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A RP-LC method with evaporative light scattering detection for the assay of simethicone in pharmaceutical formulations

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Abstract

A reversed-phase liquid chromatographic method has been developed and validated for the determination of the polydimethylsiloxane (PDMS) component of Simethicone, which is used as an anti-foaming agent in pharmaceutical formulations. The method involves acidification to neutralise antacid components of the formulation, then a single extraction of the PDMS with dichloromethane. This is followed by separation with a reversed-phase column using an acetonitrile–chloroform solvent gradient, and quantification by an evaporative light scattering detector. An assay precision of 3% was achieved in intraday and interday determinations. No interference was found from the aluminium and magnesium hydroxide components of antacid formulations.

Keywords: Simethicone; Evaporative light scattering detector; Reversed-phase liquid chromatography; Antacid formulations; Polydimethylsiloxane

1. Introduction

The anti-foaming agent Simethicone is a complex mixture of high molecular weight polydimethylsiloxane (PDMS) oligomers (approximately 92%) with particulate silicon dioxide added to enhance the defoaming properties of the silicone oil. The PDMS is characterised in terms of its viscosity of about 60 000 centiStokes, corresponding to an average molecular weight of about 27 000 Daltons. Simethicone is an ingredient in a number of pharmaceutical formulations, in particular those designed to release gastrointestinal gas for the relief of indigestion and heartburn.

As a raw material, Simethicone can be used either directly in solid formulations as Simethicone USP, but is also available in the form of an emulsion (Simethicone 30% Emulsion USP) for inclusion in liquid suspension formulations. Because of the complexity of Simethicone, and its lack of easily used analytical characteristics, the analysis of this agent has not been well developed.

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UV detection is not possible due to the absence of an appropriate chromophore in PDMS. The method given in the USP [1] relies on measurement of the infra-red absorption band of PDMS in carbon tetrachloride solution at 1265 cm^{-1} and provides a satisfactory, albeit manipulatively demanding, quantification procedure for the PDMS content extracted from simple formulations. However, it has been reported that interferences caused by matrix components are highly variable. In particular, those formulations that contain the antacid components, aluminium hydroxide and magnesium hydroxide returned an apparently high PDMS content [2]. Hence the IR method is not robust, nor does it have the characteristics that are adaptable for product stability indication.

The alternative approach to the determination of Simethicone, with a particular view to stability indication, involves separation by chromatography. The only chromatographic method reported to date is based on molecular fractionation by gel permeation chromatography (GPC) [3]. An adequate separation of the PDMS component of Simethicone was achieved on a GPC column with a fractionation range of $1000–1 \times 10^6$ Daltons, using toluene as the mobile phase and a refractive index detector. The disadvantages of this system are the poor resolution of the GPC column and the incompatibility of the refractive index detector to solvent gradients that might be applied in an attempt to improve the resolution.

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 (ELSD). The LC-ELSD method has been applied to the assay of the PDMS content of Simethicone USP and Simethicone 30% Emulsion USP, and then to the determination of the Simethicone content of both solid and liquid formulations that also contain the antacid ingredients, aluminium and magnesium hydroxides.

2. Experimental

2.1. Materials

Simethicone USP and Simethicone 30% Emulsion USP were supplied by Dow Corning Australia Pty Ltd, Sydney. PDMS USP Reference Standard was obtained through Selby-Biolab Pty Ltd, Sydney.

Dichloromethane and chloroform (Selby-Biolab Pty Ltd, Sydney, AR grade) were distilled before use. Acetonitrile (Mallinkrodt UltimAR grade) was used for chromatography. Hydrochloric acid and sodium sulphate (anhydrous) were BDH Chemicals AR grade. High purity nitrogen gas was obtained from BOC Gases Australia, Sydney. All water was double distilled in an all glass apparatus.

2.2. Formulations

The following formulations were obtained from Pfizer Consumer Healthcare Research and Development (Sydney).

(A) Mylanta Liquid Antacid suspension containing in each 10 ml magnesium hydroxide (400 mg), aluminium hydroxide (400 mg) and Simethicone (40 mg added in the form of Simethicone 30% Emulsion USP), together with emulsifying and flavouring agents.

(B) Mylanta Liquid Antacid 'Placebo' suspension containing magnesium hydroxide (400 mg), aluminium hydroxide (400 mg) and excipients as in A, but no Simethicone Emulsion.

(C) Mylanta Antacid Tablets containing in each tablet magnesium hydroxide (200 mg), aluminium hydroxide (200 mg) and Simethicone (20 mg added in the form of Simethicone USP), together with tablet binding and flavouring agents.

