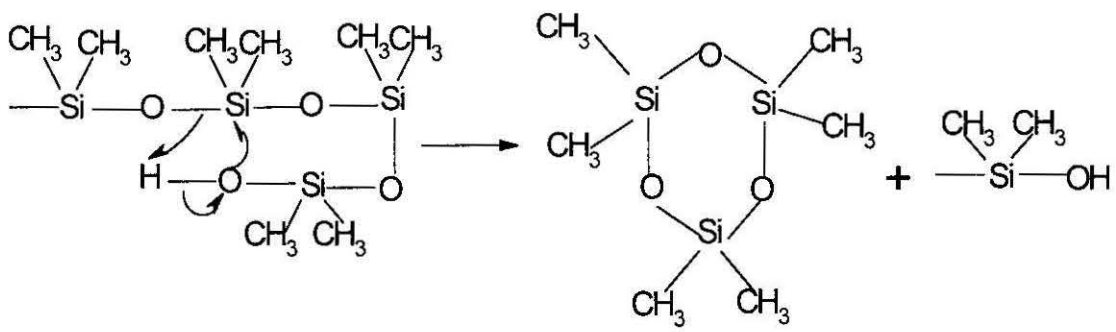


Figure 1.5 Reaction A - Formation of cyclic siloxane trimer involving terminal hydroxyl group

Reaction A shows the hydroxyl groups reacting with the third Si atom from the chain end leading to a trimer. Higher oligomers can be formed by analogous reaction with points further from the chain ends. Although end-capping (blocking of free Si-OH groups) imparts stability to the polymer, when degradation occurs, the products are the same as for hydroxyl terminated chains. Thus a comparable mechanism may be suggested in which a second silicon atom replaces the hydrogen atom in the four-membered ring transition state but also requiring participation of an OH donor (Grassie, 1985).

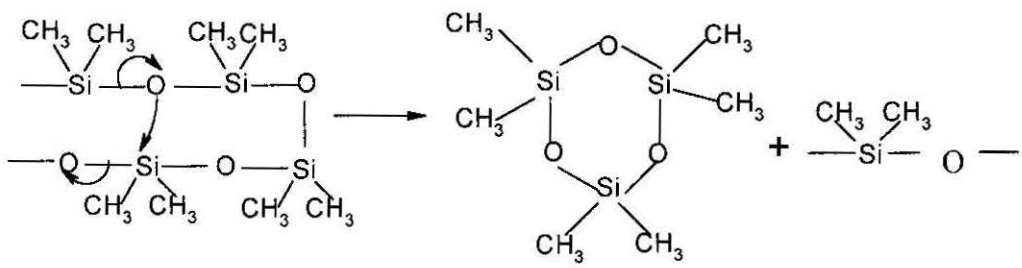


Figure 1.6 Reaction B - Formation of cyclic siloxane trimer from a mid-chain reaction

Acceleration of the degradation reaction by KOH is probably due to hydroxyl ions providing the driving force for the polymer-breaking reaction.

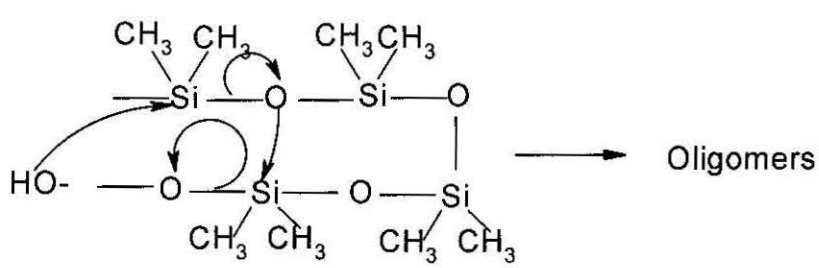


Figure 1.7 Hydroxide ion catalysis of PDMS degradation

When the reaction was carried out in the presence of KOH, mass spectrometry revealed a minor product with a molecular weight of 430. The only reasonable structure corresponds to product C (Fig 1.8)

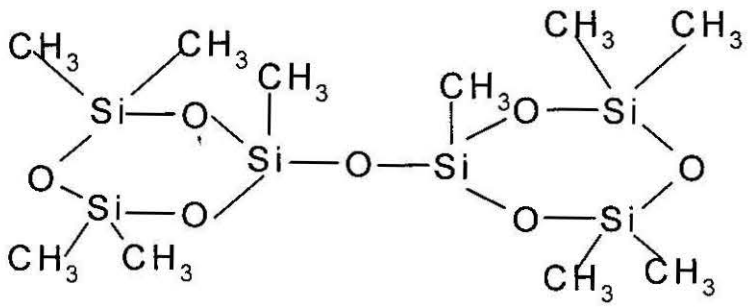
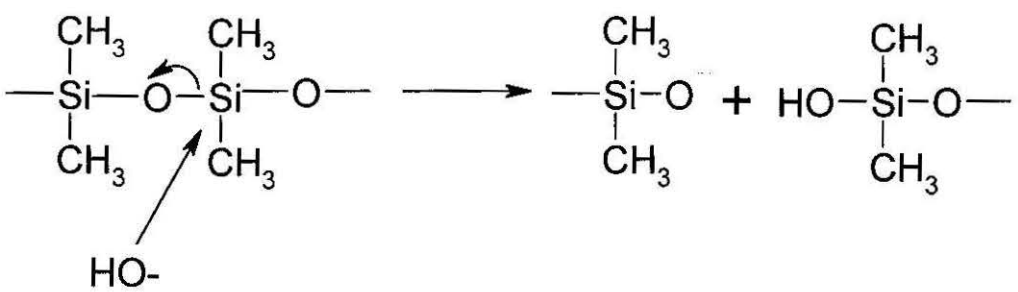


Figure 1.8 Postulated structure of product C

It has been proposed that the first step in its formation involves a siloxyl ion which reacts to form methane, which is also formed in the hydroxyl ion catalyzed reaction. A similar reaction further along the chain would liberate product C (Grassie, 1972)



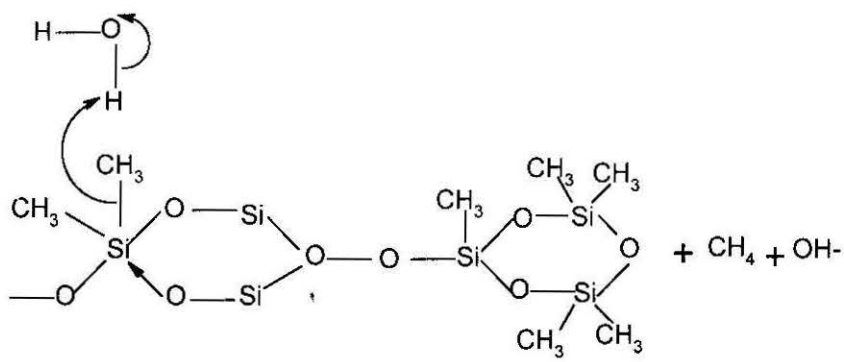


Figure 1.9 Reaction D

The replacement of a portion of the methyl groups by phenyl groups is used to improve the thermal stability of silicones. The behaviour of poly(methyl phenylsiloxane) provides a clue to the reasons for this. Benzene is formed in small amounts and the polymer becomes insoluble on heating to 150°C (David, 1975). In addition, thermogravimetric analysis of this polymer demonstrated that the higher the molecular weight, the lower is the stability and that the stability is decreased by end-capping.

In the hydrolysis reaction of PDMS, it is assumed that normal hydrolysis of Si-O-Si bond is occurring, producing silanol (SiOH) groups on the surface of the polymer. Because the remaining polymer is unchanged beneath this layer, this hydrolysis would affect only the surface state (Wallace et al, 1993).

Due to the insolubility of PDMS in water and its high adsorption coefficient, liquid PDMS discharged as effluent will adsorb to particulate matter and, therefore, will become a component of sewage sludge during waste water treatment. The subsequent environmental fate of PDMS will depend on the fate of the sludge. Due to increasing practices of soil amendment with sewage sludge the principal environment compartment receiving PDMS

fluids is the soil. Degradation of PDMS is a common process taking place in many different types of soils. It occurs through a unique combination of environmental degradation process (Grassie, 1966). The initial hydrolysis product, dimethylsilanediol (DMSD), is either biodegraded, or evaporated into the atmosphere, where it is subsequently oxidized in the presence of sunlight. The end products in both cases are expected to be CO_2, SiO_2 and H_2O (Xu et al, 1998).

1.5 Analysis of Polydimethylsiloxane

Although Simethicone is a widely used ingredient in pharmaceutical formulations, it has been generally regarded as similar to an excipient, rather than an active component. Hence methodology for the chemical analysis of the quantity of Simethicone in such formulations is limited in the pharmaceutical literature (Kala et al, 1997). Another factor may be that the quantitation of this type of ingredient frequently proves to be quite challenging to the analytical chemist (Hidaca et al, 1970). Often the ingredient is present in low concentration in a formulation of complex composition. For example it may contain ingredients such as water, glycerin, stearic acid, mineral oil, acetylated lanolin alcohol, salts and preservatives, none of which have readily identifiable analytical characteristics (Khafi et al, 1996).

The BP describes a test for the identification of dimethicone by viscosity and infrared absorption spectrophotometry, but no quantitative tests are given (BP, 2000). The USP (2000) describes assays for the silicon dioxide content of Simethicone by gravimetry, and the PDMS content by Fourier Transform Infrared Subtraction (FTIR) spectrometry. Consequently FTIR has become the principal method for quantitation of PDMS (Torrado et al, 1999).

The only reported chromatographic analysis of PDMS involves the use of gel permeation (Andersson et al, 1989). The advantages and limitations of these methods are described below.

1.5.1 Gel Permeation Chromatography of PDMS

Gel Permeation Chromatography (GPC) is a technique for the separation of components of a molecule according to their molecular size. The range of molecular weights which can be resolved into individual components is determined by the pore size of the gel stationary phase. As applied to the determination of PDMS in Simethicone Emulsion, the sample preparation is straightforward (Andersson et al, 1989). An aliquot of the emulsion was accurately weighed and dissolved in methyl isobutyl ketone. After ultrasonication and centrifugation, the supernatant was injected on to the GPC column. The analytical column (300 mm × 7.7 mm i.d.) contained polystyrene-divinylbenzene, 10 μm particles, of mixed pore size with a molecular weight working range of 1000-10^6 (PL-gel; Polymer Laboratories, Amerst, U.S.A.). The mobile phase of 100% toluene was delivered at a flow rate of 1.0 mL/min. The injection volume was 30 μL. Detection of PDMS was achieved using a refractive index detector. The separation of PDMS by this procedure is shown in Figure 1.10. Several formulations containing Dimeticone were assayed after construction of a standard curve with PDMS reference standards.

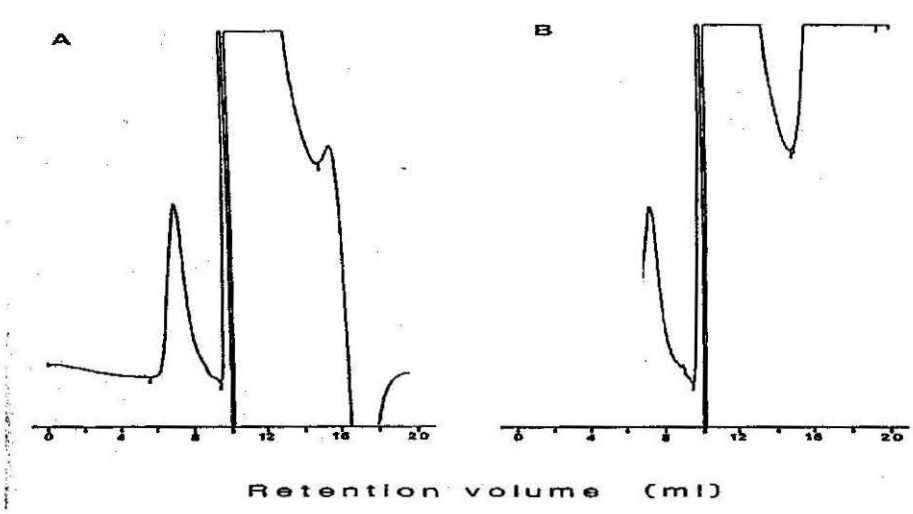


Figure 1.10 Separation of PDMS (retention volume 7.2 ml) by gel permeation chromatography (A) PDMS Standard and (B) Sample (from Andersson et al, 1989).

In the above chromatograms, A shows the separation of standard PDMS as in Dimeticone, while B shows the separation of PDMS in a pharmaceutical formulation containing Dimeticone. The void volume, V_o, of the column was determined by use of a high-molecular weight PDMS (MW > 10^6) and was found to be 5.5 ml. The total permeation volume, Vt, determined by use of chloroform (dissolved in the mobile phase) was found to be 13.5 ml. The retention volume, V_R, for the PDMS in pharmaceutical samples was 7.20 ± 0.032 ml (n = 32) (Andersson et al, 1989).

The GPC method utilizes a refractive index detector which has disadvantages due to its poor sensitivity. The RI detector is also much less suitable for gradient elution due to the refractive index change that occurs as the solvent composition changes. It is also sensitive to fluctuations in cell pressure, flow rate and the temperature of the mobile phase.

1.5.2 Fourier Transform Infrared Spectrophotometry (FTIR)

The British Pharmacopoeia (BP, 2000) includes FTIR as a component of the identification procedure for PDMS in Dimeticone. The FTIR spectrum is compared to one obtained with a certified standard sample. However the BP advises that the region of the spectrum from 750 to 850 cm^{-1} should be disregarded since slight difference may be observed depending on the degree of polymerization of the PDMS. This is also stated in the USP (2000) which presents an assay for analysis of PDMS in Simethicone by infra red spectrometry. This assay quantifies the amount of PDMS, and two additional tests are described to determine the silicon dioxide content and the defoaming activity.

The USP procedure involves the addition of 50 ml of dilute hydrochloric acid to neutralise the antacid components of the formulation, followed by extraction of the PDMS with 25 mL of carbon tetrachloride. After extraction, the organic layer is dried with anhydrous sodium sulfate and examined in a 0.5 mm path liquid cell by FTIR. The infrared spectrum

of PDMS (Fig 1.11) exhibits a characteristic doublet at 1097.6 and 1014.6 cm^{-1} due to stretching and bending of Si-O bonds in addition to a sharp band around 1265 cm^{-1} due to CH_3-Si vibrations. Quantitative use of the 1265 cm^{-1} band is precluded in other solvents by very strong solvent absorption in that area (Durkin, 1998).

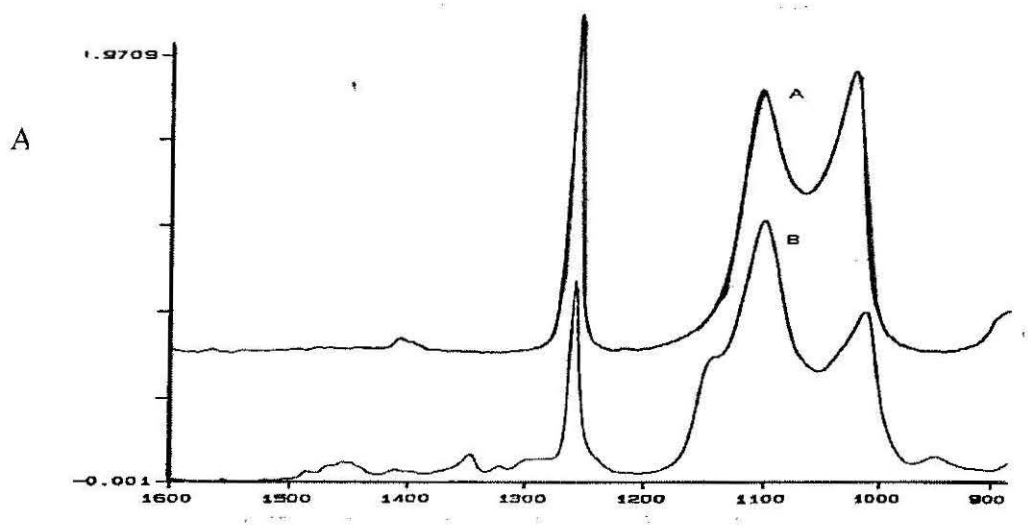


Fig 1.11 IR Spectrum of standard PDMS (A) and formulation E (B) (from Torrado et al, 1999)

A modification of the USP procedure for Simethicone was developed and validated by Torrado et al (1997). This method eliminated the acidic aqueous phase by direct dissolution of the formulation in the solvent carbon tetrachloride with the aid of a lengthy sonication period. The sample then was filtered into the 0.5 mm IR cells and examined at the wavelength of maximum absorbance of 7.9 μm (1265 cm^{-1}). A blank is prepared with carbon tetrachloride and used to zero the instrument.

