Industrial Multi-Shot Gin Manufacture

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Abstract

This study investigates whether a concentrated gin 'hotshot' could be developed to increase production efficiency and still output. This concentrated 'hotshot' would also decrease transport costs and be later diluted to final strength. This 'hotshot' must have the same characteristics as the original gin once diluted with water and ethanol but, after distillation should be far more concentrated in flavour compounds. Recent literature published at the end of this study call this technique multi-shot gin. Since this was found at the end of this project the title and aim were renamed from gin 'hotshot' to 'multi-shot' gin to match industry standard.

There are many different gas chromatography (GC) methods to analyse the volatile organic compounds (VOC) present in gin samples. An analytical method was developed to analyse the VOC via gas chromatography – flame ionization detector (GC-FID) and gas chromatography – mass spectrometry (GC-MS). This method utilised both internal and external standardisation techniques to determine the concentration of the key analytes within the gin.

Out of the tested solvents, chloroform, hexane, dichloromethane and dimethyl sulfoxide it was found that hexane produced the highest extraction yield for the key analytes of interest. The highest yield was found using 1 mL of hexane to 3 mL of gin sample and extracted by gently agitating for 17 hours. GC-MS analysis was performed to identify 28 total volatile organic compounds within the gin. The key compounds of interest were found to be α-pinene, β-myrcene, limonene, γ-terpinene, and citronellal and their concentration in the original gin specimen was found to be 116 ± 5 mg/L, 57 ± 3 mg/L, 18 ± 1 mg/L, 8 ± 1 mg/L and 23 ± 1 mg/L respectively.

During gin manufacture the complex flavour extraction process can be broken down into several smaller micro-processes that were focused on in this study: maceration, macerate distillation, condensed vapour percolation and vapour infusion. Analyte extraction parameters were investigated and as the botanical ratio increased, the concentration of the analytes in the distillate increased but with gradually declining effectiveness for maceration and the condensed vapour percolation. Vapour infusion had not yet reached the threshold of declining returns.

The results from the research conducted for this project show that a concentrated 'hotshot' is possible. Vapour infusion was found to be the most efficient extraction process and can be combined with macerate distillation for a hybrid method to maximise analyte extraction.

Keywords: Gin; Gas chromatography; Volatile organic compounds; Multi-shot; Distillation

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Table of Contents

Abstract i

Acknowledgments ii

Table of Contents iii

List of Figures vii

List of Tables ix

List of Abbreviations xiv

Chapter 1: Introduction 1

1.1 Project Overview 1

1.2 Research Aim 2

1.2.1 Research Questions 2

1.2.2 Specific Objectives 3

1.3 Implications of Recent Literature 3

1.4 Thesis Structure 4

Chapter 2: Literature Review 5

2.1 Introduction 5

2.2 Background of Gin 5

2.3 Gin Manufacture 7

2.3.1 Neutral Spirit 7

2.3.2 Maceration 8

2.3.3 Vapour Infusion 8

2.3.4 Vacuum Distillation 9

2.3.5 Low or No Alcohol Gin 9

2.4 Distillation 10

2.4.1 Gin Distillation Process 10

2.4.2 Still Design 12

2.4.3 Cuts 14

2.5 Volatile Organic Compounds Present in Gin 15
2.5.1 Odour Threshold 17
2.6 Volatile Organic Compound Extraction 18
2.6.1 Maceration 18
2.6.2 Hydro-Distillation 19
2.6.3 Vapour/Steam Distillation 21
2.7 Analysis 22
2.7.1 Ethanol and Water 23
2.7.2 Volatile Compounds 23
2.8 Partition Coefficient 28
2.9 Sample Preparation 29
2.9.1 Water Removal 29
2.9.2 Liquid-Liquid Extraction 30
2.9.3 Blanks and Samples 31
2.10 Calibration of System 31
2.10.1 External Standardisation 31
2.10.2 Internal Standardisation 32
2.10.3 Kovats Retention Index 33
2.11 Conclusions and Research Gaps 34
2.12 Coda to Literature Review: Emergence of Recent Work on “Multi-Shot Gin” 35
Chapter 3: Key Analyte Determination and Calibration of Equipment 36
3.1 Introduction 36
3.2 Gas Chromatography – Mass Spectrometry Method Development 36
3.2.1 Gas Chromatography-Mass Spectrometry 36
3.2.2 Gas Chromatography – Flame Ionization Detector 38
3.3 Determination of Key Analytes 40
3.3.1 Materials and Methods 40
3.4 Internal Standard 41