(D) Mylanta Antacid Tablets 'Placebo' containing magnesium hydroxide (200 mg) and aluminium hydroxide (200 mg) and excipients as in C, but no Simethicone.

2.3. Instrumentation

A Shimadzu LC-10 Avp Liquid Chromatograph system was used in conjunction with an Alltech model 500 ELSD (Alltech Associates, Sydney). The column was an Alltima C8 (250 × 4.6 mm) with 5 μm packing (Alltech Associates, Sydney). For the quantitative determination of PDMS, an acetonitrile–chloroform solvent gradient was used as follows:

Time minutes	Acetonitrile (%)	Chloroform (%)
0	45	55
5	15	85
10	45	55
15	45	55

2.4. Methods

Stock solutions of PDMS were prepared by dissolving an accurately weighed amount (approximately 0.1 g) of PDMS (USP Reference Standard) in 50 ml of chloroform. A series of standard solutions was obtained by dilution of the stock solution with chloroform. A Simethicone standard solution was prepared in a similar fashion and filtered through a 0.45 μm syringe filter to remove the particulate silicon dioxide.

The PDMS was extracted from Simethicone, Simethicone Emulsion, and the antacid formulations according to the USP procedure as follows.

An amount of the mixture under test was accurately weighed into a 100 ml round bottom flask, then 25 ml dichloromethane and 25 ml diluted HCl (2:1) were added by pipette. The mixture was shaken vigorously for 5 min then allowed to stand for 1 h. Five millilitre of the bottom layer was removed to a stoppered tube and dried with 0.5 g of anhydrous sodium sulphate. The dried solution was filtered through a 0.45 μm nylon syringe driven filter into an HPLC injection vial.

For Simethicone USP and Simethicone 30% Emulsion USP, the amounts taken were such as to contain about 40 mg of PDMS, and the standard curve was prepared with the diluted standard solutions of PDMS.

For the liquid formulation, about 10 ml was taken, and the standard curve was prepared by adding weighed amounts of Simethicone 30% Emulsion USP (approximately 67, 100, 133, 167 and 200 mg) to 10 ml of the Liquid Antacid Placebo. This represented amounts of Simethicone ranging from 50 to 150% of the labelled content (40 mg) in 10 ml of the formulation.

For the solid formulation, a 1 g sample of powder obtained by crushing ten tablets was accurately weighed into a 100 ml round bottom flask, then 25 ml dichloromethane and 25 ml diluted HCl (1:1) were added. The mixture was treated in the same way as for the liquid formulation. For the standard curve, 400 mg of Simethicone USP was dissolved in 25 ml of dichloromethane. Five portions of 1 g of Antacid Tablet Placebo were weighed into separate 100 ml round bottom flasks, then 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the Simethicone USP stock solution was added, followed by 24.0, 23.5, 23.0, 22.5 and 22.0 ml dichloromethane, respectively. 25 ml of diluted HCl (1:1) was added to each flask and the mixtures treated as above.

3. Results

3.1. Chromatographic separation of simethicone

It has been reported [4] that PDMS (as Dimethicone) having a viscosity of 200 cS could be separated into its oligomers using a reversed phase column and an acetonitrile–chloroform gradient (25–75% chloroform over 20 min). Using the same conditions, we were also able to achieve such a separation of a low viscosity PDMS (average molecular weight about 2000 Daltons). However, the PDMS component in Simethicone has a viscosity rating of 160 000 centiStokes, and a molecular weight averaging 27 000 Daltons. The individual oligomers in the high molecular weight polymer were not resolved by the above gradient, nor by extended variations. The molecular weight difference between successive oligomers is only 78 Daltons, i.e. about 4% of the average for the low viscosity sample, but less than 0.3% of the average for the high viscosity PDMS. Clearly the system

does not have the power to resolve the oligomers of the high viscosity PDMS as used in Simethicone. Nonetheless, the ability of the reverse phase column to retain the PDMS does provide the basis for a quantitative determination of the PDMS content in pharmaceutical formulations containing Simethicone.

USP Reference Standard PDMS was used to establish chromatographic conditions more suitable for the quantitative assay of PDMS in Simethicone. A linear solvent gradient (1.0 ml/min) starting at 45 acetonitrile–55% chloroform for 5 min, then ramping to 15–85% over 5 min led to the elution of a slightly asymmetric peak due to the PDMS with a retention time of 9.2 min (Fig. 1A). The asymmetry is probably due to the fact that PDMS is not a single compound but a mixture of oligomers. An extract of Simethicone 30% Emulsion USP produced a similar chromatogram.