All formulations showed a spectrum similar to the one obtained with standard PDMS, as shown in Figure 1.12 for USP standard PDMS and two of the formulations tested, B and

D. The FTIR method was used for the quantitation of Simethicone in lotions, capsules and tablets. For the samples tested, reliable results are generated only when a simple matrix was not involved. It was found that quantitation of PDMS in complex emulsions and suspensions such as those containing antacid components requires careful sample handling and precise background subtraction of the sample matrix (Torrado et al, 1997).

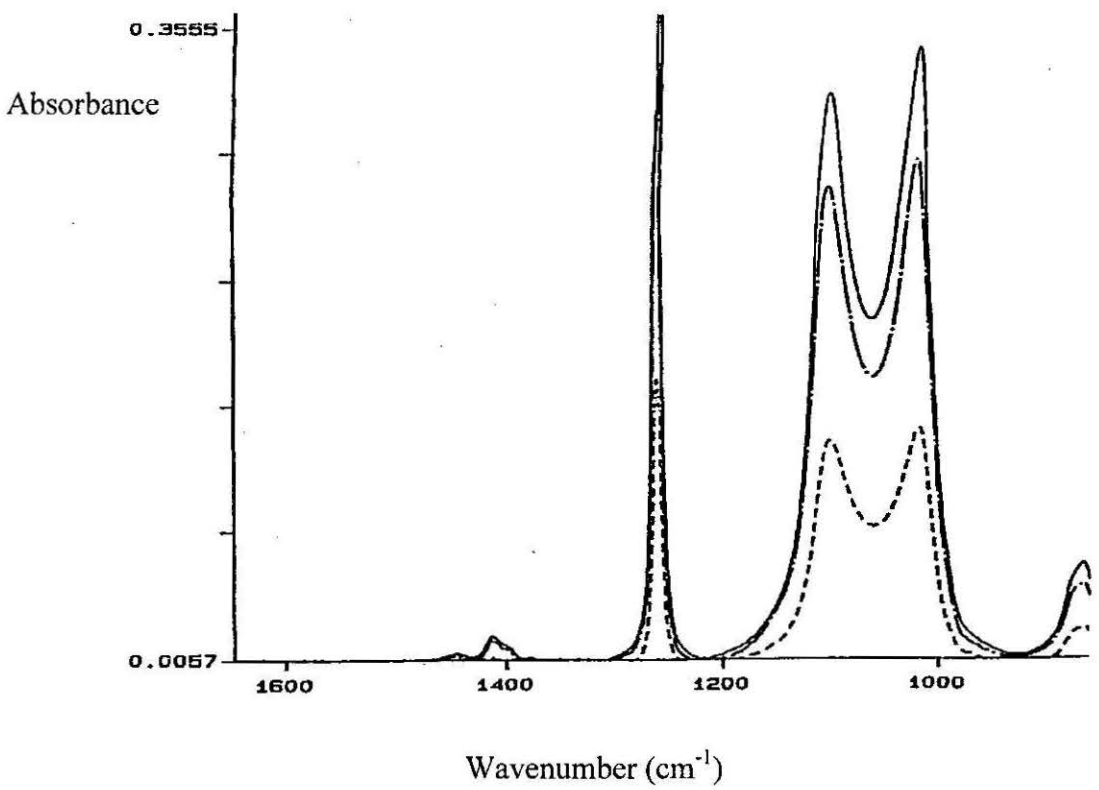


Figure 1.12 FTIR spectroscopy of standard simethicone (-), formulation B (-.-) and formulation D (- - -) (from Torrado et al,1999).

In the case of the more complex formulations, the peak at 1014.6 cm^{-1} is recommended for the quantitation of PDMS since there is less interference from both solvent and blank formulation at this wavenumber.

With a more complex procedure involving the use of liquid–liquid extraction followed by column liquid-solid extraction prior to FTIR analysis and matrix subtraction, a slightly positive bias due to matrix interference was still obtained. With the USP reference standard PDMS (Figure 1.11), the peak at 1097.6 cm^{-1} for formulation E is just slightly smaller than the peak at 1014.6 cm^{-1}, while the sample spectrum shows the opposite. This is most probably due to blank matrix absorption. Where the blank interference is constant, it still produces more significant quantitation errors at lower concentrations (Sabo et al, 1983).

1.5.3 Deficiencies of the USP method for analysis of PDMS

Although the FTIR method appears to provide an adequate quantitation procedure for PDMS, the interferences caused by matrix components are highly variable, and the handling of the small volume IR cells with extremely volatile solvents requires great care. A further and very important consideration is that these methods are not stability indicating, a requirement that emerges in regard to shelf-life determination of therapeutic goods. For the latter purpose, a chromatographic method appears desirable but the separation power needs to be greater than reported by the published gel permeation technique.

For greater sensitivity and selectivity in development, the chromatographic method involved would be enhanced by a detection technique that has applicability to compounds without UV-absorbing properties, and operating over a wider separating range than the refractive index detector. A description of current possibilities follows.

1.5.4 Detection of substances without UV chromophore in liquid chromatographic analysis

Ultraviolet absorption detection (UVD) is the simplest form of detection of the separated components in liquid chromatography and the most universally applicable in pharmaceutical analysis, but clearly requires that the components have suitable UV absorbance characteristics. Many analyses of weak UV absorbers have been developed with UVD at shorter wavelengths (less than 220 nm) where solvent and impurity absorptions are prone to occur, so that gradient elution becomes more difficult as the baseline changes steeply.

Indirect detection by means of chemical reaction to add a UV absorbing group has also been used, but only to a limited extent since an appropriate reactive group is required. Other methods require the molecule to have the appropriate properties to respond, such as fluorescence (Lafosse et al, 1987), or electrochemical detection involving oxidation or reduction of the solute (Leung, 1989). Refractive index detection (RI) and more recently, the evaporative light scattering detector (ELSD) are being used routinely for drugs which are weakly absorbing or contain no UV chromophores (Asmus and Ebert, 1984).

The refractive index detector , being a bulk property detector, has a significantly lower sensitivity than the UV detector but more nearly approaches the universal detector in the sense that it will detect any solute that has a refractive index different from that of the mobile phase. It also finds a major application in the detection of solutes that do not have UV chromophores (McCrossen et al, 1998). The refractive index detector has a relatively narrow linear dynamic range of two to three orders of magnitude and it is thus less suitable than the UV detector for the quantitative analysis of a mixture having solutes present over a wide concentration range. Due to the change in refractive index as the solvent composition changes, the detector is much less suitable for gradient elution development than the UV detector. The RI detector is also very sensitive to fluctuations in cell pressure, flow rate and the temperature of the mobile phase. If the ambient

temperature changes during a chromatogram then significant drift will almost surely result.

Thus the refractive index detector, although relatively simple to operate, is probably the most difficult instrument to use at maximum sensitivity due to its general instability under the conditions. If the pump employed is reciprocating in action, this often produces short term noise in the detector output and may in fact be the limiting factor with respect to sensitivity.

The mass spectrometer may be classed as a universal detector, but it has an upper mass limit because it relies on the solute molecule being ionized in the gas phase. Thus non-volatile high molecular weight compounds such as polymers are not detected unless initially degraded to lower molecular weight units (generally less than 2000 Daltons).

1.5.5 The Evaporative Light Scattering Detector

A new alternative for routine LC detection of solutes without a significant a UV chromophore is the Evaporative Light Scattering Detector (ELSD) (Conforti et al, 1993). Unlike the RID, low-wavelength UVD and mass detection, the ELSD is able to employ multisolvent gradients for improved resolution and faster separations, without the baseline problems common to RID and UV detection (Colin et al, 1983).

The detection principle of the ELSD involves nebulization of the column effluent to form an aerosol, whereupon the solvent vaporizes to produce a cloud of solute droplets, which are detected by light scattering (Colin et al, 1984). The major components of the system are the nebulizer, the heated drift tube, and the light scattering cell as shown in schematic form in Figure 1.13.

Evaporative Light Scattering Detector

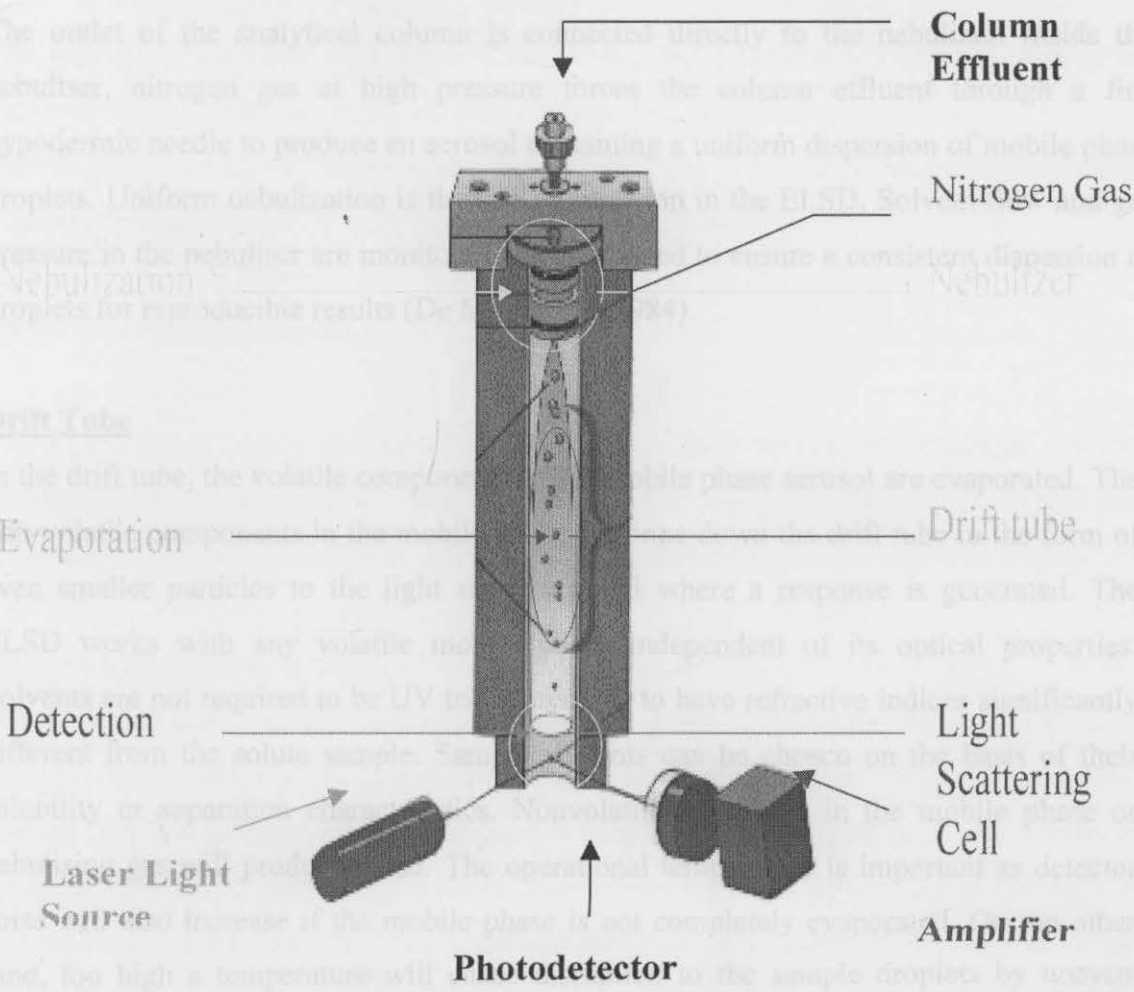


Fig 1.13 Key features of the Evaporative Light Scattering Detector

Nebulizer

The outlet of the analytical column is connected directly to the nebulizer. Inside the nebuliser, nitrogen gas at high pressure forces the column effluent through a fine hypodermic needle to produce an aerosol containing a uniform dispersion of mobile phase droplets. Uniform nebulization is the key to precision in the ELSD. Solvent flow and gas pressure in the nebuliser are monitored and controlled to ensure a consistent dispersion of droplets for reproducible results (De Mann et al, 1984).

Drift Tube

In the drift tube, the volatile components of the mobile phase aerosol are evaporated. The non-volatile components in the mobile phase continue down the drift tube in the form of even smaller particles to the light scattering cell where a response is generated. The ELSD works with any volatile mobile phase independent of its optical properties. Solvents are not required to be UV transparent, or to have refractive indices significantly different from the solute sample. Sample solvents can be chosen on the basis of their solubility or separation characteristics. Nonvolatile impurities in the mobile phase or nebulising gas will produce noise. The operational temperature is important as detector noise will also increase if the mobile phase is not completely evaporated. On the other hand, too high a temperature will cause disruption to the sample droplets by uneven evaporation. The optimum temperature for the drift tube is determined by observing the signal to noise ratio with respect to temperature.

Light Scattering Cell

The nebulized column effluent, depleted of volatile components, enters the light scattering cell where the solute particles are irradiated with the light from a laser diode. The scattered light is detected at 90° to the incident laser beam by photodiode, producing an electrical

signal which is sent to the analog outputs for collection. A light trap is located 180° from the laser to collect any light not scattered by particles in the aerosol stream.

The amount of light scattered depends on the following factors: (1) The intensity of the incident light. (2) The angle of observation. (3) The ratio of the particle radius to wavelength. (4) The number of scattering particles. (5) The size of the scattered particles (Guiochon et al, 1988).

ELSD Operating Parameters

The ELSD has a number of operating parameters, which need to be optimized for each application. A compromise between the mobile phase flow, nebulizer gas flow, and drift tube temperature must be chosen to maximize the sensitivity (Herbreteau et al, 1990).

< 1 > Nebulizer gas flow rate

The nebulizer gas flow rate determines the mobile phase droplet size, flow rates from 0.5 to 4.00 standard litres per minute (SLPM) can be selected. A higher flow rate will produce smaller droplets to enhance evaporation, but these scatter less light, reducing sensitivity. Thus the lowest acceptable gas flow rate producing the largest signals should be determined. A stable gas flow rate is critical for good reproducibility. It has been stated that a secondary pressure regulator may be needed if the gas source is unstable (Oppenheimer and Mourey, 1985).

< 2 > Mobile phase flow rate

The mobile phase flow rate has a direct effect on the choice of the gas flow rate and drift tube temperature. A higher mobile phase flow rate requires a higher gas flow rate and a higher drift tube temperature. It is therefore advantageous to use the lowest mobile phase flow rate possible, and this can be assisted by the use of narrow bore columns without affecting retention times.

< 3 > Drift tube temperature

The selection of the drift tube temperature depends mainly on the volatility of the mobile phase, but it is also affected by the mobile phase and gas flow rates. Aqueous solvents require higher temperatures than organic solvents while a lower gas flow rate produces larger droplets, and therefore, requires higher temperatures. The lowest temperature that produces an acceptable, low noise baseline should be used. When gradient elution of the multi-component mixture is being used, a compromise may have to be reached with respect to the drift tube temperature. At this stage, no instrument has been developed whereby the drift tube temperature can be programmed to change in the course of an analysis.

The advantages and limitations of ELSD

The ELSD works with any volatile mobile phase independent of its optical properties. The mobile phase is not limited to the UV transparent, or to those which have refractive indices significantly different from sample. Mobile phases are chosen only based on sample solubility and volatility (Oppenheimer and Mourey, 1985).

The ELSD offers the following advantages:

1. The baseline is not affected by solvent, column and laboratory temperature
2. Gradient elution shows no significant baseline shift, so it can be used for improved resolution of components
3. A functional group is not required in the analyte.
4. The detector generally responds to sample concentration (mass) directly.

The chromatographic requirements of the ELSD are similar to those for a LC-MS system, so that the ELSD is useful for developing the chromatographic conditions off-line prior to the LC-MS. The universal response of the ELSD gives a closer representation of sample mass than the UV detector for unknown substances and impurities (Letter, 1992). All sample types are detected with nearly equivalent response factors. Impurities identified during chemical characterization are detected according to their actual mass

concentrations (Letter, 1993). Unknown components can be approximately quantified by comparison to internal standards because the mass responses are nearly equivalent.

Disadvantages:

The major limitation of the ELSD is that the sensitivity of the detector is largely affected by the volatility of the analyte. The sample must be less volatile than the mobile phase and not evaporated at the selected drift tube temperature. Also, the ELSD is not able to operate with non-volatile solvents, such as salt-buffer solutions. Volatile buffer components, such as acetic acid, trifluoroacetic acid (TFA) or formic acid combined with ammonium hydroxide may be used as long as the concentration is below 0.1%.

