An Investigation into the Leaching of Volatiles from a Container Closure System into Ophthalmic Solutions

by

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of

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is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Date: _____

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Abstract:

In this study, two separate methods were developed and validated for two different products to track three leachables; cyclohexanone, propylene glycol monoethyl ether acetate (PGMEEA), and propylene glycol monomethyl ether acetate (PGMMEA). These leachables were recently discovered to be leaching from an ink that is applied during labelling to a plastic vial containing the sample, a process known as tampoprinting.

Due to the volatile nature of these leachables the best technique of tracking them is by gas chromatography (GC). This analytical separation technique utilises a system that vaporises liquid samples containing organic compounds of interest. The sample travels through a column where the sample components are separated and then detected producing a quantitative measurement of each component.

In chapter 2, a direct injection gas chromatography method was developed for Product Z to track the three leachables of interest. The method was then fully validated in accordance with the current United States Pharmacopeia (USP) Category II for leachables and in line with all requirements under International Conference on Harmonisation (ICH) Q2 (R1).

In chapter 3, direct injection gas chromatography could not be utilised for Product X due to the different nature of its sample matrix to Product Z. Instead, a method to track the Tampoprint leachables was developed using headspace GC, a slightly different technique whereby volatile compounds from the sample are evaporated from the liquid before being injected onto the GC column. This prevents much of the sample matrix from going onto the column and thus produces much cleaner chromatography. This method was then also fully validated in accordance with USP requirements and ICH guidelines.

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List of Abbreviations:

AET	Analytical Evaluation Threshold
API	Active Pharmaceutical Ingredient
BPA	Bisphenol A
CYX	Cyclohexanone
DB	Dual Bond
DL	Detection Limit
DSS	Detector Sensitivity Solution
EMEA	European Agency for the evaluation of Medical Products
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FID	Flame Ionisation Detector
GC	Gas Chromatography
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HS-GC	Headspace Gas Chromatography
HSPH	Harvard School of Public Health
ICH	International Conference on Harmonisation

I-DSS	Intermediate Detector Sensitivity Solution
IS	Intermediate Standard
ITX	Isopropilthioxanthone
IUL	Individual Unidentified Leachable
LC	Liquid Chromatography
LDPE	Low Density Polyethylene
LIS	Leachable Identification Solution
LRS	Laboratory Reference Standard
MeOH	Methanol
min	Minutes
mL	Millilitre
mm	Millimetre
MS	Mass Spectrometry
Nm	Nanometres
NMR	Nuclear Magnetic Resonance
NLT	Not Less Than
NMT	Not More Than
NWDA	National Wholesale Druggists' Association

OINDP	Orally Inhaled and Nasal Drug Products
PAP	Polyfluoroalkyl Phosphoric Acid
PDA	Photodiode Array
PGMEEA	Propylene Glycol Monoethyl Ether Acetate
PGMMEA	Propylene Glycol Monomethyl Ether Acetate
PFCA	Perfluorinated Carboxylic Acid
ppm	Part Per Million
PQRI	Product Quality Research Institute
psi	Pounds Per Square Inch
QbD	Quality by Design
QL	Quantitation Limit
R&D	Research and Development
RH	Relative Humidity
Rs	Resolution
SCT	Safety Concern Threshold
S-ID	Stock Identification Solution
SOP	Standard Operating Procedure
SS	Stock Standard

TOF	Time Of Flight
UD	Unit Dose
USP	United States Pharmacopeia
UV	Ultra-Violet
v	Velocity
V	Voltage
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organisation
WS	Working Standard

Glossary of Terms:

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Analytical Procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation.

Asymmetry / Tailing Factor (T_f)

A measure of how close a chromatographic peak is to a symmetrical shape. As a peak slopes the tailing factor increases.

$T_f = ac / 2ab$

where ac is the peak width at 5% of the peak height, and ab is the front half-width measured from the leading edge to a perpendicular dropped from the peak apex.

An ideal chromatography peak is a sharp symmetrical shape, a Gaussian peak, on a flat baseline. A peak can deviate from this ideal in several different ways. It can become asymmetrical, flatten and become broader, or the baseline can rise. One of the common shifts away from a Gaussian peak is when the back half of the peak falls away. If the peak were split into two, vertically, the latter half would be wider than the first half of the peak. This effect is most clearly seen close to the baseline and is known as peak tailing. In Gas Chromatography (GC) tailing can be a result of a poorly installed column, inlet contamination, column blockages, solvent polarity mismatch or a low split ratio.

Fronting peaks are the opposite of tailing peaks in that the first half of the peak would be wider than the latter half. They usually occur when the sample capacity of the analytical column is exceeded. This overloading effect usually results from injecting too much sample. Fronting peaks may also be a result of poor column installation or co-elution.

Capacity Factor (k)

Expression that measures the degree of retention of an analyte relative to an unretained peak, where t_R is the retention time for the sample peak and t_0 is the retention time for an unretained peak. A measurement of capacity will help determine whether retention shifts are due to the column (capacity factor is changing with retention time changes) or the system (capacity factor remains constant with retention time changes). Thus the higher the capacity factor, the longer the retention time.

$$K = \frac{t_R - t_0}{t_0}$$

Conventional Ultra Violet-Visible (UV-Vis) Detector

A detector analyses individual wavelengths as they are scanned across the full spectrum or spectrum of interest for a particular sample.

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Typically the detection limit will be equal to 3 times the standard deviation of the blank. For validation purposes the detection limit may often be set at one third of the quantitation limit as concentration levels can become so small they are regarded as zero.

Distribution Coefficient (Log D)

The ratio of the sum of the concentrations of all forms of the compound (ionised plus un-ionised) in each of the two phases. It is a measure of how hydrophobic or hydrophilic a given compound is taking into account the ionised state of a compound. Thus, Log D is always expressed as a function of pH.

$$\text{Log D} = \text{Log} \left([\text{Analyte}]_{\text{octanol}} / ([\text{Analyte}_{\text{ionised}}]_{\text{water}} + [\text{Analyte}_{\text{neutral}}]_{\text{water}}) \right)$$

Extractable

Chemicals that migrate from the product-contact component into a solvent at accelerated conditions (such as heat, time, pH, ionic strength, organic solvent content).

Flame Ionisation Detector (FID)

Flame ionisation detectors (FID) are the most generally applicable and most widely used GC detectors. In an FID, the sample is directed at an air-hydrogen flame after exiting the column. At the high temperature of the air-hydrogen flame, the sample undergoes pyrolysis, or chemical decomposition through intense heating. Pyrolised hydrocarbons release ions and electrons that carry current. A high-impedance picoammeter measures this current to monitor the sample's elution.

It is advantageous to use FID as the detector is unaffected by flow rate, non-combustible gases and water. These properties allow FID high sensitivity and low noise. The unit is both reliable and relatively easy to use.

Flow Rate

The speed at which the carrier gas travels through the column. The higher the flow rate the shorter the retention time.

GC Column

A glass or metal tube through which the sample can travel as a gas. The choice of column depends on the sample and the active measured. The main chemical attribute regarded when choosing a column is the polarity of the mixture. The polarity of the sample must closely match the polarity of the column stationary phase to increase resolution and separation while reducing run time. The separation and run time also depends on the film thickness (of the stationary phase), the column diameter and the column length.

Headspace

Using the known temperature of the sample, the bottle volume, the concentrations of gas in the headspace (as determined by GC), and Henry's law constant, the concentration of the original water sample is calculated. Total gas concentration (TC) in the original water sample is calculated by determining the concentration of headspace and converting this to the partial pressure and then solving for the aqueous concentration which partitioned in the gas phase (CAH) and the concentration remaining in the aqueous phase (CA). The total concentration of gas in original sample (TC) is the sum of the concentration partitioned in the gas phase (CAH) and the concentration remaining in the aqueous phase (CA).

TC = CAH + CA

Henry's law states that the mole fraction of a dissolved gas (Xg) is equal to the partial pressure of the gas (pg) at equilibrium divided by Henry's law constant (H). Gas solubility coefficients are used to calculate Henry's law constant.

Xg = Pg/H

After manipulating equations and substituting volumes of each phase, the molar concentration of water (55.5 mol/L) and the molecular weight of the gas analyte (MW), a final equation is solved for.

TC = [(55.5 mol/L)* Pg/H*〖10〗^3 mg/g] + ([Vh/((Vb-Vh))]*
Cg*MW(g/mol)/22.4(L/mol) *[273K/((T+273K)]* 〖10〗^3 mg/g)

Where Vb is the bottle volume and Vh is the volume of headspace. Cg is the volumetric concentration of gas.

Headspace Sampling

Sampling from the vapour phase, which is on top, of a sample container which contains the liquid or solid sample. Sampling may be conducted at room temperature or higher temperature depending on the volatility of the analyte molecules.

Isothermal Programming

In isothermal programming, the temperature of the column is held constant throughout the entire separation. The optimum column temperature for isothermal operation is about the middle point of the boiling range of the sample. However, isothermal programming works best only if the boiling point range of the sample is narrow. If a low isothermal column temperature is used with a wide boiling point range, the low boiling fractions are well resolved but the high boiling fractions are slow to elute with extensive band broadening. If the temperature is increased closer to the boiling points of the higher boiling components, the higher boiling components elute as sharp peaks but the lower boiling components elute so quickly there is no separation.

Leachable

Chemicals that migrate from the product-contact component into a formulated drug during normal storage/usage conditions.

Liquid Stationary Phase

A thin layer of liquid that coats the inside of the GC column and interacts with gaseous sample compounds. This layer is typically 0.25 – 3.0 µm in depth and may solubilise the analyte molecules but does not chemically react with analyte molecules.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Mass Spectrometry

An analytical technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio.

Mobile Phase / Carrier Gas

Moving gaseous phase that can carry the vaporised sample molecules through the column. In GC the mobile phase gas does not interact with the sample molecules but merely carries them through the column.

Noise

This is a measure of the short time variation of the baseline of a chromatogram. It can be caused by electric signal fluctuations, lamp instability, temperature fluctuations and other factors. It is usually measured as the distance from top of a small peak on the baseline to the bottom of the next peak. Noise is the factor which limits detector sensitivity. In trace analysis, the operator must be able to distinguish between noise spikes and component spikes.

Partition Coefficient (Log P)

The ratio of concentrations of an un-ionised compound in the two phases of a mixture of immiscible solvents at equilibrium. Generally, the two solvents chosen are water (aqueous phase) and octanol (hydrophobic organic phase). The compound to be measured is added to the water/octanol system. If the compound is acidic or basic, the pH is adjusted to ensure the compound is neutralised. Once equilibrium has been achieved, the compound is quantified in each solvent, typically via UV-Vis and the logarithm of the concentration ratio is calculated. The measurement is expressed with the following equation:

$\text{Log P} = \text{Log } ([\text{Analyte}]_{\text{octanol}} / [\text{Analyte}_{\text{neutral}}]_{\text{water}})$

pH

The pH of a solution is a measure of the acidity of the solution. It is defined as:

$\text{pH} = -\log_{10} ([H_3O^+])$

where $[H_3O^+]$ is the concentration of hydronium ions in the solution.

Photodiode Array (PDA) UV-Vis Spectrophotometer

UV–Vis spectrophotometers that use PDA detectors are able to simultaneously analyse a full spectrum.

pKa

The pKa of a solution describes the acidity of that solution, based on the inherent properties of the acid involved and the concentration of that acid.

$\text{pKa} = -\log_{10}(\text{Ka})$

where $\text{Ka} = [H+][A-]/[HA]$

HA = acid

A- = conjugate base

H+ = aqueous proton

Placebo

A placebo is lab-scale product made as per the manufacturing procedure but with the omission of at least one active ingredient contained within the full formulation. It is used to determine if any baseline interference can be detected at the expected

retention time of the omitted active ingredient. When testing for leachables the placebo may refer to a freshly made product that hasn't been subjected to possible causes of leachables and will most often be packaged in glass containers.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment.

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The quantitation limit can also be obtained from precision studies. For this approach, decreasing analyte concentrations are analysed repeatedly and the relative standard

deviation is plotted against the corresponding concentration (precision function). If a predefined limit is exceeded, the corresponding concentration is established as the quantitation limit However, in practice, due to the high variability of standard deviations the true precision function is much more difficult to draw unless a large number of concentrations is included.
The QL can be specifically calculated using the actual precision of the analytical procedure at this concentration. The calculation is based on the compatibility between analytical variability and specification acceptance limits. QL can be regarded as the maximum true impurity content of the manufactured batch, i.e., as the basic limit

$$QL=AL-\frac{(st_{df,95\%})_{validation}}{\sqrt{n_{assay}}}$$

AL = Acceptance limit of the specification for the impurity.
s = Precision standard deviation at QL, preferably under intermediate or reproducibility conditions. AL and s equal same unit (e.g., percentage with respect to active, mg, mg/ml).
N_{assay} = Number of repeated, independent determinations in routine analyses, as far as the mean is the reportable result, i.e., is compared with the acceptance limits. If each individual determination is defined as the reportable result, n=1 has to be used.
t_{df} = Student t-factor for the degrees of freedom during determination of the precision, usually at 95% level of statistical confidence.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Resolution

A characteristic of the separation of two adjacent peaks. It may be expressed according to the equation:

$$RAB = 2 \frac{|dR(B) - dR(A)|}{|w(B) + w(A)|}$$

where *R*AB is the resolution, *d*R(A) and *d*R(B) are the retention distances (time or volume) of each eluted component A and B, and *w*(A) and *w*(B) are the respective widths of each peak at its base.

Retention Time (t_R)

The time taken after injection for the analyte molecules to reach the detector. This dependent on the column flow rate, column capacity, extra column dead volume and the retardation factor of the molecules on the stationary phase.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Run Time

The time it takes for a complete chromatography cycle, i.e. from the time the sample is injected onto the column, until all products have been eluted from the system.

Selectivity (α)

A quantity which describes the separation of two species (A and B) on the column using the capacity factor (k)

$$\alpha = k(B) / k(A)$$

When calculating the selectivity factor, species A elutes faster than species B. The selectivity factor is always greater than one.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix.

Split Injection

This allows a portion of the injected sample to enter the column and the rest will be vented to waste. The split is carried out in the injection chamber just prior to the column entry. This is used so that the column will not be overloaded and hence allow sharp peaks to be obtained.

Split Ratio

The ratio of gas that flows through the column and the split line.

Split Ratio = (Column Flow Rate / Total System Flow Rate)

Splitless Injection

All injected molecules are carried onto the column.

Stationary Phase

A static surface that may interact with the molecules of a sample as they travel through the column. In GC this may be a solid or a liquid.

System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Temperature Programming

In the temperature programming method, the column temperature is either increased continuously or in steps as the separation progresses. This method is well suited to separating a mixture with a broad boiling point range. The analysis begins at a low temperature to resolve the low boiling components and increases during the

separation to resolve the less volatile, high boiling components of the sample. Rates of 5-7°C/minute are typical for temperature programming separations.

Theoretical Plates (N)

The number of theoretical plates is an index used to determine the performance and efficiency of columns. It is calculated using the below equation

$$N = 16\left(\frac{tr}{W}\right)^2$$

where tr = retention time and W = peak width.

Alternatively, efficiency can be calculated using:

$$N = 5.54\left(\frac{tr}{W_{0.5}}\right)^2$$

where tr = retention time and $W_{0.5}$ = peak width at half peak height.

Chapter 1: An Introduction to Container Closure Systems and Testing of Leachables in Pharmaceutical Products

1.1 Introduction

Health systems rely on the continuous availability of safe, affordable pharmaceuticals (medicines, vaccines, diagnostics, and other medical supplies) of assured quality (Strengthening Pharmaceutical Systems 2011). It is the job of pharmaceutical companies to ensure that medicines and drugs are supplied free of contaminants, poisons, toxins and leachables. With the global pharmaceuticals market worth US$300 billion a year, the figure is expected to rise to US$400 billion within three years (World Health Organisation 2015). One common source of contaminants however, is the leaching of chemicals from the packaging into the drug product.

The packaging components that hold and protect the drug product are known as the container closure system and include bottles, ampoules, vials, stopper lids, screw caps, stopper overseals, cardboard containers, plastic trays, paper inserts, overwraps and labels. The components can be divided mainly into primary and secondary packaging components with primary components being any of those in direct contact with the drug product and secondary components those that will never be in contact with the drug product (FDA 1999).

The primary container's chief purpose is to protect the drug product throughout its handling and storage. Most drug product primary components comprise of a bottle and cap, pouch or a blister that may be made from a variety of materials, including plastic, glass, metal, and laminated flexible materials. Primary container components must meet the requirements set out by the Food and Drug Administration (FDA) and the United States Pharmacopeia (USP).

Figure 1.1: An Example of Primary Packaging Components (Gerresheimer 2015)

Although many types of materials can be used in a primary container closure system, the three most common are glass, polymers and elastomers. One may expect the manufacturer of any component of a container closure system to be able to provide a complete list of the formulation and process used to manufacture the component, but this may not always be the case. The two main reasons for manufacturers not providing this information are:

1. The manufacturer may consider the information to be proprietary or the manufacturer may not have the information.
2. The manufacturers of polymer container closure systems may use upstream suppliers that do not place strict controls over their processes.

For example, a resin manufacturer will set specifications for their product on its physical characteristics only and then sell the same resin to a manufacturer of a pharmaceutical container closure system and a manufacturer of lawn furniture. In this example, the resin manufacturer may not have needed to keep accurate records on the amounts and type of antioxidants used as long as the resin met the manufacturer's specifications, but these antioxidants do have the potential to leach into a drug product (NSF International 2014).

The secondary container components may be divided into critical and non-critical. A critical component such as a pouch may contain a primary component and provide protection against such things as light, gas and moisture that the primary component could not. It is a vital part of the container closure system for product stability. The

most common type of non-critical secondary component is the folding cardboard carton. Its job is to hold the primary container or containers for the final market and will usually contain any extras required for administering the drug product as well as paper inserts giving instruction on use. Generally labels and barcodes will be found on this component whilst it also serves as extra protection during storage and transport of the product.

Figure 1.2: An Example of the Folding Cardboard Carton for Eye Drop Solutions (Drugs.com 2015)

Additional packaging may be required for the final exterior package and can include a case made of corrugated fibreboard and a wrapper. The final package will often be transported on wood pallets which are frequently treated with fungicide and have

been known to be the cause of previously seen leachables (Koschier *et al* 2011). All labels necessary for the National Wholesale Druggists' Association (NWDA) will be affixed to the exterior packaging (United States Pharmacopeia 37 2015, Good Packaging Practices).

1.2 Extractables and Leachables

Although every component of the container closure system is intended to protect the drug product from contaminants, they can invariably be the principal source of monomers and polymer additives such as antioxidants, plasticisers, stabilisers, dyes, metal catalysts and other harmful chemicals leaching into the drug product.

Extractables and leachables are often mentioned concurrently. Extractables can be defined as chemicals that migrate from the product-contact component into a solvent at accelerated conditions (such as heat, time, pH, ionic strength, organic solvent content). Leachables are a subset of extractables. They are chemicals that migrate from the product-contact component into a formulated drug during normal storage/usage conditions (Feilden 2008).

The need to investigate extractables and track leachables is an important challenge faced by the pharmaceutical industry. Analytical methods are required to test for and track leachables but before that method development extraction studies need to be conducted to determine what extractables could become leachables over time. The European Agency for the evaluation of Medical Products (EMEA) and the U.S. FDA are increasing scrutiny on potential extractables and leachables in drug product container and closure systems. Recently, the FDA Food Safety Modernisation Act was updated with particular relevance for extractables stating that "A drug or device shall be deemed to be adulterated... if its container is composed, in whole or in part, of any poisonous or deleterious substance which may render the contents injurious to health" (Code of Federal Regulations 2015). This definition plainly highlights the need for extractable studies within regulatory expectations.

Extraction studies are designed to mimic both intended use and worst-case-scenario models to identify the extractables and leachables that could migrate into the drug product. The toxicology of each potential leachable migration must also be established (Beierschmitt 2009; Northup 2008; Nicholas 2006; Osterberg 2005). A

toxicological study is conducted following extraction experiments performed on the packaging components and patient impact is evaluated. Extraction experiments are performed under exaggerated conditions of temperature and time in the laboratory using common, neat solvents that bracket the solvating power of the drug (Moffat 2010). The formulation matrix is the preferred solvent as it replicates what is most likely to happen in the final marketed product. Reliable identification and sensitive analytical techniques such as Mass Spectrophotometry (MS), Gas Chromatography (GC) and Liquid Chromatography (LC) are essential in identifying extractables and possible leachables.

Whilst these studies are performed during the development phase, the only way to truly measure potential extractables and leachables is over the shelf life of the product with applicable toxicological data to support. Two types of Biological Reactivity tests (in vivo and in vitro) are stipulated by the USP to assess the toxicity of possible extractables and leachables (United States Pharmacopeia 37 2015).

Robust carcinogenic, mutagenic or genotoxic studies may not need to be performed throughout a stability study on every identified leachable if supporting data is already available. The molecular structure, known toxicity of a leachable or known toxicity of closely related compounds may be satisfactory to assess the safety threshold. Toxicologists may reference various databases such as INCHEM® (International Programme on Chemical Safety 2015), ExPub® (Chemical Hazard Information for EH&S Professionals 2015) and TOXNET® (Toxicology Data Network, Untied States National Library of Medicine 2015) as part of their data search.

When container closure systems are developed for a pharmaceutical product, various vendors will be screened by companies to assess suitability for each component. The toxicological and analytical data will ultimately be what decides the final selection (Laschi *et al* 2009; Corredor *et al* 2009; Ball 2007; Markovic 2009; Wakankar *et al* 2010; Alarcon *et al* 2007a; Alarcon *et al* 2007b; Feilden 2008; Vega-Mercado 2004). Jenke *et al* (2007) outlines what is expected of the packaging component supplier and final product vendor. He defines a collaborative strategy between both parties to enable a knowledge sharing platform while protecting the confidential information belonging to each individual group. Separately, Pan *et al* (2008) and Castner *et al* (2009) suggest approaches using GC, LC, MS and Ultra Violet (UV) with Photodiode Array (PDA) for the determination of leachables in liquid drug. Further

evidence for leachable identification is used by Castner *et al* (2009) with Log D partitioning data based on the solution pH and analyte pKa.

A typical extractable and leachable study plan can be seen in Figure 1.3.

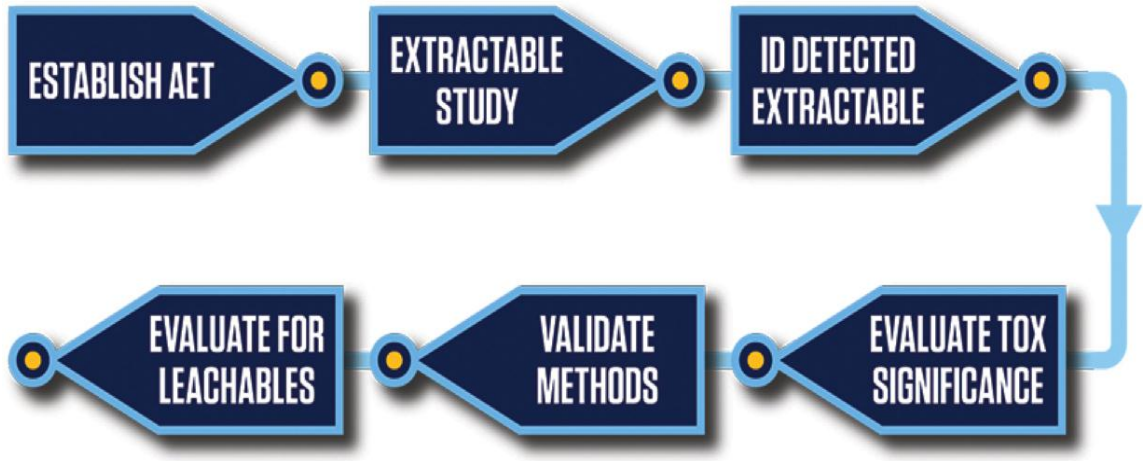


Figure 1.3: An Example of an Extractable and Leachable Study Plan
(Evans Analytical Group 2015)

The study needs to be able to detect substances of concern from process equipment and the container closure system and then be able to track them throughout the product's shelf life. The European Agency for the evaluation of Medical Products (EMEA) Guideline states that " It should be determined whether any of the extractables are also leachables present in the formulation at the end of the shelf life of the product or to the point equilibrium is reached if sooner" (Cartwright *et al* 2010).