Analysis of dichloromethane extracts of acidified antacid formulations (liquid and solid) gave a peak at the PDMS retention time of 9.2 min, as well as a peak at 3.2 min corresponding to some of the other components and excipients, as shown in Fig. 1B and C. Chromatography of the dichloromethane extract of acidified 'placebo' antacid formulations (containing all components except Simethicone) gave no peak in the region between 7 and 12 min, confirming the specificity of the separation for PDMS (Fig. 1D).

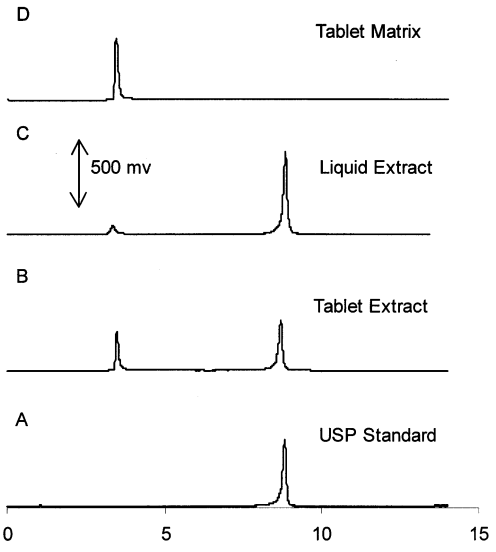

Fig. 1. Chromatograms of Polydimethylsiloxane obtained by reversed-phase liquid chromatography with detection by evaporative light scattering. The ordinate is the detector response in millivolts. (A) USP Reference Standard Polydimethylsiloxane, (B) Mylanta Antacid Tablet extract, (C) Mylanta Liquid Antacid Suspension extract, (D) Extract of Placebo Matrix for Mylanta Antacid Tablet

Operational parameters for the ELSD were varied and it was found that optimal response was achieved with the nitrogen gas flow to the nebulizer set at 2.0 l/min and the drift tube temperature at 95 °C. Note that these parameters are instrument dependent. On a later version, the Alltech model 2000, the optimal response was achieved with a nitrogen flow rate of 1.6 l/min and drift tube temperature of 70 °C.

3.2. Precision of the ELSD for the detection of PDMS

On a number of different days, a series of ten injections of the same solution of PDMS in dichloromethane was made to determine the detector precision. It was found that the retention time was reproducible to better than 0.2% but the peak area recorded by the ELSD had a relative standard deviation (RSD) that ranged between 1.2 and 4%. The factors that influenced the signal size were investigated. It was found that small fluctuations in the nitrogen gas flow rate to the nebulizer had a significant bearing on the reproducibility. The nitrogen flow rate was closely monitored in subsequent experiments. Cleanliness of the nebulizer and drift tube was also important to prevent build-up of analyte and variability of aerosol formation from the mobile phase. For PDMS as analyte, more consistent results were obtained when the drift tube and nebulizer were rinsed with chloroform after every 250 injections.

Connection of an ultraviolet detector (UVD) in series with the ELSD and the use of several UV-absorbing analytes revealed the significant difference in reproducibility between the two detectors. Using paracetamol as analyte, the UVD registered a peak area RSD of 0.2% over ten injections, while the ELSD had a peak area RSD of 1.3% for the very same injections. Thus the inherently more complex method of detection in

the ELSD involving nebulization of the mobile phase, evaporation of the volatile components and light scattering detection of solute particles, is the reason for a lesser instrumental precision of the ELSD compared with the UVD. In the case of PDMS as analyte, the peak shape was slightly asymmetric and this factor contributed to a higher peak area RSD when automatic baseline selection was used in the data handling.

3.3. Linearity of the PDMS assay

The linearity of response of the HPLC–ELSD system was evaluated using a series of five standard solutions of PDMS USP Reference Standard in dichloromethane covering the concentration range 0.2–2 mg/ml. The response was found to be linear in this range with correlation coefficient of 0.9994, but the intercept was significantly less than zero. The regression equation for the linear portion of the standard curve was found to be:

$$Y = (7.98 \pm 0.16)X - (0.62 \pm 0.15)$$

The non-linearity or sigmoidal nature of the response at low and high concentrations of analyte is a feature of the ELSD due to the concentration-dependent changes in aerosol particle size distributions [5,6]. The lower concentration limit of the linear range was deemed to be 0.2 mg of PDMS per ml of solution for this detector. The upper limit of the linear region for PDMS was not investigated. For the linear range, the RSD of the slope was found to be 1.8–2.0% over six determinations.