3.5 Calibration 42
3.5.1 External and Internal Standardisation 42
3.6 Key Analyte Concentration in Gin Specimen 46
3.6.1 Error 47
3.7 Conclusions 48
Chapter 4: Gin Sample Preparation and Gas Chromatography Method Development 49
4.1 Introduction 49
4.2 Sample Preparation 49
4.2.1 Possible Approaches 49
4.2.2 Liquid-Liquid Extraction Investigation 50
4.2.3 Optimal Extraction Method for Sample Preparation 58
4.3 Conclusions 58
Chapter 5: Maceration and Distillation Investigation 60
5.1 Introduction 60
5.2 Ethanol Contamination 60
5.3 Maceration 61
5.3.1 Materials and Methods 61
5.3.2 Results and Discussion 63
5.4 Macerate Distillation 70
5.4.1 Materials and Methods 70
5.4.2 Results and Discussion 72
5.5 Condensed Vapour Percolation 74
5.5.1 Materials and Methods 75
5.5.2 Results and Discussion 78
5.6 Vapour Infusion 80
5.6.1 Materials and Methods 80
5.6.2 Results and Discussion 83
5.7 Ethanol Concentration and Distillate Volume 87

5.7.1 Materials and Methods 87
5.7.2 Results and Discussion 88
5.8 Analyte Extraction Effectiveness 89
5.8.1 Materials and Methods 90
5.8.2 Results and Discussion 90
5.9 Conclusion 92
Chapter 6: Conclusions and Recommended Further Research 93
6.1 Conclusions 93
6.2 Recommendations for Future Work 94
References 96
Appendices 108
Appendix A 108
A.1.1 Composition of Essential Oil from Botanicals 108
A.1.2 Chemical Properties 112
A.1.3 GC-MS Compound Identification Data 113
A.1.4 External Standard Calibration 114
A.1.5 Internal Standard Calibration 120
A.1.6 Concentration of Analytes in Gin 121
A.1.7 Ethanol Contamination 122
A.1.8 Maceration 122
A.1.9 Macerate Distillation 127
A.1.10 Condensed Vapour Percolation Extraction 130
A.1.11 Vapour Infusion 133
A.1.12 Process Analyte Extraction Effectiveness 137
A.1.13 Moisture content 138

List of Figures

Figure 1: This is a simple pot still diagram that shows 8 possible components. Pot stills vary greatly in design, and some will not share the same components or even look alike. 12
Figure 2: Simplistic column still schematic showing an example with copper chip packing within the column, due to the copper chips more distillations can be achieved in one pass leading to a purer more concentrated spirit (image from Veitch, F. P., 1911). 14
Figure 3: Simplistic line diagram of gas chromatograph. The carrier gas passes through a flow controller and into the column within the GC oven. The sample is injected into the column and the different compounds elute and are registered by the detector which converts the data into a chromatograph (image by Offnfopt, 2015). 24
Figure 4: Simplified diagram of a GC-MS machine showing the pathway of the carrier gas. The sample will be injected into the column. A computer will be connected to the mass analyser which will be used to analyse the data and identify the compounds (image by Kkmurray, 2018). 26
Figure 5: The two different time-temperature programmes; short: 23 minutes and long: 43.5 minutes long. The longer method was more successful at eluting a greater number of compounds for identification. 37
Figure 6: The time-temperature programme used for GC-FID analysis. It was adapted from the method used for GC-MS analysis, by increasing the temperature ramp and reducing the time. 39
Figure 7: Triplicate injections of a single combined standard overlayed on each other. The first peak is the solvent hexane, and the following six peaks are the standard compounds. In order they are: α-pinene, β-myrcene, limonene, γ-terpinene, 2-octanol, and citronellal. There are two smaller peaks after 15-minutes which are deemed to be due to contamination. The increase after 24 minutes is due to the increase in temperature at the end of the program. 45
Figure 8: Calibration curve for α-pinene. Standard solutions were injected in triplicate and the points are superimposed. Graph had a function of y=169237x + 340644 and a R^2=0.98. 45
Figure 9: Maceration experimental trial. Bottle on the left has macerated for 1 hour, bottle on the right has macerated for 48 hours. 62
Figure 10: Concentration of key analytes after 72 hours in macerated solution for three experiments of different solvent concentration. These were analysed on the GC-FID with single injections from single sample. 67
Figure 11: The experimental apparatus for macerate distillation was comprised of a lab jack, heating mantle, thermometer and Quickfit glassware. There is little chance for reflux since most of the condensate flow directly out of the condenser into the collection vessel. 70
Figure 12: Concentration of key analytes in distillate after distillation using berries maceration for three different lengths of time. The samples were analysed by GC-FID with single injection of single sample. Citronellal showed no increase and was not included on this graph. 73