First and foremost Analytical Evaluation Thresholds (AETs) need to be calculated. This is gained by establishing the Safety Concern Threshold (SCT) set out by the Product Quality Research Institute (PQRI) relevant to the product. From the SCT, the sample preparation and daily exposure are assessed and an AET value generated. Extractables can then be tested for and put forward for toxicological assessment. Based on the levels of extractable(s) observed and related toxicological evaluation, method(s) can be established and validated for long term leachable evaluation (Product Quality Research Institution 2006).

A typical leachable study to monitor potential migratory chemical species throughout the shelf life of the product might be as per Table 1.1:

Time (months)	0	1	2	3	6	9	12	18	24	36
Real Time Data	x			x	x	x	x	x	x	x
Accelerated data		x	x	x	x	x	x			

Table 1.1: A Typical Leachables Test Schedule

Real time data is based on the normal storage conditions of the product whereas accelerated data tests the drug product at higher temperatures and/or humidity. If a correlation between extractable and leachable profiles can be established, control of leachables could be accomplished via testing and limits on extractables.

1.3 Leachables in Industry

Over the years, there have been many incidents involving chemicals leaching into foods and drug products from container closure systems. In 2008, Canada banned the use of the chemical bisphenol A (BPA) in polycarbonate baby bottles while some polycarbonate bottle manufacturers voluntarily eliminated BPA from their products (Wang *et al* 2010). Numerous studies have shown that BPA acts as an endocrine-disruptor in animals, including early onset of sexual maturation, altered development and tissue organisation of the mammary gland and decreased sperm production in offspring. In one particular study on the effects of BPA, Karin B. Michels, Associate Professor of Epidemiology at Harvard School of Public Health (HSPH) and Harvard Medical School said “We found that drinking cold liquids from polycarbonate bottles for just one week increased urinary BPA levels by more than two-thirds. If you heat those bottles, as is the case with baby bottles, we would expect the levels to be considerably higher. This would be of concern since infants may be particularly susceptible to BPA’s endocrine-disrupting potential” (Carwile *et al* 2009).

Carbon black, common in tyres and industrial rubber products is used as an additive to make rubber supple. In the 1980s it was shown that cancer-causing polynuclear

aromatic hydrocarbons leached from carbon-black-containing rubber used in products such as asthma inhalers and baby-bottle nipples (Grilli 2015). It still has many uses including pigmentation and UV protection but is no longer used as part of container closure systems.

Plastic is by far the most common packaging material and leachables can include everything from additives used to make plastic strong or malleable, to leftover monomer building blocks. However, leachables can be produced from all sorts of packaging materials. The waxy wrapping that lines popcorn bags and is used to wrap burgers has been shown to leach polyfluoroalkyl phosphoric acids (PAPs). A study by Scott Mabury, a University of Toronto chemistry professor, described how these PAPs can accumulate in the body after being absorbed by humans (D'eon *et al* 2010). They can then become carcinogenic and hormone disrupting when metabolised and turned into perfluorinated carboxylic acids (PFCAs). European, Canadian and US governments now intend to extensively track PAPs and their dangers when exposed to humans.

Inks used for printing are also a major concern. In 2005, Nestle were forced to recall millions of litres of infant formula across Europe when isopropilthioxanthone (ITX), a printing-ink component, was found in the product. The European Food Safety Authority (EFSA) had found that levels of ITX in the product did not pose a health risk but that did not prevent a number of European countries demanding the recall such is the fear of a consumer backlash. The chemical's use in the formula's packaging has since been phased out by Tetra Pak, the firm that supplied the packaging to Nestle (Laksin *et al* 2007).

Recently in Germany, a printing ink component, 4-methylbenzophenone had been found by the EFSA to be leaching from the outside of the cardboard box holders of chocolate muesli into the cereal. 4-methylbenzophenone is a photo-initiator, a highly photoactive compound included in the formulations of UV curing printing inks usually applied to packaging surfaces (Luis Aparicio *et al* 2015). It has similar properties to benzophenone and is often partly left behind during the printing process. If a functional barrier like aluminium foil is not present and due to its high volatility 4-methylbenzophenone may migrate from the cardboard through any plastic barriers and into the food (Choi *et al* 2002; Pastorelli *et al* 2008; Song *et al* 2003; Feigenbaum *et al* 2005). The EFSA concluded that based on its knowledge on the toxicity of benzophenone, people should not be at risk to short term consumption

of cereals contaminated by 4-methylbenzophenone. However, if the chemical's use was to be continued, more data would need to be gathered for a full risk assessment (European Food Safety Authority 2009).

Other examples of leaching were described at a 2009 leachables convention in Barcelona by Ingrid Markovic, a regulator at FDA's Centre for Drug Evaluation & Research. She discussed how two different pharmaceutical companies encountered problems with the packaging of their liquid protein drugs. In the first case, a solvent from the epoxy glue used to stick a syringe barrel and metal needle together had leached into the drug product and aggregated the protein into clumps. In the other case, tungsten oxide salt residue had leached into the drug product after using a tungsten filament on the syringe needle which again led to aggregation of the protein (Everts 2009).

1.4 Tampoprinting

Pad printing, also known as tampoprinting, was first developed and used to print watch dials in the Swiss watch industry (TAMPOPRINT AG 2015). Machines were manually operated and used pads made from gelatine and oil based inks that were slow drying. The process was developed further by the Germans during the 1960s with the use of mechanical machines and pads made from silicone rubber. Inks were also improved which meant that printing could be achieved on many different materials and products.

Nowadays, there is a large variety of inks available meaning nearly all materials can be printed on and due to the inks quick drying properties very detailed designs of many different colours can be accomplished. All shapes of products can also benefit from modern day tampoprinting thanks to the suppleness and elasticity of the silicone rubber pads.

It is most commonly used to print on plastics, metals and ceramics for use in the industrial, pharmaceutical, automotive, white goods and retail markets (Tampo Limited 2015).

The tampoprinting cycle begins by filling the engraved area of a printing plate, known as the cliché, with low viscosity ink. The low viscosity allows the ink to flow evenly.

The surface of the cliché is then cleared of excess ink leaving just the engraved area with any ink. The transfer pad then presses over the engraved part of the cliché in an even, rolling action, pushing air out of the way as it compresses. As the solvents evaporate from the top of the ink within the engraved surface of the cliché, the increased stickiness of the exposed ink surface enables it to adhere to the pad.

When the pad moves away from the cliché the exposed surface of the ink film on the pad starts drying making it stickier so when the pad is pressed onto the surface of the product being imprinted, the stickiness of the ink's surface allows the ink to leave the pad and now adhere to the product. The pads design means the image can be rolled onto the product ensuring that air is not trapped and good image transfer can be achieved.

Lastly, the pad moves from the product surface back to its starting position ready for the following transfer (ACC Silicones 2015).

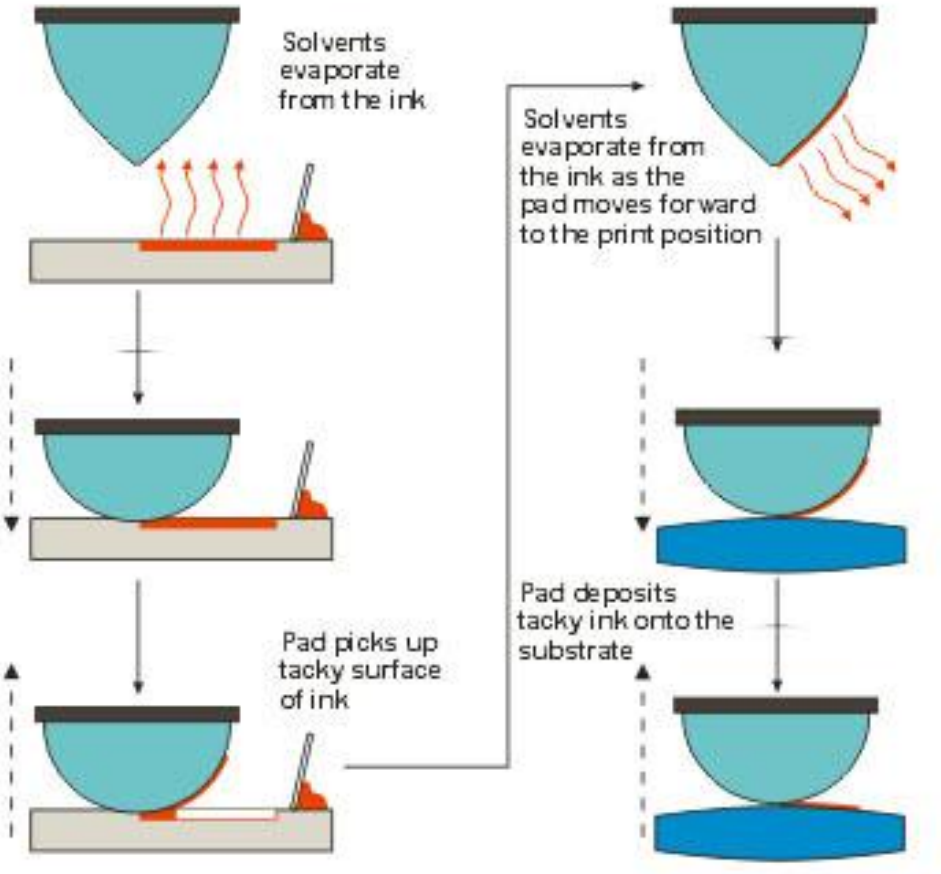


Figure 1.4: A Schematic of the Tampoprint Process (PDS International Limited 2015)

The characteristics of each component involved in the process are very important. Hardness is the defining characteristic when choosing the material from which to make the cliché. The harder the material, the longer it will last so a photopolymer plate is popular for short to medium production runs whereas steel plates are more common in medium to long runs. For very long runs a thick steel cliché is required. Engravings in the cliché are usually 25 to 30 microns in depth allowing a dried ink film thickness of 2 microns up to 20 microns depending on the depth of colour required.

The shape, hardness and surface of the pad will determine its ability to transfer an image effectively. Any changes in the hardness of the pad can dramatically affect print quality. Harder pads are generally more efficient in transferring ink but will not compress properly if too hard. A good balance is therefore always required to ensure high quality printing (ACC Silicones 2015).

Inks used for tampoprinting will generally be a mixture of a coloured ink with the addition of a thinner and a hardener. The thinner is made up of solvents that influence the flow and viscosity of the ink as well as its ability to adhere to the pad and transfer from the pad to the work piece. The thinner will also affect the drying speed of the ink with quick and slow drying solvents available. The hardener is added to the mixture to create a better consistency and allow the ink and thinner to form effectively.

There are many benefits of tampoprinting. They include high levels of quality with excellent repeatability due to microprocessor controlled machines and the ability to turn plain pieces of work into more visually pleasing pieces and hence increase their value. The ability to use an assortment of coloured inks without the need for drying is an immense time saver. Also, machines are generally very easy to operate with few parts to change whilst a wide variety of materials of all shapes and sizes can be printed on (Irish Micro Moulding 2015).

1.5 Tampoprinting at Allergan

In Allergan tampoprinting is performed on unit dose (UD) vials only. The UD vials are made from low density polyethylene (LDPE), a virtually unbreakable yet quite flexible and chemically unreactive plastic (Dynalab 2015). They arrive at the tampoprint line filled with product and sealed ready to be labelled.

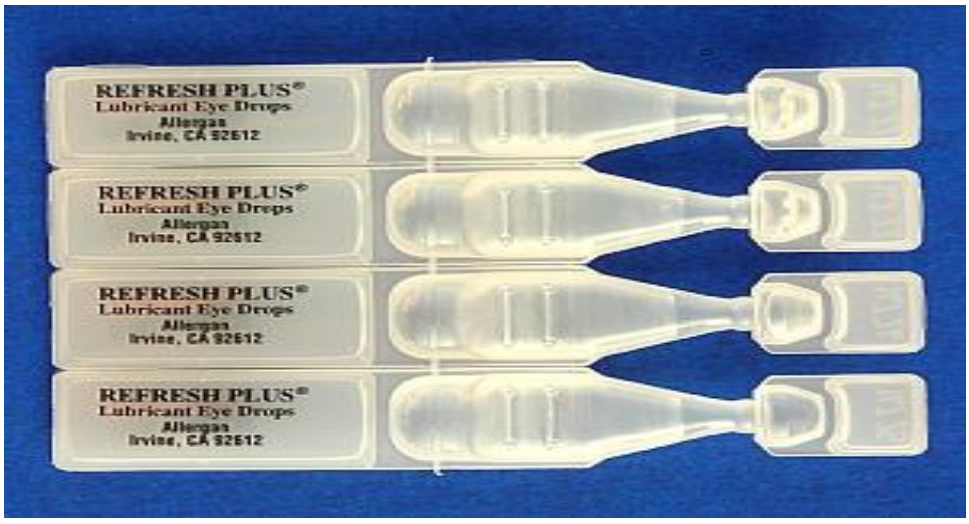


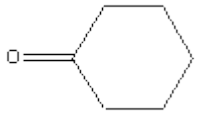
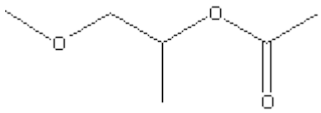
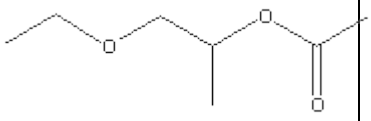
Figure 1.5: UD Vials after Tampoprinting

The vials are sorted and stacked on the belt of the machine before lasers ensure they are correctly aligned and in the correct position for printing. The printing machine consists of two printers; the first printer that the vials arrive at applies the lot number and expiry of the product plus a 2D code that is unique to each vial. The vials continue along the machine to the next printer which performs the tampoprinting. A thick steel cliché is used due to the high volume of printing with engravings that are etched using a laser for high precision and definition. The ink mixture is made up using 10 parts ink, 1 part hardener and 6 parts thinner and mixed for 12 hours before use. The vials are tampoprinted with the ink mixture on the opposite side from the initial printing before travelling through an infrared drying station to cure the ink and prevent smudging. The vials are then stacked and prepared for packaging.

Over time it has been observed that solvents present in the ink thinner can leach from the Tampoprint ink into the product. Tampoprint ink thinner purchased from Tampoprint International, is routinely qualified as a laboratory reference standard (LRS) within Allergan. This reference standard is utilised by analytical laboratories as a leachables identification solution (LIS) in GC finished product assays to identify the tampoprint related container-closure leachables cyclohexanone and PGMEEA. Historically, test results of the tampoprint ink thinner had detected a mixture of approximately 20:80 (cyclohexanone:PGMEEA) by area percent but recently a third component was identified in new lots of tampoprint ink thinner. New lots of the tampoprint ink thinner were consequently tested by proton/carbon nuclear magnetic resonance (NMR) and results revealed the third compound was present at approximately 20% (by proton mole ratio) in the ink thinner in addition to the expected proton signals for cyclohexanone and PGMEEA which were seen at approximately 30% and 50% respectively. GC tests were then conducted that showed the new component to be present at approximately 15% by area percent while cyclohexanone had increased from 20% to 33% and PGMEEA had decreased from 80% to 52%.

Tampoprint International was notified that a third component had been observed in new lots of the ink thinner and they verified that the component was PGMMEA. Going forward it is important that any methods used for the analysis of Tampoprint related leachables assesses the presence of all three ink thinner components; cyclohexanone, PGMMEA and PGMEEA. The chemical structures and properties for each leachable can be seen in Table 1.2.

Table 1.2: Chemical Structure of Cyclohexanone, PGMMEA and PGMEEA

Name	Properties	Analyte Structure
Cyclohexanone (Pubchem 2015)	Molecular Weight = 98.143 g/mol Molecular Formula = $C_6H_{10}O$ Boiling Point = 156˚C Melting Point = -31˚C	
Propylene glycol monomethyl ether acetate (PGMMEA) (Haltermann 2015)	Molecular Weight = 132.16 g/mol Molecular Formula = $C_6H_{12}O_3$ Boiling Point = 146˚C Freezing Point = -66˚C	
Propylene glycol monoethyl ether acetate (PGMEEA) (NIOSH 2015)	Molecular Weight = 146.184 g/mol Molecular Formula = $C_7H_{14}O_3$ Boiling Point = 160˚C Melting Point = -89˚C	

1.6 Analysis of Leachables by GC and HS-GC

Leachables in final product are most commonly detected using GC and LC techniques with GC utilised for the quantitation of volatiles and LC employed for non-volatiles. A testing schedule for every lot produced in Allergan is created from when the final product is packaged and released right through its shelf life to final expiry usually at 3 to 6 month intervals. The packaging of the product will be the main consideration in developing test methods to track possible leachables that can appear during the lifetime of the product. If certain components such as those used in the Tampoprint ink thinner are known to leach into the product then a method is required to track those leachables. As the tampoprint related components are known volatiles the best technique to use is GC. In Allergan, the two main types of GC in use are direct injection GC and headspace GC.

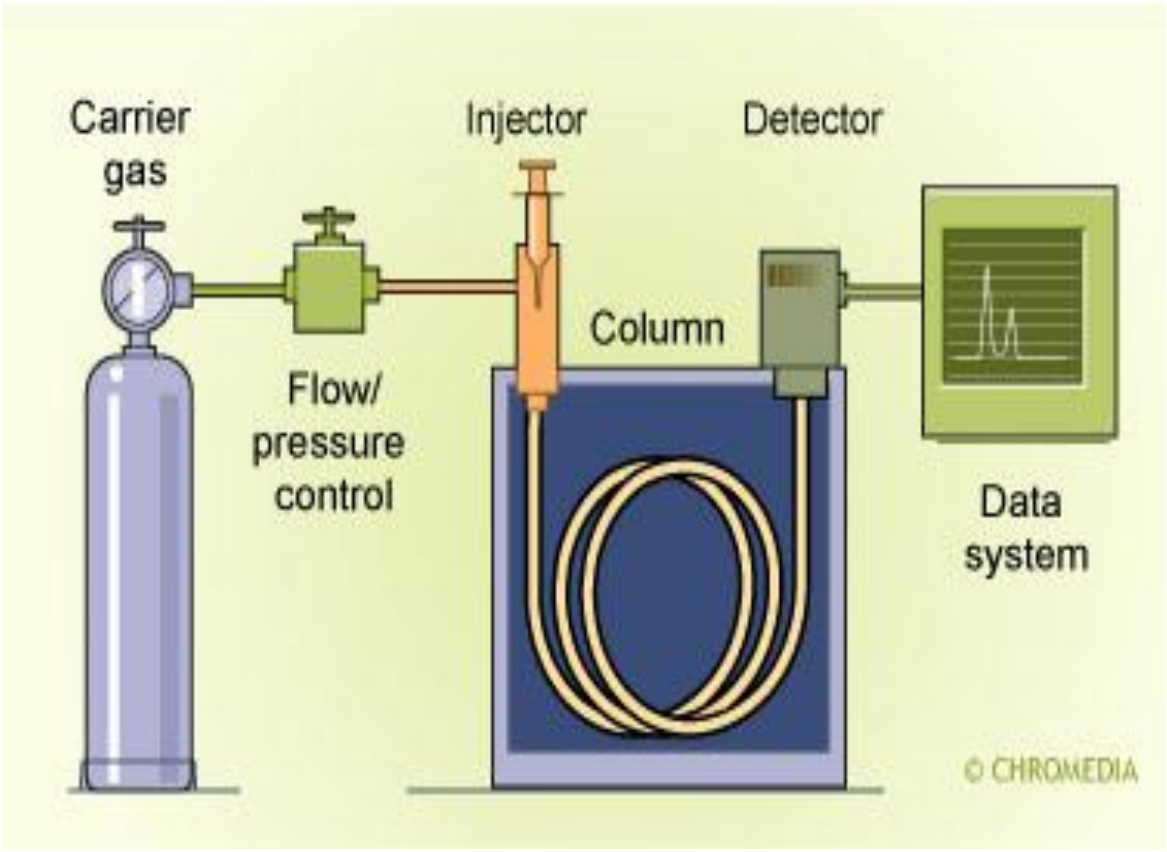


Figure 1.6: A Schematic of a Typical GC System (Chromedia 2015)

In direct injection GC the sample is transferred from a vial to the GC system via a micro syringe injector. Generally no more than a few microliters of the sample are required for injection. The injector port is typically set to a temperature about 50°C hotter than the boiling point of the least volatile component in the sample. This ensures vaporisation and only a gas form of the sample reaches the chromatographic column.

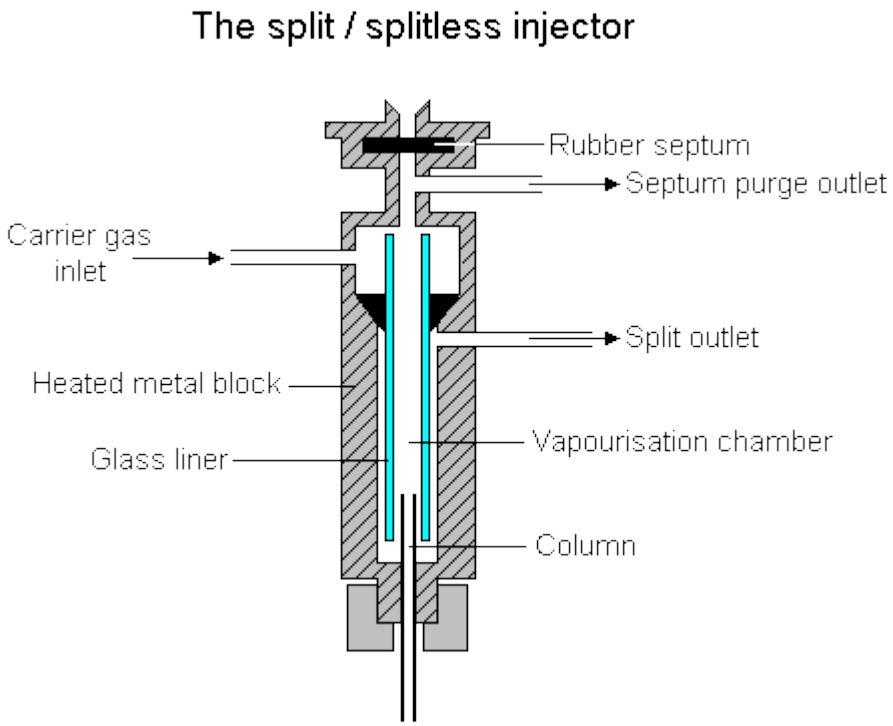


Figure 1.7: The Split/Splitless Injector (Sheffield Hallam University 2015)

The sample is carried along through the system via the mobile phase which is a chemically inert gas such as helium or nitrogen. Hydrogen is also commonly used, however due to safety issues arising from its flammability, none of the GC systems within Allergan are set up to use Hydrogen as a carrier gas. It is therefore left to decide between Helium and Nitrogen. The most obvious choice is Helium due to its superior properties in diffusion speed. The diffusion speed of Hydrogen is about 4 times higher than that of Nitrogen allowing for faster chromatography and better separation (Grob 1997).

Once at the column the components in the sample can be separated by the column. The most common type of column is a capillary column which is made up of a thin fused silica tube and a polyimide coating. They are strong yet flexible with the ability to be wound into coils and have low reactivity. The inside of the tube is coated with a chemically bonded stationary phase which interacts with the components in the sample. The other column option is a packed column. They are usually stainless steel and shorter with a larger diameter than capillary columns. They have the advantage of a higher sample capacity and are more durable whilst also costing less. However, for analysis of low ppm concentrations the capillary column is far superior to the

packed column. The capillary column has many more theoretical plates allowing for much better detection and separation of peaks. Capillary columns also heat and cool to temperature faster within the oven and require less gas to the detector.

