ALMAC

Partnering to Advance Human Health

Case study

Validation of a GC-FID headspace method for quantitative detection of silicone oils (siloxane polymers) within the lyophilisation life cycle

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Introduction

With biological entities increasingly targeting new therapeutic areas, the use of lyophilisation to ensure adequate shelf life during stability and storage remains an important consideration.

An important aspect of the lyophilisation process is the transfer of heat from the stainless-steel shelves to the vial or tray containing the entity. Commonly, silicone oils (siloxane polymers) are used to control this transfer due to their high thermal stability allowing them to function within a wide temperature range.

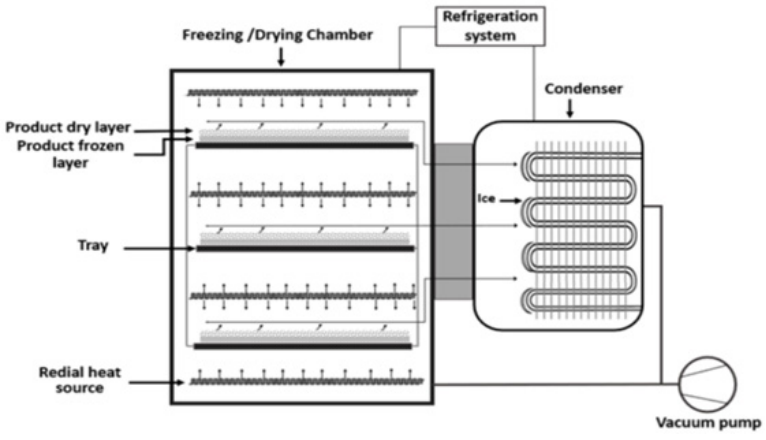
The potential for siloxane polymers to enter the product chamber may arise through leaks in the system due to general wear and tear associated with years of use, or leaks introduced into the system after improper cleaning and maintenance procedures.

A method was developed utilising Gas Chromatography (GC), a Flame Ionisation Detector (FID) and Headspace (HS) sampling technique for determination of eight siloxane polymers. This method can be used as an additional control within equipment cleaning assessment or can be part of additional tests on the lyophilised product.

Lyophilisation

Lyophilisation is a preservation process that works by removing water from the desired frozen product. This is carried out at a reduced temperature giving it popularity among thermally sensitive Active Pharmaceutical Products (APIs), Antibody-drug conjugates (ADCs) and Antibiotics. The process of lyophilisation requires sublimation from the frozen state directly into gas, combining elements of freezing, heating and condensing aided by a vacuum system. An overview of this process can be seen in Figure 1.

Figure 1 Lyophilisation Overview



Silicone oils are commonly used to control the heat transfer process in many lyophilisation systems. Common siloxane polymers contained within these silicone oils are included in Table 1 and were assessed during the development and validation of a quantitative GC-HS-FID method.

Table 1 Siloxane Polymers Evaluated

Siloxane
Octamethyltrisiloxane
Octamethylcyclotetrasiloxane
Decamethyltetrasiloxane
Decamethylcyclopentasiloxane
Dodecamethylpentasiloxane
Tetradecamethylheptasiloxane
Hexadecamethyloctasiloxane
Octadecamethyloctasiloxane

GC Method Conditions

To confirm the absence of siloxane polymers in cleaning samples or lyophilised product, a quantifiable measure for siloxanes is required. To achieve this, a method was developed which separates the eight siloxanes included in Table 1 using a GC-HS-FID setup alongside a DB-WAX 30m x 0.530mm, 1µm column.

Individual siloxane materials were sourced for use as qualitative standards to ensure response factor was not a concern.