The ELSD is therefore a powerful new tool for HPLC and GPC in that it can detect lipids, carbohydrates, polymers, fatty acids, oils, and other difficult samples precisely and accurately

1.6. Aim and Scope of the Project

The use of Simethicone as a therapeutic ingredient in pharmaceutical formulations has led to a requirement for efficient stability-indicating analytical methods for its quantitation. To date, the standard method for the analysis and quantitation of PDMS has been infrared spectroscopy (USP, 2000). If the matrix is simple, reliable results for the amount of PDMS are generated by this method. However, emulsions and suspensions containing PDMS require efficient extraction but still show background effects due to the sample matrix, particularly when antacid components are present (Olivie and Atkinson, 1996). Additionally the method is not stability indicating. An alternative is therefore needed for the analysis of simethicone as part of the quality control procedure in the pharmaceutical industry (Mihaly et al, 1982).

The objective of this project was to develop and validate a stability-indicating assay for Simethicone in liquid and solid antacid formulations. Liquid chromatography was the method of choice with its potential for the separation of components of a mixture. Because of the lack of UV chromophore in PDMS, the key component of the instrument is the evaporative light scattering detector (ELSD). When fully validated, the assay should be suitable for routine use in the quality control laboratory. The validation process required the determination of the method precision, system precision, recovery, linearity and working range, together with selectivity and ruggedness of the assay. The additional objective was to determine whether the assay method could be used in a stability indicating fashion for purposes of shelf-life assessment of pharmaceutical formulations containing PDMS.

Chapter 2 Experimental

2.1 Materials

2.1.1 Chemicals and reagents

Hydrochloric acid (AR) and methanol (AR) were supplied by Asia Pacific Specialty Chemicals Limited (Sydney). Dichloromethane (AR) and chloroform (AR) were supplied by Selby Scientific Limited (Sydney). Acetonitrile (HPLC grade) and sodium sulfate anhydrous (AR) were from Mallinckrodt, Australia. Other chemicals used were of analytical reagent grade (Merck, BDH and Ajax Chemicals, Sydney). Paracetamol was of analytical grade and was supplied by Sigma-Aldrich (Sydney, Australia). Water was double-distilled from an all-glass apparatus. Nitrogen gas of high purity grade was supplied by BOC (Sydney). The gas was passed through an in-line filter during use.

2.1.2 Silicone polymer samples

The following materials were provided by Pfizer Warner-Lambert Pty Ltd

- **Polydimethylsiloxane USP reference standard 350**: a clear, viscous oil, it is a mixture of fully methylated linear silicone polymers containing repeating units of dimethylsiloxane. In the following, this material is referred as USP PDMS.
- **Simethicone Emulsion**: manufactured by Dow Corning Corporation. It is composed of PDMS, silica, stearate emulsifiers, benzoic acid ester preservatives and water. It is a thick white paste with a slight characteristic odour and with viscosity of 25000 centistokes. One batch Log # 161097-D, was used in all experiments. The content of PDMS in this batch was 27.8% (Dow Corning Corporation).

- **Mylanta Liquid Antacid Suspension**: a formulation of Simethicone and antacids in water, manufactured by Pfizer Warner-Lambert Pty Ltd according to the following recipe:

Magnesium Hydroxide	400 mg/ 10 mL
Aluminum Hydroxide	400 mg/ 10 mL
Simethicone	40 mg/ 10 mL
Butyl Hydroxybenzoate	0.2 mg/ mL
Propyl Hydroxybenzoate	0.3 mg/ mL

Various batches of the formulation were used.

- **"Mylanta Placebo" Matrix:** contained all of the ingredients of Mylanta liquid antacid suspension except Simethicone.

2.2 Apparatus

The HPLC system was supplied by Shimadzu Scientific Instruments (Sydney) and consisted of a Shimadzu LC-10ATVP liquid chromatograph pump and solvent delivery system. A Shimazdu SPD-M10 AVP variable wavelength diode array detector and Alltech evaporative light scattering detector model 500 (Alltech Associates, Sydney) were used together with a SPD-10ADVP Autoinjector. The system was operated through a Shimadzu CBM–10A communication module and the software used was LC-10 workstation. The data handling system included a Daewoo Pentium computer and a Hewlett Packard Deskjet 560 printer. For all HPLC analyses, an Alltima C8 reversed-phase column (Alltech Associates, Sydney) with dimensions 250 x 4.6 mm was used. The column packing was of 5 μm particle size.

2.3 Methods

2.3.1 Mobile phase and sample preparation

The mobile phase compositions for the different analysis were as follows:

1. For the analysis of paracetamol, an isocratic mobile phase of 30% water and 70% methanol was used at a flow rate of 1.0 mL/min.

2. For analysis of PDMS, various binary gradient elution programs were used based on acetonitrile and chloroform. In general, the programs began with a solvent mixture of about equal parts then the chloroform content was increased. The main gradient program used was initiated at 55% chloroform (45% acetonitrile). The concentration of chloroform was ramped up to 85% over 5 minutes, then the concentration of chloroform was taken down to 55% in 5 minutes. The system was equilibrated at 55% chloroform for another 5 minutes. The flow rate was 1.0 mL/min.

Sample preparation:

1. Paracetamol standard solution

A stock solution of paracetamol at a concentration 2.00 mg/mL in double distilled water was prepared with a small amount of methanol (2%) to aid dissolution. From the stock solution, a series of dilutions (1.6, 1.28, 1.0, 0.5, 0.26 mg/mL) were prepared for injection into the HPLC system.

2. PDMS standard solutions

Approximately 100 mg of USP PDMS was accurately weighed into a 25 mL volumetric flask. The sample was dissolved with chloroform and made up to the mark. 1.0, 2.0, 3.0 , 4.0, and 5.0 mL of the diluted solution were transferred into separate 10.0 mL volumetric

flasks and diluted with chloroform, mixed well and used as the standard solutions. For each sample, the area of the PDMS peak (average of the three injections) was plotted against the PDMS concentration to produce the PDMS standard curve.

3. Analysis of PDMS in Mylanta Liquid antacid suspension

The principle of all PDMS analysis methods was the same. The sample was treated with concentrated hydrochloric acid to neutralize the anti-acid components. Dichloromethane was then used as extraction solvent for the separation of PDMS. The detailed procedure is as follows:

The Mylanta Liquid sample was mixed thoroughly. Approximately 10.0 mL of the sample was accurately weighed into a 50 mL beaker, then 15 mL of hydrochloric acid added. The solution was transferred into a 50 mL separating funnel. The beaker was washed with 15 mL dichloromethane and the washings transferred into the same separating funnel. The combined mixture was shaken vigorously for 2 minutes and then allowed to separate into two layers. The bottom layer was collected into a 100 mL volumetric flask and the top layer was extracted with another two portions of 15 mL dichloromethane. The dichloromethane portions were combined, 5.0 gram of anhydrous sodium sulfate was added and the solution was allowed to stand at room temperature for 2 hours with occasional shaking. The dried solution was filtered through a 0.5 cm diameter Whatman No.1 filter paper and collected in a 100 mL round bottom flask. The filter was washed with another 5 mL of dichloromethane. The combined dichloromethane was evaporated to dryness under reduced pressure on the rotary evaporator. The residue was redissolved with chloroform to a final volume of 25.0 mL. Aliquots of this solution were filtered through a 0.45 μm Gelman PTFE membrane syringe filter unit into the HPLC injection vial. For each sample, the peak area of the PDMS peak in the HPLC-ELSD system was obtained as the average from three injections.

4. Blank placebo matrix sample preparation

Approximately 10 mL of the Placebo matrix was weighed into a separating funnel, and the same extraction procedure as in (3) was followed.

5. Standard samples for Mylanta analysis

Five different amounts (approximately 67, 100, 133, 167, 200 mg) of Simethicone Emulsion were accurately weighed into 50 mL beakers, and 10.0 mL Mylanta placebo matrix was added and mixed. The extraction procedure with dichloromethane was followed exactly as described above in (3). After extraction, evaporation and redissolving in chloroform, the five samples were filtered and analyzed by the HPLC-ELSD system. The peak area (average of three injections) was plotted versus the amount of Simethicone Emulsion taken to produce the Simethicone Emulsion Recovery Standard Curve.

6. PDMS content of Simethicone Emulsion

Approximately 133 mg Simethicone Emulsion was weighed accurately and treated with the same procedure as described in (3), and analyzed with the PDMS standard curve obtained in (2).

Chapter 3 Results

3.1 Optimisation of ELSD parameters for HPLC analysis

Achieving optimised performance of the ELSD depends on setting the parameters appropriate to the application. The optimum drift tube temperature and gas flow rate settings depends on the volatility of the mobile phase and its flow rate. The higher the volatility, the lower the gas flow rate and drift tube temperature required. It is important that the temperature of the drift tube be sufficient to enable the mobile phase to vaporise quickly and produce a stable baseline. The gas flow rate is varied to adjust the size of the droplet and lead to the maximum detector response. For the initial characterisation of the ELSD, paracetamol was used as analyte. Paracetamol was chosen in order that the response of the ELSD could be compared with that of the UV detector. The Alltima C8 column was used with the mobile phase of water : methanol (30:70) at a flow rate of 1.0 mL/min. When desired, eluant from the column could be passed first through the UVD set at 265nm and then to the ELSD. The nebulizer gas flow rate to the ELSD was set at 2.0 litres per minute while the drift tube temperature was varied from 90 to 110°C and the average peak area at each temperature was recorded.

Table 3.1 Peak areas recorded (arbitrary units) for Paracetamol at various ELSD drift tube temperatures (gas flow rate 2.0 L/min, mobile flow rate 1.0 mL/min).

Drift tube Temp (^{0}C)	Peak Area Injection 1	Peak Area Injection 2	Peak Area Injection 3	Average peak area ± SD
90	25.0	25.2	25.7	25.3 ± 0.4
95	25.0	27.5	26.5	26.3 ± 1.3
100	20.0	20.0	19.0	19.7 ± 0.6
105	16.0	16.7	16.8	16.5 ± 0.4
110	9.0	9.6	9.8	9.5 ± 0.4
115	5.5	5.3	5.5	5.4 ± 0.1

From Table 3.1, it can be seen that when the temperature was set at 95^{0}C, the detector gave the maximum response. Hence the drift tube temperature was set at 95^{0}C for subsequent experiments with paracetamol as analyte. Since the detection process involves the scattering of light by the solute in particle form after evaporation of the mobile phase, the size of the sample particle can affect the detector response. The nebulization process acts to regulate the column effluent, and the factors that affect the process are the gas and mobile phase flow rates. The nitrogen gas flow rate to the ELSD was varied from 1.5 L/min to 4.0 L/min. The detector responses are compared in Table 3.2

Table 3.2 Peak Area recorded (arbitrary units) for Paracetamol at various gas flow rates for the ELSD (drift tube temperature 95°C), other conditions as in Table 3.1.

Gas flow rate (L/min)	Peak Area 1	Peak Area 2	Average Peak Area ± SD
1.5	23.8	24.0	23.9 ± 0.1
2.0	25.0	27.0	26.0 ± 1.4
2.5	27.0	24.8	25.9 ± 1.1
3.0	25.0	23.5	24.5 ± 1.1
3.5	19.0	21.0	20.0 ± 1.4
3.9	16.0	17.0	16.5 ± 0.5

Although the variations between individual chromatograms were rather large, a gas flow rate of 2.00 L/min was chosen for use when the drift tube temperature was 95°C. In the next step, the mobile phase flow rate was varied from 0.8 to 1.0 mL/min. No significant difference in the peak area was found, so the mobile phase was set at 1.0 mL/min in subsequent experiments.

Comparison of ELSD and UVD response levels

The HPLC system was set up with the UV detector and ELSD in series so that the same amount of eluate passed through both detectors. Paracetamol was used as analyte to check the detector response, with the conditions as established above (gas flow rate 2.0 L/min, mobile phase flow rate 1.0 mL/min, drift tube temperature 95°C. The wavelength of the UV detector was set at 265 nm to obtain a suitable peak size. The standard curves from the UVD and ELSD were generated using peak area versus concentration of paracetamol in mg/mL, as shown in Figs 3.1 and 3.2.

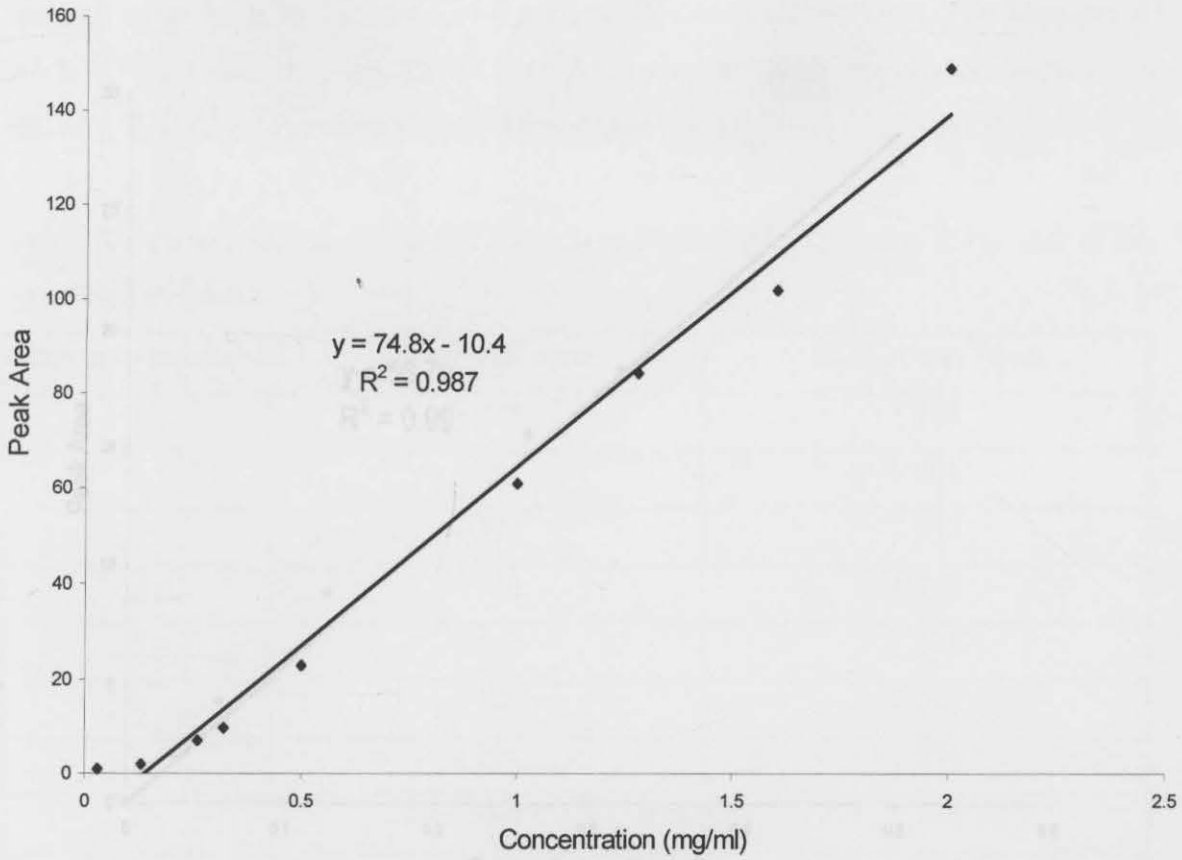


Fig 3.1 Standard curve for Paracetamol Analysis using ELSD

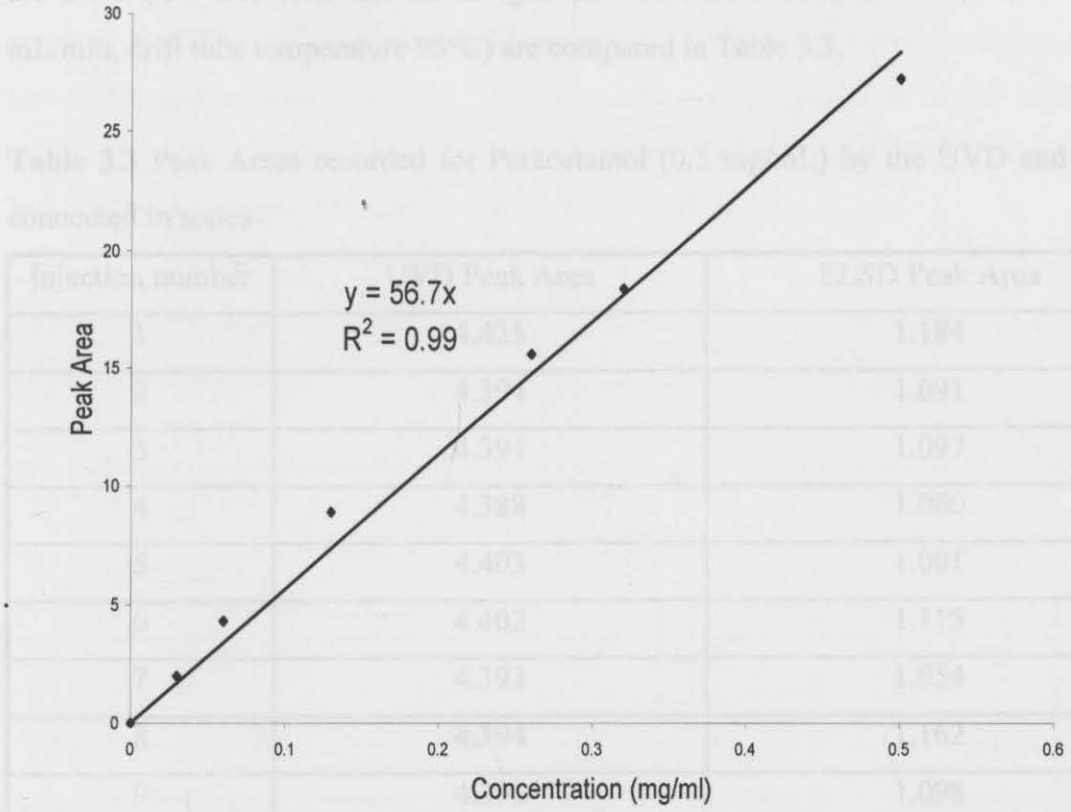


Fig 3.2 Standard curve for Paracetamol analysis using UV detector at 265 nm

Because paracetamol has a relatively high molar absorptivity, the UVD is capable of detecting the drug in concentrations below 0.1 mg/mL as seen in Fig 3.2. On the other hand, the response of the ELSD shows a non-linear section at paracetamol concentrations below about 0.2 mg/mL. The standard curve is better fitted by a second order polynomial at low concentration. This is presumably due to the poor light scattering characteristics of the very small solute droplets formed at this concentration level. A linear response was obtained above about 0.2 mg/mL. The extent of the linear range was not tested.