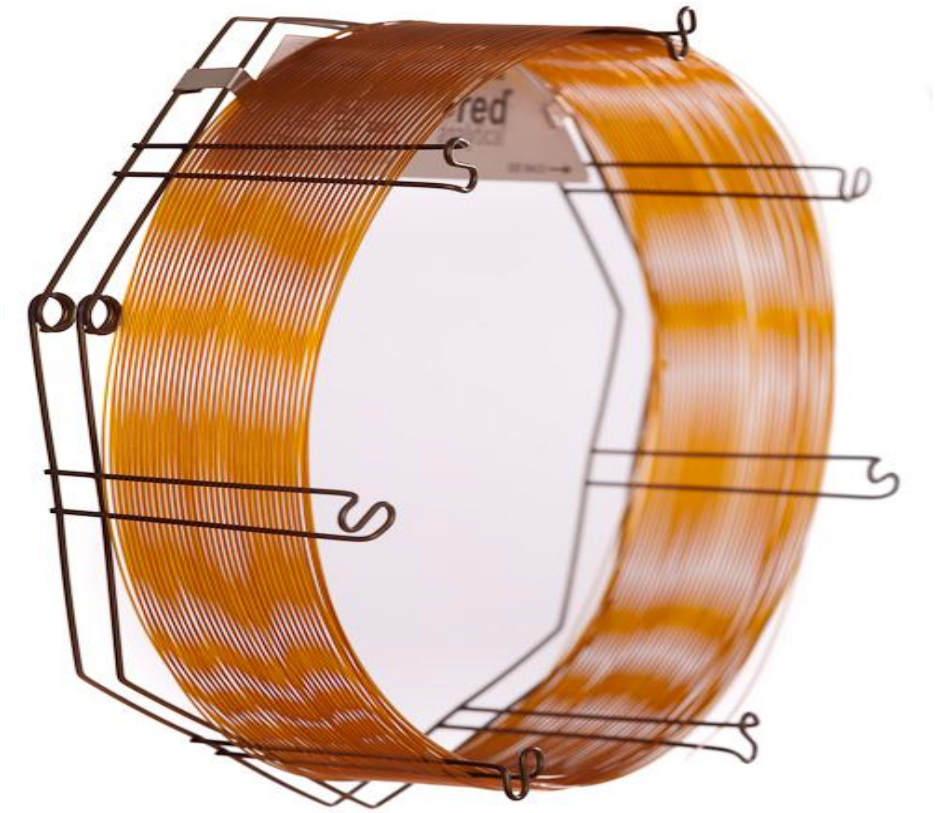


Figure 1.8: A Fused Silica GC Column (Dot-Red Analytical 2015)

There are two basic types of fused silica capillary columns; the Wall Coated Open Tubular (WCOT) column and the Porous Layer Open Tubular (PLOT) column. WCOT columns are most commonly used for GC analysis and consist of a liquid film coated to the deactivated wall of the column. In PLOT columns the stationary

phase is a solid substance, most commonly Divinylbenzene and Aluminia, that is coated to the column wall and used for very specific analysis (Agilent 2015).

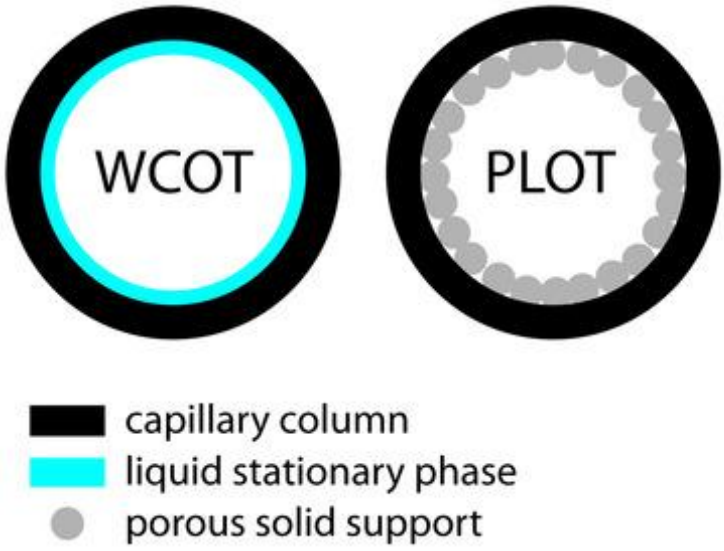


Figure 1.9: Schematic of Wall Coated Open Tubular (WCOT) column and the Porous Layer Open Tubular (PLOT) Column (UC Davis ChemWiki 2015)

The column is set up in the GC oven which dictates the temperature of the column, the lower the temperature the slower the sample components take to travel through the column and the lower the quality of separation between the components. However, if the temperature is too high the sample components travel through the column too quickly and are unable to interact with the stationary phase. Most GC methods will therefore incorporate a temperature gradient whereby the column oven is increased in temperature over a certain time allowing full separation of components with shorter run times. Components with higher boiling points will be seen later than those with lower boiling points as the oven temperature rises. The column length is also an important factor with shorter columns providing shorter run times but poorer separation to longer columns. The speed that the mobile phase flows through the column also needs to be considered, if it flows too fast the run time is again reduced but there is less time for the sample components to interact with the stationary phase and poor separation can result (UCLA 2014).

At the end of the column the mobile phase carrying the sample reaches the detector with sample components arriving at varying times depending on how long they were retained on the column. The best type of detector for the analysis of organic compounds is the flame ionisation detector (FID) due to its high sensitivity, ease of use and robustness. There is also very low background noise as minimal ions are created when carbon is absent. Hydrogen and air are pumped through the detector and mix with the sample before igniting. Electrons and ions are produced from any burning organic compounds and conduct electricity with the current measured by the detector via a collector electrode above the flame. The changes in current can be visualised as a chromatogram and allows the sample components to be quantitated using software such as Empower (Sheffield Hallam University 2015). A diagram of the FID can be seen in Figure 1.7.

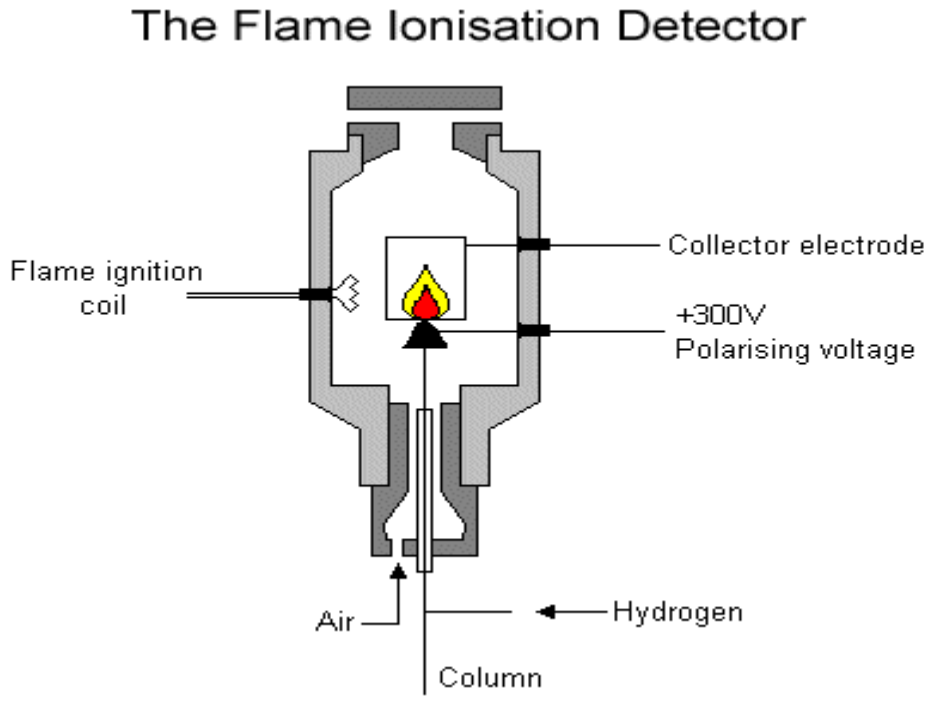
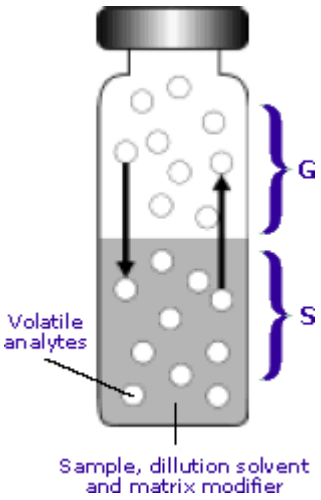


Figure 1.10: An FID Detector (Sheffield Hallam University 2015)

Headspace GC differs from direct injection GC in how the sample is injected onto the column. The sample vials used for headspace GC are typically 10 to 20 mL in volume compared to the smaller 2 mL vials used for direct injection GC. Typically, a stipulated amount of sample is added to a headspace vial occupying less than 50% of

the vial volume. The vial is then heated in a vial oven allowing any volatiles within the sample to move into the gas portion, also known as the "headspace" of the sample vial. This means that volatile sample components can be separated from non-volatile sample components within the vial. Once a state of equilibrium is reached between the gas phase and sample phase a portion of the gas will be removed from the vial and injected onto the column. Once the sample reaches the column the same process takes place as that previously described for direct injection GC.

Headspace GC is therefore an effective technique for the determination of volatile components within a complex sample matrix that would otherwise require sample extraction or be difficult to directly analyse (Labhut.com 2015).



G = The gas phase/headspace

The gas phase/headspace sits above the sample phase and is occupied by sample volatiles once the vial is sealed.

S = The sample phase

Volatiles within the sample phase move between the headspace and the sample phase until a state of equilibrium is reached as depicted by the arrows. An aliquot of the headspace is then removed and injected onto the column.

Figure 1.11: Phases of the Headspace Vial (Labhut.com 2015)

Headspace GC often employs a standard addition method. This is a universal procedure in headspace measurement and has been recommended since the early days of quantitative headspace analysis. The determination is carried out in an identical matrix; thus, no response (calibration) factors are needed for the calculation. Peak area and amount of the analyte are proportional; from this relationship, one can then directly calculate the original amount present (Kolb 2006).

1.7 Summary/Concluding Remarks

The objectives of this thesis are to develop and validate methods capable of the detection and quantitation of three known leachables in two separate ophthalmic formulations. Cyclohexanone, PGMMEA and PGMEEA are solvents from the ink thinner used during the Tampoprint process when labelling the LDPE vials that contain the ophthalmic solutions. Once packaged, these solvents are known to leach from the outside of the vial into the solution. It is a requirement for companies to track any leachables that may be present within their products. Hence, these developed methods will be used for the duration of each products shelf life to ensure that the amount of each leachable remains at a safe level below its threshold limit. Each method will also be capable of detecting unknown volatiles from the container closure system that may show up during the shelf life of each product.

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Chapter 2: Validation of a Direct Injection Gas Chromatography Method for the Analysis of Tampoprinting Leachates

2.1 Introduction

Although there are a number of published papers to show cycloheaxanone, propylene glycol monoethyl ether acetate (PGMEEA), and propylene glycol monomethyl ether acetate (PGMMEA) have been tested by GC (Ulsaker *et al* 1977; Dugard *et al* 1984), there is very little to show that there are any methods available to test the three analytes together.

This method uses direct injection gas chromatography to detect and quantitate the concentrations of the Tampoprint-related leachables cyclohexanone and PGMEEA, and PGMMEA. It also estimates the concentration of other potential volatile and semi-volatile container closure leachable components by comparison of gas chromatographic (GC) profiles of ophthalmic solution samples stored in plastic container/closure systems versus identical samples stored in glass (or unlabelled plastic containers) as a control. Comparison of these two chromatographic 'finger prints' forms the basis of evaluating the presence of volatile and semi-volatile leachables. If gas chromatographic profiles are the same for test and control samples, it is strong evidence that no such leachables are present in the ophthalmic solution.

The Tampoprint-related leachables that are seen in Product Z have not previously been tested and thus a new method is required.

This method will be validated in accordance with the current USP Category II (United States Pharmacopeia 37 2015) for cyclohexanone, PGMMEA and PGMEEA. The validation will also meet all requirements under ICH guidelines Q2 (R1) (International Conference of Harmonisation 2005).

The proposed method validation tests that will be carried out on the specified leachables cyclohexanone and PGMMEA are: accuracy, linearity, range, precision (repeatability and intermediate), specificity, detection limit (DL), quantitation limit (QL), robustness and standard stability. As no PGMEEA standard is available, the validation tests that will be carried out on the specified leachable PGMEEA are: precision (repeatability and intermediate), robustness and specificity. Since there is no standard material available for PGMEEA and due to its structural similarity PGMMEA will be used to estimate PGMEEA in samples.

2.2 Experimental

2.2.1 Initial Development

An initial investigation into previously validated leachables methods provided a number of options for sample preparation and GC conditions. Although the leachables for this particular project had not been tested before it was believed that their properties were ideal for one particular method which utilised a DB5 GC column (5%-phenyl 95%-dimethylpolysiloxane). The boiling point range of 146 - 160˚C for the three leachables lies in the middle of the recommended temperature range of the DB5 column. Also the DB5 column is a low polarity column and is ideal for the separation of the relatively low polarity leachables. A sample preparation of 1:1 using acetonitrile as sample and standard diluent was to be utilised.

An external toxicological assessment was performed for each of the proposed leachables. The report concluded that each of the leachables were safe up to a limit of 20 ppm. As the sample preparation required a dilution of 1:1, standards were required to be prepared at a final working concentration of 10 ppm.

Standards ranging in concentration from 0.5 to 30 ppm were made and tested on the DB5 column utilising the following GC conditions; an oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 7.0 mL/minute. The inlet was set at 250˚C with a purge flow of 25 mL/minute for 1.5 minutes. The detector was set at 250˚C and the makeup gas was helium at 30 mL/minute. Hydrogen was used for the fuel flow at 30 mL/minute and air was used for the oxidiser flow at 300 mL/minute.

The chromatograms were assessed and showed good peak shape and resolution.

2.2.2 Reagents

Analytical grade cyclohexanone and propylene glycol monomethyl ether acetate (PGMMEA) were purchased from Sigma Aldrich (Poole, UK). Tampoprint thinner, VDL-1015 was purchased from Tampoprint (Stuttgart, Germany).

Solvents used included acetonitrile (HPLC grade) which was purchased from Labscan (Dublin, Ireland). Ultrapure water was obtained from a millipore Milli-Q

water purification unit (Millipore, Bedford, MA, USA). High purity grade compressed air, hydrogen and helium were purchased from BOC (Surrey, UK).

2.2.3 Instrumentation and Apparatus

An Agilent 7890 GC system with FID and Agilent DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness (part number 125-503J) were utilised for all testing. A deactivated fused silica tubing guard column (Phenomenex, part number 7CK-G000-00-GZK) was attached to the column. Graphite ferrules by Agilent were used to attach the column to the GC system (part number 5080-8773). GC auto injector vials and caps were from Agilent (part number 5182-0866). The liner was an Agilent capillary single taper splitless liner, 4 mm, deactivated borosilicate glass (part number 5062-3587). The injector septa and o-ring were also from Agilent (part number 5183-4757 and 5188-5365 respectively). The GC utilised an oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 7.0 mL/minute. The inlet was set at 250˚C with a purge flow of 25 mL/minute for 1.5 minutes. The detector was set at 250˚C and the makeup gas was helium at 30 mL/minute. Hydrogen was used for the fuel flow at 30 mL/minute and air was used for the oxidiser flow at 300 mL/minute. All settings were controlled using Empower software.

Glassware was provided by Schott (Mainz, Germany) and consisted of 0.5 mL, 1 mL, 2 mL, 3 mL, 5 mL and 10 mL volumetric pipettes (Class A) as well as 10 mL, 20 mL, 50 mL and 100 mL amber volumetric flasks (Class A). All glassware was washed in a Hamo LS2000 glass washer using RBS 50 pF detergent and RBS R 60 acid. Both the detergent and acid were purchased from Chemical Products R. Borghgraef S.A. (Brussels, Belgium).

Pasteur pipettes made from low density polyethylene were obtained from VWR (part number 16001-170).

2.2.4 Standard Preparation

A stock standard (SS) was prepared by pipetting 1 mL of cyclohexanone and 1 mL of PGMMEA into a 100 mL amber volumetric flask containing approximately 70 mL of acetonitrile. The volumetric flask was brought to volume with acetonitrile and

mixed using a magnetic stirrer for 15 minutes. Nominal concentration was 9470 ppm for cyclohexanone and 9700 ppm for PGMMEA.

An intermediate standard (IS) was prepared by pipetting 1 mL of the stock standard solution into a 100 mL amber volumetric flask, containing approximately 70 mL of acetonitrile. The volumetric flask was then brought to volume with acetonitrile and mixed using a magnetic stirrer for 15 minutes. Nominal concentration was 94.7 ppm for cyclohexanone and 97.0 ppm for PGMMEA.

A working standard (WS) was prepared by pipetting 10 mL of the intermediate standard solution into a 100 mL amber volumetric flask, containing approximately 70 mL of acetonitrile. The volumetric flask was brought to volume with acetonitrile and mixed using a magnetic stirrer for 15 minutes. Nominal cyclohexanone concentration was 9.47 ppm. Nominal PGMMEA concentration was 9.70 ppm. A typical chromatogram of the working standard solution can be seen in Figure 2.1. Working standard concentrations are based on the cyclohexanone density of 0.947 g/mL and the PGMMEA density of 0.970 g/mL.

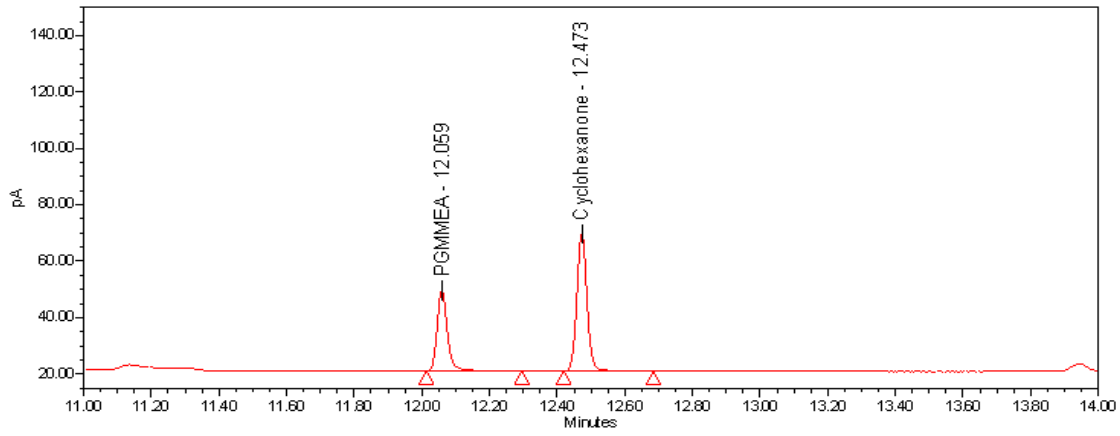

Figure 2.1: *GC-FID of WS (cyclohexanone 9.47 ppm; PGMMEA 9.70 ppm). GC Conditions: Agilent 7890 GC FID, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), splitless injection, injection volume 1µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 7.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (30:300).*

A PGMMEA Detector Sensitivity Solution (DSS) was prepared by pipetting 2 mL of the working standard solution into a 20 mL amber volumetric flask containing

approximately 10 mL of acetonitrile before diluting to volume with acetonitrile. The solution was mixed using a magnetic stirrer for 15 minutes. Nominal PGMMEA concentration was 0.97 ppm.

A cyclohexanone detector sensitivity solution (DSS) was prepared by pipetting 0.5 mL of the working standard solution into a 20 mL amber volumetric flask, containing approximately 10 mL of acetonitrile before diluting to volume with acetonitrile. The solution was mixed using a magnetic stirrer for 15 minutes. Nominal cyclohexanone concentration was 0.24 ppm.

A leachable identification working solution (LIS) was prepared by pipetting 1 mL of the tampoprint thinner solution into a 100 mL amber volumetric flask containing approximately 70 mL of acetonitrile. The volumetric flask was then brought to volume with acetonitrile and mixed using a magnetic stirrer for 15 minutes. 1 mL of this solution was pipetted into a 50 mL amber volumetric flask, containing approximately 30 mL of acetonitrile. The volumetric flask was then brought to volume with acetonitrile and mixed using a magnetic stirrer for 15 minutes. 5 mL of this solution was then pipetted into a 50 mL amber volumetric flask, containing approximately 30 mL of acetonitrile. The volumetric flask was then brought to volume with acetonitrile and mixed using a magnetic stirrer for 15 minutes. See Figure 2.2 for a typical chromatogram of the leachable identification working solution.

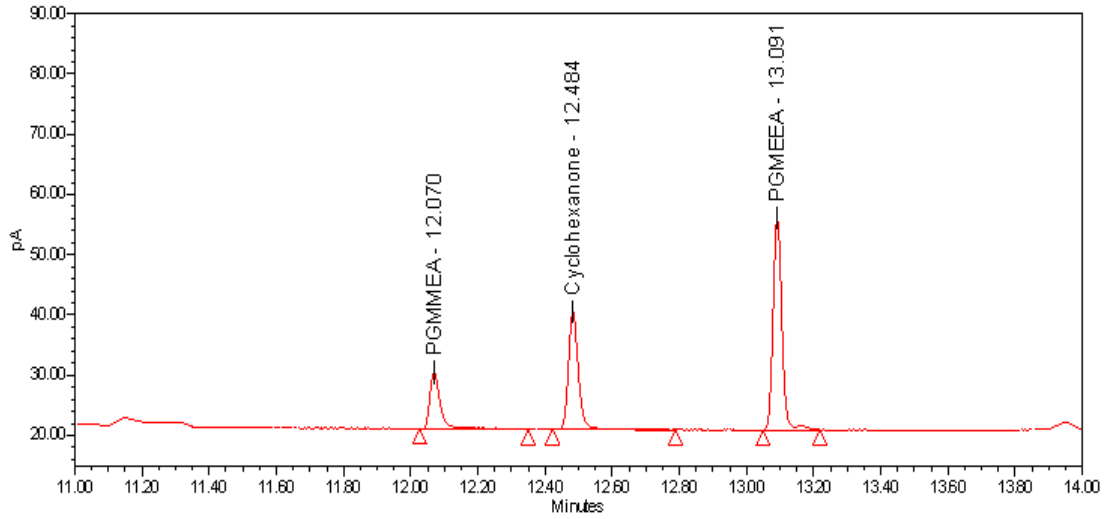

Figure 2.2: *GC-FID of LIS. GC Conditions: Agilent 7890 GC FID, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), splitless injection, injection volume 1µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 7.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (30:300).*

A blank was used to identify any system related peaks unrelated to the sample. The blanks consisted of GC vials filled by pasteur pipette with the acetonitrile that was used to dilute the samples and standards.

2.2.5 Sample Preparation

2.2.5.1 Product Z Control Samples

Product Z control samples are Product Z samples stored in glass containers, glass ampoules, unlabelled bottles or bottles with blank labels depending on the nature of the leachable study. Product Z control sample was pooled into a glass container from a number of glass ampoules. A 1:1 dilution of the control sample was then performed with acetonitrile in a volumetric flask. The volumetric flask was then vortexed for 15 seconds before leaving the volumetric flask to stand for 15 minutes to allow the contents to settle. (A white precipitate became visible at the base of the volumetric flask.) A GC vial was then filled from the volumetric flask using a pasteur pipette ensuring no precipitate was removed from the bottom of the flask.

2.2.5.2 Product Z Test Samples

Product Z test sample was prepared in the same manner as the control sample by pooling sample from final product plastic bottles (with Tampoprint labelling) into a glass container. See Figure 2.3 for a typical chromatogram of a Product Z sample.