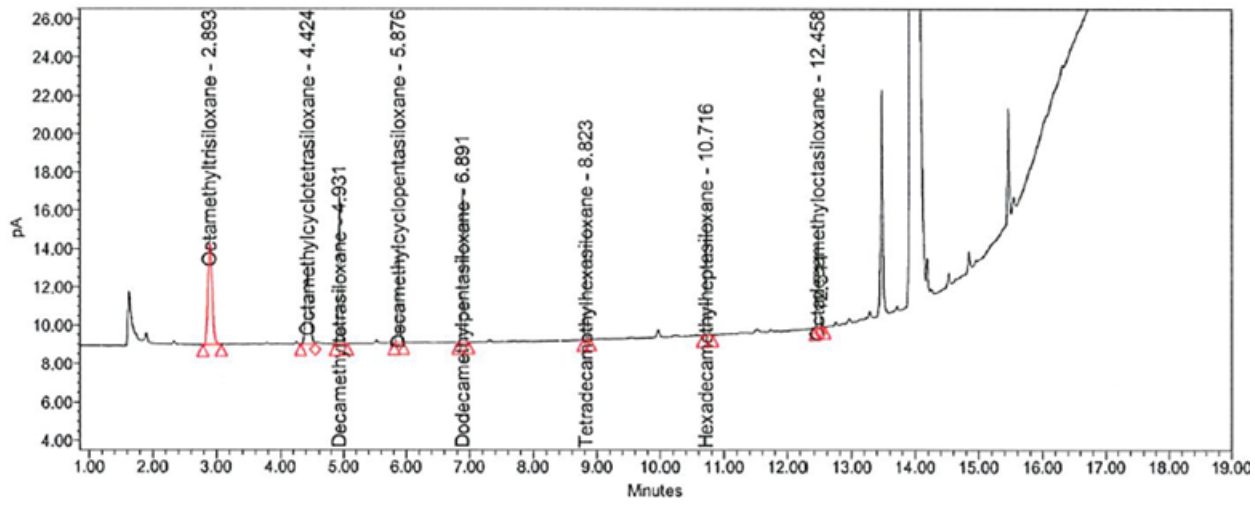
The GC-Headspace sampling technique allows for reduced potential interference around the siloxane polymers of interest through not introducing solutions directly into the GC instrument injection port. This in turn maximises the potential of the method to be used across multiple solution matrices for investigation or contamination control purposes.

Validation

Method validation was performed on the mixed siloxane polymers solution for specificity, linearity, sensitivity (limit of quantification), and solution stability in the absence of any additional matrix.

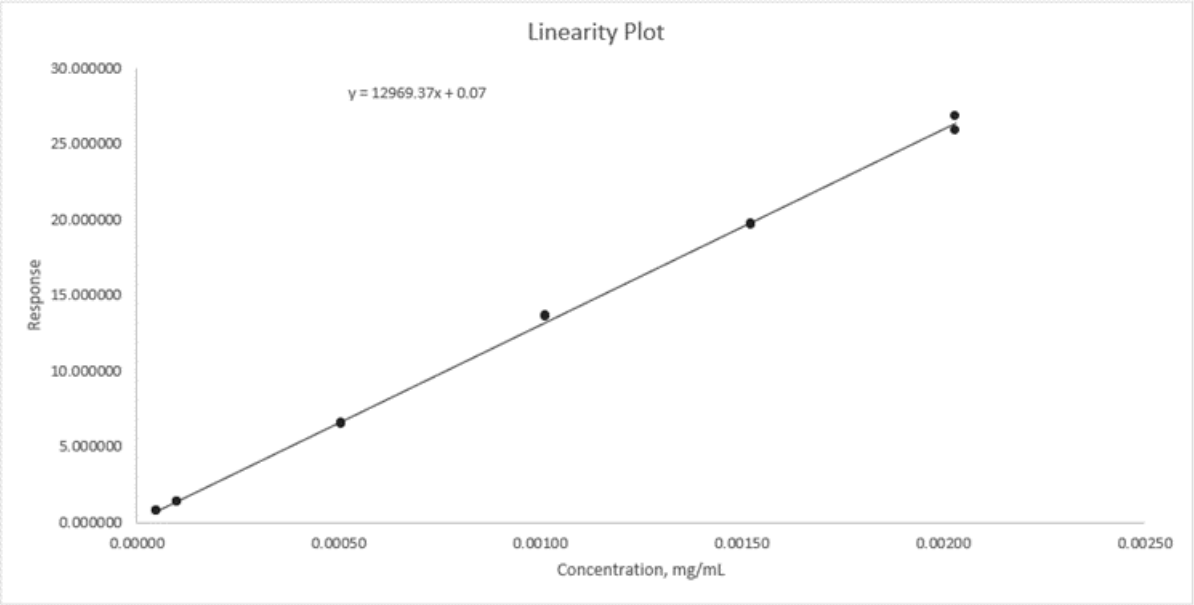
The specificity of the method was demonstrated by injecting a blank diluent mixed standard and each individual siloxane to demonstrate adequate resolution. Example chromatography for the separation of siloxane polymers is shown in Figure 2.