System reproducibility test

The reproducibility of the HPLC system with the ELSD connected after the UVD was checked by making 10 injections of 0.5 mg/mL paracetamol solution. The response of the UVD (λ = 265 nm) and ELSD (gas flow rate 2.0 L/min, mobile phase flow 1.0 mL/min, drift tube temperature 95°C) are compared in Table 3.3.

Table 3.3 Peak Areas recorded for Paracetamol (0.5 mg/mL) by the UVD and ELSD connected in series

Injection number	UVD Peak Area	ELSD Peak Area
1	4.425	1.184
2	4.394	1.091
3	4.394	1.097
4	4.388	1.060
5	4.403	1.001
6	4.402	1.115
7	4.393	1.054
8	4.394	1.162
9	4.396	1.098
10	4.40	0.956
Average	4.399	1.082
RSD (%)	0.23%	5.8%

The relative standard deviation (RSD) of 0.23% achieved by UVD was much smaller than the value of 5.8% obtained for the ELSD. Since these results were obtained from the same injections, it is indicative that the injection process was satisfactory while the response of the ELSD is much more variable than that of the UVD. The reasons for this variation were examined in relation to the analysis of Simethicone, described in the following section.

3.2 Development of a reversed-phase HPLC method for Simethicone

3.2.1 Analysis of Simethicone by Isocratic Elution

The reversed-phase C8 column and isocratic elution with acetonitrile-chloroform (30:70) at a flow rate of 1.0 mL/min was used in an attempt to develop a simple chromatographic system for the analysis of PDMS. This produced a single peak for PDMS solution in chloroform with retention time of 4.2 min. A series of dilutions of PDMS USP Reference Standard was analysed with the results shown in Table 3.4.

Table 3.4 Peak Areas recorded using the ELSD for PDMS (USP Reference Standard) solutions in chloroform

Solution ID	PDMS Concentration (mg/mL)	Peak Area 1	Peak Area 2	Peak Area 3	Average Peak Area
U1	0.2148	0.80	0.80	0.80	0.80 ± 0.0
U2	0.4296	1.80	1.80	1.70	1.76 ± 0.06
U3	0.5370	2.20	2.10	2.15	2.15 ± 0.05
U4	0.8592	3.50	3.50	3.60	3.53 ± 0.06
U5	1.074	4.50	4.40	4.50	4.43 ± 0.06

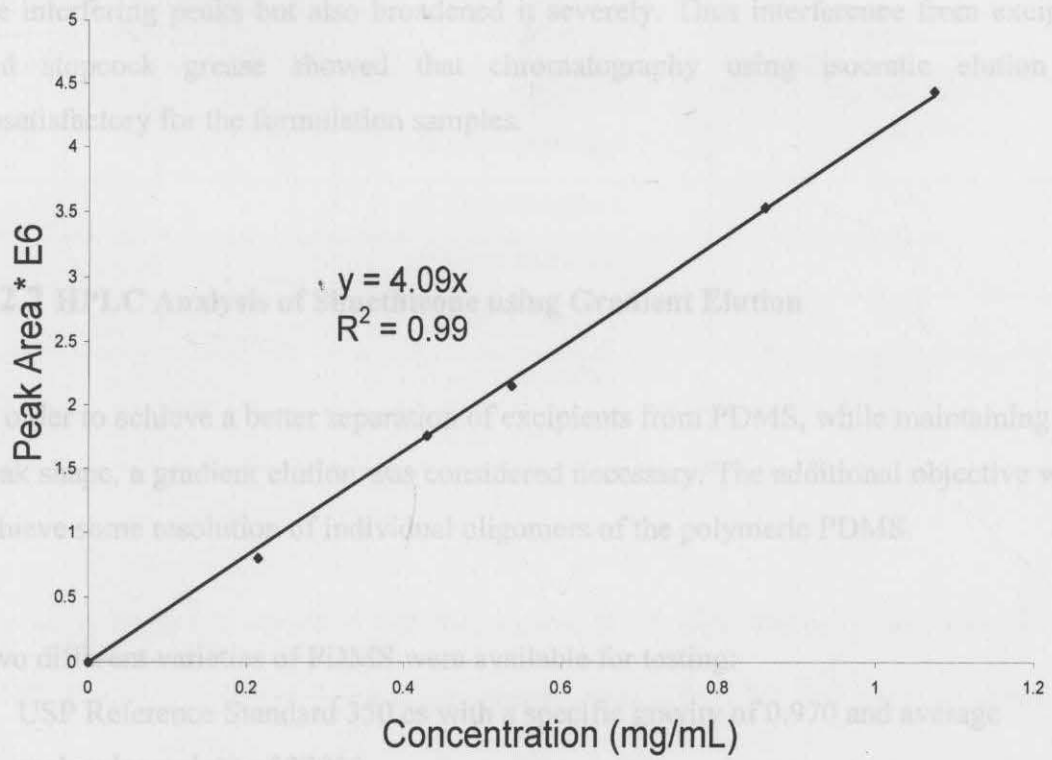


Fig 3.3 ELSD Peak Area for PDMS (USP Reference Standard) Solutions in Chloroform

A good linear response with correlation coefficient of 0.99 was obtained for solutions in the concentration range 0.2 to 1.2 mg/mL of PDMS. The specificity of the isocratic HPLC assay in relation to the analysis of Simethicone in formulations was then tested in two ways. First, a sample of placebo matrix (containing no Simethicone) was acidified with HCl and extracted with chloroform. The chloroform extract was injected into the HPLC with the result that a peak was observed at a retention time of 3.6 min, which is close to that of PDMS (4.2 min). In the second test, an HCl solution was extracted with chloroform. This extract also produced a peak at short retention time overlapping with the PDMS peak, subsequently identified as being due to grease used on the stopcock of

the separating funnel. The use of an isocratic mobile phase containing of 60% chloroform and 40% acetonitrile extended the retention of the PDMS peak away from the interfering peaks but also broadened it severely. Thus interference from excipients and stopcock grease showed that chromatography using isocratic elution was unsatisfactory for the formulation samples.

3.2.2 HPLC Analysis of Simethicone using Gradient Elution

In order to achieve a better separation of excipients from PDMS, while maintaining good peak shape, a gradient elution was considered necessary. The additional objective was to achieve some resolution of individual oligomers of the polymeric PDMS.

Two different varieties of PDMS were available for testing:

1. USP Reference Standard 350 cs with a specific gravity of 0.970 and average molecular weight of 27000.
2. USP Reference Standard 200 cs with an average molecular weight of 1800

Both the USP standards were dissolved with chloroform directly and diluted to a concentration of 2.0 mg/mL for injection into the HPLC.

The ELSD parameters were finalised as follows using the same procedure as detailed for paracetamol in section 3.1. The mobile phase flow rate was 1.0 mL/min, the gas flow rate was set at 2.0 L/min, and the drift tube temperature was 95 ^{0}C.

The gradient elution program shown in Table 3.5 was found to achieve a separation of PDMS for quantitative analysis of PDMS.

Table 3.5 Gradient elution program for PDMS analysis

Solvent A-Acetonitrile: Solvent B- Chloroform

Time (min)	Function	Value
1	T.Flow B.Concentration	1.0 mL/min 25%
4	B.Concentration	25%
5	B.Concentration	75%
25	B.Concentration	75%
40	B.Concentration	25%
50	B.Concentration	25%

The chromatograms obtained for the separation of high viscosity and low viscosity PDMS are shown in Fig 3.5 and 3.6, respectively. The USP Reference Standard with high viscosity (high molecular weight) could only be eluted as one complex peak whereas the USP PDMS 200 cs was separated into its constituent oligomers. This can be explained on the basis of the large difference in molecular weight for the oligomers of the low viscosity PDMS. Individual oligomers differ by 78 Daltons, the mass of the $(CH_3)_2$-Si-O repeating unit. The average molecular weight of the 200 cs PDMS is about 1800, so that each oligomer is approximately 4% different in mass and capable of being resolved in the HPLC system. On the other hand, the high molecular weight PDMS (as used in Mylanta Liquid) has an average chain of 27000 Daltons, with 0.2% difference between oligomers. This small difference appears to be beyond the resolution capabilities of the present HPLC system.

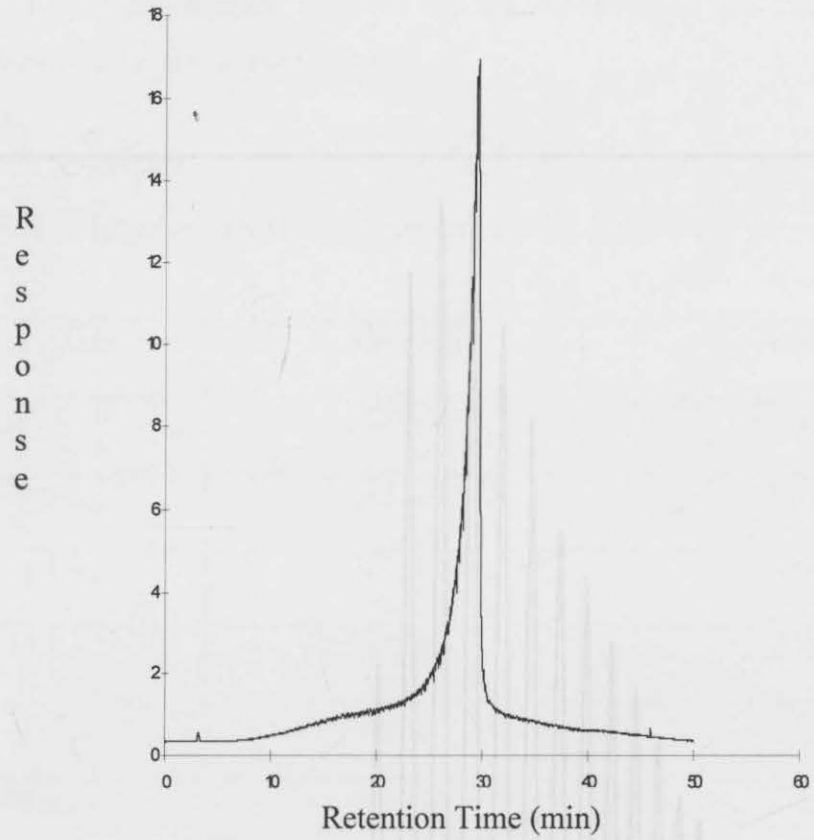


Fig 3.4 Separation of PDMS 350 cs (high molecular weight-high viscosity) by gradient elution

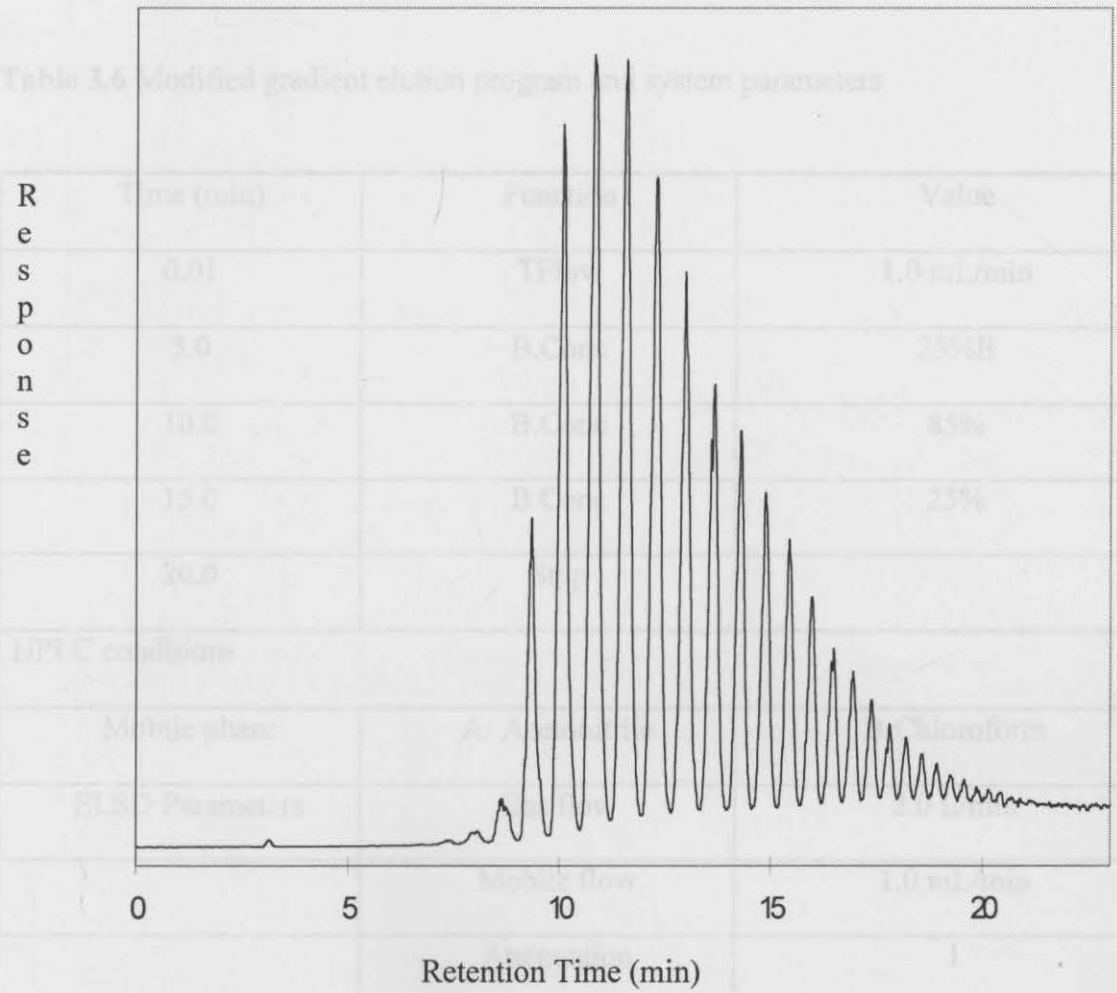


Fig 3.5 Separation of PDMS low viscosity 200 cs (low molecular weight) by gradient elution

While the gradient elution program was not able to resolve the individual oligomers of the high molecular weight PDMS, it was apparent that quantification of total PDMS in Mylanta Liquid Suspension could be achieved in this way. To lessen the retention time, the gradient elution program was modified to that given in Table 3.6. The resulting chromatogram is shown in Fig 3.6. With the faster elution program, the resolution of PDMS from all the excipients was achieved and the retention time was shortened and the peak shape was suitable for quantification.