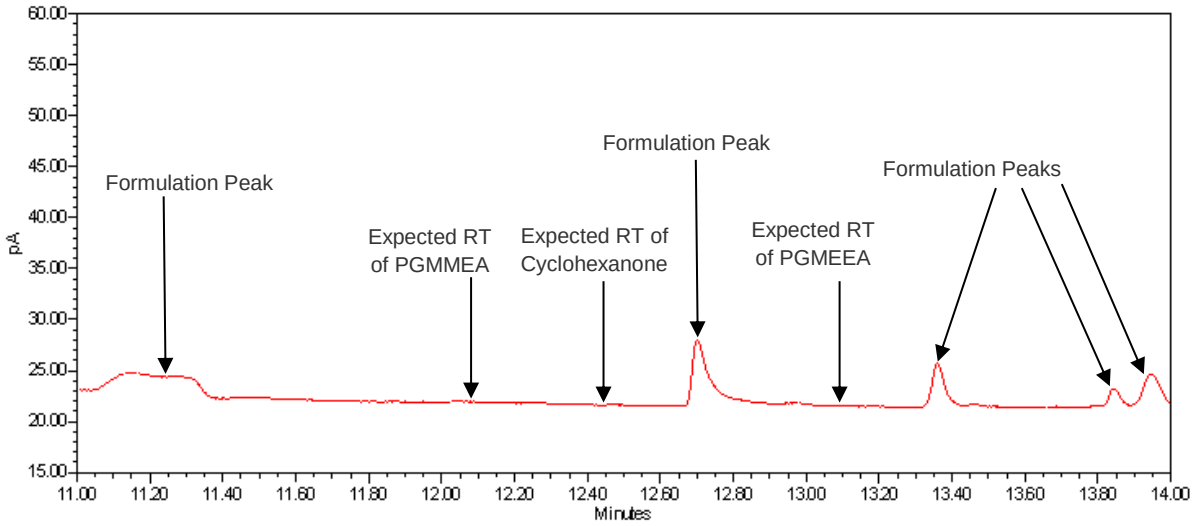

Figure 2.3: *GC-FID of Product Z Control. GC Conditions: Agilent 7890 GC FID, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), splitless injection, injection volume 1µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 7.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (30:300).*

2.2.6 Development Testing

Before validation could be performed it was important to carry out a series of verification tests to ensure that no problems would be encountered during full validation. These tests were used to ascertain acceptance criteria for system suitability that would be required at the start of each validation test. Retention time, tailing factor, % RSDs, signal-to-noise values and resolution of the cyclohexanone and PGMMEA peaks in the working standard (WS) and detector sensitivity solution (DSS) were tracked. The tests included altering a number of the GC conditions (robustness) as well as using different systems and different analysts on different days (intermediate precision). Mini accuracy and linearity tests were also run using spiked product to ensure that all the required levels could achieve acceptable results.

The limit of detection and the limit of quantitation were also tested to confirm they could be achieved.

At the end of development all testing had achieved satisfactory results and full validation could then commence.

2.2.7 System Suitability

Prior to every test it was required to show that the GC system was capable of performing as required. An example of an injection sequence for determining system suitability is shown in Table 2.2.

Note: It was important to ensure that enough blank injections were made such that no carryover was observed in two blank injections prior to further injections.

Table 2.1 Example of System Suitability Injection Sequence

Sample	No. of Injections
Blank	Minimum of 2
LIS	1
Cyclohexanone DSS	1
PGMMEA DSS	1
WS-1	5

The 5 separate injections of WS-1 were used to satisfy the system suitability requirements for retention times, tailing factors and peak area % RSD given in Table 2.2.

Each DSS was injected once. This injection of the DSS was used to satisfy the system suitability requirements for DSS signal-to-noise ratio given in Table 2.2.

The analysis could not be carried out if the system suitability criteria listed in Table 2.2 could not be met. When system suitability criteria could not be met, appropriate corrective action had to be taken and the system suitability was repeated. Definitions of each term in Table 2.2 can be found in the current USP (United States Pharmacopeia 37 2015).

Table 2.2 System Suitability Criteria

System Suitability Parameter	PGMMEA	Cyclohexanone
Retention Time (WS-1) (n = 5)	12.0 ± 1.0 min	12.5 ± 1.0 min
Tailing Factor (WS-1) (n = 5)	≤ 2.0	≤ 2.0
% RSD for Peak Area (WS-1) (n = 5)	NMT 5%	NMT 5%
Resolution between Cyclohexanone and PGMMEA	NLT 5	
DSS Signal-to-Noise (n = 1)	NLT 20:1	NLT 10:1

2.2.8 Method Validation

The validation tests that were carried out on the specified leachables cyclohexanone and PGMMEA were: accuracy, linearity, range, precision (repeatability and intermediate), specificity, standard and sample stability, detection limit (DL), quantitation limit (QL) and robustness. As no PGMEEA standard was available, the validation tests that were carried out on the specified leachable PGMEEA were: precision (repeatability and intermediate), robustness and specificity. For this method unknown leachables are estimated using cyclohexanone. Since there was no standard material available for PGMEEA and due to its structural similarity to PGMMEA, PGMMEA was used to estimate PGMEEA in samples.

The first test conducted was specificity because if the method was not specific more development would have been required. Following on from that the standard and sample stability was conducted. This would enable standards to be used over a number of days during the rest of validation and thus would save on preparing fresh standards every day. Robustness was then carried out followed by the rest of the validation tests.

2.2.8.1 Specificity

To demonstrate the specificity of this method in regards to leachable interferences, Product Z laboratory scale preparation was stressed using the following conditions and the samples were tested using the method to determine if there are any sample interferences at the retention times of cyclohexanone, PGMMEA and PGMEEA.

- Adjusted the pH to approximately 2.5 with 5 N HCl and stored at ambient temperature in the dark.
- Adjusted the pH to approximately 11.5 with 5 N NaOH and stored at ambient temperature in the dark.
- Stored at a temperature of 70^{o}C in the dark
- Added 5 drops of 30% hydrogen peroxide to 60 mL of sample and stored at ambient temperature in the dark.
- Placed in clear glass and stored in an ICH compliant light chamber until ICH light stress guidelines (International Conference of Harmonisation 2005) have been met.

The pH adjusted, heat and hydrogen peroxide stressed samples were stored under ambient laboratory conditions and pulled at day 14 and then stored at 2 - 8ºC and protected from light until time of analysis. The light stressed samples were pulled once ICH guidelines had been met. Control samples were untreated and unstressed and then stored in the dark at 2 - 8°C. All control samples were from the same batch as the treated and stressed samples.

2.2.8.2 Stock Standard Stability

Stock standard solutions containing cyclohexanone at 1.0% (v/v) and PGMMEA at 1.0% (v/v) were prepared in amber glassware on day-0. These solutions were assayed in triplicate and stored under ambient laboratory temperature and lighting. Fresh working standards were prepared for each day of the study from the stock standards prepared on day-0. These standards were then re-assayed against freshly prepared working standards on day-1, day-3 and day-8 in order to determine the chemical stability of the stock standard solutions in amber glassware. A summary of the data is presented in Tables 2.4 and 2.5.

2.2.8.3 Intermediate Standard Stability

Stock standard solutions containing cyclohexanone at 1.0% (v/v) and PGMMEA at 1.0% (v/v) were prepared in amber glassware. Separate intermediate standard solutions containing cyclohexanone and PGMMEA at 0.01% (v/v) were prepared in amber glassware from these stock standards and analysed in triplicate on day-0.

These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared working standards on day-1, day-3 and day-8 in order to determine the chemical stability of the intermediate standard solutions in amber glassware. A summary of the data is presented in Tables 2.6 and 2.7.

2.2.8.4 Working Standard Stability

Stock standard solutions containing cyclohexanone at 1.0% (v/v) and PGMMEA at 1.0% (v/v) were prepared in amber glassware. Separate working standard solutions containing cyclohexanone and PGMMEA at 0.001% (v/v) were prepared in amber glassware from these stock standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared working standards on day-1, day-3 and day-8 in order to determine the chemical stability of the working standard solutions in amber glassware. A summary of the data is presented in Tables 2.8 and 2.9.

2.2.8.5 Cyclohexanone DSS Stability

Working standard solutions were freshly prepared in amber glassware. Separate cyclohexanone DSS solutions were prepared in amber glassware from these working standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared cyclohexanone DSS on day-1, day-3 and day-8 in order to determine the chemical stability of the cyclohexanone DSS solution in amber glassware. A summary of the data is presented in Table 2.10.

2.2.8.6 PGMMEA DSS Stability

Working standard solutions were freshly prepared in amber glassware. Separate PGMMEA-DSS solutions were prepared in amber glassware from these working standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared PGMMEA-DSS on day-1, day-3 and day-8 in order to determine the chemical stability of the PGMMEA-DSS solution in amber glassware. A summary of the data is presented in Table 2.11.

2.2.8.7 Leachable Identification Working Solution Stability

Leachable identification working solution was freshly prepared in amber glassware as per section 2.2.4 and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed on day-1, day-3 and day-8 in order to determine the chemical stability of the leachable identification working solution in amber glassware.

2.2.8.8 Sample Stability

Sample solutions of Product Z diluted 1:1 with acetonitrile were freshly prepared in amber vials on day-0 as per section 2.2.5.2. These solutions were assayed in triplicate and stored under ambient laboratory temperature and lighting. Freshly prepared standards were used to reanalyse the test sample preparations after day-1, day-3 and day-8.

2.2.8.9 Robustness

Robustness is the reproducibility of the test results for cyclohexanone, PGMMEA and PGMEEA obtained by analysis of Product Z product under deliberate variations of the nominal test conditions. Product Z lab scale preparation was spiked with Tampoprint thinner solution containing approximately 6.5 ppm cyclohexanone, 6.5 ppm PGMMEA and 19 ppm PGMEEA. The method ruggedness (robustness) validation is demonstrated from the intermediate precision data. Additional robustness studies were conducted by analysing the same spiked sample (n = 6) using different injector temperatures, detector temperatures, final column temperatures and carrier gas flow as shown in Table 2.3.

Each test condition in Table 2.3 was then evaluated for:

All System Suitability data: Report value
Retention time and relative retention time of PGMEEA
Mean cyclohexanone, PGMMEA and PGMEEA, ppm data (n = 6)
Standard deviation of cyclohexanone, PGMMEA and PGMEEA, ppm data (n = 6)
% RSD of cyclohexanone, PGMMEA, and PGMEEA, ppm data (n = 6)
Ratio of the means cyclohexanone, PGMMEA and PGMEEA, ppm value versus nominal conditions (Test 1) where, X_1 = Test 1 and X_2 = Test 2 - 9

Table 2.3 Robustness Test Conditions

	Injector Temperature (ºC)	Detector Temperature (ºC)	Final Oven Temperature (ºC)	Carrier Gas Flow (mL/min)
Test 1 *	250	250	245	7.0
Test 2	**240**	250	245	7.0
Test 3	**260**	250	245	7.0
Test 4	250	**240**	245	7.0
Test 5	250	**260**	245	7.0
Test 6	250	250	**235**	7.0
Test 7	250	250	**255**	7.0
Test 8	250	250	245	**6.5**
Test 9	250	250	245	**7.5**

*Represents nominal analysis conditions of the method

The method robustness tests 1 - 9 given in Table 2.3 were conducted and the resultant data is provided in Tables 2.12 to 2.16. The mean cyclohexanone (ppm), PGMMEA (ppm) and PGMEEA (ppm) data from robustness test 1 was used to determine the ratio of the means results for robustness tests 2 – 9 in Tables 2.14 to 2.16.

2.2.8.10 Accuracy of Cyclohexanone

Product Z lab scale preparation was spiked with cyclohexanone at approximately 0.5, 1, 2, 10, 20 and 30 ppm. These levels are equivalent to 2.5%, 5%, 10%, 25%, 50%, 100% and 150% of cyclohexanone proposed product specification, respectively. This was achieved by preparing a cyclohexanone stock solution of 10000 ppm in acetonitrile. A 100 ppm spiked solution was then prepared by adding 1 mL of the stock to a 100 mL volumetric flask and brought to volume with Product Z lab scale formulation. Each spiked preparation was then made by diluting the 100 ppm solution in sample. 30 ppm was prepared by adding 3 mL to a 10 mL volumetric flask and brought to volume with sample. 20 ppm was prepared by adding 2 mL to a 10 mL volumetric flask and brought to volume with sample. 10 ppm was prepared by adding 1 mL to a 10 mL volumetric flask and brought to volume with sample. 2 ppm was prepared by adding 2 mL to a 100 mL volumetric flask and brought to volume with sample. 1 ppm was prepared by adding 1 mL to a 100 mL volumetric flask and brought to volume with sample. 0.5 ppm was prepared by adding 1 mL to a 200 mL volumetric flask and brought to volume with sample. Three separate preparations were analysed for the 5%, 10%, 25%, 50% and 150% levels, whereas six separate preparations were analysed for the 2.5% and 100% levels. The ppm concentration of

cyclohexanone was calculated for each preparation. The cyclohexanone accuracy results are reported in Table 2.18.

2.2.8.11 Accuracy of PGMMEA

Product Z lab scale preparation was spiked with PGMMEA at approximately 2, 5, 10, 20 and 30 ppm PGMMEA. These accuracy levels are equivalent to 10%, 25%, 50%, 100%, and 150% of PGMMEA proposed product specification, respectively. Spiked sample concentrations were prepared in the same manner as 2.2.9.10. Three separate preparations were analysed for the 25%, 50% and 150% levels, whereas six separate preparations were analysed for the 10% and 100% levels. The ppm concentration of PGMMEA was calculated for each preparation. The PGMMEA accuracy results are reported in Table 2.19.

2.2.8.12 Cyclohexanone Linearity

To determine linearity for cyclohexanone, a linearity curve was generated from 0.5 to 30.0 ppm cyclohexanone. This range is equivalent to approximately 2.5 to 150% of the cyclohexanone proposed product specification. Each concentration point was run in triplicate, except for the 2.5% and 100% target solution which were run 6 times (separate preparations). The y-intercept at the origin for peak area data is less than 5% of the y-intercept at label strength, indicating that a single point calibration can be used. This specification is an in-house guideline as regulations require that the y-intercept only be reported. Table 2.19 contains the linearity data. A summary of the data can be seen in Table 2.20.

2.2.8.13 PGMMEA Linearity

To determine linearity for PGMMEA, a linearity curve was generated from 2.0 to 30.0 ppm PGMMEA. This range is equivalent to approximately 10% to 150% of the PGMMEA proposed product specification. Each concentration point was run in triplicate, except for the 10% and 100% target solution which were run 6 times (separate preparations). The y-intercept at the origin for peak area data is less than 5% of the y-intercept at label strength, indicating that a single point calibration can be used. Table 2.21 contains the linearity data. A summary of the data can be seen in Table 2.22.

2.2.8.14 Cyclohexanone and PGMMEA Standard Precision (Repeatability)

Standard precision was determined by injecting 6 replicate injections of cyclohexanone and PGMMEA working standard. A summary of the data is shown in Table 2.23.

2.2.8.15 Cyclohexanone, PGMMEA and PGMEEA Sample Precision (Repeatability)

Sample precision was determined by injecting a Tampoprint thinner spiked Product Z lab scale preparation containing approximately 6.5 ppm cyclohexanone, 6.5 ppm PGMMEA and 19 ppm PGMEEA. A summary of the data is shown in Table 2.24.

2.2.8.16 Cyclohexanone, PGMMEA and PGMEEA Intermediate Precision

As defined in the glossary, intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment. In this case, six separate preparations of Product Z, spiked with Tampoprint thinner solution containing approximately 6.5 ppm cyclohexanone and PGMMEA and 19 ppm PGMEEA were analysed and the amount of cyclohexanone, PGMMEA and PGMEEA (ppm) determined by operator-A. On day-2 (operator-A) analysed the same spiked sample to provide information concerning day-to-day precision. The same instrument and column were used on both days. A summary of the data is shown in Tables 2.25 to 2.27.

A second operator (operator-B) analysed the same spiked product to provide information concerning operator-to-operator precision. Operator B used a different instrument and different column lot number to operator A. A summary of the data is shown in Tables 2.25 to 2.27.

2.2.8.17 Range

The range is the interval between the lower and upper concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range was established using data for accuracy, linearity and precision.

2.2.8.18 Detection Limit (DL) Determination for Cyclohexanone and PGMMEA

The DL of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Typically the DL will be equal to 3 times the standard deviation of the blank. For validation purposes the detection limit may often be set at one third of the quantitation limit as concentration levels can become so small they are regarded as zero.

The DL of cyclohexanone and the DL of PGMMEA in Product Z were determined by preparing a single solution of Product Z lab scale preparation spiked at 0.2 ppm with cyclohexanone and 1.0 ppm with PGMMEA. From this solution 3 separate preparations were analysed. A summary of the DL data is given in Table 2.28.

2.2.8.19 QL Determination for Cyclohexanone and PGMMEA

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The QL can be specifically calculated using the actual precision of the analytical procedure at this concentration. The calculation is based on the compatibility between analytical variability and specification acceptance limits. QL can be regarded as the maximum true impurity content of the manufactured batch, i.e., as the basic limit

$$QL=AL-\frac{(st_{df,95\%})_{validation}}{\sqrt{n_{assay}}}$$

AL = Acceptance limit of the specification for the impurity.
s = Precision standard deviation at QL, preferably under intermediate or reproducibility conditions. AL and s equal same unit (e.g., percentage weight per volume).

N_{assay} = Number of repeated, independent determinations in routine analyses, as far as the mean is the reportable result, i.e., is compared with the acceptance limits. If each individual determination is defined as the reportable result, n=1 has to be used.

t_{df} = Student t-factor for the degrees of freedom during determination of the precision, usually at 95% level of statistical confidence.

The QL of cyclohexanone and the QL of PGMMEA in Product Z were determined by preparing a single solution of Product Z lab scale preparation spiked at 0.5 ppm with cyclohexanone and 2.0 ppm with PGMMEA. From this solution 6 separate preparations were analysed. A summary of the QL data is given in Table 2.29.

The QL was set at 0.5 ppm and 2.0 ppm for cyclohexanone and PGMMEA respectively as these were the predetermined reporting thresholds. It is possible that the method could accurately quantitate at lower concentrations but is not necessary for the purpose of this method.

2.2.9 Sample Analysis

Injections of the working standard (WS-1) were used for bracketing after every six samples.

2.3 Results and Discussion

2.3.1 Rationale for Procedure

This method has been validated for the quantitation of the specified Tampoprint leachables cyclohexanone, PGMMEA and PGMEEA. Results from all the validation tests are recorded in the following tables (Tables 2.4 to 2.29).

This method was validated to the current USP Category II (United States Pharmacopeia 37 2015) for cyclohexanone, PGMMEA and PGMEEA. The validation also complies with ICH Q2 (R1) (International Conference of Harmonisation 2005) for assay and impurities.

2.3.2 Specificity

There were no peaks observed at the retention times of PGMMEA and PGMEEA. There was a peak detected at the retention time of cyclohexanone in the samples stressed by acid. However, this peak had a signal to noise of considerably less than 10:1 and therefore meets the acceptance. No significant change in the chromatographic profile of the stressed samples was seen when compared with that

of the control. The method is deemed to be specific for the determination of container closure leachables in Product Z.

2.3.3 Standard Stability

2.3.3.1 Stock Standard Stability

Table 2.4 Cyclohexanone Stock Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean Cyclohexanone ppm	9.67	9.50	9.00	9.38
SD ppm	1.3×10^{-1}	7.5×10^{-2}	3.1×10^{-2}	5.0×10^{-2}
% RSD	1.4	0.7	0.3	0.5
n	3	3	3	3
Ratio of the means Cyclohexanone ppm values (vs. day-0)	N/A	0.982	0.931	0.970

Table 2.5 PGMMEA Stock Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean PGMMEA ppm	9.77	9.89	9.55	9.52
SD ppm	1.4×10^{-1}	1.5×10^{-1}	5.1×10^{-2}	4.9×10^{-2}
% RSD	1.4	1.5	0.5	0.5
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	1.012	0.977	0.974

The acceptance criterion for this study is that the ratio of mean values for cyclohexanone and PGMMEA for the stock standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the stock standard is stable for up to 8 days when stored in amber glassware.

2.3.3.2 Intermediate Standard Stability

Table 2.6 Cyclohexanone Intermediate Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean Cyclohexanone ppm	9.67	9.41	8.82	9.28
SD ppm	1.3×10^{-1}	1.2×10^{-1}	1.7×10^{-2}	2.3×10^{-2}
% RSD	1.4	1.3	0.1	0.2
n	3	3	3	3
Ratio of the means Cyclohexanone ppm values (vs. day-0)	N/A	0.973	0.912	0.959

Table 2.7 PGMMEA Intermediate Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean PGMMEA ppm	9.77	9.77	9.33	9.48
SD ppm	1.4×10^{-1}	1.3×10^{-1}	8.3×10^{-2}	7.0×10^{-2}
% RSD	1.4	1.3	0.8	0.7
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	1.000	0.954	0.973

The acceptance criterion for this study is that the ratio of mean values for the cyclohexanone and the PGMMEA for the intermediate standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the cyclohexanone intermediate standard is stable for up to 8 days when stored in amber glassware.

2.3.3.3 Working Standard Stability

Table 2.8 Cyclohexanone Working Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean Cyclohexanone ppm	9.67	9.20	8.78	9.19
SD ppm	1.3×10^{-1}	1.1×10^{-1}	3.3×10^{-2}	2.4×10^{-2}
% RSD	1.4	1.2	0.3	0.2
n	3	3	3	3
Ratio of the means Cyclohexanone ppm values (vs. day-0)	N/A	0.951	0.907	0.950

Table 2.9 PGMMEA Working Standard Stability – Amber Glassware

	Day-0	Day-1	Day-3	Day-8
Mean PGMMEA ppm	9.77	9.50	9.34	9.49
SD ppm	1.4×10^{-1}	2.3×10^{-1}	2.7×10^{-2}	4.9×10^{-2}
% RSD	1.4	2.4	0.2	0.5
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	0.972	0.955	0.971

The acceptance criterion for this study is that the ratio of mean values for the cyclohexanone and the PGMMEA for the working standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the cyclohexanone working standard is stable for up to 8 days when stored in amber glassware.

2.3.3.4 Cyclohexanone DSS Stability

Table 2.10 Cyclohexanone DSS Stability in Amber Glassware (n = 3)

Parameter	Day-0	Day-1	Day-3	Day-8
Mean S/N DSS	13.9:1	12.3:1	16.9	20.5
S/N SD	7.5×10^{-1}	1.1×10^{0}	1.2×10^{0}	8.9×10^{-1}
S/N % RSD	5.3	9.6	7.5	4.3
Mean Cyclohexanone peak area	2.56	2.29	2.66	2.65
SD peak area	1.1×10^{-1}	9.1×10^{-2}	1.0×10^{-1}	2.0×10^{-1}
% RSD	4.4	3.9	3.9	7.8
Mean % Recovery DSS	101.0	93.8	93.1	102.9
% Recovery SD	4.4×10^{0}	3.7×10^{0}	3.6×10^{0}	8.0×10^{0}
% Recovery % RSD	4.4	3.9	3.9	7.8
Ratio of the means % Recovery values (vs. day-0)	N/A	0.928	0.921	1.018

The acceptance criterion for this study is that the ratio of mean for the cyclohexanone DSS data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the cyclohexanone DSS is stable for up to 8 days when stored in amber glassware. However, in line with the PGMMEA DSS stability study, the cyclohexanone DSS must be prepared fresh on day of analysis.

2.3.3.5 PGMMEA DSS Stability

Table 2.11 PGMMEA DSS Stability in Amber Glassware (n = 3)

Parameter	Day-0	Day-1	Day-3	Day-8
Mean S/N DSS	26.1:1	17.5:1	25.4	38.7
S/N SD	9.5×10^{0}	1.2×10^{0}	6.6×10^{-1}	1.9×10^{0}
S/N % RSD	3.6	7.0	2.6	4.9
Mean PGMMEA peak area	5.20	3.81	5.24	5.86
SD peak area	1.4×10^{-1}	2.4×10^{-1}	3.0×10^{-1}	1.4×10^{-1}
% RSD	2.7	6.3	5.7	2.4
Mean % Recovery DSS	89.9	74.7	86.4	95.6
% Recovery SD	2.4×10^{0}	4.8×10^{0}	5.0×10^{0}	2.3×10^{0}
% Recovery % RSD	2.7	6.4	5.7	2.4
Ratio of the means % Recovery values (vs. day-0)	N/A	0.830	0.961	1.063

The acceptance criterion for this study is that the ratio of mean for the PGMMEA DSS data remains within 0.7 and 1.3 throughout the testing period. However, the mean PGMMEA DSS % recovery fails to meet the 80.0 – 120.0% recovery

acceptance criterion which is required for system suitability. Based on the data, the PGMMEA DSS must be prepared fresh on the day of analysis.

2.3.3.6 Leachable Identification Working Solution Stability

The acceptance criterion for this study is that there is no significant change in the chromatographic profile of the sample solution between day-0 and day-1, day-3 and day-8. No deterioration of chromatography or shift in retention time has occurred for the cyclohexanone, PGMMEA and PGMEEA peaks throughout the testing period. Based on the data, the leachable identification working solution is stable for up to 8 days when stored in amber glassware.

2.3.4 Sample Stability

The acceptance criterion for this study is that there is no significant change in the chromatographic profile of the sample solution between day-0 and day-1 and day-3. Chromatography for the sample changed on day-8 testing, with an increase in the number of peaks detected, most noticeably at the retention time of the cyclohexanone. Based on the data, the sample preparation is stable for up to 3 days when stored in amber glassware.