Figure 2 Example Siloxane Separation



The linearity of each individual siloxane was confirmed over a range of approximately 0.0001 – 0.004 mg (0.00005 – 0.002 mg/mL), except for Hexadecamethylheptasiloxane and Octadecamethyloctasiloxane where the range was approximately 0.0001 – 0.003 mg (0.00005 – 0.0015 mg/mL). Each siloxane was found to have a y-intercept value ≤10% of the response area found at the target value of 100ppm (of a 20 mg sample) calculated from the regression line. A correlation coefficient of ≥0.99 was shown and residuals were shown to be randomly distributed. An example linearity plot is shown in Figure 3.

Figure 3 Example Linearity Plot (Decamethylcyclopentasiloxane)



The Limit of Quantitation (LOQ)/sensitivity was determined as 5ppm (0.0001 mg or 0.00005 mg/mL in solution) where a sample weight of 20mg was to be assessed in a final addition of 2 mL of N-methyl-pyrrolidone (NMP):Water (80:20) for each siloxane polymer with the exception of Octamethylcyclotetrasiloxane. The LOQ for Octamethylcyclotetrasiloxane was determined to be 10ppm. Each siloxane polymer met the USP criteria of signal to noise ratio of ≥10:1 at these concentrations.

A solution stability evaluation was able to determine a period of 29 hours within which the % change in each individual siloxane content did not exceed the range of 75-125%.

Use of method within the lyophilisation process lifecycle

When using lyophilisation within a GMP environment the equipment is subjected to a cleaning regime that may include but is not limited to first use cleaning, post maintenance cleaning, cleaning after processing and mid lot cleaning.

Post cleaning of equipment, generation of a sample for analysis may typically involve swabbing using a suitable material and extraction solvent, typically over a 1dm^2 area as per Figure 4 to enable quantitative analysis.

Figure 4 Typical Swabbing Technique

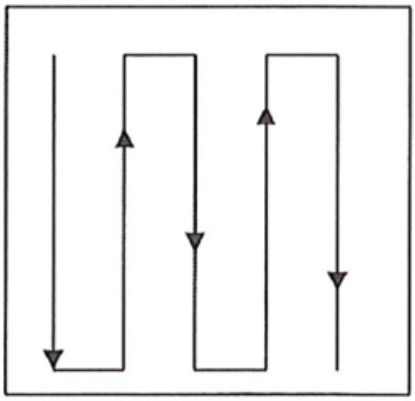


Figure A

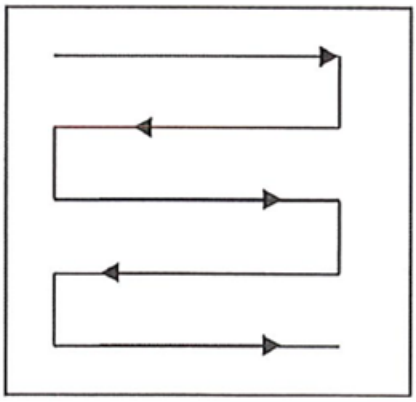


Figure B

To incorporate this into a siloxane polymer specific assessment the extraction solvent of choice should be switched to NMP:Water (80:20)

To use this method for determination of siloxane polymer content in the presence of additional matrixes (such as final lyophilised product) an additional specificity and accuracy check should be performed to ensure appropriate recovery is met. This would rule out additional interference or unwanted interactions between the siloxanes and the sample matrix.

Summary

A GC-FID Headspace method for low level siloxane quantification has been validated to enable incorporation into a strategy for contamination prevention and assessment within the lyophilisation process life cycle.

Author: Damien Higgins, Analytical Team Leader