Figure 13A and 13B: Shows the reactor column used to house the botanical basket, the column is wrapped in heat trace. The stainless-steel caps are also visible at either end of the column. The botanical basket is also visible within the column on the right. 75
Figure 14: Condensed vapour percolation experimental apparatus. The glass column had a botanical basket at the top just below the vapour inlet. Top of shot is the condenser where the vapour condensed before falling over the botanicals and out the bottom of the column into the measuring cylinder. Since the column is not above the kettle there is a lower level of reflux than steam distillation. 76
Figure 15: Analyte concentration in distillate segments in full botanical ratio experiment. 79
Figure 16: Concentration of key analytes in total distillate of all botanical ratios for condensed steam distillation. Total distillate was 238 mL. Samples were analysed by GC-FID and were single injection from single sample. 79
Figure 17: Vapour infusion experimental apparatus. In this arrangement the vapour entered the bottom of the column before passing over the botanicals and into the condenser at the top of the frame. Condensate can fall back into the kettle since the column is directly above it and this leads to a higher level of reflux. 81
Figure 18: Concentration of key analytes in distillate for full botanical ratio for each segment from vapour infusion experiments. 83
Figure 19: Concentration of α-pinene in distillate segments for all botanical ratios for vapour infusion distillation experiments. The samples were collected in 40 mL segments with the total distillate sample taken from the full 238 mL yield. Analysed by GC-FID and were single injection from single sample. 85
Figure 20: Concentration of β-myrcene in distillate segments for all botanical ratios for aqueous-ethanol vapour distillation experiments. The samples were collected in 40 mL segments with the total distillate sample taken from the full 238 mL yield. Analysed by GC-FID and were single injection from single sample. 86
Figure 21: The ethanol concentration of the three separate process as a function of time. The ethanol concentration was calculated for two trials of each process that were then averaged, and that value displayed on this chart. 88
Figure 22: Rate of distillate produced as a function of time across the three processes. Maceration distillation had a much quicker production rate and condensed vapour percolation, and vapour infusion were very similar. 89
Figure 23: Calibration curve for β-myrcene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=102091x + 32741 and a R^2=0.998 119
Figure 24: Calibration curve for limonene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=156370x + 66915 and a R^2=0.9979. 119
Figure 25: Calibration curve for γ-terpinene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=159770x + 84731 and a R^2=0.9996. 120
Figure 26: Calibration curve for citronellal. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=137536x + 85892 and a R^2=0.9993. 120