2.3.5 Robustness

Table 2.12 Cyclohexanone Robustness System Suitability Data

	Retention Time Cyclohexanone (min)	Tailing Factor Cyclohexanone	Resolution between Cyclohexanone and PGMMEA	Signal-to-Noise Cyclohexanone	% RSD Cyclohexanone Peak Area
Acceptance Criteria	Report	Report	Report	Report	≤ 5%
Test 1 *	12.47	1.06	7.6	15.6:1	0.4
Test 2	12.47	1.07	7.5	15.1:1	0.4
Test 3	12.47	1.06	7.6	10.7:1	0.4
Test 4	12.47	1.07	7.6	15.3:1	0.2
Test 5	12.47	1.08	7.6	14.9:1	0.5
Test 6	12.46	1.07	7.6	14.0:1	0.2
Test 7	12.47	1.09	7.6	12.9:1	0.5
Test 8	12.65	1.08	7.8	12.9:1	0.2
Test 9	12.29	1.08	7.4	12.3:1	0.4

*Represents nominal analysis conditions of the method

Retention Time (t_R)

The time taken after injection for the analyte molecules to reach the detector. This dependent on the column flow rate, column capacity, extra column dead volume and the retardation factor of the molecules on the stationary phase.

Tailing Factor

A measure of how close a chromatographic peak is to a symmetrical shape. As a peak slopes the tailing factor increases.

$T_f = ac / 2ab$

where ac is the peak width at 5% of the peak height, and ab is the front half-width measured from the leading edge to a perpendicular dropped from the peak apex.

Resolution

A characteristic of the separation of two adjacent peaks. It may be expressed according to the equation:

$$R\text{AB} = 2\,\frac{|d\text{R(B)} - d\text{R(A)}|}{|w\text{(B)} + w\text{(A)}|}$$

where *R*AB is the resolution, *d*R(A) and *d*R(B) are the retention distances (time or volume) of each eluted component A and B, and *w*(A) and *w*(B) are the respective widths of each peak at its base.

Table 2.13 PGMMEA Robustness System Suitability Data

	Retention Time PGMMEA (min)	Tailing Factor PGMMEA	% RSD PGMMEA Peak Area	Signal-to-Noise PGMMEA
Acceptance Criteria	Report	Report	≤ 5%	Report
Test 1 *	12.05	1.26	0.4	29.0:1
Test 2	12.05	1.25	0.6	30.1:1
Test 3	12.05	1.22	0.4	24.5:1
Test 4	12.05	1.23	0.6	32.2:1
Test 5	12.05	1.24	0.6	28.2:1
Test 6	12.05	1.22	0.4	30.6:1
Test 7	12.05	1.25	0.4	27.2:1
Test 8	12.23	1.24	0.2	25.1:1
Test 9	11.88	1.24	0.3	23.5:1

*Represents nominal analysis conditions of the method

Table 2.14 Cyclohexanone Robustness Data

	Mean Cyclohexanone (ppm)	SD Cyclohexanone (ppm)	% RSD Cyclohexanone (ppm)	Ratio of the Means Cyclohexanone
Acceptance Criteria	Report	Report	≤ 5%	0.7 – 1.3
Test 1 *	7.06	1.1×10^{-1}	1.6	N/A
Test 2	7.52	1.8×10^{-1}	2.4	1.1
Test 3	6.95	1.4×10^{-1}	2.1	1.0
Test 4	7.10	1.2×10^{-1}	1.6	1.0
Test 5	7.11	1.1×10^{-1}	1.6	1.0
Test 6	6.98	1.5×10^{-1}	2.2	1.0
Test 7	6.93	1.4×10^{-1}	2.0	1.0
Test 8	6.72	8.6×10^{-2}	1.2	1.0
Test 9	7.18	8.3×10^{-2}	1.1	1.0

*Represents nominal analysis conditions of the method

Table 2.15 PGMMEA Robustness Data

	Mean PGMMEA (ppm)	SD PGMMEA (ppm)	% RSD PGMMEA (ppm)	Ratio of the Means PGMMEA
Acceptance Criteria	Report	Report	≤ 5%	0.7 – 1.3
Test 1 *	6.14	7.6×10^{-2}	1.2	N/A
Test 2	6.55	1.0×10^{-1}	1.5	1.1
Test 3	6.12	1.4×10^{-1}	2.3	1.0
Test 4	6.25	1.3×10^{-1}	2.1	1.0
Test 5	6.29	1.6×10^{-1}	2.5	1.0
Test 6	6.13	1.4×10^{-1}	2.4	1.0
Test 7	6.21	1.8×10^{-1}	2.9	1.0
Test 8	6.24	9.0×10^{-2}	1.4	1.0
Test 9	6.46	8.3×10^{-2}	1.2	1.1

*Represents nominal analysis conditions of the method

Table 2.16 PGMEEA Robustness Data

	Retention Time PGMEEA (min)	Relative Retention Time PGMEEA	Mean PGMEEA (ppm)	SD PGMEEA (ppm)	% RSD PGMEEA (ppm)	Ratio of the Means PGMEEA
Acceptance Criteria	Report	Report	Report	Report	≤ 5%	0.7 – 1.3
Test 1 *	13.07	1.04	19.12	2.8×10^{-1}	1.4	N/A
Test 2	13.07	1.04	20.57	5.2×10^{-1}	2.5	1.1
Test 3	13.07	1.04	18.96	4.0×10^{-1}	2.1	1.0
Test 4	13.07	1.04	19.64	2.9×10^{-1}	1.5	1.0
Test 5	13.07	1.04	19.76	3.4×10^{-1}	1.7	1.0
Test 6	13.07	1.04	19.30	4.0×10^{-1}	2.0	1.0
Test 7	13.07	1.04	19.50	4.1×10^{-1}	2.1	1.0
Test 8	13.23	1.04	19.12	1.8×10^{-1}	0.9	1.0
Test 9	12.91	1.05	20.34	2.3×10^{-1}	1.1	1.1

*Represents nominal analysis conditions of the method

Conclusion of Robustness Study

The data indicates that all System Suitability criteria were met for all challenges. The method is capable of withstanding:

- ± 10°C variation in injector temperature
- ± 10°C variation in detector temperature
- ± 10°C variation in final oven temperature
- ± 0.5 mL/min change in carrier gas flow

Overall, the method is considered robust and suitable for routine use where accurate and reliable quantitative data is required for cyclohexanone, PGMMEA, PGMEEA and other potential leachables.

The information derived from the precision section provides an additional measure of method ruggedness. Using different analysts, on different days and different columns will yield chromatography and quantitative cyclohexanone, PGMMEA and PGMEEA values that are very consistent. This demonstrates that the method is sufficiently rugged, reproducible and robust under conditions of routine laboratory usage.

2.3.6 Accuracy

2.3.6.1 Product Z Placebo Interferences

Three replicate injections of Product Z lab scale preparation showed no interferences with a S/N ratio greater than 10:1 at the location of the cyclohexanone, PGMMEA and PGMEEA peaks. This lack of placebo (i.e. lab-scale product made as per the manufacturing procedure but with the omission of at least one active ingredient contained within the full formulation) interference meets the acceptance criteria.

2.3.6.2 Accuracy Data for Cyclohexanone

Table 2.17 Accuracy Data for Cyclohexanone

Conc. ppm	**0.5**	**1**	**2**	**5**	**10**	**20**	**30**
Conc. ppm	0.48 0.47 0.50 0.47 0.49 0.47	0.97 0.98 0.94	1.76 1.78 1.77	3.94 4.32 4.30	8.53 8.57 8.28	17.30 17.45 17.26 16.44 15.89 16.47	26.78 26.32 24.98
Mean Conc. ppm	0.49	0.97	1.78	4.19	8.46	16.81	26.03
SD, Conc. ppm	1.2×10^{-2}	1.8×10^{-2}	1.2×10^{-2}	2.1×10^{-1}	1.5×10^{-1}	6.2×10^{-1}	9.3×10^{-1}
% RSD, Conc. ppm	2.6	1.9	0.7	5.0	1.8	3.7	3.5
% Recovery	102.74 100.42 107.18 101.05 104.22 100.00	103.06 103.69 100.00	93.13 94.45 93.66	83.29 91.23 90.93	90.09 90.51 87.46	91.37 92.15 91.15 86.83 83.92 86.97	94.26 92.67 87.93
Mean % Recovery	102.6	102.3	93.7	88.5	89.4	88.7	91.6
SD, % Recovery	2.7×10^{0}	1.9×10^{0}	6.6×10^{-1}	4.5×10^{0}	1.6×10^{0}	3.2×10^{0}	3.2×10^{0}
% RSD, % Recovery	2.6	1.9	0.7	5.0	1.8	3.7	3.5
n	6	3	3	3	3	6	3

The mean accuracy values obtained meets the acceptance criteria of 80.0% - 120.0%. The individual accuracy requirement of 70.0% - 130.0% was also met for each individual preparation.

2.3.6.3 Accuracy Data for PGMMEA

Table 2.18 Accuracy Data for PGMMEA

Conc. ppm	**2**	**5**	**10**	**20**	**30**
Conc. ppm	1.93 1.90 1.91 1.94 1.88 1.70	4.15 4.52 4.46	8.77 8.79 8.49	17.61 17.66 17.48 16.77 16.18 16.75	27.32 26.91 25.45
Mean Conc. ppm	1.88	4.38	8.69	17.08	26.56
SD, Conc. ppm	8.8×10^{-2}	1.9×10^{-1}	1.6×10^{-1}	6.0×10^{-1}	9.8×10^{-1}
% RSD, Conc. ppm	4.7	4.5	1.9	3.5	3.6
% Recovery	99.58 98.35 98.50 100.46 97.26 87.93	85.64 93.23 92.02	90.41 90.65 87.55	90.81 91.07 90.11 86.48 83.40 86.38	93.88 92.48 87.47
Mean % Recovery	97.0	90.3	89.5	88.0	91.3
SD, % Recovery	4.5×10^{0}	4.0×10^{0}	1.7×10^{0}	3.0×10^{0}	3.3×10^{0}
% RSD, % Recovery	4.7	4.5	1.9	3.5	3.6
n	6	3	3	6	3

The mean accuracy values obtained meets the acceptance criteria of 80.0% - 120.0%. The individual accuracy requirement of 70.0% - 130.0% was also met for each individual preparation.

2.3.7 Linearity

2.3.7.1 Cyclohexanone Linearity

Table 2.19 Cyclohexanone Linearity Concentrations and Peak Areas

Concentration (ppm)	Peak Area	% Specification Limit
0.5	2.594	2.5
0.5	2.700	2.5
0.5	2.621	2.5
0.5	2.780	2.5
0.5	2.605	2.5
0.5	2.665	2.5
1.0	5.268	5
1.0	5.459	5
1.0	5.427	5
2.0	9.709	10
2.0	9.791	10
2.0	9.651	10
5.0	23.717	25
5.0	23.793	25
5.0	21.721	25
10.0	45.632	50
10.0	47.222	50
10.0	47.002	50
20.0	90.731	100
20.0	87.541	100
20.0	90.576	100
20.0	95.114	100
20.0	96.159	100
20.0	95.345	100
30.0	137.593	150
30.0	145.009	150
30.0	147.494	150

Table 2.20 Linearity for Cyclohexanone Data Summary

Parameter	Values
Correlation Coefficient, r	0.998
Y-intercept, Y_0	-0.028
Y at label strength, Y_{100}	92.6
% Y_0/Y_{100} = (Y_0/Y_{100}) x 100%	0.03
Slope, m	4.97
Regression Equation	y = 4.97(x) + (-0.028)

Linearity requirements for cyclohexanone have been established and meet the acceptance criteria of not more than (NMT) 2% for y intercept. See Figure 2.4 for the cyclohexanone linearity curve (Label Strength = 20 ppm).

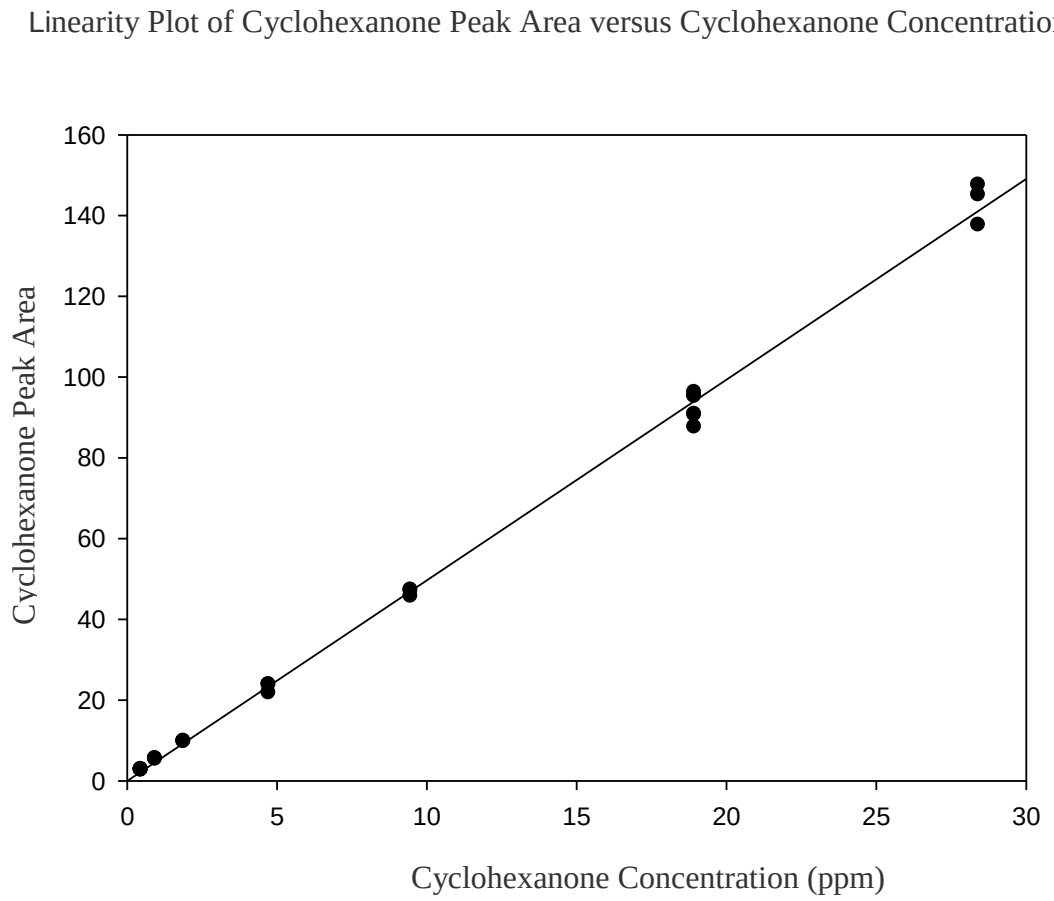

Figure 2.4: Linearity Plot of Cyclohexanone from 0.5 – 30.0 ppm, Equivalent to 2.5 – 150% of Cyclohexanone Product Specification

The graph in Figure 2.4 shows a best fit line for the average of each set of replicates. There are 6 replicates for the 0.5 and 20 ppm concentrations and 3 replicates for the rest.

2.3.7.2 PGMMEA Linearity

Table 2.21 PGMMEA Linearity Concentrations and Peak Areas

Concentration (ppm)	Peak Area	% Specification Limit
2.0	5.423	10
2.0	5.998	10
2.0	6.196	10
2.0	6.041	10
2.0	6.030	10
2.0	6.107	10
5.0	14.186	25
5.0	14.374	25
5.0	13.203	25
10.0	26.990	50
10.0	27.948	50
10.0	27.870	50
20.0	53.195	100
20.0	51.362	100
20.0	53.260	100
20.0	55.561	100
20.0	56.149	100
20.0	55.988	100
30.0	80.803	150
30.0	85.432	150
30.0	86.723	150

Table 2.22 Linearity for PGMMEA Data Summary

Parameter	Values
Correlation Coefficient, r	0.998
Y-intercept, Y_0	0.056
Y at label strength, Y_{100}	54.3
% Y_0/Y_{100} = (Y_0/Y_{100}) x 100%	0.10
Slope, m	2.85
Regression Equation	y = 2.85(x) + 0.056

Linearity requirements for PGMMEA have been established and meet the acceptance criteria of NMT 2% for y intercept. See Figure 2.5 for the PGMMEA linearity curve.

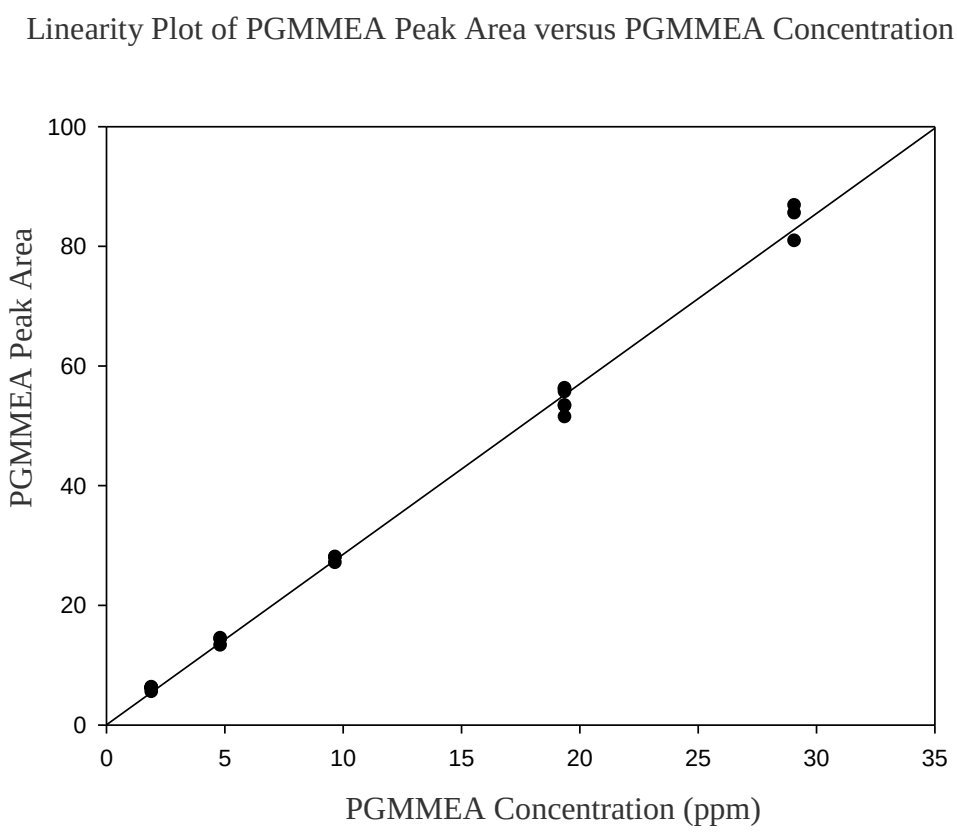

Figure 2.5: Linearity Plot of PGMMEA from 2.0 – 30.0 ppm, Equivalent to 10 – 150% of PGMMEA Product Specification

Similar to Figure 2.4, the graph in Figure 2.5 shows a best fit line for the average of each set of replicates. There are 6 replicates for the 2 and 20 ppm concentrations and 3 replicates for the remaining concentrations.

2.3.8 Precision (Repeatability and Intermediate Precision)

2.3.8.1 Cyclohexanone and PGMMEA Standard Precision (Repeatability)

Table 2.23 Cyclohexanone and PGMMEA Standard Precision Data

Parameter	Cyclohexanone Results	PGMMEA Results
Mean Peak Area	102.99	60.95
SD Peak Area	4.6×10^{-1}	2.3×10^{-1}
% RSD	0.4	0.3
Confidence interval	102.99 ± 0.48	60.95 ± 0.24
n	6	6

The % RSD meets the acceptance criterion of ≤ 5%.

2.3.8.2 Cyclohexanone, PGMMEA and PGMEEA Sample Precision (Repeatability)

Table 2.24 Cyclohexanone, PGMMEA and PGMEEA Sample Precision Data

Parameter	Cyclohexanone Results	PGMMEA Results	PGMEEA Results
Mean ppm	7.06	6.14	19.12
SD ppm	1.1×10^{-1}	7.6×10^{-2}	2.8×10^{-1}
% RSD	1.6	1.2	1.4
Confidence interval	7.06 ± 0.11	6.14 ± 0.07	19.12 ± 0.29
n	6	6	6

The % RSD meets the acceptance criterion of ≤ 5%.

2.3.8.3 Cyclohexanone, PGMMEA and PGMEEA Operator-A Day-1/Day-2 (Intermediate Precision)

Table 2.25 Cyclohexanone Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	7.06	6.68	7.74
SD ppm	1.1×10^{-1}	2.3×10^{-2}	3.5×10^{-1}
% RSD	1.6	0.3	4.5
Confidence interval	7.06 ± 0.11	6.68 ± 0.02	7.74 ± 0.36
n	6	6	6
Ratio of the means, operator-A day-2/day-1	0.9		
Ratio of the means, operator-B/operator-A day-1	1.1		

Table 2.26 PGMMEA Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	6.14	6.31	6.57
SD ppm	7.6×10^{-2}	5.3×10^{-2}	2.5×10^{-1}
% RSD	1.2	0.8	3.9
Confidence interval	6.14 ± 0.07	6.31 ± 0.05	6.57 ± 0.26
n	6	6	6
Ratio of the means, operator-A day-2/day-1	1.0		
Ratio of the means, operator-B/operator-A day-1	1.1		

Table 2.27 PGMEEA Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	19.12	19.80	22.68
SD ppm	2.8×10^{-1}	9.7×10^{-2}	1.0×10^{0}
% RSD	1.4	0.4	4.6
Confidence interval	19.12 ± 0.29	19.80 ± 0.10	22.68 ± 1.00
n	6	6	6
Ratio of the means, operator-A day-2/day-1	1.0		
Ratio of the means, operator-B/operator-A day-1	1.2		

The ratio of the means value (ppm) for cyclohexanone, PGMMEA and PGMEEA meets the acceptance criterion of between 0.7 and 1.3 for cyclohexanone, PGMMEA and PGMEEA intermediate precision. The % RSD values meet the acceptance criterion of % RSD ≤ 5.0 for n = 6.

2.3.9 Range

The range for cyclohexanone has been established from 0.5 ppm to 30.0 ppm which corresponds to 2.5 to 150% of the proposed product specification. The range for PGMMEA has been established from 2.0 ppm to 30.0 ppm which corresponds to 10 to 150% of the working standard concentration. This was achieved through measurements for accuracy, linearity and precision.

2.3.10 Detection Limit (DL)

DL Determination for Cyclohexanone and PGMMEA

Table 2.28 DL of Cyclohexanone and PGMMEA in Product Z

Parameter	Cyclohexanone Results	PGMMEA Results
Mean ppm	0.21	0.86
Mean S/N Ratio	4.9:1	9.3:1
% RSD of Peak Height	3.9	2.1
n	3	3

Based upon the data shown in Table 2.28, the DL for cyclohexanone is determined to be 0.2 ppm and the DL for PGMMEA is determined to be 0.9 ppm.

2.3.11 Quantitation Limit (QL)

QL Determination for Cyclohexanone and PGMMEA

Table 2.29 QL of Cyclohexanone and PGMMEA in Product Z

Parameter	Cyclohexanone Results	PGMMEA Results
Mean ppm	0.49	1.88
Mean % Recovery	102.6	97.0
Mean S/N Ratio	11.3:1	23.9:1
% RSD of Peak Height	2.9	4.3
n	6	6

Based upon the data shown in Table 2.29, the QL for cyclohexanone is determined to be 0.5 ppm and the QL for PGMMEA is determined to be 1.9 ppm.

Mean % recovery of both cyclohexanone and PGMMEA also meets the acceptance criteria of 100.0% ± 20.0%.

2.4 Conclusion

The concentration of container closure leachables are determined by diluting the samples 1:1 with acetonitrile and injecting onto the gas chromatographic DB-5 capillary column. Volatile and semi-volatile organic compounds are detected using a flame ionisation detector (FID). A temperature program is incorporated which allows the separation of potential volatile compounds from higher boiling compounds. The ability of the FID to detect virtually any volatile or semi-volatile organic compound with a carbon-hydrogen bond makes it ideal for this 'finger printing' comparison test which will detect a wide variety of potential organic leachables and is sensitive down to less than 1 ppm for common organic solvents. Cyclohexanone is quantitated and unknown leachables are estimated against cyclohexanone in the standard; PGMMEA is quantitated and PGMEEA is estimated against PGMMEA in the standard.