Table 3.6 Modified gradient elution program and system parameters

Time (min)	Function	Value
0.01	TFlow	1.0 mL/min
5.0	B.Conc	25%B
10.0	B.Conc	85%
15.0	B.Conc	25%
20.0	Stop	
HPLC conditions		
Mobile phase	A: Acetonitrile	B:Chloroform
ELSD Parameters	Gas flow	2.0 L/min
	Mobile flow	1.0 mL/min
	Attenuation	1
	Drift tube temperature	95°C
	Output attenuation	11

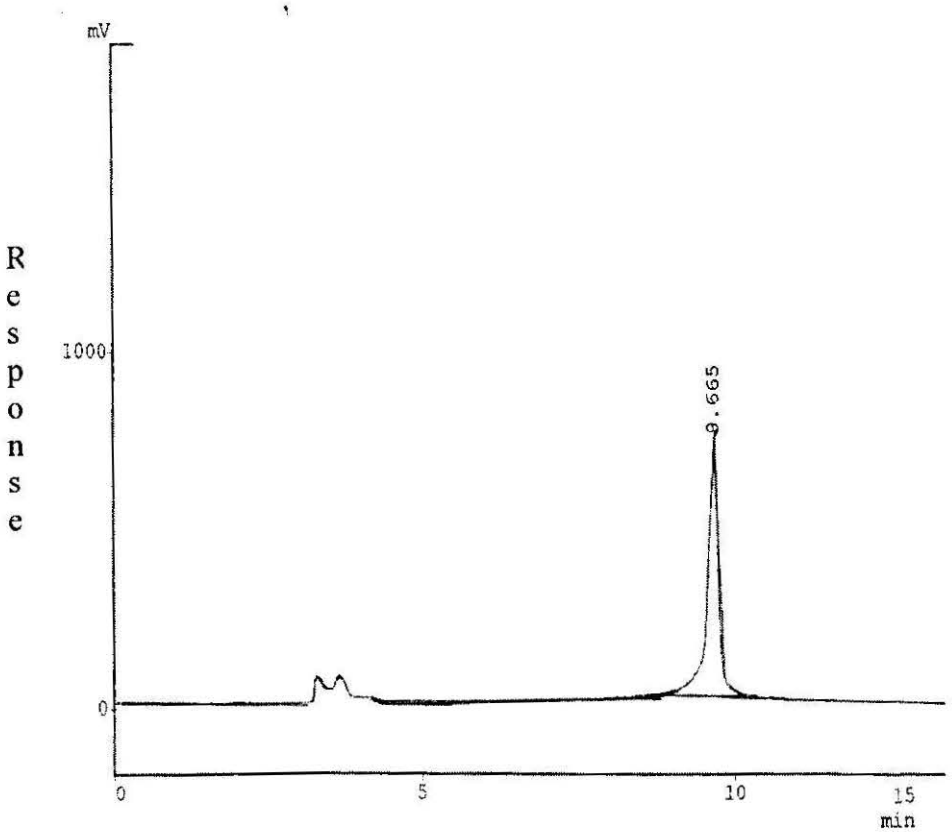


Fig 3.6 Chromatogram of USP Reference Standard PDMS (high viscosity) using the gradient elution program in Table 3.6.

In order to validate the assay procedure, a series of tests were performed to determine the system reproducibility and precision.

3.2.3 System precision and reproducibility

Since the detection principle of the ELSD involves nebulization of the column effluent to form an aerosol, the nebulization process is the key to obtaining satisfactory reproducibility and precision.

Ten injections of 0.21 mg/mL PDMS USP reference standard solution were made with the HPLC-ELSD system operating with the gradient elution program in Table 3.6.
The retention time and peak area for each injection are reported in Table 3.7.

Table 3.7 System precision test using USP Reference PDMS (0.21 mg/mL)

Injection Number	Retention Time (min)	Peak Area * E6
1	9.33	6.87
2	9.34	7.59
3	9.36	5.96
4	9.34	6.56
5	9.34	7.38
6	9.34	6.89
7	9.33	6.49
8	9.33	7.17
9	9.35	6.43
10	9.33	7.02
Average	9.34	6.84
SD	0.009	0.49
RSD%	0.10%	7.2%

PA*E6 = Integrated Peak Area (in arbitrary units) x 10^6

While the retention time of the peak was reproducible to 0.1%, the peak area variation of approximately 7% was considered unacceptable. From the results obtained with paracetamol using the UVD in series with the ELSD, it was clear that the large variation in peak area was due to the ELSD and not the HPLC system. Different ways were explored to improve the system precision, including washing the complete system thoroughly, and testing the precision of another ELSD on loan from Alltech Associates. Different solutes, such as caffeine, paracetamol and USP Reference polydimethylsiloxane, were examined, but there was no significant difference in the relative standard deviation between detectors and samples.

A build-up of retained sample in the drift tube and nebulizer is a possible cause of the variation of response of the ELSD. Cleaning the drift tube and nebulizer at frequent intervals was found to improve the reproducibility. Premature evaporation of the solvent will lead to blockage of the very fine needle of the nebulizer, as well as material adhering to the inner surface of the drift tube, thereby affecting the precision and reproducibility. A routine clean up procedure was established by soaking the drift tube in chloroform for 2 hours, followed by washing with methanol, this procedure was carried out once every 2 weeks and the system precision was improved to about 4%, as shown in Table 3.8.

Table 3.8 HPLC-ELSD System precision using PDMS (0.4228 mg/mL) after cleaning the drift tube and nebulizer

Injection No	Retention time (min)	Peak Area*E6
1	9.32	11.34
2	9.34	10.86
3	9.36	10.24
4	9.39	10.21
5	9.35	11.54
6	9.34	10.62
7	9.34	10.71
8	9.34	11.13
9	9.34	10.56
10	9.30	11.17
Average	9.34	10.84
SD	0.023	0.45
RSD	0.25%	4.2%

Another important factor, in terms of reproducible operation of the ELSD is the nitrogen gas flow to the nebulizer. A varying gas flow rate to the ELSD will cause the size of the sample droplets to change leading to a variation in response. The gas flow controller was found to be leaking and was replaced. The system precision was checked again with the results shown in Table 3.9, indicating an improved precision of 2.6% RSD.

Table 3.9 PDMS System precision test after changing the flow controller (PDMS solution of 0.21 mg/mL)

Injection No	Retention time (min)	Peak Area*E6
1	9.34	7.10
2	9.34	7.46
3	9.35	7.12
4	9.35	7.53
5	9.35	7.30
6	9.34	7.56
7	9.35	7.33
8	9.35	7.57
9	9.34	7.57
10	9.35	7.20
Average	9.35	7.38
SD	0.003	0.19
RSD	0.04%	2.6%

Repeated experiments showed that an expectation of about 3% RSD is achievable for PDMS detection by the ELSD. This was confirmed by an independent experiment, carried out by the detector manufacturers, Alltech Associates.

It was found that by continuous monitoring and manual adjustment of the gas flow rate for every injection, the RSD could be reduced to about 1.5%. Since this requirement is contrary to the concept of an automated analytical procedure for batch analysis, the instrument manufacturer was notified. Newer models of the ELSD from Alltech Associates are reported to have incorporated a gas flow controller with improved characteristics.

The precision of the HPLC system was also checked by making six injections of USP PDMS reference standard solution on each of the six different days. The peak area and retention time recorded as shown in Table 3.10. The purpose of the precision test is to determine the intraday and interday variations of the equipment parameters, such as injection volume, mobile phase flow rate and nitrogen gas flow to the ELSD.

Table 3.10 System precision test (USP PDMS in chloroform)

PDMS 0.432 mg/mL (31/8/99)			PDMS 0.434 mg/mL (4/8/99)			PDMS 0.40 mg/mL (31/8/99)		
ID	Peak Area *E6	Retention Time (min)	ID	Peak Area *E6	Retention Time (min)	ID	Peak Area *E6	Retention Time (min)
1	4.08	9.28	1	3.57	9.26	1	2.71	9.00
2	3.77	9.26	2	3.38	9.27	2	2.57	9.02
3	3.90	9.26	3	3.73	9.27	3	2.73	9.01
4	3.90	9.26	4	3.63	9.28	4	2.78	9.02
5	3.75	9.26	5	3.46	9.28	5	2.69	9.01
6	3.73	9.26	6	3.48	9.26	6	2.69	9.01
Ave	3.86	9.26	Ave	3.54	9.27	Ave	2.70	9.01
SD	0.13	0.008	SD	0.13	0.009	SD	0.07	0.008
RSD	3.5%	0.09%	RSD	3.6%	0.10%	RSD	2.6%	0.08%

Table 3.11 System precision test (inter-day)

PDMS 0 .40 mg/mL (3/9/99)			PDMS 1.2 mg/mL (29/9/99)			PDMS 1.73 mg/mL (21/10/99)		
ID	Peak Area *E6	Retentior Time (min)	ID	Peak Area *E6	Retention Time (min)	ID	Peak Area *E6	Retentior Time (min)
1	2.71	9.0	1	8.22	9.32	1	19.09	9.16
2	2.57	9.02	2	8.31	9.32	2	19.60	9.16
3	2.73	9.01	3	8.28	9.31	3	19.66	9.16
4	2.78	9.02	4	7.74	9.31	4	19.15	9.16
5	2.61	9.01	5	8.17	9.32	5	18.98	9.15
6	2.69	9.01	6	8.19	9.31	6	19.32	9.15
Ave	2.68	9.01	Ave	8.15	9.32	Ave	19.30	9.16
SD	0.08	0.007	SD	0.21	0.005	SD	0.28	0.005
RSD	3.0%	0.08%	RSD	2.6%	0.06%	RSD	1.4%	0.06%

From replicate injections, the RSD of the peak area varied between 2% and 4% thereby suggesting that the acceptance criteria for this measurement should be 3%. The retention time RSD was consistently within 0.1%. The acceptance criteria of < 0.5% was adopted for the retention time variation. The HPLC- ELSD system was considered to be suitable for the development of the Simethicone analysis with these acceptance criteria.

An alternative approach, to provide an analytical procedure that would take account of small variations of the gas flow and other parameters, is the use of an internal standard. A number of possible materials for use as internal standard have been investigated, such as

long chain and cyclic hydrocarbons (tetradecane, octadecane, hexadecane, anthracene, etc), but no suitable substances have been identified due to their elution at the same retention time as the excipients from Mylanta formulation. Thus the Simethicone assay development was attempted on the basis of obtaining a reproducible extraction of the PDMS from the antacid formulation, together with a closely monitored operation of the ELSD. The acceptance criterion for the instrument precision was set at 3%. For each batch of samples that were analysed by the HPLC-ELSD system, an instrument precision test was run and results of this were required to meet the acceptance criterion.

3.3. PDMS content of Simethicone 30% Emulsion

The first step in developing the assay method was to determine the PDMS content of the Simethicone 30% Emulsion used as the raw material in the antacid formulation. The Emulsion was acidified and the PDMS was extracted with dichloromethane. The separation of PDMS from the other components extracted from Simethicone Emulsion is shown in Figure 3.7. The peak with retention time of 9.35 minutes was identified in shape and retention time as that obtained with the PDMS standard. The peak at 3.2 minutes can be attributed to other components of Simethicone Emulsion such as emulsifiers that are also extracted under these conditions.

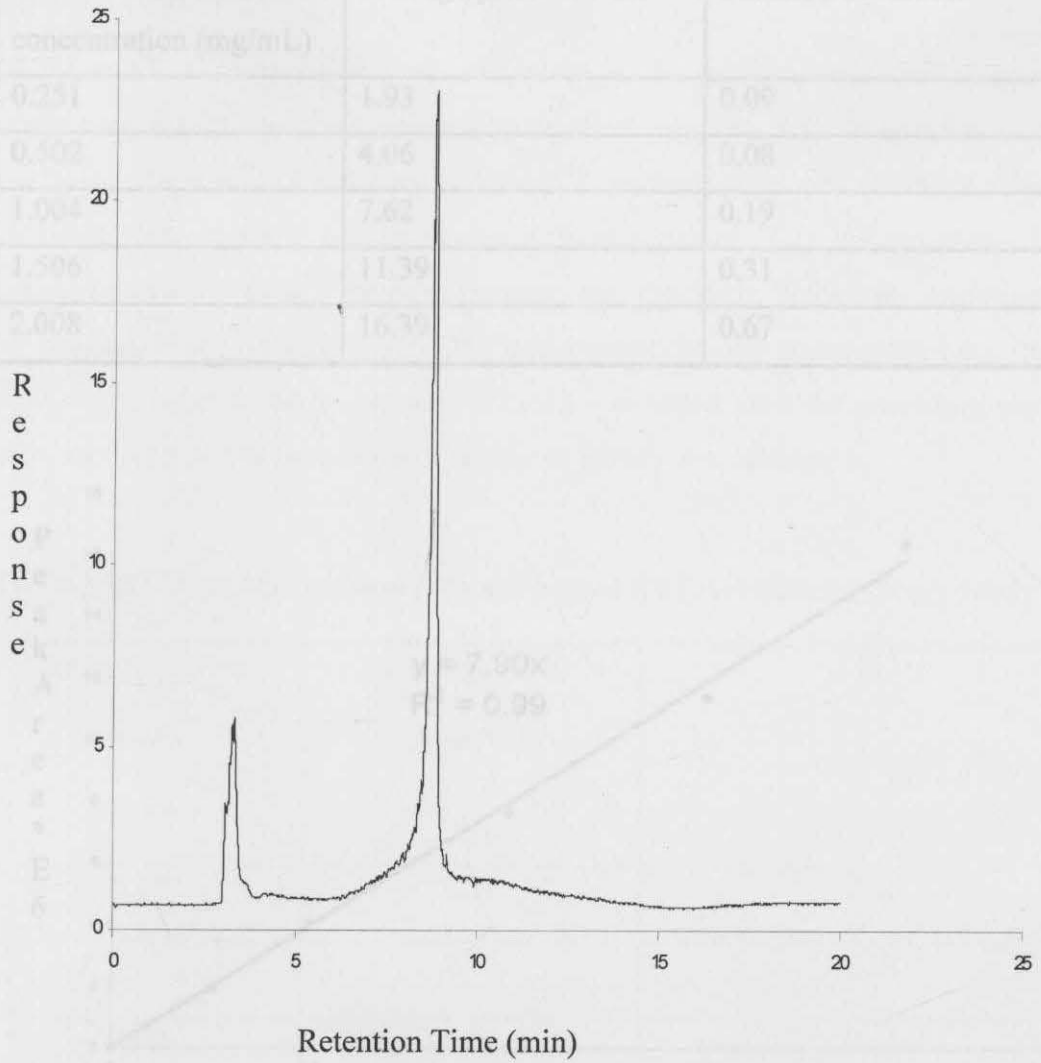


Fig 3.7 HPLC chromatogram of Simethicone Emulsion Extract in chloroform

A standard curve was generated by measuring the peak area for a series of solutions of PDMS USP Reference Standard in chloroform, as given in Table 3.12 and Figure 3.8.

Table 3.12 Peak Areas for the PDMS USP Reference Solutions

USP reference standard concentration (mg/mL)	Average peak area*E6	Standard deviation
0.251	1.93	0.09
0.502	4.06	0.08
1.004	7.62	0.19
1.506	11.39	0.31
2.008	16.39	0.67

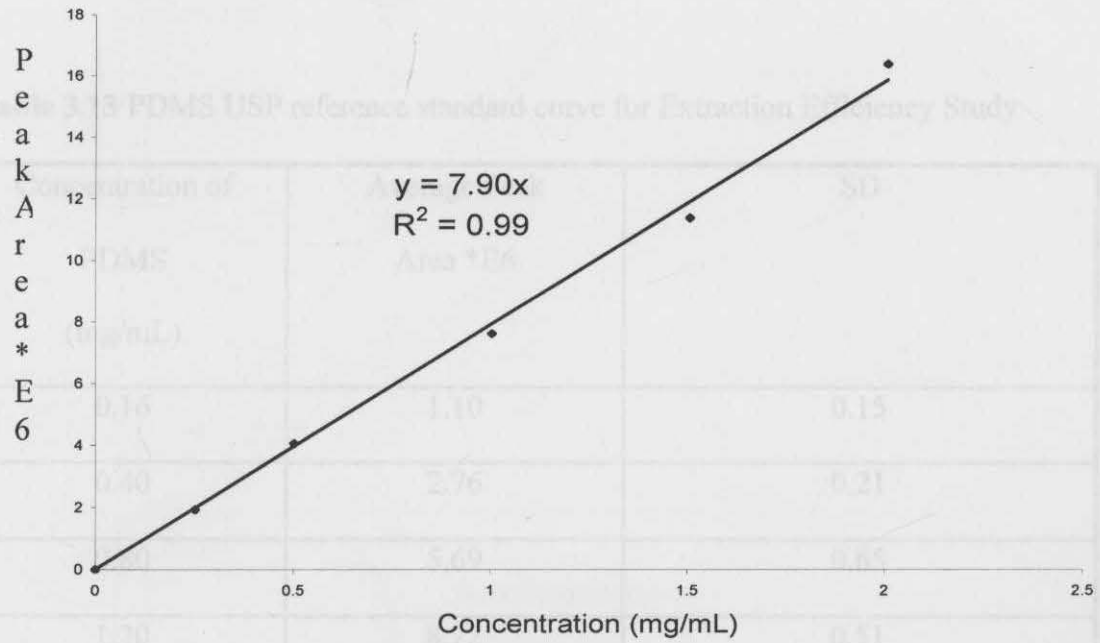


Figure 3.8 USP reference PDMS standard curve- Peak Area * E6 versus concentration.