List of Tables

Table 1: Composition by mass of the most common VOC found within the botanicals. The compounds are expressed as a percentage of total essential oil from each botanical. These were the only compounds present across four or more botanicals with the compound present in the highest concentration being α-pinene, limonene and linalool. 16
Table 2: Odour threshold data for five of the main compounds found by Clutton & Evans, (1978). 17
Table 3: Odour threshold data for four of the main compounds calculated by Hodel et al., (2020). 17
Table 4: Odour threshold data for three of the main compounds calculated by Buck et al., (2020). 18
Table 5: Number of compounds identified using GC-MS analysis from hexane extraction between the different time-temperature programme using the NIST library. A full list of the identified compounds can be found in Appendix A.1.3, Table 43. 38
Table 6: This table lists the compounds with the biggest peak areas identified in hexane. Based on this table the key analytes were determined based on economic viability and availability. 41
Table 7: This table displays the varying concentrations of each external standard and the constant internal standard concentration which was used for internal and external standardisation calibration. 43
Table 8: The average retention time for the external and internal standard on the gas chromatography – flame ionization detector equipment. 44
Table 9: Relative response factor for each external standard calculated from the response to the internal standard 2-octanol. 46
Table 10: Three 40% gin samples were extracted into hexane and the average concentration of the key analytes is shown. Triplicate injections of each sample were performed and error was calculated using standard error. 47
Table 11: To determine the most effective solvent to extract gin volatiles, fours solvents were tested, with and without the addition of NaCl. Raw gin without any solvent was also tested at 40% ABV and 80% ABV. The following table displays the volumes and masses. 51
Table 12: To investigate extraction time and extraction efficiency a spiked ethanol solution was prepared with approximate concentrations of 115 mg/L. This was called a mock gin. 52
Table 13: The number of compounds identified and relative response area across four different solvents. The addition of NaCl was also trialled which did not show an increase in peak area. 53
Table 14: The addition of salt during extraction with hexane was also trialled before analysis on the GC-FID and it was shown to not be beneficial to increase peak area. 54
Table 15: The ratio of solvent to gin was trialled with three different ratios. The results are expressed as efficiency by amount recovered into hexane. The 1:3 ratio proved to have the highest recovery efficiency. 54

Table 16: The extraction efficiency expressed as percent recovery of target analyte. Extraction duration results for 1-, 5-, 17- and 24-hour periods. 55
Table 17: The average extraction efficiency from the mock gin solution. 56
Table 18: The spiked ethanol solution was spiked with analytes pre-extraction and the final concentration was lower than the expected concentration which indicates that the loss is due to extraction efficiency as the values correlate with earlier values. 57
Table 19: The concentrations of analytes in the 'pure' ethanol solution that proved that the ethanol had been contaminated. 60
Table 20: Maceration experimental data for botanical ratio and solvent concentration. The mass of juniper berries and volumes of ethanol and water used are listed. Juniper berry expressed as grams per litre of aqueous ethanol solution. 63
Table 21: Concentration of key analytes over time for largest botanical ratio: 63.1 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol. 64
Table 22: Concentration of key analytes over time for botanical ratio: 33.0 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol. 64
Table 23: Concentration of key analytes over time for smallest botanical ratio: 16.6 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol 65
Table 24: Concentration of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry. 66
Table 25: Key analyte concentration in macerated solution over time with 16.6 g/L of juniper berry in 78% ABV aqueous ethanol 66
Table 26: Concentration of key analytes over time in macerated solution with 16.6 g/L of crushed juniper berries in 58% ABV aqueous ethanol. 68
Table 27: Concentration of key analytes over time in macerated solution with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol. 69
Table 28: Weights and volumes of juniper berry, ethanol and water for macerate distillation experiments comparing botanical ratio. Juniper berry expressed as grams per litre of aqueous ethanol solution. 71
Table 29: The effect of maceration time was compared by comparing 0 hours, 24 hours and 72 hours to the same botanical and solvent ratio. The solvent ratio remained constant for each trial at 350 mL 60% ABV. 71
Table 30: Concentration of key analytes in distillate after distillation using berries macerated over different lengths of time. The berries were in the kettle and were in direct contact with the boiling liquid. 72
Table 31: Key analytes concentration in macerate distillate for 8.3 g/L, 16.6 g/L, 33.1 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 58% ABV aqueous ethanol. 74
Table 32: The distillate segment that each sample was taken from, and the volume needed. Each sample was taken from a 40 mL distillate segment other than the final distillate sample which was of the entire yield. The kettle was sampled pre- and post-boil. GC analysis was used to quantify the analyte concentration and the HPLC analysis calculated the ABV. 77