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United States Pharmacopeia 37 (2015) *General Chapters: <1225> Validation of Compendial Procedures.*

Chapter 3: Validation of a Headspace Gas Chromatography Method for the Analysis of Tampoprinting Leachates

3.1 Introduction

This method uses headspace gas chromatography to detect and quantitate the concentrations of the Tampoprint-related leachables cyclohexanone and propylene glycol monoethyl ether acetate (PGMEEA), and estimate propylene glycol monomethyl ether acetate (PGMMEA). It also estimates the concentration of other potential volatile and semi-volatile container closure leachable components by comparison of gas chromatographic (GC) profiles of ophthalmic solution samples stored in plastic container/closure systems versus identical samples stored in glass (or unlabelled plastic containers) as a control. Comparison of these two chromatographic 'finger prints' forms the basis of evaluating the presence of volatile and semi-volatile leachables. If gas chromatographic profiles are the same for test and control samples, it is strong evidence that no such leachables are present in the ophthalmic solution.

The Tampoprint-related leachables that are seen in Product X have not previously been tested for this product and thus a new method is required.

This method will be validated in accordance with the current USP Category II (United States Pharmacopeia 37 2015) for cyclohexanone, PGMMEA and PGMEEA. The validation will also meet all requirements under ICH guidelines Q2 (R1) (International Conference of Harmonisation 2005).

The proposed method validation tests that will be carried out on the specified leachables cyclohexanone and PGMMEA are: accuracy, linearity, range, precision (repeatability and intermediate), specificity, detection limit (DL), quantitation limit (QL), robustness and standard stability. As no PGMEEA standard is available, the validation tests that will be carried out on the specified leachable PGMEEA are: precision (repeatability and intermediate), robustness and specificity. Since there is no standard material available for PGMEEA and due to its structural similarity PGMMEA will be used to estimate PGMEEA in samples.

3.2 Experimental

3.2.1 Initial Development

After developing and validating the method for Product Z, the same method was trialled on Product X. However, the sample matrix was quite different and resulted in a lot of interference with the cyclohexanone and PGMMEA peaks. Accuracy results were also poor and it was decided to move away from the direct injection GC method and try a headspace (HS) GC method instead.

As the external toxicological assessment had previously found that each of the leachables were safe up to a limit of 20 ppm, standards were required to be prepared at a final working concentration of 20 ppm.

A particular headspace GC method that was used in the laboratory to test different leachables was trialled using cyclohexanone and PGMMEA standards. The GC conditions employed were an oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 5.0 mL/minute. The inlet was set at 250˚C with a split ratio of 1.0. The detector was set at 250˚C and the makeup gas was helium at 20 mL/minute. Hydrogen was used for the fuel flow at 45 mL/minute and air was used for the oxidiser flow at 450 mL/minute. The headspace sampler utilised a vial oven temperature of 80˚C, a loop temperature of 130˚C and a transfer line temperature of 140˚C. Agitation was set to high and vial pressurisation at 16 psi. Oven stabilisation was set at 1 minute, vial equilibration at 20 minutes, vial pressurisation at 0.4 minutes, loop fill at 0.2 minutes, loop equilibration at 0.2 minutes and sample inject at 0.5 minutes.

The baseline was a lot better with much less interference but peak shape was poor. The method utilised an Agilent DB-WAX column so it was changed for the DB5 column as that had previously shown good chromatography for the cyclohexanone and PGMMEA. The results were much improved with good peak shape and resolution so it was decided to proceed with the DB5 column.

3.2.2 Reagents

Analytical grade cyclohexanone and propylene glycol monomethyl ether acetate (PGMMEA) were purchased from Sigma Aldrich (Poole, UK). Tampoprint thinner, VDL-1015 was purchased from Tampoprint (Stuttgart, Germany).

Solvents used included acetonitrile (HPLC grade) which was purchased from Labscan (Dublin, Ireland) and dimethylacetamide purchased from VWR (Pennsylvania, USA). Ultrapure water was obtained from a Millipore Milli-Q water purification unit (Millipore, Bedford, MA, USA). High purity grade compressed air, hydrogen and helium were purchased from BOC (Surrey, UK).

3.2.3 Instrumentation and Apparatus

An Agilent 7890 GC system with FID, Agilent G1888 headspace sampler and Agilent DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness (part number 125-503J) were utilised for all testing. A deactivated fused silica tubing guard column (Phenomenex, part number 7CK-G000-00-GZK) was attached to the column. Graphite ferrules by Agilent were used to attach the column to the GC system (part number 5080-8773). 10 mL amber headspace screw top vials were from Agilent (part number 5188-6538). An Agilent glass liner was used for split operation, 4 mm, non-deactivated borosilicate glass (part number 19251-60540). The injector septa and o-ring were also from Agilent (part number 5183-4757 and 5188-5365 respectively). The GC utilised an oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 5.0 mL/minute. The inlet was set at 250˚C with a split ratio of 1.0. The detector was set at 250˚C and the makeup gas was helium at 20 mL/minute. Hydrogen was used for the fuel flow at 45 mL/minute and air was used for the oxidiser flow at 450 mL/minute. The headspace sampler utilised a vial oven temperature of 80˚C, a loop temperature of 130˚C and a transfer line temperature of 140˚C. Agitation was set to high and vial pressurisation at 16 psi. Oven stabilisation was set at 1 minute, vial equilibration at 20 minutes, vial pressurisation at 0.4 minutes, loop fill at 0.2 minutes, loop equilibration at 0.2 minutes and sample inject at 0.5 minutes.

Glassware was provided by Schott (Mainz, Germany) and consisted of 0.5 mL, 1 mL, 2 mL, 3 mL, and 4 mL volumetric pipettes (Class A) as well as 20 mL amber volumetric flasks (Class A). All glassware was washed in a Hamo LS2000 glass washer using RBS 50 pF detergent and RBS R 60 acid. Both the detergent and acid were purchased from Chemical Products R. Borghgraef S.A. (Brussels, Belgium).

A calibrated micro syringe capable of dispensing 6 µL was purchased from Agilent (part number 5181-3354).

3.2.4 Standard Preparation

A stock standard (SS) was prepared by pipetting 1 mL of cyclohexanone and 1 mL of PGMMEA into a 20 mL amber volumetric flask containing 4 mL of dimethylacetamide (as solvent). The flask was gently swirled and then brought to volume with deionised water and mixed using a magnetic stirrer for 15 minutes.

An intermediate standard was prepared by pipetting 4 mL of the stock standard solution into a 20 mL amber volumetric flask, containing approximately 10 mL of deionised water. The volumetric flask was brought to volume with deionised water and mixed using a magnetic stirrer for 15 minutes.

Working standards were prepared by transferring a portion of the intermediate standard solution into a 4 mL amber HPLC vial and capping. 3 mL of Product X was then pipetted into the headspace vial. Using a calibrated micro syringe, the septum of the HPLC vial was pierced and 6 µL of the intermediate standard solution removed and dispensed into the headspace vial containing 3 mL of Product X before capping immediately. The nominal concentration of the working standard solution was 18.94 ppm for cyclohexanone and 19.4 ppm for PGMMEA. A typical chromatogram of the working standard solution can be seen in Figure 3.1. Working standard concentrations are based on the cyclohexanone density of 0.947 g/mL and the PGMMEA density of 0.970 g/mL.

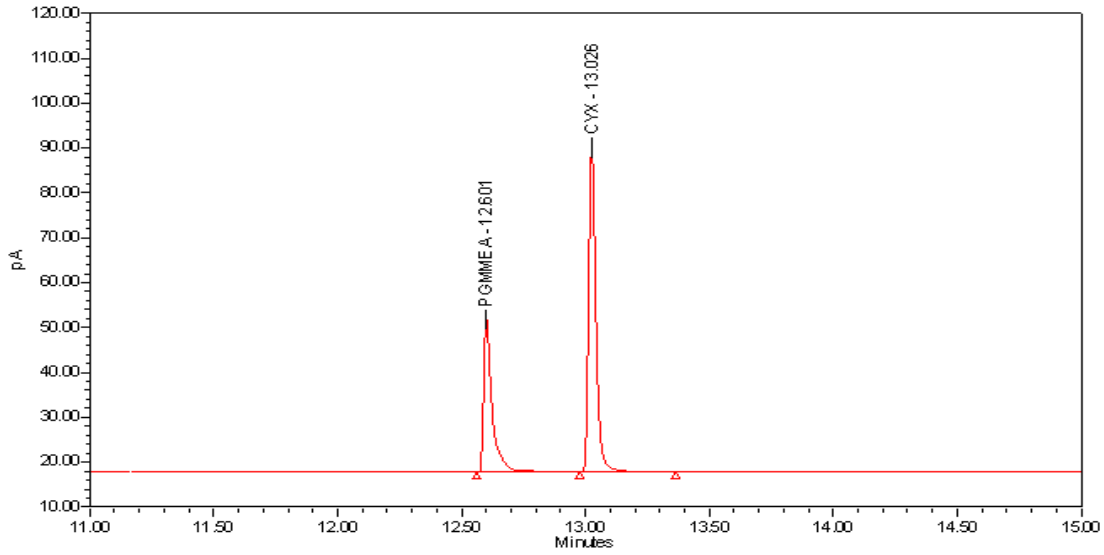


Figure 3.1: *GC-FID of WS (cyclohexanone 18.94 ppm; PGMMEA 19.40 ppm). GC Conditions: Agilent 7890 GC FID, Agilent G1888 headspace sampler, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), split injection with split ratio of 1.0, injection volume 1000µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 5.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (45:450). Vial oven temperature 80˚C, loop temperature 130˚C, transfer line temperature 140˚C. High agitation, vial pressurisation 16 psi, oven stabilisation 1 minute, vial equilibration 20 minutes, vial pressurisation 0.4 minutes, loop fill 0.2 minutes, loop equilibration 0.2 minutes, sample inject 0.5 minutes.*

A PGMMEA intermediate detector sensitivity solution (I-DSS) was prepared by transferring 2 mL of the intermediate standard solution into a 20 mL amber volumetric flask containing approximately 10 mL of deionised water and diluting to volume with deionised water. It was mixed using a magnetic stirrer for 15 minutes.

The PGMMEA detector sensitivity solution (DSS) was then prepared by transferring a portion of the PGMMEA intermediate detector sensitivity solution into a 4 mL amber HPLC vial and capping. 3 mL of Product X was then pipetted into a headspace vial. Using a calibrated micro syringe, the septum of the HPLC vial was pierced and 6 µL of the intermediate detector sensitivity solution removed and dispensed into the headspace vial containing 3 mL of Product X before capping immediately. The nominal PGMMEA concentration of the working DSS was 1.94 ppm.

A cyclohexanone intermediate detector sensitivity solution was prepared in a similar manner to the PGMMEA I-DSS using 0.5 mL of the intermediate standard solution rather than 2 mL.

A cyclohexanone detector sensitivity solution was prepared in the same way as the PGMMEA DSS. The nominal cyclohexanone concentration of the working DSS was 0.473 ppm.

A Tampoprint thinner stock solution was prepared by transferring 4 mL of dimethylacetamide into a 20 mL amber volumetric flask. 1 mL of the Tampoprint thinner solution was then pipetted into the same 20 mL amber volumetric flask. The flask was gently swirled and brought to volume with deionised water before mixing using a magnetic stirrer for 15 minutes.

A leachable identification stock solution (S-ID) was then prepared by transferring 4 mL of the tampoprint thinner stock solution into a 20 mL amber volumetric flask, containing approximately 10 mL of deionised water. The volumetric flask was brought to volume with deionised water and mixed using a magnetic stirrer for 15 minutes.

A leachable identification working solution (LIS) was then prepared by transferring a portion of the S-ID into a 4 mL amber HPLC vial and capping. 3 mL of Product X was then pipetted into a headspace vial. Using a calibrated micro syringe, the septum of the HPLC vial was pierced and 6 µL of the S-ID removed and dispensed into the headspace vial containing 3 mL of Product X before capping immediately. A typical chromatogram of the LIS can be seen in Figure 3.2.

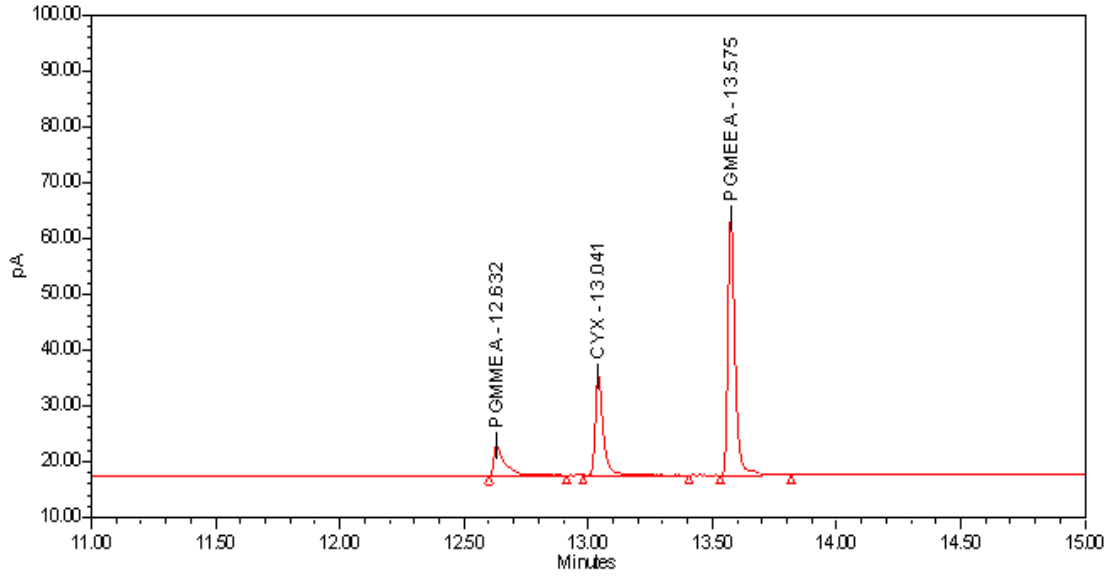


Figure 3.2: *GC-FID of LIS. GC Conditions: Agilent 7890 GC FID, Agilent G1888 headspace sampler, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), split injection with split ratio of 1.0, injection volume 1000µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 5.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (45:450). Vial oven temperature 80˚C, loop temperature 130˚C, transfer line temperature 140˚C. High agitation, vial pressurisation 16 psi, oven stabilisation 1 minute, vial equilibration 20 minutes, vial pressurisation 0.4 minutes, loop fill 0.2 minutes, loop equilibration 0.2 minutes, sample inject 0.5 minutes.*

Calibration controls were prepared by transferring 3 mL of Product X into a headspace vial and capping immediately. These controls were run due to the possibility of the presence of interference at the retention time of cyclohexanone or PGMMEA due to the sensitivity of the method. As standards are added to the sample the control allows for any standard peaks seen in the un-spiked sample to be calculated and taken away from the standard amount to give an accurate value.

Blanks (to capture possible laboratory contaminants) were prepared by capping empty headspace vials and were used to identify any system related peaks unrelated to the sample.

3.2.5 Sample Preparation

3.2.5.1 Product X Control Samples

Product X control samples are Product X samples stored in glass containers, glass ampoules, unlabelled bottles or bottles with blank labels depending on the nature of the leachable study. Product X control sample was pooled into a glass container from a number of glass ampoules before pipetting 3 mL of Product X control sample into headspace vials and capping immediately.

3.2.5.2 Product X Test Sample

Product X test sample was prepared in the same manner as the control sample by pooling sample from final product plastic bottles (with Tampoprint labelling) into a glass container. 3 mL of Product X sample was then pipetted into headspace vials and capped immediately. See Figure 3.3 for a typical chromatogram of a Product X control sample.

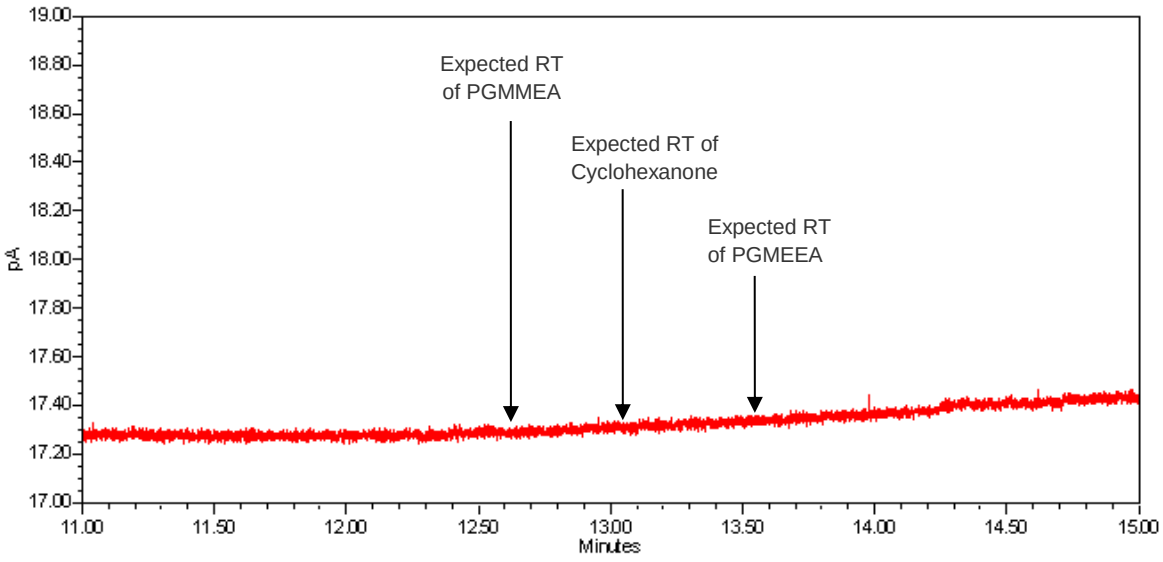


Figure 3.3: *GC-FID of Product X Control. GC Conditions: Agilent 7890 GC FID, Agilent G1888 headspace sampler, Agilent Capillary DB-5 column, 30 m x 0.53 mm x 1.0 µm film thickness, guard column (Phenomenex deactivated fused-silica tubing), split injection with split ratio of 1.0, injection volume 1000µL. The GC oven temperature programme starting at 35˚C for 8 minutes before ramping at 15˚C/minute and holding at 245˚C for 10 minutes. Total run time was 32 minutes with a constant flow of helium at 5.0 mL/minute. Injection temperature 250˚C, detector temperature 250˚C. Hydrogen: Air flame (45:450). Vial oven temperature 80˚C, loop temperature 130˚C, transfer line temperature 140˚C. High agitation, vial pressurisation 16 psi, oven stabilisation 1 minute, vial equilibration 20 minutes, vial pressurisation 0.4 minutes, loop fill 0.2 minutes, loop equilibration 0.2 minutes, sample inject 0.5 minutes.*

3.2.6 Development Testing

Before validation could be performed it was important to carry out a series of verification tests to ensure that no problems would be encountered during full validation. These tests were used to ascertain acceptance criteria for system suitability that would be required at the start of each validation test. Retention time, tailing factor, % RSDs, signal-to-noise values and resolution of the cyclohexanone and PGMMEA peaks in the working standard and DSS were tracked. The tests included altering a number of the GC conditions (robustness) as well as using different systems and different analysts on different days (intermediate precision). Mini accuracy and linearity tests were also run using spiked product to ensure that all the required levels could achieve acceptable results. The limit of detection and the limit of quantitation were also tested to confirm they could be achieved.

At the end of development all testing had achieved satisfactory results and full validation could then commence.

3.2.7 System Suitability

Prior to every test it was required to show that the GC system was capable of performing as required. An example of an injection sequence for determining system suitability is shown in Table 3.1.

Note: It was important to ensure that enough blank injections were made such that no carryover was observed in two blank injections prior to further injections.

Table 3.1 Example of System Suitability Injection Sequence

Sample	No. of Injections
Blank	Minimum of 2
LIS	1
Cyclohexanone DSS	1
PGMMEA DSS	1
Calibration Control	1
WS-1	5

The 5 separate injections of WS-1 were used to satisfy the system suitability requirements for retention times, tailing factors and peak area % RSD given in Table 3.2.

Each DSS was injected once. This injection of the DSS was used to satisfy the system suitability requirements for DSS signal-to-noise ratio for cyclohexanone and PGMMEA given in Table 3.2.

The analysis could not be carried out if the system suitability criteria listed in Table 3.2 could not be met. When system suitability criteria could not be met, appropriate corrective action had to be taken and the system suitability was repeated. Definitions of each term in Table 3.2 can be found in the current USP (United States Pharmacopeia 37 2015).

Table 3.2 System Suitability Criteria

System Suitability Parameter	PGMMEA	Cyclohexanone
Retention Time (WS-1) (n = 5)	12.5 ± 1.0 min	13.0 ± 1.0 min
Tailing Factor (WS-1) (n = 5)	≤ 2.0	≤ 2.0
% RSD for Peak Area (WS-1) (n = 5)	NMT 10%	NMT 10%
Resolution between Cyclohexanone and PGMMEA	NLT 5	
DSS Signal-to-Noise (n = 1)	NLT 20:1	NLT 10:1

3.2.8 Method Validation

The validation tests that were carried out on the specified leachables cyclohexanone and PGMMEA were: accuracy, linearity, range, precision (repeatability and intermediate), specificity, standard and sample stability, detection limit (DL), quantitation limit (QL), and robustness. As no PGMEEA standard was available, the validation tests that were carried out on the specified leachable PGMEEA were: precision (repeatability and intermediate), robustness and specificity. For this method unknown leachables are estimated using cyclohexanone. Since there was no standard material available for PGMEEA and due to its structural similarity to PGMMEA, PGMMEA was used to estimate PGMEEA in samples. The first test conducted was

specificity because if the method was not specific more development would have been required. Next, the standard and sample stability was conducted. This would enable standards to be used over a number of days during the rest of validation and thus would save on preparing fresh standards every day. Robustness was then carried out followed by the rest of the validation tests.

3.2.8.1 Specificity

To demonstrate the specificity of this method in regards to leachable interferences, Product X laboratory scale preparation was stressed using the following conditions.

- Adjusted the pH to approximately 2.5 with 5 N HCL and stored at ambient temperature in the dark.
- Adjusted the pH to approximately 11.5 with 5 N NaOH and stored at ambient temperature in the dark.
- Stored at a temperature of 70^{o}C in the dark
- Added 5 drops of 30% hydrogen peroxide to 60 mL of sample and stored at ambient temperature in the dark.
- Placed in clear glass and stored in an ICH compliant light chamber until ICH light stress guidelines have been met.

The pH adjusted, heat and hydrogen peroxide stressed samples were stored under ambient laboratory conditions and pulled at day-14 and then stored at 2 - 8ºC and protected from light until time of analysis. The light stressed samples were pulled once ICH guidelines had been met. Control samples were untreated and unstressed and then stored in the dark at 2 - 8°C. All control samples were from the same batch as the treated and stressed samples.

The samples were tested using the method to determine if there are any sample interferences at the retention times of cyclohexanone, PGMMEA and PGMEEA.

3.2.8.2 Stock Standard Stability

Stock standard solutions containing cyclohexanone at 1.0% (v/v) and PGMMEA at 1.0% (v/v) were prepared (in a similar manner as described in Section 3.2.4) in

amber glassware on day-0. These solutions were assayed in triplicate and stored under ambient laboratory temperature and lighting. Fresh working standards were prepared for each day of the study from the stock standards prepared on day-0. These standards were then re-assayed against freshly prepared working standards on day-2, day-3 and day-7 in order to determine the chemical stability of the stock standard solutions in amber glassware. A summary of the data is presented in Tables 3.4 and 3.5.

3.2.8.3 Intermediate Standard Stability

Separate intermediate standard solutions containing cyclohexanone and PGMMEA were prepared (in a similar manner as described in Section 3.2.4) in amber glassware from freshly prepared stock standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared working standards on day-2, day-3 and day-7 in order to determine the chemical stability of the intermediate standard solutions in amber glassware. A summary of the data is presented in Tables 3.6 and 3.7.

3.2.8.4 Working Standard Stability

Sufficient working standard solutions containing cyclohexanone and PGMMEA were prepared (in a similar manner as described in Section 3.2.4) in amber headspace vials from freshly prepared intermediate standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared working standards on day-2, day-3 and day-7 in order to determine the chemical stability of the working standard solutions in amber headspace vials. A summary of the data is presented in Tables 3.8 and 3.9.