3.3.1 Extraction Efficiency Test

PDMS Recovery

In order to study the effect of extraction of the analyte, recovery is computed by comparing responses of replicates of extracted samples with those of extracted blank matrix to which analyte has been added at the same nominal concentration. Although it is desirable that recovery has to be the highest possible, it is not necessary to achieve complete recovery to provide good accuracy and precision, if adequate reproducibility can be attained. USP reference standard PDMS sample (40 mg) was weighed and dissolved in dichloromethane, hydrochloric acid (15 mL) was added, then the procedure was followed as in section 2.3. The percentage recovery of PDMS was calculated.

Table 3.13 PDMS USP reference standard curve for Extraction Efficiency Study

Concentration of PDMS (mg/mL)	Average Peak Area *E6	SD
0.16	1.10	0.15
0.40	2.76	0.21
0.80	5.69	0.65
1.20	8.72	0.51
1.60	11.17	0.74

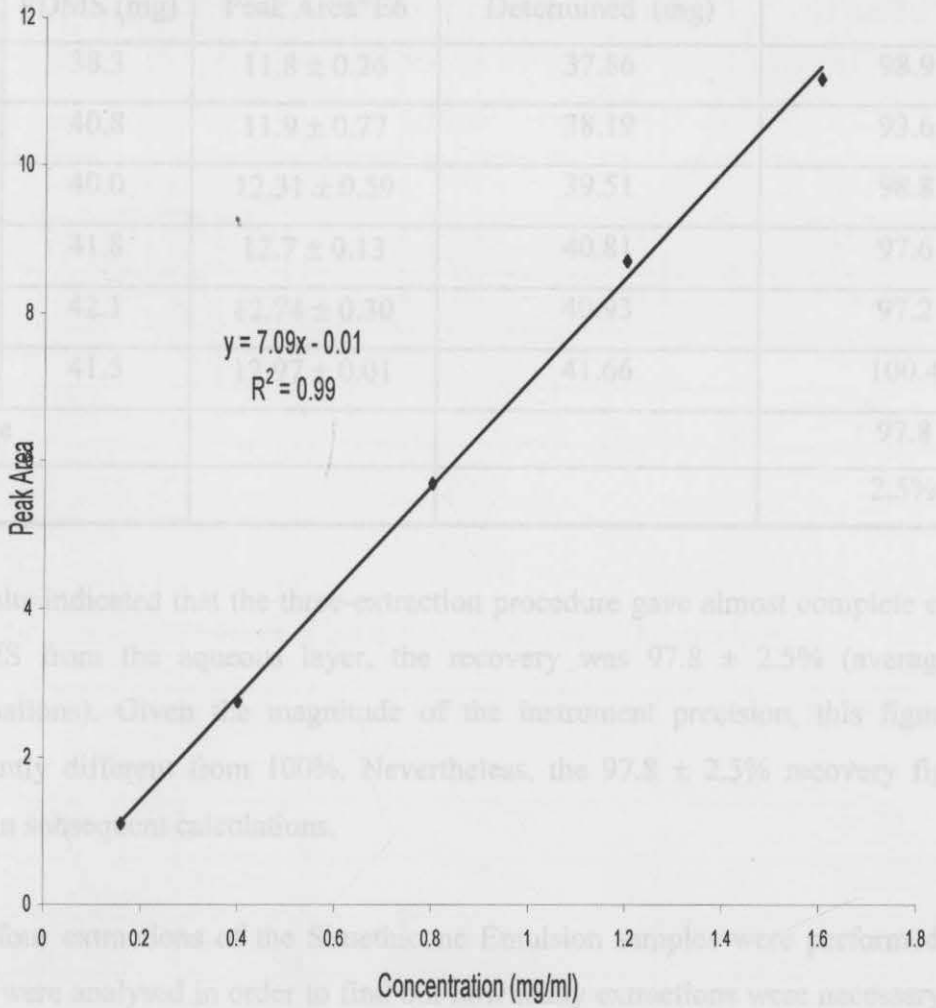


Figure 3.9 USP reference PDMS standard curve for Extraction Efficiency Study

Table 3.14 Analysis of USP PDMS extraction samples (three extractions)

ID	Weight PDMS (mg)	Average Peak Area*E6	Total PDMS Determined (mg)	% Recovery
UE1	38.3	11.8 ± 0.26	37.86	98.9
UE2	40.8	11.9 ± 0.77	38.19	93.6
UE3	40.0	12.31 ± 0.59	39.51	98.8
UE4	41.8	12.7 ± 0.13	40.81	97.6
UE5	42.1	12.74 ± 0.30	40.93	97.2
UE6	41.5	12.97 ± 0.01	41.66	100.4
Average				97.8
RSD				2.5%

The results indicated that the three-extraction procedure gave almost complete extraction of PDMS from the aqueous layer, the recovery was 97.8 ± 2.5% (average of six determinations). Given the magnitude of the instrument precision, this figure is not significantly different from 100%. Nevertheless, the 97.8 ± 2.5% recovery figure was applied in subsequent calculations.

Two to four extractions of the Simethicone Emulsion samples were performed and the samples were analysed in order to find out how many extractions were necessary to get a complete extraction of PDMS from the formulations.

Sixteen samples of 1.5 gram of Simethicone Emulsion were weighed out into separation funnels individually, five of them were extracted twice, five were extracted four times and the remaining six were extracted three times. The reconstituted sample solutions were analysed against the PDMS USP reference standard curve, and the percentage of PDMS in the Simethicone Emulsion was calculated.

Table 3.15 Analysis of PDMS in Simethicone Emulsion (2 extractions)

ID	Simethicone emulsion Amount taken (g)	% PDMS Found
S1	1.5056	25.38
S2	1.5080	25.11
S3	1.5067	23.92
S4	1.5095	26.86
S5	1.5062	25.29
Average		25.90
RSD		4.2%

Table 3.16 Analysis of PDMS in Simethicone emulsion (3 extractions)

ID	Simethicone taken (mg)	Total PDMS Found (mg)	% PDMS
S1	133.1	34.77	26.72
S2	133.9	35.95	27.47
S3	133.4	34.26	26.28
S4	133.9	34.68	26.50
S5	133.5	34.57	26.49
S6	133.9	35.34	27.00
Ave		34.93	26.74
SD		0.60	0.43
RSD		1.6%	1.6%

Table 3.17 Analysis of PDMS in Simethicone Emulsion (4 extractions)

ID	Simethicone emulsion Amount taken (g)	% PDMS Found
S1	1.5058	24.37
S2	1.5025	27.01
S3	1.5077	25.16
S4	1.5037	26.99
S5	1.5072	26.67
Average	1.5054	26.04
RSD		4.1%

In summary, Simethicone Emulsion that had undergone two, three and four extractions gave a PDMS content of 25.9, 26.7 and 26.0%. No significant difference in the PDMS content of the Simethicone Emulsion between the procedures involving two, three or four extractions could be seen. Hence, in subsequent work the samples were prepared using three extractions.

In a further determination of the reproducibility of the assay for PDMS in Simethicone Emulsion, six more samples of Simethicone Emulsion were prepared with 3 extractions and analysed.

Table 3.18 USP PDMS Standard Curve for Simethicone Emulsion Assay

ID	USP Reference PDMS Concentration (mg/mL)	Average Peak Area*E6	SD
U11	0.41	2.94	0.05
U12	0.41	3.37	0.05
U21	0.81	6.51	0.05
U22	0.81	6.75	0.49
U31	1.22	9.57	0.33
U32	1.22	10.40	0.11
U41	1.62	13.02	0.47
U42	1.63	13.34	0.33
U51	2.03	15.63	0.10
U52	2.03	16.38	0.27

Table 3.19 Analysis of PDMS in Simethicone Emulsion (Reproducibility Study)

ID	Simethicone Emulsion taken (mg)	PDMS Found (mg)	PDMS content %
S1	133.9	36.45	27.85
S2	133.5	35.63	27.31
S3	134.0	36.22	27.65
S4	133.2	35.01	26.89
S5	133.7	35.91	27.48
S6	134.4	36.67	27.91
Ave			27.51
SD			0.38
RSD			1.4%

Combining the results of the assays using three extractions, the analysis of Simethicone Emulsion (Batch Number HH077242) gave the average PDMS content of (27.1 ± 0.4)%. Individual assays had a RSD of 1.4 to 2.8%, ie, within the acceptance criteria. The Certificate of Analysis from the manufacturer (Dow Corning Company) for this batch of Simethicone Emulsion gave a PDMS content of 28.5% as determined by the USP method (infrared analysis). The RSD is not stated on the Certificate of Analysis, but it would need to be about 5% (ie, ± 1.4) in order that the value recorded here by the HPLC method is not significantly different. The specification limits for PDMS content of Simethicone Emulsion are given on the Certificate of Analysis as low 27.8, high 31.6, but this is not necessarily indicative of the RSD for the USP analysis.

3.4. PDMS content of Mylanta Liquid Suspension

3.4.1 Separation of PDMS from Mylanta Liquid Suspension

The basis of the assay of the Simethicone content of the Mylanta Liquid Suspension was to construct a recovery standard curve by assaying known amounts of Simethicone Emulsion added to Mylanta Placebo Matrix. This was done by taking approximately 67, 100, 133, 167 and 200 mg of the Simethicone Emulsion and mixing with 10 g Placebo Matrix, representing 50, 75, 100, 125 and 150% of the nominal Simethicone Emulsion content of the formulation. After extraction (3-fold) the solution were analysed by the HPLC-ELSD system. The peak areas obtained were the average of three injections.
Approximately 10 mL of Mylanta Liquid Suspension was extracted the same way. A typical chromatogram is shown in Fig 3.10.

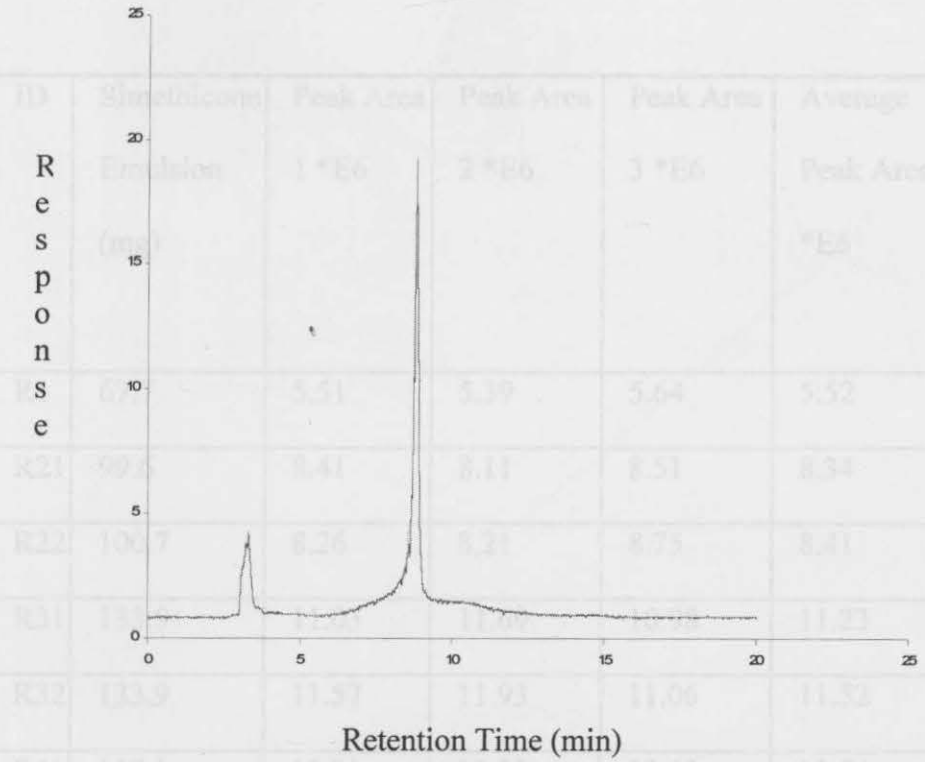


Figure 3.10 Separation of PDMS in the Extract from Mylanta Antacid Suspension

Mylanta Assay

In the procedure, six replicate aliquots from the same composite sample of Mylanta Liquid Suspension were extracted and assayed according to the gradient elution program. The same batch of Simethicone Emulsion was used throughout this project for standard curves, and Mylanta Liquid Suspension (Batch number MP01C – prepared 30/3/99 – stored at 40°C) was used for purposes of validation of the assay. Then the assay results for different batches of Mylanta Liquid Suspension were compared.

Table 3.20 Simethicone Recovery Standard Curve 4/8/99

ID	Simethicone Emulsion (mg)	Peak Area 1 *E6	Peak Area 2 *E6	Peak Area 3 *E6	Average Peak Area *E6	SD
R1	67.7	5.51	5.39	5.64	5.52	0.13
R21	99.6	8.41	8.11	8.51	8.34	0.20
R22	100.7	8.26	8.21	8.75	8.41	0.27
R31	133.9	11.03	11.69	10.98	11.23	0.36
R32	133.9	11.57	11.93	11.06	11.52	0.44
R41	167.1	13.81	13.33	13.49	13.54	0.11
R51	201.5	17.87	17.14	17.13	17.38	0.14
R52	201.4	17.62	16.75	17.16	17.18	0.24

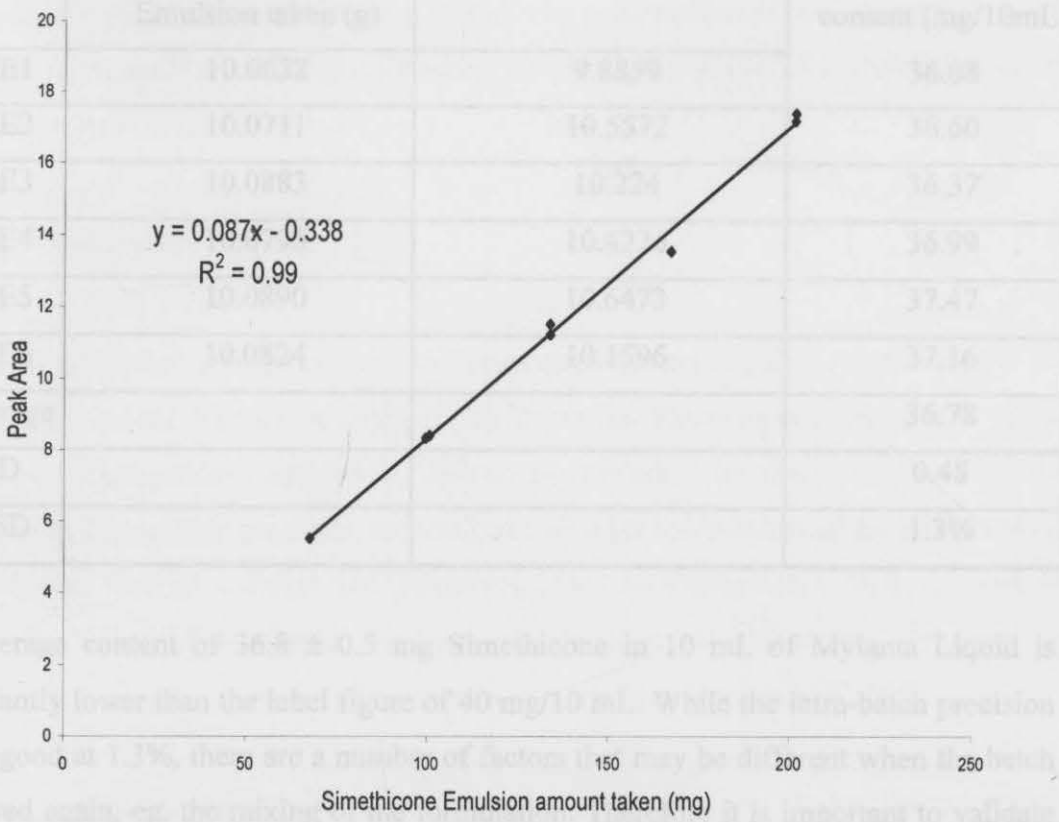


Figure 3.11 Simethicone Recovery Standard Curve 4/8/99

Table 3.21 Analysis of Mylanta Liquid Suspension B#MP01C

(Manufactured 30/3/99 – stored at 40°C – Specific gravity 1.08)

ID	Mylanta Liquid Emulsion taken (g)	Average Peak Area *E6	Simethicone content (mg/10mL)
ME1	10.0632	9.8859	36.08
ME2	10.0711	10.5572	36.60
ME3	10.0883	10.224	36.37
ME4	10.0795	10.4226	36.99
ME5	10.0890	10.6473	37.47
ME6	10.0824	10.1596	37.16
Average			36.78
SD			0.48
RSD			1.3%

The average content of 36.8 ± 0.5 mg Simethicone in 10 mL of Mylanta Liquid is significantly lower than the label figure of 40 mg/10 mL. While the intra-batch precision is very good at 1.3%, there are a number of factors that may be different when the batch is assayed again, eg, the mixing of the formulation. Therefore it is important to validate the assay procedure by a series of determinations on different days, to ensure that it is capable of a precision whereby the discrepancy of the content as shown here is verifiable.