Table 33: The entire botanical ratio for condensed vapour percolation is listed below. The botanical ratio was doubled in between trials while keeping the solvent ratio the same. The solvent ratio remained constant for each trial at 350 mL 60% ABV. 77
Table 34: The distillate segment that each sample was taken from, and the volume needed. Each sample was taken from a 40 mL distillate segment other than the final distillate sample which was of the entire yield. The kettle was sampled pre- and post-boil. GC analysed was used to quantify the analyte concentration and the HPLC analysis calculated the ABV. 82
Table 35: The entire botanical ratio for vapour infusion trials is listed below. The botanical ratio was increased in between trials while keeping the solvent ratio the same. The solvent ratio remained constant for each trial at 350 mL 60% ABV. Juniper berry expressed as grams per litre of aqueous ethanol solution. 82
Table 36: Concentration of the key analytes in the total distillate yield of 238 mL for all botanical ratios for vapour infusion experiments. 84
Table 37: Average ABV of the final distillate of the three micro processes. 89
Table 38: The weight of the berries used for analyte extraction effectiveness calculation along with the weight of hexane. 90
Table 39: The mass of juniper berry used across effectiveness trials. 90
Table 40: Percentage of analyte recovered in distillate from the three processes. 91
Table 41: Composition of essential oil of common botanicals shown in alphabetical order expressed in mass percentage. This table shows that while many botanicals are composed of the same compounds the majority are only present in one or two botanicals. A small fraction is still unidentified for each botanical other than orange powder. 108
Table 42: Chemical properties of the standards and solvents used in this study. All data sourced from https://pubchem.ncbi.nlm.nih.gov/. 112
Table 43: Gin extracted into hexane and analysis via Shimadzhu GC-MS with a stepped time-temperature programme that took 43-minutes identified 28 compounds. 113
Table 44: Gin extracted into hexane and analysis via Shimadzhu GC-MS with a single ramped time-temperature programme that took 23-minutes identified 20 compounds. 114
Table 45: For initial retention time determination a single analyte was diluted in hexane and analysed via GC-FID. The following concentrations were used. 114
Table 46: Concentrations of five external standards and one internals standard for the first combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times. 115
Table 47: Relative response factor for the internal standard for combined standard solution A. 115
Table 48: Concentrations of five external standards and one internals standard for the second combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times. 116
Table 49: Relative response factor for the internal standard for combined standard solution B. 116

Table 50: Concentrations of five external standards and one internals standard for the third combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times. 116
Table 51: Relative response factor for the internal standard for combined standard solution C. 117
Table 52: Concentrations of five external standards and one internals standard for the fourth combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times. 117
Table 53: Relative response factor for the internal standard for combined standard solution D. 117
Table 54: Concentrations of five external standards and one internals standard for the fifth combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times. 118
Table 55: Relative response factor for the internal standard for combined standard solution E. 118
Table 56: Multiple internal standard solutions were prepared over the course of this project, and they are shown below. 120
Table 57: The concentration and response area for 2-octanol that was used to determine the calibration curve for the Agilent GC-FID. 121
Table 58: Concentration of internal standard within the three gin samples. 121
Table 59: Peak areas and concentrations for analytes in Juno gin. 121
Table 60: This table shows the compounds that were identified in the ethanol contamination investigation. Several compounds were identified but were unable to be quantified due to lack of standards and are labelled as trace amounts. 122
Table 61: Sample properties and internal standard concentration data. 122
Table 62: Sample properties and internal standard concentration data for crushed and vacuum botanical trials. 123
Table 63: Peak area of key analytes in 78% ABV macerated solution with 16.6 g/L of juniper berry. 124
Table 64: Key analyte concentration in macerated solution over time with 16.6 g/L of juniper berry at 78% ABV. 124
Table 65: Peak area of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry. 125
Table 66: Concentration of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry. 125
Table 67: Sample properties and internal standard concentration data for crushed and vacuum botanical trials. 125
Table 68: Peak areas of key analytes in macerated solution over time with 16.6 g/L of crushed juniper berry in 58% ABV aqueous ethanol. 126
Table 69: Concentration of key analytes in macerated solution with 16.6 g/L g of crushed juniper berries in 58% ABV aqueous ethanol. 126
Table 70: Peak areas of key analytes in macerated solution over time with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol. 127
Table 71: Concentration of key analytes in macerated solution over time with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol. 127