3.2.8.5 Cyclohexanone Intermediate DSS Stability

Intermediate standard solutions were freshly prepared (in a similar manner as described in Section 3.2.4) in amber glassware. Separate cyclohexanone intermediate DSS solutions were prepared from these working standards and analysed in triplicate

on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared cyclohexanone intermediate DSS solutions on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the cyclohexanone intermediate DSS solution in amber glassware. A summary of the data is presented in Table 3.10.

3.2.8.6 PGMMEA Intermediate DSS Stability

Intermediate standard solutions were freshly prepared (in a similar manner as described in Section 3.2.4) in amber glassware. Separate PGMMEA intermediate DSS solutions were prepared from these working standards and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared PGMMEA intermediate DSS solutions on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the PGMMEA DSS solution in amber glassware. A summary of the data is presented in Table 3.11.

3.2.8.7 Cyclohexanone DSS Stability

Sufficient cyclohexanone DSS solutions were prepared (in a similar manner as described in Section 3.2.4) in amber headspace vials from a freshly prepared cyclohexanone intermediate DSS and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly prepared cyclohexanone DSS solutions on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the cyclohexanone DSS solution in amber headspace vials. A summary of the data is presented in Table 3.12.

3.2.8.8 PGMMEA Intermediate DSS Stability

Sufficient PGMMEA DSS solutions were prepared (in a similar manner as described in Section 3.2.4) in amber headspace vials from a freshly prepared PGMMEA intermediate DSS and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed against freshly

prepared PGMMEA DSS solutions on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the PGMMEA DSS solution in amber headspace vials. A summary of the data is presented in Table 3.13.

3.2.8.9 Leachable Identification Stock Solution Stability

Leachable identification stock solution was freshly prepared (in a similar manner as described in Section 3.2.4) in amber glassware in triplicate on day-0. These solutions were assayed in triplicate and stored under ambient laboratory temperature and lighting. Fresh working ID solutions were prepared for each day of the study from the stock ID solutions prepared on day-0. These working ID solutions were then re-assayed against freshly prepared working ID solutions on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the leachable identification stock solution in amber glassware.

3.2.8.10 Leachable Identification Working Solution Stability

Leachable identification working solutions were freshly prepared (in a similar manner as described in Section 3.2.4) in amber headspace vials and analysed in triplicate on day-0. These solutions were stored under ambient laboratory temperature and lighting and re-assayed on day-1, day-2, day-3 and day-7 in order to determine the chemical stability of the leachable identification working solution in amber glassware.

3.2.8.11 Sample Stability

Sample solutions of Product X lab scale preparation were freshly prepared (in a similar manner as described in Section 3.2.4) in amber headspace vials on day-0 by adding 3 mL of Product X to a vial and capping. Sufficient samples were prepared on day-0 to complete the stability study. These solutions were assayed in triplicate and stored under ambient laboratory temperature and lighting. Freshly prepared standards were used to reanalyse the test sample preparations after day-1, day-2, day-3 and day-7.

3.2.8.12 Robustness

Robustness is the reproducibility of the test results for cyclohexanone, PGMMEA and PGMEEA obtained by analysis of Product X product under deliberate variations of the nominal test conditions. Product X lab scale preparation was spiked with tampoprint thinner solution containing approximately 13 ppm cyclohexanone, 13 ppm PGMMEA and 18 ppm PGMEEA. The method ruggedness (robustness) validation is initially demonstrated from the intermediate precision data. Additional robustness studies were conducted by analysing the same spiked sample (n = 6) using different vial incubation temperatures, transfer line temperatures, final column oven temperatures and carrier gas flow rates as shown in Table 3.3.

Each test condition in Table 3.3 was then evaluated for:

All system suitability data: Report value
Retention time and relative retention time of PGMEEA
Mean cyclohexanone, PGMMEA and PGMEEA, ppm data (n = 6)
Standard deviation of cyclohexanone, PGMMEA and PGMEEA, ppm data (n = 6)
% RSD of cyclohexanone, PGMMEA, and PGMEEA, ppm data (n = 6)
Ratio of the means of cyclohexanone, PGMMEA and PGMEEA, ppm value versus nominal conditions (Test 1) where, X_1 = Test 1 and X_2 = Test 2 - 9

Table 3.3 Robustness Test Conditions

	Vial Oven Temperature (ºC)	**Transfer Line Temperature (ºC)**	**Final Column Oven Temperature (ºC)**	**Carrier Gas Flow (mL/min)**
Test 1 *	80	140	245	5.0
Test 2	**75**	140	245	5.0
Test 3	**85**	140	245	5.0
Test 4	80	**135**	245	5.0
Test 5	80	**145**	245	5.0
Test 6	80	140	**235**	5.0
Test 7	80	140	**255**	5.0
Test 8	80	140	245	**4.5**
Test 9	80	140	245	**5.5**

*Represents nominal analysis conditions of the method

The method robustness tests 1 - 9 given in Table 3.3 were conducted and the resultant data is provided in Tables 3.14 to 3.18. The mean cyclohexanone (ppm), PGMMEA (ppm) and PGMEEA (ppm) data from robustness test 1 was used to determine the ratio of the means results for robustness tests 2 – 9 in Tables 3.16 to 3.18.

3.2.8.13 Accuracy of Cyclohexanone

Product X lab scale preparation was spiked with cyclohexanone at approximately 0.5, 1, 2, 10, 20 and 30 ppm. These levels are equivalent to 2.5%, 5%, 10%, 25%, 50%, 100% and 150% of cyclohexanone proposed product specification, respectively. Three separate preparations were analysed for the 5%, 10%, 25%, 50% and 150% levels, whereas six separate preparations were analysed for the 2.5% and 100% levels. The ppm concentration of cyclohexanone was calculated for each preparation. The cyclohexanone accuracy results are reported in Table 3.19.

3.2.8.14 Accuracy of PGMMEA

Product X lab scale preparation was spiked with PGMMEA at approximately 2, 5, 10, 20 and 30 ppm PGMMEA. These accuracy levels are equivalent to 10%, 25%, 50%, 100%, and 150% of PGMMEA proposed product specification, respectively. Three separate preparations were analysed for the 25%, 50% and 150% levels, whereas six separate preparations were analysed for the 10% and 100% levels. The ppm concentration of PGMMEA was calculated for each preparation. The PGMMEA accuracy results are reported in Table 3.20.

3.2.8.15 Cyclohexanone Linearity

To determine linearity for cyclohexanone, a linearity curve was generated from 0.5 to 30.0 ppm cyclohexanone. This range is equivalent to approximately 2.5 to 150% of the cyclohexanone proposed product specification. Each concentration point was run in triplicate, except for the 2.5% and 100% target solution which were run 6 times (separate preparations). The y-intercept at the origin for peak area data is less than

5% of the y-intercept at label strength, indicating that a single point calibration can be used. Table 3.21 contains the linearity data. A summary of the data can be seen in Table 3.22.

3.2.8.16 PGMMEA Linearity

To determine linearity for PGMMEA, a linearity curve was generated from 2.0 to 30.0 ppm PGMMEA. This range is equivalent to approximately 10% to 150% of the PGMMEA proposed product specification. Each concentration point was run in triplicate, except for the 10% and 100% target solution which were run 6 times (separate preparations). The y-intercept at the origin for peak area data is less than 5% of the y-intercept at label strength (this is an in-house specification as regulatory agencies do not set a specification other than to report value), indicating that a single point calibration can be used. Table 3.23 contains the linearity data. A summary of the data can be seen in Table 3.24.

3.2.8.17 Cyclohexanone and PGMMEA Standard Precision (Repeatability)

Standard precision was determined by injecting 6 replicate injections of cyclohexanone and PGMMEA working standard. A summary of the data is shown in Table 3.25.

3.2.8.18 Cyclohexanone, PGMMEA and PGMEEA Sample Precision (Repeatability)

Sample precision was determined by injecting a Tampoprint thinner spiked Product X laboratory scale preparation containing approximately 13 ppm cyclohexanone, 13 ppm PGMMEA and 18 ppm PGMEEA. A summary of the data is shown in Table 3.26.

3.2.8.19 Cyclohexanone, PGMMEA and PGMEEA Operator-A Day-1/Day-2 (Intermediate Precision)

Six separate preparations of Product X, spiked with Tampoprint thinner solution containing approximately 13 ppm cyclohexanone and PGMMEA and 18 ppm PGMEEA were analysed and the amount of cyclohexanone, PGMMEA and PGMEEA (ppm) determined by operator-A. On day-2 (operator-A) analysed the same spiked sample to provide information concerning day-to-day precision. The same instrument and column were used on both days. A summary of the data is shown in Tables 3.27 to 3.29.

A second operator (operator-B) analysed the same spiked product to provide information concerning operator-to-operator precision. Operator B used a different instrument and different column lot number to operator A. A summary of the data is shown in Tables 3.27 to 3.29.

3.2.8.20 Range

The range is the interval between the lower and upper concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range was established through accuracy, linearity and precision.

3.2.8.21 DL Determination for Cyclohexanone and PGMMEA

The DL of cyclohexanone and the DL of PGMMEA in Product X were determined by preparing a single solution of Product X lab scale preparation spiked at 0.2 ppm with cyclohexanone and 1.0 ppm with PGMMEA. From this solution 3 separate preparations were analysed. A summary of DL data is given in Table 3.30.

3.2.8.22 QL Determination for Cyclohexanone and PGMMEA

The QL of cyclohexanone and the QL of PGMMEA in Product X were determined by preparing a single solution of Product X lab scale preparation spiked at 0.5 ppm

with cyclohexanone and 2.0 ppm with PGMMEA. From this solution 6 separate preparations were analysed. A summary of the QL data is given in Table 3.31.

3.2.9 Sample Analysis

Injections of the working standard (WS-1) were used for bracketing after every six samples.

3.3 Results and Discussion

3.3.1 Rationale for Procedure

This method has been validated for the quantitation of the specified Tampoprint leachables cyclohexanone, PGMMEA and PGMEEA. Results from all the validation tests are recorded in the following tables.

This method was validated to the current USP Category II (United States Pharmacopeia 37 2015) for cyclohexanone, PGMMEA and PGMEEA. The validation also complies with ICH Q2 (R1) (International Conference of Harmonisation 2005) for assay and impurities.

3.3.2 Specificity

There were no peaks observed at the retention times of PGMMEA and PGMEEA. There was a peak detected at the retention time of cyclohexanone in the stressed samples. However, this peak had a signal to noise of considerably less than 10:1 and therefore meets the acceptance criteria. No significant change in the chromatographic profile of the stressed samples was seen when compared with that of the control. The method is deemed to be specific for the determination of container closure leachables in Product X.

3.3.3 Standard Stability

3.3.3.1 Stock Standard Stability

Table 3.4 Cyclohexanone Stock Standard Stability – Amber Glassware

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean cyclohexanone ppm	18.58	19.20	19.11	19.09
SD ppm	5.4×10^{-1}	7.4×10^{-1}	2.8×10^{-1}	4.1×10^{-1}
% RSD ppm	2.9	3.8	1.4	2.1
n	3	3	3	3
Ratio of the means cyclohexanone ppm values (vs. day-0)	N/A	1.033	1.028	1.027

Table 3.5 PGMMEA Stock Standard Stability – Amber Glassware

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean PGMMEA ppm	18.97	19.95	19.89	19.92
SD ppm	5.5×10^{-1}	7.9×10^{-1}	3.5×10^{-1}	5.7×10^{-1}
% RSD ppm	2.9	3.9	1.7	2.9
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	1.051	1.048	1.050

The acceptance criterion for this study is that the ratio of mean values for cyclohexanone and PGMMEA for the stock standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the stock standard is stable for up to 7 days when stored in amber glassware.

3.3.3.2 Intermediate Standard Stability

Table 3.6 Cyclohexanone Intermediate Standard Stability – Amber Glassware

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean cyclohexanone ppm	18.58	18.64	18.16	18.90
SD ppm	5.4×10^{-1}	1.1×10^{0}	1.9×10^{-1}	8.2×10^{-1}
% RSD ppm	2.9	5.9	1.0	4.3
n	3	3	3	3
Ratio of the means cyclohexanone ppm values (vs. day-0)	N/A	1.003	0.977	1.017

Table 3.7 PGMMEA Intermediate Standard Stability – Amber Glassware

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean PGMMEA ppm	18.97	19.35	18.86	19.75
SD ppm	5.5×10^{-1}	1.1×10^{0}	2.3×10^{-1}	9.4×10^{-1}
% RSD ppm	2.9	6.0	1.2	4.8
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	1.020	0.994	1.041

The acceptance criterion for this study is that the ratio of mean values for the cyclohexanone and the PGMMEA for the intermediate standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the intermediate standard is stable for up to 7 days when stored in amber glassware.

3.3.3.3 Working Standard Stability

Table 3.8 Cyclohexanone Working Standard Stability – Amber Headspace Vials

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean cyclohexanone ppm	18.58	18.23	18.42	19.26
SD ppm	5.4×10^{-1}	4.7×10^{-1}	3.8×10^{-1}	7.5×10^{-1}
% RSD ppm	2.9	2.6	2.0	3.9
n	3	3	3	3
Ratio of the means cyclohexanone ppm values (vs. day-0)	N/A	0.981	0.991	1.036

Table 3.9 PGMMEA Working Standard Stability – Amber Headspace Vials

	Day-0	**Day-2**	**Day-3**	**Day-7**
Mean PGMMEA ppm	18.97	18.81	19.02	20.03
SD ppm	5.5×10^{-1}	5.5×10^{-1}	4.9×10^{-1}	7.6×10^{-1}
% RSD ppm	2.9	2.9	2.6	3.7
n	3	3	3	3
Ratio of the means PGMMEA ppm values (vs. day-0)	N/A	0.991	1.002	1.055

The acceptance criterion for this study is that the ratio of mean values for the cyclohexanone and the PGMMEA for the working standard data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the working standard is stable for up to 7 days when stored in amber headspace vials.

3.3.3.4 Cyclohexanone Intermediate DSS Stability

Table 3.10 Cyclohexanone Intermediate DSS Stability in Amber Glassware (n = 3)

	Day-0	**Day-1**	**Day-2**	**Day-3**	**Day-7**
Mean S/N DSS	23.3:1	24.5:1	26.8:1	29.1:1	18.9:1
S/N SD	6.0×10^{0}	6.1×10^{-1}	1.0×10^{0}	4.4×10^{0}	6.4×10^{-1}
S/N % RSD	25.9	2.4	4.0	15.2	3.4
Mean cyclohexanone peak area	3.92	3.20	3.37	3.66	3.37
SD peak area	1.5×10^{-1}	9.4×10^{-2}	1.8×10^{-1}	1.7×10^{-1}	1.7×10^{-1}
% RSD peak area	3.9	2.9	5.3	4.7	5.2
Mean % Recovery DSS	108.4	102.0	112.6	119.4	110.0
% Recovery SD	4.2×10^{0}	2.9×10^{0}	6.0×10^{0}	5.7×10^{0}	5.7×10^{0}
% Recovery % RSD	3.9	2.9	5.3	4.7	5.2
Ratio of the means % Recovery values (vs. day-0)	N/A	0.940	1.038	1.101	1.014

The acceptance criterion for this study is that the ratio of means for the cyclohexanone intermediate DSS data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the cyclohexanone intermediate DSS is stable for up to 7 days when stored in amber glassware.

3.3.3.5 PGMMEA Intermediate DSS Stability

Table 3.11 PGMMEA Intermediate DSS Stability in Amber Glassware (n = 3)

	Day-0	**Day-1**	**Day-2**	**Day-3**	**Day-7**
Mean S/N DSS	32.8:1	50.9:1	54.1:1	47.5:1	34.5:1
S/N SD	1.7×10^{0}	2.7×10^{0}	7.7×10^{0}	7.0×10^{-1}	5.7×10^{-1}
S/N % RSD	5.3	5.4	14.3	14.7	1.6
Mean PGMMEA peak area	7.61	6.27	6.60	6.58	6.63
SD peak area	3.0×10^{-1}	2.2×10^{-1}	8.0×10^{-2}	2.7×10^{-1}	1.3×10^{-1}
% RSD peak area	4.0	3.5	1.2	4.2	2.0
Mean % Recovery DSS	94.4	102.1	105.4	102.2	102.5
% Recovery SD	3.8×10^{0}	3.6×10^{0}	1.2×10^{0}	4.3×10^{0}	2.1×10^{0}
% Recovery % RSD	4.0	3.5	1.2	4.2	2.0
Ratio of the means % Recovery values (vs. day-0)	N/A	1.081	1.116	1.082	1.085

The acceptance criterion for this study is that the ratio of means for the intermediate PGMMEA DSS data remains within 0.7 and 1.3 throughout the testing period. Based on the data, the intermediate PGMMEA DSS is stable for up to 7 days when stored in amber glassware.

3.3.3.6 Cyclohexanone DSS Stability

Table 3.12 Cyclohexanone DSS Stability in Amber Headspace Vials (n = 3)

	Day-0	**Day-1**
Mean S/N DSS	23.3:1	13.9:1
S/N SD	6.0×10^{0}	1.4×10^{0}
S/N % RSD	25.9	3.9
Mean cyclohexanone peak area	3.922	4.130
SD peak area	1.5×10^{-1}	5.5×10^{-2}
% RSD peak area	3.9	1.3
Mean % Recovery DSS	108.41	131.60
% Recovery SD	4.2×10^{0}	1.7×10^{0}
% Recovery % RSD	3.9	1.3
Ratio of the means % Recovery values (vs. day-0)	N/A	1.213

The acceptance criterion for this study is that the ratio of means for the cyclohexanone DSS data remains within 0.7 and 1.3 throughout the testing period. However, the mean cyclohexanone DSS % recovery fails to meet the 80.0 – 120.0% recovery acceptance criterion. Based on the data, the cyclohexanone DSS must be prepared fresh on the day of analysis.

3.3.3.7 PGMMEA DSS Stability

Table 3.13 PGMMEA DSS Stability in Amber Headspace Vials (n = 3)

	Day-0	**Day-1**
Mean S/N DSS	32.8:1	78.8:1
S/N SD	1.7×10^{0}	5.4×10^{0}
S/N % RSD	5.3	6.9
Mean PGMMEA peak area	7.609	8.640
SD peak area	3.0×10^{-1}	5.7×10^{-1}
% RSD peak area	4.0	6.6
Mean % Recovery DSS	94.37	140.73
% Recovery SD	3.8×10^{0}	9.32×10^{0}
% Recovery % RSD	4.0	6.6
Ratio of the means % Recovery values (vs. day-0)	N/A	1.491

The acceptance criterion for this study is that the ratio of means for the PGMMEA DSS data remains within 0.7 and 1.3 throughout the testing period. However, the mean PGMMEA DSS % recovery fails to meet the 80.0 – 120.0% recovery acceptance criterion. The ratio of the means value of 1.5 also fails. Based on the data, the PGMMEA DSS must be prepared fresh on the day of analysis.

3.3.3.8 Leachable Identification Working Solution Stability

The acceptance criterion for this study is that there is no significant change in the chromatographic profile of the sample solution between day-0 and day-1, day-3 and day-7. No deterioration of chromatography or shift in retention time has occurred for the cyclohexanone, PGMMEA and PGMEEA peaks throughout the testing period. Based on the data, the leachable identification working solution is stable for up to 7 days when stored in amber glassware.

3.3.4 Sample Stability

The acceptance criterion for this study is that there is no significant change in the chromatographic profile of the sample solution between day-0 and day-1, day-2, day-3 and day-7. Sample degradation has not produced any additional peaks that are

visible on the baseline that could interfere with the detection of any potential leachables.

Based on test results, sample preparations of Product X are stable for 7 days when stored at ambient laboratory temperature and lighting in amber headspace vials.

3.3.5 Robustness

Table 3.14 Cyclohexanone Robustness System Suitability Data

	Retention Time Cyclohexanone (min)	Tailing Factor Cyclohexanone	Resolution between Cyclohexanone and PGMMEA	Signal-to-Noise Cyclohexanone	% RSD Cyclohexanone Peak Area
Acceptance Criteria	Report	Report	Report	Report	≤ 10%
Test 1 *	13.01	1.37	7.7	24.5:1	1.2
Test 2	13.00	1.38	7.4	14.6:1	0.6
Test 3	12.99	1.37	7.6	17.1:1	5.7
Test 4	12.98	1.34	7.7	17.5:1	2.4
Test 5	12.98	1.34	7.6	22.9:1	6.4
Test 6	12.97	1.35	7.6	17.2:1	3.7
Test 7	12.97	1.33	7.7	13.9:1	5.2
Test 8	13.27	1.27	7.8	15.1:1	2.1
Test 9	12.70	1.38	7.4	18.6:1	3.0

*Represents nominal analysis conditions of the method

Retention Time (t_R)

The time taken after injection for the analyte molecules to reach the detector. This dependent on the column flow rate, column capacity, extra column dead volume and the retardation factor of the molecules on the stationary phase.

Tailing Factor

A measure of how close a chromatographic peak is to a symmetrical shape. As a peak slopes the tailing factor increases.

$T_f = ac / 2ab$

where ac is the peak width at 5% of the peak height, and ab is the front half-width measured from the leading edge to a perpendicular dropped from the peak apex.

Resolution

A characteristic of the separation of two adjacent peaks. It may be expressed according to the equation:

$$RAB = 2\,\frac{|dR(B) - dR(A)|}{|w(B) + w(A)|}$$

where *R*AB is the resolution, *d*R(A) and *d*R(B) are the retention distances (time or volume) of each eluted component A and B, and *w*(A) and *w*(B) are the respective widths of each peak at its base.

Table 3.15 PGMMEA Robustness System Suitability Data

	Retention Time PGMMEA (min)	**Tailing Factor PGMMEA**	**% RSD PGMMEA Peak Area**	**Signal-to-Noise PGMMEA**
Acceptance Criteria	Report	Report	≤ 10%	Report
Test 1 *	12.58	2.21	1.2	24.7:1
Test 2	12.58	2.19	0.9	21.7:1
Test 3	12.56	2.13	5.7	27.6:1
Test 4	12.55	2.02	2.3	29.0:1
Test 5	12.55	2.02	6.9	28.4:1
Test 6	12.55	2.00	4.2	26.9:1
Test 7	12.53	1.92	5.3	26.6:1
Test 8	12.83	1.79	2.5	32.6:1
Test 9	12.29	2.03	2.9	31.5:1

*Represents nominal analysis conditions of the method

Table 3.16 Cyclohexanone Robustness Data

	Mean Cyclohexanone (ppm)	**SD Cyclohexanone (ppm)**	**% RSD Cyclohexanone (ppm)**	**Ratio of the Means Cyclohexanone**
Acceptance Criteria	Report	Report	≤ 10%	0.7 – 1.3
Test 1 *	13.85	4.1×10^{-1}	3.0	N/A
Test 2	14.18	3.9×10^{-1}	2.7	1.0
Test 3	14.79	9.1×10^{-1}	6.2	1.1
Test 4	13.31	2.1×10^{-1}	1.6	1.0
Test 5	15.08	5.0×10^{0}	3.3	1.1
Test 6	15.03	1.6×10^{-1}	1.0	1.1
Test 7	14.30	2.9×10^{-1}	2.0	1.0
Test 8	14.19	5.8×10^{-1}	4.1	1.0
Test 9	13.84	4.3×10^{-2}	3.1	1.0

*Represents nominal analysis conditions of the method

Table 3.17 PGMMEA Robustness Data

	Mean PGMMEA (ppm)	SD PGMMEA (ppm)	% RSD PGMMEA (ppm)	Ratio of the Means PGMMEA
Acceptance Criteria	Report	Report	≤ 10%	0.7 – 1.3
Test 1 *	13.31	4.5×10^{-1}	3.4	N/A
Test 2	13.60	3.8×10^{-1}	2.8	1.0
Test 3	14.24	9.7×10^{-1}	6.8	1.1
Test 4	12.78	1.7×10^{-1}	1.3	1.0
Test 5	14.64	5.3×10^{-1}	3.6	1.1
Test 6	13.92	2.0×10^{-1}	1.4	1.0
Test 7	13.69	2.7×10^{-1}	2.0	1.0
Test 8	13.69	6.4×10^{-1}	4.7	1.0
Test 9	13.26	4.7×10^{-2}	3.5	1.0

*Represents nominal analysis conditions of the method

Table 3.18 PGMEEA Robustness Data

	Retention Time PGMEEA (min)	Relative Retention Time PGMEEA	Mean PGMEEA (ppm)	SD PGMEEA (ppm)	% RSD PGMEEA (ppm)	Ratio of the Means PGMEEA
Acceptance Criteria	Report	Report	Report	Report	≤ 10%	0.7 – 1.3
Test 1 *	13.56	1.04	18.09	5.5×10^{-1}	3.0	N/A
Test 2	13.55	1.04	17.29	3.3×10^{-1}	1.9	1.0
Test 3	13.54	1.04	18.95	1.4×10^{0}	7.7	1.0
Test 4	13.54	1.04	16.95	6.2×10^{-1}	3.6	1.0
Test 5	13.53	1.04	19.53	6.7×10^{-1}	3.4	1.1
Test 6	13.53	1.03	19.65	5.4×10^{-1}	2.7	1.1
Test 7	13.53	1.04	18.28	3.9×10^{-1}	2.1	1.0
Test 8	13.80	1.03	18.40	9.1×10^{0}	5.3	1.0
Test 9	13.30	1.04	17.76	7.2×10^{0}	4.0	1.0

*Represents nominal analysis conditions of the method

Conclusion of Robustness Study

The data indicates that all System Suitability criteria were met for all challenges. The method is capable of withstanding:

- ± 5°C variation in vial oven temperature
- ± 5°C variation in transfer line temperature
- ± 10°C variation in final oven temperature
- ± 0.5 mL/min change in carrier gas flow

Overall, the method is considered robust and suitable for routine use where accurate and reliable quantitative data is required for cyclohexanone, PGMMEA, PGMEEA and other potential leachables.