3.4.2 Validation of the assay method

The ultimate goal of the method validation process is to provide evidence that the method does what it is intended to do (Bressolle et al, 1996). All of the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, detection and data evaluation and the use of the sample

matrix as that of the intended samples. The extent to which these variables affect the assay result is found by repeating the full procedure several times on different days.

The validation of the analytical method includes an examination of the selectivity, method precision, reproducibility, linearity, recovery, solution ruggedness, and where appropriate, membrane binding in a filtration step.

3.4.2.1 Selectivity

The term selectivity and specificity are often used interchangeably in relation to an analytical procedure. Specificity is the ability to assess unequivocally the analyte in the presence of endogenous compounds. Selectivity includes the ability to separate the analyte from degradation products, metabolites and other components of the formulation. The simplest test to validate the selectivity for chromatographic analysis was to demonstrate a lack of response in the blank sample matrix. In this project, the retention time of endogenous components of the matrix was compared to that of PDMS.

A 10 mL sample of the placebo matrix was analysed according to the method with 3 extractions. No peaks were detected with retention time between 7 and 12 minutes in the region corresponding to PDMS, confirming that there is no interference from excipients or reagents (Fig 3.12).

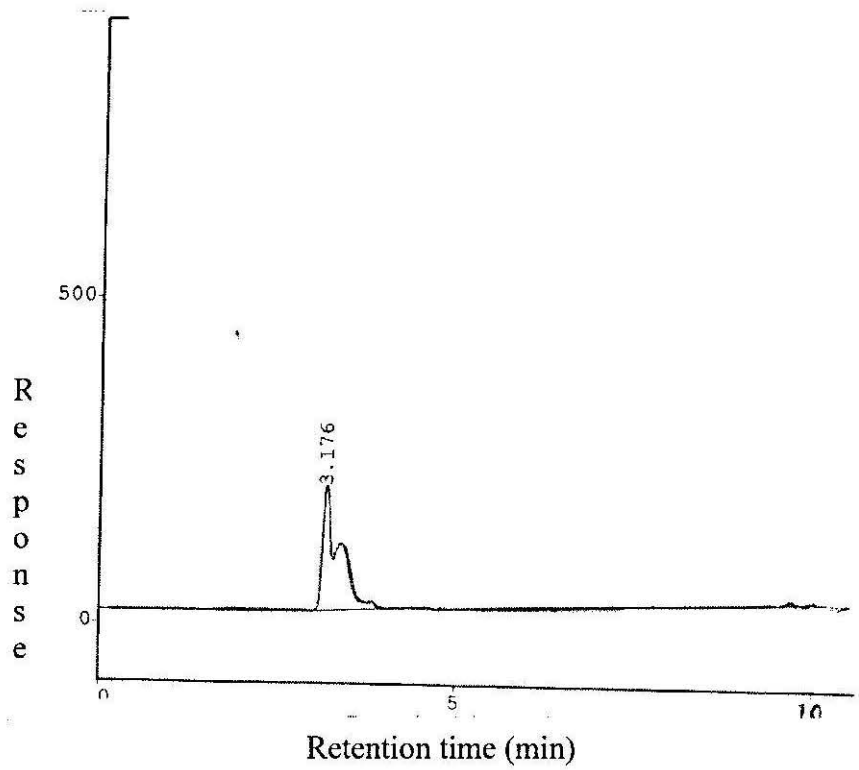


Figure 3.12 Blank Chromatogram of extract of placebo matrix

3.4.2.2 Method precision

Precision is usually assessed on a within-batch and a between-batch basis. Between-batch assessment is not always carried out with a single batch per day, and some batches may be of sufficient size that more than one day is required for analysis.

The accuracy and precision should be determined with a minimum of five determinations per sample (excluding blank sample matrix) from an equivalent biological matrix. The precision around the mean value should not exceed 15% and the mean value should be within ± 15% deviation of the nominal value, ie, the bias should be less than 15% for acceptable accuracy (Hartmann et al, 1997).

In order to validate the method and check the method precision, the same batch of Mylanta Antacid Suspension was re-assayed twice, with the results shown in Table 3.22. For each set, this included the full preparation of the recovery standard curve using Simethicone Emulsion and Placebo matrix.

Table 3.22 Method precision test results

ID	Mylanta Suspension taken (mg)	Simethicone Content (mg/10 mL)
Test 1 Equation of the recovery standard curve: $Y = 0.069X - 0.808$, $R^2 = 0.99$		
ME1	10.0399	38.6
ME2	10.0743	39.9
ME3	10.0177	36.5
ME4	10.3560	36.8
ME5	10.0733	36.2
ME6	10.4234	36.9
Average		37.5
SD		1.4
RSD		3.8%
Test 2 Equation of the recovery standard curve: $Y = 0.065X + 1.461$, $R^2 = 0.99$		
ME1	10.0287	37.0
ME2	10.0566	34.7
ME3	10.0990	35.8
ME4	10.0430	37.0
ME5	10.0830	35.5
ME6	10.0819	37.4
Average		36.3
SD		1.0
RSD		2.9%

From Tables 3.21 and 3.22 three complete assays of the same batch of Mylanta Liquid Suspension gave the Simethicone content of 36.8, 37.5 and 36.3 mg/10 mL, with the RSD varying from 1.3 to 3.8%. It can be concluded that the precision of the assay is 4%.

3.4.2.3 Linearity and working range

It is necessary to use a sufficient number of standards to define adequately the relationship between concentration and response. Although some analytical procedures may require the use of non-linear calibration, it is customary to use a linear model, with the standard parameter estimation procedure based on the "least squares" methodology. In this approach, the independent variable (x) is concentration, the dependent variable (y) is the instrument response, and the computation procedure implicitly assumes that the measurement error is the same and normally distributed for each sample (y).

Linearity of the method was demonstrated by showing that the slope of the linear calibration curve is statistically different from 0, the intercept is not statistically different from 0 and that the regression coefficient is not statistically different from 1.

In the assay development, two types of calibration curve are required. The first is the PDMS standard curve for the PDMS content of Simethicone Emulsion, and the second is the Simethicone Recovery standard curve for the Simethicone content of Mylanta Suspension. The PDMS Standard curves for the Simethicone Emulsion assays reported above were linear in the PDMS concentration range 0.4 to 2.0 mg/mL

Table 3.23 Equation parameters for PDMS standard curves

Date and Test		Correlation Coefficient	Slope	Y-intercept
23/4/99	Simethicone Emulsion assay	0.999	8.627	0.582
2/7/99	Simethicone Emulsion assay	0.997	6.701	0.173
10/8/99	Simethicone Emulsion assay	0.998	7.551	0.182
23/8/99	Simethicone Emulsion assay	0.997	7.953	0.096

The correlation coefficient was always better than 0.996, so the acceptance criteria of < 0.996 was established for the PDMS standard curve.

In the Mylanta Suspension assay, the standard curves for Simethicone recovery were linear in the concentration range of 50 to 150 nominal % content for Simethicone Emulsion in Mylanta Suspension. From the results in Table 3.24, in the range of 50 to 150% of the nominal Simethicone content of the sample, the slope was linear.

Table 3.24 Equation Parameters for Simethicone Recovery Standard curve

Date and Test		Correlation Coefficient	Slope	Y-intercept
4/8/99	Recovery standard curve	0.998	0.087	-0.338
3/9/99	Recovery standard curve	0.998	0.071	-0.894
15/11/99	Recovery standard curve	0.996	0.065	1.461

On the basis of the parameters in Table 3.24, the acceptance criteria of the correlation coefficient was set at < 0.995.

3.4.2.4 Ruggedness of the Method

The ruggedness of the method can be assessed by studying the eventual effect of different sets of conditions on the method. This is done through cross-validation. Typical reasons for cross-validation should include transfer of the method from one analyst to another, significant instrumental or procedural modification (in HPLC, the difference in chromatographic performance between columns of the same designation is the most common source of chromatographic variability) and a significant time lapse between periods of operation. In this project, it was not possible to test the effect of different analysts, instrumentation or laboratories, but one important ruggedness factor related to the stability of the samples once prepared. In the normal conduct of the assay, the reconstituted extracts of PDMS were subjected to the HPLC analysis as soon as each batch was ready. Each batch consisted of 2 solvent blanks, 2 PDMS reference solutions, 6 Simethicone recovery standards and 6 Mylanta extracts, a total of 16 samples. As each sample was injected 3 times, the total elapsed time for the batch analysis was about 24 hours. Since the samples contained the volatile solvent chloroform, it was important to establish that the samples were stable for that length of time, and also to determine if they could be stored in circumstances when the HPLC instrumentation was not available for immediate analysis. This was checked as follows.

Test 1 PDMS Standard solution stability

The stability of PDMS standard solution was based on triplicate determination of PDMS samples at three concentration levels (low, medium and high) at multiple time points after the start of storage to allow "trends" to be detected. The issue is not whether there is a trend in degradation, but whether the study samples are adequately preserved at the time of analysis.

Separate sealed vials of PDMS solutions in chloroform were stored at 4°C for 0, 24, 48, 72, 96 hours and then examined by the HPLC procedure.

Table 3.25 Standard PDMS solution stability

Sample used: USP reference standard PDMS solution 1.2 mg/mL					
ID	0 h	24 h	48 h	72 h	96 h
1	8.22	7.56	8.17	7.66	8.26
2	8.31	8.26	7.86	7.89	8.29
3	8.28	8.11	8.24	8.21	8.76
4	7.74	7.84	7.95	8.19	8.66
5	8.17	7.79	8.47	7.93	7.98
6	8.19	8.09	8.04	8.12	8.14
7	8.27	7.77	8.04	7.95	8.38
8	8.45	8.30	8.45	8.06	7.90
9	8.23	8.24	7.86	8.47	8.18
10	8.33	8.08	8.22	8.52	8.11
Ave	**8.22**	**8.00**	**8.13**	**8.10**	**8.27**
SD	0.19	0.25	0.22	0.26	0.27
Deviation from initial value	0	-2.7%	-1.6%	-1.5%	+0.6%

The variation in the peak area for PDMS in chloroform solution was in all cases less than 3%, ie, within the acceptance criteria for the system precision. Hence it can be concluded that the PDMS solution in chloroform was stable over 96 hours of this experiment. Similar results were gained for other concentrations tested.

Test 2 - Stability test of Mylanta Emulsion Extract samples (Performed on 4/9/99)

The samples were prepared for the complete assay (Simethicone recovery standards and Mylanta Suspension extracts). Each reconstituted solution was subdivided into two HPLC vials, one set of which were analysed immediately, while the other was stored at 4°C for 24 hours and then analysed. The result of the immediate analysis is given in Table 3.21 as part of the method precision test, and the result for the 24 hour stored samples is shown in Table 3.26.

Table 3.26 24 Hour stability results of Mylanta Extracts

ID	Mylanta Suspension taken (g)	Simethicone Content Found (mg/10 mL)
ME1	10.0399	36.28
ME2	10.0743	37.73
ME3	10.0177	34.67
ME4	10.3560	36.23
ME5	10.0733	35.63
ME6	10.4234	36.19
Average		36.1
SD		1.0
RSD		2.8%

The reconstituted samples assayed twenty-four hours after preparation gave a Simethicone content of 36.1 ± 1.0 mg/10 mL, compared to the value of 36.8 ± 0.5 mg/10 mL when assayed immediately (Table 3.21). The difference of 0.7 represents a 2% variation, which is well within the method precision of 4%. Additionally, the difference is not significant according to the Student's t-test based on the RSD of the data sets.

3.4.2.5 Membrane binding test

Since the sample preparation procedure involved a filtration step, it is possible that some of the PDMS could adhere to the filter membrane and housing. In order to test if binding occurred, a PDMS solution in chloroform was analysed before and after filtering. The USP Reference Standard PDMS solution was prepared and two aliquots were filtered through two different 0.45 µm filters (polypropylene housing-PTFE membranes and Millex membrane). The filtered and unfiltered samples were analysed (Table 3.25).

Table 3.27 Effect of filtering on PDMS Content

Sample used : PDMS 0.960 mg/mL in chloroform			
ID	Peak Area – unfiltered	Peak Area - PTFE filtered	Peak Area – Millex filtered
1	4.62	4.84	4.69
2	4.80	4.81	4.83
3	4.63	4.90	4.79
4	4.74	4.70	4.72
5	4.73	4.56	4.59
6	4.63	4.72	4.79
Average	4.69	4.76	4.73
SD	0.07	0.12	0.09
Difference %	0	+1.5	+0.9

The solution concentration was not affected by filtering through the PTFE or Nylon (Millex) membranes. The difference between the filtered and unfiltered solutions was within the instrument precision acceptance criteria (≤ 3%). Thus it can be concluded that there is no significant loss of PDMS during the filtration step.

Stability of Mylanta Antacid Suspension

In the assay validation it was found that the Simethicone content of one batch of Mylanta Antacid Suspension was 36.1 mg/10 mL, ie, lower than the label claim of 40 mg/10 mL. Thus several different batches of Mylanta Antacid Suspension were analysed and the results are shown in Table 3.28.

Table 3.28 Analysis of Mylanta Antacid Suspension – Batches D, F and J

ID		Mylanta Antacid Suspension Taken (g)	Average Peak Area*E6	Specific Gravity	Simethicone Content (mg)	Average (mg/10ml)	RSD
Equation of the recovery standard curve: $Y = 0.066X + 0.86$, $R^2 = 0.99$							
D	D1	10.0521	8.4	1.081	41.85	42.02	1.9%
	D2	10.0368	8.6	1.081	42.89		
	D3	10.0272	8.28	1.081	41.34		
Equation of recovery standard curve: $Y = 0.066x + 0.86$, $R^2 = 0.99$							
F	F1	10.0358	9.17	1.082	36.57	38.69	2.8%
	F2	10.0427	9.53	1.082	38.15		
	F3	10.0308	9.64	1.082	38.67		
Equation of the recovery standard curve: $Y = 0.064X + 0.04$							
N	N1	10.23	8.75	1.083	38.91		
	N2	10.24	9.02	1.083	40.06	39.21	1.89%
	N3	10.25	8.72	1.083	38.68		

The above batches of the formulation were all stored at the same temperature (5°C) but for different storage times as shown in Table 3.29.

Table 3.29 Storage conditions of Mylanta Antacid Suspension

Batch Number	Storage condition	Storage Time	Simethicone content determined (mg/10 mL)
N	5°C	2 months	39.5
D	5°C	22 months	42.0
F	5°C	22 months	38.7

From Table 3.29 it can be seen that the variation of Simethicone content of Mylanta Suspension stored at 5°C is greater than the assay precision, so it is a measure of the batch-to-batch content variation.

Several batches that had been stored at elevated temperatures were analysed and the results are shown in Table 3.30.