3.4. PDMS content of simethicone USP and simethicone 30% emulsion USP

The USP assay method for the PDMS content of Simethicone specifies an extraction step after mixing the sample with an aqueous acid solution, and subsequent drying of the organic extract with sodium sulfate. The PDMS content by HPLC using the USP extraction procedure was found to be $87.2 \pm 2.8\%$ from six determinations. The Certificate of Analysis supplied with this batch of Simethicone USP listed the PDMS content as 92.5%-presumably determined by the IR method, which has a reported precision of 4.4% on an intra-day basis and 5–6% for inter-day reproducibility [2].

Since the PDMS content measured by the HPLC method was about two standard deviations below the certified value, the possibility of incomplete recovery in the extraction procedure was investigated. A more direct approach was taken by dissolving a known amount of Simethicone USP in dichloromethane, then filtering through a 0.45 μm filter before injecting into the HPLC. The PDMS content by HPLC by this direct injection method was found to be $88.0 \pm 1.5\%$ from a total of twelve determinations, thereby verifying that the extraction process was complete.

The PDMS content of Simethicone 30% Emulsion USP was determined by the extraction method to be $27.1 \pm 0.4\%$, a value that is lower than the figure of 28.5% given in the Certificate of Analysis by the manufacturer (Dow Corning Company). The reason for the significant difference between the outcomes from the HPLC and IR methods is not apparent. It is presumed that the PDMS in the USP Reference Standard is identical to that used in making Simethicone.

3.5. Recovery

The method involves a single extraction step with dichloromethane to remove the PDMS from the acidified formulation, as is also used in the USP FTIR method. The standard curve for the assay is set up by addition of known amounts of appropriate Simethicone raw material to the Placebo formulation (contains all ingredients except Simethicone), followed by acidification and extraction. The effectiveness of the extraction procedure was tested by performing one, two or three extractions on a known mixture of 133 mg of Simethicone 30% Emulsion with 10 ml of the Placebo liquid formulation. Mean recoveries were 97.7 ± 2.5, 98.3 ± 3.5 and $95.6 \pm 3.8\%$ for one, two and three extractions respectively. Since the recovery using one extraction was within one standard deviation of 100%, as well as not being significantly different from the double and triple extraction recoveries, the single extraction method was considered appropriate to quantify Simethicone at these concentrations.

3.6. Precision

The Simethicone content of one production batch of Mylanta Liquid formulation was determined 6 times with a mean result of 39.9 ± 1.2 mg/10 ml (RSD 3.1%), in good agreement with the label claim of 40 mg/10 ml. When the same method was applied to the analysis of a batch of Mylanta Tablets that had been stored at room temperature for 4 months, the Simethicone content was found to be 17.3 ± 0.5 mg per tablet (RSD 3.0%). Although this result was significantly different from the label claim of 20 mg per tablet, the same precision was achieved for the method applied to both formulations. The reason for the low value is not apparent, and is being investigated. However, the Simethicone content is within the $\pm 15\%$ variation allowed by the USP [1]. Since the standard curve for the determination was constructed on the basis of the amount of Simethicone, as opposed to the amount of PDMS, the discrepancy in the tablet analysis is not due to the low measured content of PDMS in the Simethicone.

Analysis of the same batches on different days, each with their individual standard curves, gave results of 40.6 ± 1.2 and 39.2 ± 0.6 mg/10 ml for the Liquid and 17.7 ± 0.4 and 16.7 ± 0.3 mg per tablet for the Tablet formulation. Three other production batches of Mylanta Liquid that had been stored at room temperature for no longer than 3 months, were analysed for their Simethicone content. It was determined to be 40.4, 41.2 and 39.8 mg/10 ml, i.e. all were within 3% of the label value. The overall interday precision of 3% is similar to the instrumental precision. Hence it is suggested that the method precision are limited by the instrumental variation for this analyte.

4. Conclusions

The reversed-phase liquid chromatography separation with detection by the ELSD was found to be a useful means for quantifying Simethicone in pharmaceutical formulations. The PDMS component of Simethicone is efficiently extracted from the acidified formulation by a single extraction with dichloromethane, as given in the USP monograph. The precision of the LC-ELSD method (3% for both intra- and inter-day analyses) is somewhat better than that achieved by the modified infrared analysis (4.4% intra-day and 5–6% inter-day) [2]. More importantly, the LC-ELSD technique provides a specific separation of the PDMS component of Simethicone from other ingredients of the formulations. Thus it is not prone to interference from the magnesium and aluminium hydroxide components of complex antacid formulations, as found for the infrared assay [2].

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