The information derived from the precision section provides an additional measure of method ruggedness. Using different analysts, on different days and different columns will yield chromatography and quantitative cyclohexanone, PGMMEA and PGMEEA values that are very consistent. This demonstrates that the method is sufficiently rugged, reproducible and robust under conditions of routine laboratory usage.

3.3.6 Accuracy

3.3.6.1 Product X Placebo Interferences

Three replicate injections of Product X lab scale preparation showed no interferences with a S/N ratio greater than 10:1 at the location of the cyclohexanone, PGMMEA and PGMEEA peaks. This lack of placebo interference meets the acceptance criteria.

3.3.6.2 Accuracy Data for Cyclohexanone

Table 3.19 Accuracy Data for Cyclohexanone

Cyclohexanone Conc. ppm	**0.5**	**1**	**2**	**5**	**10**	**20**	**30**
Conc. ppm	0.48 0.50 0.53 0.47 0.45 0.48	0.94 0.93 0.99	1.87 1.85 1.89	4.58 4.73 4.75	9.44 9.05 8.64	20.02 19.24 18.93 18.39 19.06 19.09	29.72 29.21 28.30
Mean Conc. ppm	0.49	0.96	1.87	4.69	9.05	19.13	29.08
SD, Conc. ppm	2.5×10^{-2}	3.1×10^{-2}	1.8×10^{-2}	9.0×10^{-2}	4.0×10^{-1}	5.2×10^{-1}	7.1×10^{-1}
% RSD, Conc. ppm	5.1	3.2	0.9	1.9	4.4	2.4	2.4
% Recovery	102.42 105.59 112.14 100.73 96.30 102.21	99.36 98.73 104.75	98.94 98.04 99.94	96.87 100.04 100.33	99.71 95.58 91.23	105.71 101.59 99.98 97.09 100.65 100.83	104.62 102.83 99.63
Mean % Recovery	103.2	100.9	99.0	99.1	95.5	101.0	102.4
SD, % Recovery	5.3×10^{0}	3.3×10^{0}	9.5×10^{-1}	1.9×10^{0}	4.2×10^{0}	2.7×10^{0}	2.5×10^{0}
% RSD, % Recovery	5.1	3.2	0.9	1.9	4.4	2.7	2.4
n	6	3	3	3	3	6	3

The mean accuracy values obtained meets the acceptance criteria of 80.0% - 120.0%. The individual accuracy requirement of 70.0% - 130.0% was also met for each individual preparation.

3.3.6.3 Accuracy Data for PGMMEA

Table 3.20 Accuracy Data for PGMMEA

PGMMEA Conc. ppm	**2**	**5**	**10**	**20**	**30**
Conc. ppm	1.83 1.86 1.87 1.89 1.77 1.94	4.58 4.72 4.69	9.48 9.13 8.65	20.72 19.60 19.18 18.54 19.33 19.32	30.36 30.09 28.79
Mean Conc. ppm	1.86	4.67	9.09	19.45	29.75
SD, Conc. ppm	5.6×10^{-2}	7.3×10^{-2}	4.1×10^{-1}	7.1×10^{-1}	8.4×10^{-1}
% RSD, Conc. ppm	3.0	1.5	4.6	3.6	2.8
% Recovery	94.74 96.08 96.70 97.42 91.39 100.15	94.55 97.46 96.74	97.76 94.13 89.17	106.81 101.06 98.90 95.57 99.64 99.59	104.33 103.43 98.93
Mean % Recovery	96.1	96.3	93.7	100.3	102.2
SD, % Recovery	2.9×10^{0}	1.5×10^{0}	4.3×10^{0}	3.6×10^{0}	2.8×10^{0}
% RSD, % Recovery	3.0	1.5	4.6	3.6	2.8
n	6	3	3	6	3

The mean accuracy values obtained meets the acceptance criteria of 80.0% - 120.0%. The individual accuracy requirement of 70.0% - 130.0% was also met for each individual preparation.

3.3.7 Linearity

3.3.7.1 Cyclohexanone Linearity

Table 3.21 Cyclohexanone Linearity Concentrations and Peak Areas

Concentration (ppm)	Peak Area	% Specification Limit
0.5	3.21	2.5
0.5	3.02	2.5
0.5	3.16	2.5
0.5	3.52	2.5
0.5	3.32	2.5
0.5	3.21	2.5
1.0	6.41	5
1.0	6.03	5
1.0	6.08	5
2.0	13.01	10
2.0	12.76	10
2.0	12.87	10
5.0	34.23	25
5.0	34.13	25
5.0	33.06	25
10.0	62.26	50
10.0	65.23	50
10.0	68.05	50
20.0	134.60	100
20.0	134.36	100
20.0	129.60	100
20.0	133.45	100
20.0	135.61	100
20.0	141.10	100
30.0	192.59	150
30.0	198.78	150
30.0	202.25	150

Table 3.22 Linearity for Cyclohexanone Data Summary

Parameter	Values
Correlation Coefficient, r	0.999
Y-intercept, Y_0	-0.088
Y at label strength, Y_{100}	135
% Y_0/Y_{100} = (Y_0/Y_{100}) x 100%	0.06
Slope, m	6.66
Residual Sum of Squares	170
Regression Equation	y = 6.66(x) + (-0.088)

Linearity requirements for cyclohexanone have been established and meet the acceptance criteria of NMT 2% for y intercept.

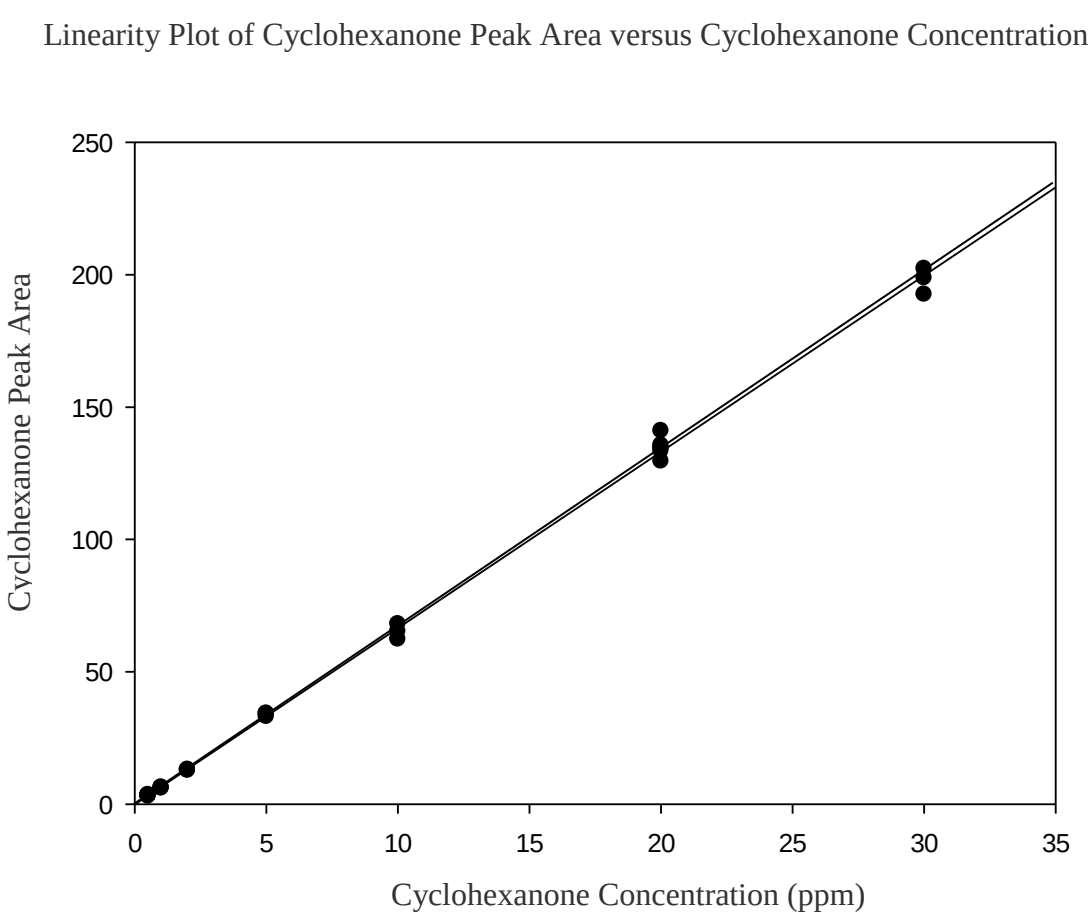


Figure 3.4: Linearity Plot of Cyclohexanone from 0.5 – 30.0 ppm, Equivalent to 2.5 – 150% of Cyclohexanone Product Specification

The graph in Figure 3.4 shows a best fit line for the average of each set of replicates. There are 6 replicates for the 0.5 and 20 ppm concentrations and 3 replicates for the remaining concentrations.

3.3.7.2 PGMMEA Linearity

Table 3.23 PGMMEA Linearity Concentrations and Peak Areas

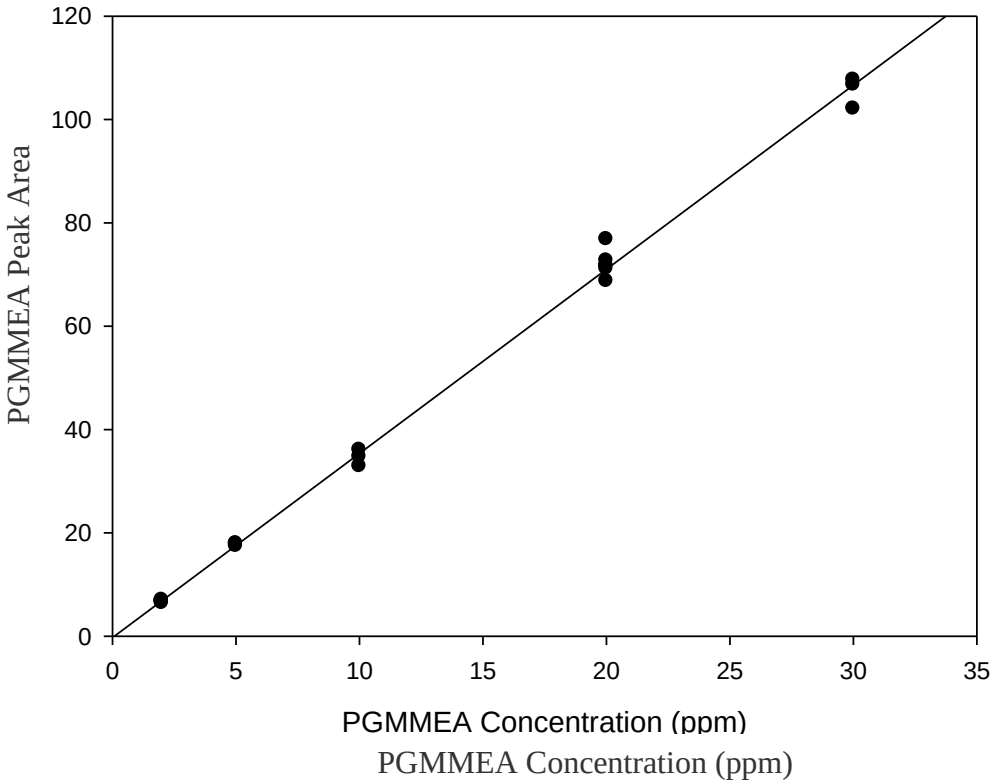
Concentration (ppm)	Peak Area	% Specification Limit
2.0	6.98	10
2.0	6.37	10
2.0	6.79	10
2.0	6.74	10
2.0	6.70	10
2.0	6.61	10
5.0	17.82	25
5.0	17.95	25
5.0	17.42	25
10.0	32.86	50
10.0	34.69	50
10.0	36.02	50
20.0	71.61	100
20.0	71.65	100
20.0	68.72	100
20.0	71.11	100
20.0	72.67	100
20.0	76.80	100
30.0	102.07	150
30.0	106.71	150
30.0	107.64	150

Table 3.24 Linearity for PGMMEA Data Summary

Parameter	Values
Correlation Coefficient, r	0.998
Y-intercept, Y_0	-0.265
Y at label strength, Y_{100}	72.1
% Y_0/Y_{100} = (Y_0/Y_{100}) x 100%	0.36
Slope, m	3.56
Residual Sum of Squares	72.1
Regression Equation	y = 3.56(x) + (-0.265)

Linearity requirements for PGMMEA have been established and meet the acceptance criteria of NMT 2% for y intercept.

Linearity Plot of PGMMEA Peak Area versus PGMMEA Concentration (ppm)



PGMMEA Concentration (ppm)

Figure 3.5: Linearity Plot of PGMMEA from 2.0 – 30.0 ppm, Equivalent to 10 – 150% of PGMMEA Product Specification

The graph in Figure 3.5 shows a best fit line for the average of each set of replicates. There are 6 replicates for the 2.0 and 20 ppm concentrations and 3 replicates for the remaining concentrations.

3.3.8 Precision (Repeatability and Intermediate Precision)

3.3.8.1 Cyclohexanone and PGMMEA Standard Precision (Repeatability)

Table 3.25 Cyclohexanone and PGMMEA Standard Precision Data

Parameter	Cyclohexanone Results	PGMMEA Results
Mean peak area	150.72	82.62
SD peak area	6.0	3.6
% RSD peak area	4.0	4.3
n	6	6
Confidence interval	150.72 ± 6.3	82.62 ± 3.7

The % RSD meets the acceptance criterion of ≤ 10%.

3.3.8.2 Cyclohexanone, PGMMEA and PGMEEA Sample Precision (Repeatability)

Table 3.26 Cyclohexanone, PGMMEA and PGMEEA Sample Precision Data

Parameter	Cyclohexanone Results	PGMMEA Results	PGMEEA Results
Mean ppm	14.43	13.82	18.12
SD ppm	4.1×10^{-1}	4.3×10^{-1}	5.9×10^{-1}
% RSD ppm	2.9	3.1	3.2
n	6	6	6
Confidence interval	14.43 ± 0.43	13.82 ± 0.45	18.12 ± 0.62

The % RSD meets the acceptance criterion of ≤ 10%.

3.3.8.3 Cyclohexanone, PGMMEA and PGMEEA Operator-A Day-1/Day-2 (Intermediate Precision)

Table 3.27 Cyclohexanone Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	14.43	13.85	15.11
SD ppm	4.1×10^{-1}	4.1×10^{-1}	7.7×10^{-1}
% RSD ppm	2.9	3.0	5.1
n	6	6	6
Confidence interval	14.43 ± 0.43	13.85 ± 0.43	15.11 ± 0.81
Ratio of the means, operator-A day-2/day-1	1.0		
Ratio of the means, operator-B/operator-A day-1	1.0		

Table 3.28 PGMMEA Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	13.82	13.31	14.13
SD ppm	4.3×10^{-1}	4.5×10^{-1}	7.3×10^{-1}
% RSD ppm	3.1	3.4	5.2
n	6	6	6
Confidence interval	13.82 ± 0.45	13.31 ± 0.47	14.13 ± 0.77
Ratio of the means, operator-A day-2/day-1	1.0		
Ratio of the means, operator-B/operator-A day-1	1.0		

Table 3.29 PGMEEA Day-to-Day Intermediate Precision Data

Parameter	Operator-A Day-1	Operator-A Day-2	Operator-B
Mean ppm	18.12	18.09	18.37
SD	5.9×10^{-1}	5.5×10^{-1}	6.4×10^{-1}
% RSD	3.2	3.0	3.4
n	6	6	6
Confidence interval	18.12 ± 0.62	18.09 ± 0.58	18.37 ± 0.67
Ratio of the means, operator-A day-2/day-1	1.0		
Ratio of the means, operator-B/operator-A day-1	1.0		

The ratio of the means value (ppm) for cyclohexanone, PGMMEA and PGMEEA meets the acceptance criterion of between 0.7 and 1.3 for cyclohexanone, PGMMEA and PGMEEA intermediate precision. The % RSD values meet the acceptance criterion of % RSD ≤ 10.0 for n = 6.

3.3.9 Range

The range for cyclohexanone has been established from 0.5 ppm to 30.0 ppm which corresponds to 2.5 to 150% of the proposed product specification. The range for PGMMEA has been established from 2.0 ppm to 30.0 ppm which corresponds to 10 to 150% of the working standard concentration. This was achieved through measurements for accuracy, linearity and precision.

3.3.10 Detection Limit (DL)

DL Determination for Cyclohexanone and PGMMEA

Table 3.30 DL of Cyclohexanone and PGMMEA in Product X

Parameter	Cyclohexanone Results	PGMMEA Results
Mean ppm	0.23	0.92
Mean S/N Ratio	6.3:1	15.5:1
% RSD of Peak Height	8.2	5.7
n	3	3

Based upon the data shown in Table 3.30, the DL for cyclohexanone is determined to be 0.2 ppm and the DL for PGMMEA is determined to be 1.0 ppm.

3.3.11 Quantitation Limit (QL)

QL Determination for Cyclohexanone and PGMMEA

Table 3.31 QL of Cyclohexanone and PGMMEA in Product X

Parameter	Cyclohexanone Results	PGMMEA Results
Mean ppm	0.49	1.86
Mean % Recovery	103.2	96.1
Mean S/N Ratio	18.1:1	38.4:1
% RSD of Peak Height	7.4	3.5
n	6	6

Based upon the data shown in Table 3.31, the QL for cyclohexanone is determined to be 0.5 ppm and the QL for PGMMEA is determined to be 1.9 ppm.

Mean % recovery of both cyclohexanone and PGMMEA also meets the acceptance criteria of 100.0% ± 20.0%.

3.4 Conclusion

The concentration of container closure leachables are determined by pipetting 3 mL of sample into a headspace vial, capping and injecting a portion of the headspace onto the gas chromatographic DB-5 capillary column. Volatile and semi-volatile organic compounds are detected using a flame ionisation detector (FID). A temperature program is incorporated which allows the separation of potential volatile compounds from higher boiling compounds. The ability of the FID to detect virtually any volatile or semi-volatile organic compound with a carbon-hydrogen bond makes it ideal for this 'finger printing' comparison test which will detect a wide variety of potential organic leachables and is sensitive down to less than 1 ppm for common organic solvents. The method employs a standard additions technique, whereby, working standards are prepared by directly spiking cyclohexanone and propylene glycol monomethyl ether acetate solution into 3 mL of sample. This technique requires the use of a 'calibration control' which is product injected neat to account for any interference that may occur at the retention time of cyclohexanone or PGMMEA in the working standards.

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Chapter 4: Final Conclusions

4.1 Discussion of Results

Two GC methods were developed and validated for the quantitation of three Tampoprint related leachables in two separate ophthalmic formulations with the ability to detect other volatiles that may turn up during the shelf life of each product. Chapter 2 sets out a method utilising direct injection GC while chapter 3 describes a method that employs HS-GC.

Direct injection GC has until recently been the only method used within Allergan for detection of volatile leachables in their products. Recently though HS-GC was also introduced with some of the more complex sample matrices making use of the technique. However, as there is more knowledge and experience of the direct injection GC it is normal to develop new methods using the direct injection GC technique. Product Z has a rather simple sample matrix and therefore the direct injection GC was capable of producing good chromatography with a clear baseline. Product X on the other hand had a much more complex sample matrix which produced a lot of baseline interference and unsatisfactory resolution of the cyclohexanone, PGMMEA and PGMEEA. It was decided to test Product X using HS-GC which gave a much improved baseline and no interference of the aforementioned leachables. A polar Agilent DB-WAX column with a polyethylene glycol stationary phase was initially used with the HS-GC but poor peak shape was noted. The non-polar DB5 column (5% phenyl 95% dimethylpolysiloxane stationary phase) was trialled and produced much better chromatography with good sharp peaks for each leachable.

Each method was validated in accordance with the current USP and ICH guidelines. These guidelines are intended to provide direction on how to accomplish validation of an analytical procedure and to demonstrate that it is fit for its intended purpose. The validation characteristics required for quantitative testing of impurities in these methods were accuracy, precision, specificity, detection limit, quantitation limit, linearity, range and robustness. System suitability parameters had to be established along with the stability of the analytical solutions to ensure that the validity of the analytical procedure is maintained whenever used.

One of the main differences between the two methods is how the standards are prepared. For direct injection the working standard is made up in a volumetric and transferred to GC vials for testing. Up to three injections can be taken from each vial meaning % RSD values tend to be quite low in comparison to the HS-GC method which incorporates a standards addition technique. This involves adding the standard to a specified aliquot of sample within a HS-GC vial. Only one injection can be made from each vial due to the loss of volatiles in the headspace after piercing the vial lid. This means that each working standard has to be individually prepared and thus creates higher % RSD results. For this reason the % RSD acceptance criteria for direct injection GC is 5% but for HS-GC it is 10%.

Overall, the direct injection GC method gave better repeatability to that of the HS-GC method. This is best illustrated when comparing the % RSDs from the system suitability results during robustness testing. Tables 2.12 and 2.13 show results for cyclohexanone and PGMMEA respectively for the direct injection GC method with % RSDs consistently below 1%. Tables 3.14 and 3.15 illustrate very inconsistent % RSDs for the HS-GC ranging from below 1% to nearly 7%. Due to the standard additions technique the HS-GC method is considerably more time consuming and also uses a lot more sample per analysis with 3 mL required for every standard preparation. Additionally, HS-GC vials are substantially more expensive than direct injection GC vials. For these reasons the direct injection GC method is therefore regarded as the preferred technique to test for volatiles within Allergan. However, the HS-GC method gave very good accuracy results as can be seen by the mean % recovery results in tables 3.19 and 3.20. Cyclohexanone and PGMMEA results were less than 100 ± 7% which is significantly better than those gained by the direct injection GC method. Tables 2.17 and 2.18 show that the mean % recovery results for cyclohexanone and PGMMEA were 100 ± 12% across the range of levels for the direct injection GC method. Furthermore, HS-GC gave a much better baseline as most of the sample matrix is not injected onto the column. Over time this also improves the longevity of the column and the GC system. It is therefore the preferable option for testing of volatiles within formulations that have a complex sample matrix.

4.2 Future Work

Future work with HS-GC may involve investigating the option of multiple injections from HS-GC vials. The option is available on some HS-GC models and would mean less individual standards needing to be prepared hence improving repeatability, reducing preparation downtime and decreasing the amount of sample required. Another option is to increase the sensitivity of the HS-GC method. If peak areas can be enlarged repeatability will usually be improved and give more accurate and reliable results. This may be achieved by investigating the different operating parameters such as the vial oven temperature, time and different columns. The addition of a salt to the standard preparation could be investigated as this can help to decrease the solubility of organic volatiles in the sample matrix and promote their transfer into the headspace thus producing larger responses.

Future work on direct injection GC may include exploring sample extractions for some of the more complex sample matrices. This could result in less sample matrix being injected onto the column and therefore give a better baseline.

Due to limited time, standard stability was only tested for up to eight days for the direct injection GC method and seven days for the HS-GC method. It is thought that stability of standards for each method could also be increased as part of future work.