Table 3.30 Analysis of different batches (A, B, M) of Mylanta Antacid Suspension

ID	Mylanta Liquid Suspension taken (g)	Average Peak Area *E6	Average Content of Simethicone (mg/10 mL)	SD	RSD
Equation: $Y = 0.058X + 0.07$, $R^2 = 0.99$					
A1	10.0392	7.07			
A2	10.0170	7.92	32.41	0.87	2.7%
A3	10.0155	7.65			
B1	10.0046	6.84			
B2	10.1431	7.11	34.71	0.44	1.3%
B3	10.0103	6.88			
M1	10.0770	7.02			
M2	10.0192	6.89	34.58	0.27	0.78%
M3	10.0592	6.91			

Table 3.31 Analysis of different batches (E, G, H) of Mylanta Liquid Suspension

ID	Mylanta Liquid Suspension taken (g)	Average Peak Area *E6	Average Simethicone Content of PDMS (mg/10 mL)	SD	RSD
Equation of the recovery standard curve: $Y = 0.066X + 0.86$, $R^2 = 0.99$					
E1	10.0256	9.59			
E2	10.0164	9.51	39.52	0.64	1.6%
E3	10.0472	9.78			
G1	10.0651	7.36			
G2	10.1107	7.58	29.59	0.40	1.4%
G3	10.0856	7.47			
H1	10.0538	8.00			
H2	10.087	8.10	32.77	0.74	2.3%
H3	10.0462	8.36			

Table 3.32 Storage conditions of Mylanta Antacid Emulsion

Batch Number	Storage time (months)	Storage condition	Simethicone content Determined (mg/10mL)
N	2	5°C	39.20
F	22	5°C	38.69
D	22	5°C	42.02
E	4.5	30°C/60%RH	39.52
A	22	30°C/60%RH	32.41
H	22	30°C/60%RH	32.77
B	4.5	40°C/75%RH	34.71
C	22	40°C	31.59
G	22	40°C	29.59

The formulations were also analysed by the USP FTIR method in the manufacturer's laboratory. The results from the two methods are compared in Table 3.35.

Table 3.33 Comparison of batch analysis results of HPLC and IR methods.

Batch ID	Storage conditions	Simethicone content (mg/10 mL)		%Agreement
		HPLC	IR	
A1	30°C/60%RH	32.5	35.3	92.1
B1	40°C	37.2	38.0	97.9
C1	30°C/60%RH	32.3	34.0	95.0
D1	40°C	33.6	33.7	99.7
E1	40°C/75%RH	37.5	37.8	99.2
F1	40°C	28.1	30.4	92.4
G1	30°C/60%RH	36.3	38.3	94.8

The comparison of the results indicated that the HPLC analysis of Simethicone in Mylanta Antacid Suspension was generally lower than the USP IR method but within the range of 90 –100% agreement. The differences appear to be within the precision of each method. Thus it can be concluded that there is a reduction of the Simethicone content with storage at the elevated temperatures of 30 and 40°C that is highly significant after 22 months of storage.

The chromatograms of the extracts obtained from different batches were compared, and a slight difference of the shape of the PDMS peak was observed from the older batches stored at higher temperature compared to the fresher batches. The chromatogram of the oldest sample stored at 40°C is shown in Fig 3.13 and when compared to the chromatogram in Fig 3.10, it can be seen to have a broader front section and some splitting of the peak. If the polymeric substance degrades with chain scission, there would be a change in the molecular weight distribution, and that presumably would

change the pattern of affinity of the molecules in the HPLC column. Thus, in principle, the HPLC method could be used as a stability-indicating assay. Expansion of the solvent elution gradient was not able to achieve anything more than a broadening of the overall peak.

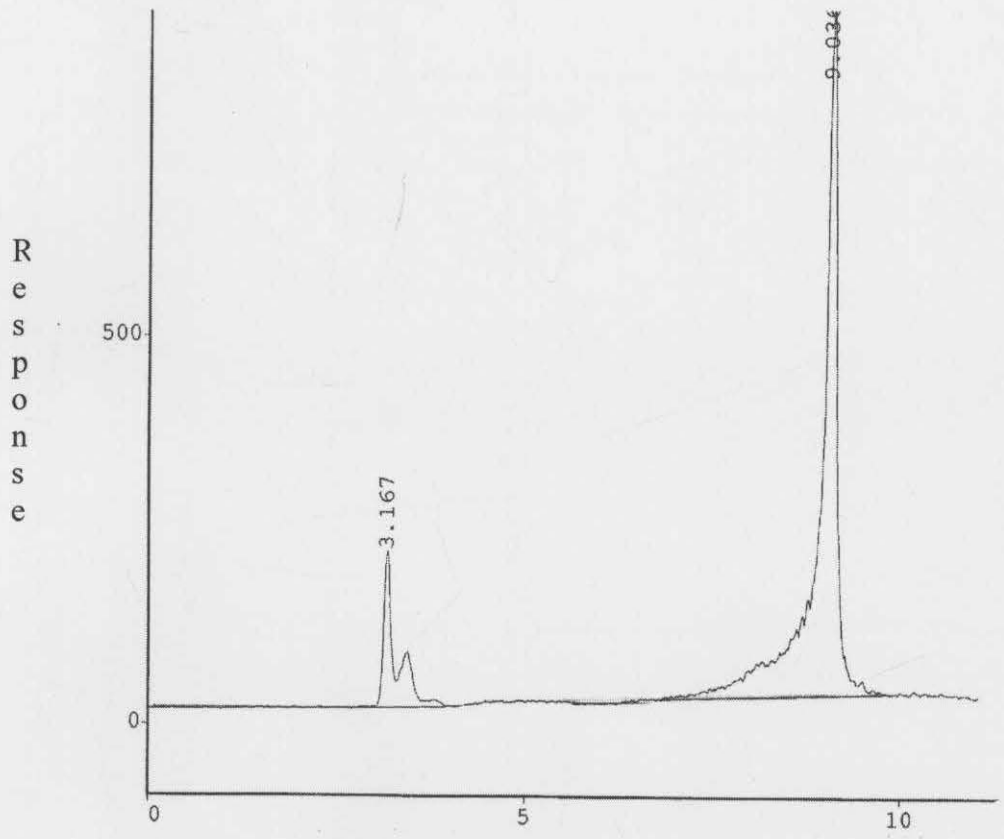
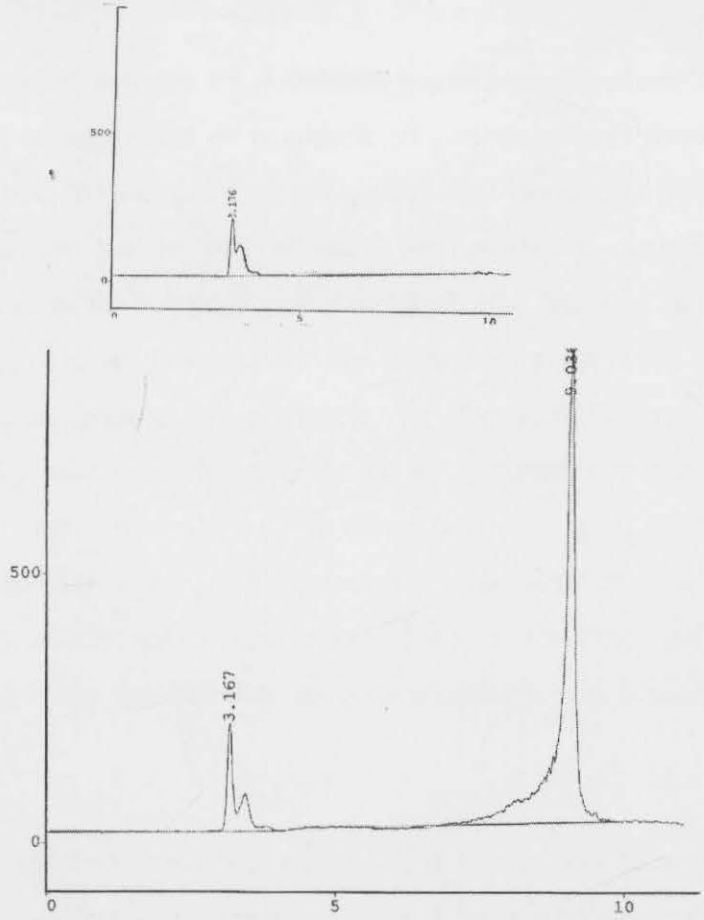


Fig 3.13 Chromatogram of Batch G of Mylanta Antacid Suspension (stored at 40°C for 22 Months)

An attempt was made to degrade the PDMS by ultrasonic irradiation. Simethicone samples were left in an ultrasonic bath for periods between 5 to 60 minutes, and then the chromatograms were compared. No difference could be detected. Another attempt to degrade the sample by physical means was by vigorously mixing the formulation for 5, 10 and 15 minutes when preparing the sample, as mixing and shear stress may cause the degradation, but no effect could be observed from the chromatogram.



P70

Chapter 4 Discussion

Simethicone is a complex mixture of polydimethylsiloxane oligomers with silicon dioxide. It is used as an ingredient of a number of pharmaceutical formulations, in particular those designed for the relief of indigestion and heartburn. Because of the complexity of Simethicone, and its lack of easily used analytical characteristics, the analysis of this component has not been well developed. UV detection is not possible due to its lack of an appropriate chromophore. The IR method given in the USP appears to provide an adequate quantitation procedure for the PDMS content, but the interferences caused by matrix components are highly variable, and the handling of small volume IR cells with extremely volatile solvents require great care. Hence the method is not robust, nor does it have the characteristics that could be used for stability indication. The only chromatography method reported to date is based on gel permeation and utilizes a refractive index detector that has poor sensitivity, and is solvent gradient incompatible.

The basis of the HPLC method developed and validated here is the extraction of PDMS from the acidified formulation and its separation on a reversed-phase column with a chloroform-acetonitrile solvent gradient. The detection and quantification is achieved using an Evaporative Light Scattering Detector.

In the preliminary experiments, the operational parameters of the ELSD were examined to optimize the response, then this was compared to the response gained from a UV detector. The ELSD and UVD were set in series so that the same amount of sample passed through both detectors. Using paracetamol as the analyte, it was found that the reproducibility of the response of the ELSD was at least ten times worse than that of the UVD. It is clear that the principle of operation of the ELSD is more complex than that of

the UVD. Since the process involves nebulization and evaporation of the HPLC column effluent, there is a far greater chance of build-up of deposits that can interfere with the consistent operation of the detector. Regular cleaning of the nebulizer and drift tube was found to be essential to achieving acceptable reproducibility.

It was also found that small variations in the nitrogen gas flow rate to the nebulizer were responsible for poor reproducibility of the ELSD. By continuous monitoring and manual adjustment of the nitrogen flow rate, the system precision of about 1.5% could be achieved. The poor control characteristics of the gas flow regulator appear to be a design fault of the particular model of the ELSD in use here. It is understood that later version have an improved gas flow regulator. The variation of gas flow can be circumvented, in principle, by the use of an internal standard in the HPLC assay procedure. In the present work, no suitable internal standard could be identified, so the validity of this approach could not be confirmed. Overall, with appropriate maintenance, the particular detector used here was found to a response precision of about 3% for successive detection of ten identical injections. In comparison, a UVD had a precision that was better than 0.3% for the same samples. In addition, for analytes such as paracetamol with high molar absorptivity, the ELSD was found to be one to two orders of magnitude less sensitive than the UVD. These characteristics confirm that the ELSD is not the first choice of detector for substances that have an appreciable UV absorption.

An adequate separation of the PDMS extracted from the Mylanta Antacid Emulsion was attained with the solvent gradient. While the separation of low molecular weight (low viscosity of 200 cs) PDMS into its oligomers was achieved, the high molecular weight part (high viscosity) was only eluted as one complex peak. This can be explained on the basis of the larger relative difference in molecular weight for the oligomers of the low viscosity PDMS. Each oligomer is approximately 3% different in mass and capable of being resolved in the HPLC system. The high viscosity PDMS only has a 0.2% difference in mass between oligomers, this small difference is beyond the resolution capabilities of the present HPLC system.

Since no suitable internal standard could be found, the efficiency of the extraction procedure was studied. Three extractions were used for the preparation of the sample. It was found that complete extractions were often hampered by poor phase separation, but this difficulty was overcome by acidifying the sample with HCl as the first step before extraction. The acidified sample showed less tendency to emulsify and gave clear interfaces in the separation.

In order to validate the method, a series of tests were performed, including the selectivity of the HPLC separation, the precision of the overall assay method, the efficiency of the recovery of PDMS, the stability of the sample solutions after reconstitution, the linearity and working range for the calibration curve and possibility of the loss of analyte by the membrane binding during the filtration. There are a variety of ways to validate selectivity, but the simplest test for chromatographic analysis is to demonstrate a lack of response at the relevant retention time in the chromatogram obtained with the blank placebo matrix. No peak was found at the same retention time of the PDMS peak, hence the matrix has no interference to the detection of PDMS.

The recovery of PDMS following extraction from a PDMS standard solution was found to be 97.8 ± 2.5%. While this figure is not significantly different from 100%, and the general level of precision of the detection was 3%, this slight correction was applied to subsequent determinations of the PDMS content of Simethicone Emulsion used as raw material in the formulation. This assay gave the PDMS content as 27.3 ± 0.4%, which was lower than the value of 28.6% on the Certificate of Analysis provided by the manufacturer. The necessary liquid handling cell for FTIR was not available to carry out a comparison analysis using the USP method. No information was available upon which to make a judgement on the precision of the USP FTIR method for PDMS quantification. It is important to note that the USP method involves a single extraction step. In the current assay developed, experiments showed there was no significant

difference when 2, 3, or 4 extractions were performed. However, 3 extractions were used in the validated method to ensure that extraction was as complete as possible, and to assist the assay precision.

The assay for the Simethicone content in the antacid formulation was based on the principle that known weights of Simethicone Emulsion (in the range of 50 to 150% of nominal content) were added to 10 g of placebo matrix for construction of the so-called "Recovery Standard Curve". These "standards" were treated in the same manner as the formulation samples, so that the area of the PDMS peak was a representation of a known amount of Simethicone. Thus the assay delivered the result as the amount of Simethicone rather than the PDMS content. On the assumption that the efficiency of extraction was identical across all samples and standards, there was no need to make any adjustment for an incomplete extraction.

In summary, a new HPLC method for determination of the content of Simethicone in Mylanta Antacid Suspension has been developed and validated. The method produced a mean Simethicone content of 36.8 ± 0.6 mg/10 mL, with a relative standard deviation of 1.7% for three different batches of the formulation. At this stage, the HPLC assay was concerned with Mylanta Antacid Suspension only. It is anticipated that there would be little difficulty in extending the method to the analysis of other formulations containing Simethicone.

The results from the HPLC assay were compared with those obtained using the USP FTIR method provided by the manufacturer. Agreement between the two sets of results ranged between 92 to 99%. It was found that the Simethicone content in Mylanta Antacid Suspension was consistently lower than that the label claim.

Several different batches of Mylanta Antacid Suspension were analyzed for Simethicone content to check the stability of the Simethicone in the formulation under different storage

conditions. The test results were compared with those from the USP FTIR method, which were provided by the manufacturer. Mylanta Antacid Suspension stored at 5°C gave a higher Simethicone content than those stored at higher temperatures of 30 and 40°C. Also, the longer the storage time, the lower the Simethicone content. This indicated that there was an apparent degradation of the Simethicone content with storage at high temperatures. Chromatograms of the newest and oldest batches were compared and it was found that the chromatogram of the oldest batch showed a slight difference indicating a change in the nature of the PDMS after storage. The resolution of the chromatographic separation was insufficient to determine whether there was a substantial change in the nature of the PDMS after storage. If degradation of the polymer chains occurred, the shape of the PDMS peak would be expected to change in the HPLC analysis. That is based on the assumption that the broad shape of the peak is caused by the presence of different oligomers. The fact is that there is a significant loss of total PDMS content of the formulation upon storage, but only a barely perceptible change in the shape of the chromatogram, suggests that some of the polymer (the lower molecular weight chains) may be lost by evaporation. This idea would require a large number of PDMS samples of differing molecular weight distribution to be examined.

An attempt was made to degrade the PDMS by sonicating the sample for up to 60 minutes using a sonication bath, but from the chromatogram, there was no effect observed. From the literature studies on the degradation of silicone polymers, it appears that severe conditions, such as temperatures greater than 150°C and strong alkaline reagents are necessary to cause the breakdown of the polymer. As an extension to this project, a fuller stability study could be undertaken to establish whether Simethicone degrades (or evaporates) under the effect of temperature and whether the presence of the alkaline components of the antacid formulation has any influence.

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