Table 72: Sample properties and internal standard concentration data for macerate distillation for different botanical ratio trials. 127
Table 73: Key analytes peak area in macerate distillate for 8.3 g/L and 16.6 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV aqueous ethanol. 128
Table 74: Key analytes concentration in macerate distillate for 8.3 g/L and 16.6 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV. 129
Table 75: Key analytes peak area in macerate distillate for 33.0 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV. 129
Table 76: Key analytes concentration in macerate distillate for 33.0 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV. 129
Table 77: Internal standard data for condensed vapour percolation trials with different botanical ratios. 130
Table 78: Key analytes peak area for condensed vapour percolation with quarter botanical recipe. 131
Table 79: Key analytes concentration for condensed vapour percolation with quarter botanical recipe. 131
Table 80: Key analytes peak area for condensed vapour percolation with half botanical recipe. 132
Table 81: Key analytes concentration for condensed vapour percolation with half botanical recipe. 132
Table 82: Key analytes peak area for condensed vapour percolation with three quarter botanical recipe. 132
Table 83: Key analytes concentration for condensed vapour percolation with three quarter botanical recipe. 132
Table 84: Key analytes peak area for condensed vapour percolation with full botanical recipe. 133
Table 85: Key analytes concentration for condensed vapour percolation with full botanical recipe. 133
Table 86: Internal standard data for vapour infusion trials with different botanical ratios. 133
Table 87: Key analytes peak area for vapour infusion with quarter botanical recipe. 135
Table 88: Key analytes concentration for vapour infusion with quarter botanical recipe. 135
Table 89: Key analytes peak area for vapour infusion with half botanical recipe. 135
Table 90: Key analytes concentration for vapour infusion with half botanical recipe. 136
Table 91: Key analytes peak area for vapour infusion with three quarters botanical recipe. 136
Table 92: Key analytes concentration for vapour infusion with three quarters botanical recipe. 136
Table 93: Key analytes peak area for vapour infusion with full botanical recipe. 136
Table 94: Key analytes concentration for vapour infusion with full botanical recipe. 137
Table 95: Internal standard data for process extraction effectiveness with 28 g of juniper berry. 137
Table 96: Key analytes peak area for process extraction effectiveness with 28 g of juniper berry. 137
Table 97: Key analytes concentration for process extraction effectiveness with 28 g of juniper berry. 138
Table 98: Moisture content analysis for juniper berries sourced from BeGin Distilling. The moisture content was conducted in triplicate and the average moisture content was 14.34% (d.b.). 138

List of Abbreviations

ABV	Alcohol by volume
COVID-19	Coronavirus disease of 2019
CAGR	Compound annual growth rate
CAR	Carboxen
CVP	Condensed vapour percolation
d.b.	Dry basis
DCM	Dichloromethane
DI-SPME	Direct immersion-solid phase microextraction
DMA	Density meter analyser
DMSO	Dimethyl sulfoxide
DVB	Divinylbenzene
EO	Essential oil
EPA	Environmental protection Agency
ESTD	External standardisation
EtOH	Ethanol
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry
HPLC	High-performance liquid chromatography
HS-SPME	Head space-solid phase microextraction
IWSR	International Wines and Spirits Record
LLE	Liquid-liquid extraction.
LOD	Limit of detection
LOQ	Limit of quantification
M-SPME	Membrane-solid phase microextraction
NA	Not available
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
NIST	National Institute of Standards and Technology
NZ	New Zealand
PDMS	Polydimethylsiloxane

RO	Reverse osmosis
RPM	Rotations per minute
SE	Standard error
SPME	Solid phase microextraction
TIC	Total ion count
USA	United States of America
USD	United States dollar
UV	Ultraviolet
VI	Vapour infusion
VOC	Volatile organic compounds
w.b.	Wet basis
XNS	Ultra-neutral spirit

Chapter 1: Introduction

1.1 Project Overview

Gin has been one of the fastest growing spirit categories over the past several years and according to recent market research by Williams and Marshall Strategy (2021), the global industry is forecast to grow from 7.9 billion USD in 2015 to 17.36 billion USD by 2025. This is represented in New Zealand with the number of gin distilleries growing from less than 50 in 2017 (Jones, 2019) to over 120 in 2022 (McDonald, 2022). Operating a small craft distillery is challenging due to time requirements, overheads and competing in a well-populated market therefore it is important to increase efficiencies and lower any possible costs.

One of the key challenges that craft distilleries face are the distribution costs associated with expansion into overseas markets. Only one glass packaging company remains manufacturing glass in New Zealand, and its primary focus is on the production of wine bottles (Boni, 2000). This has led to most of New Zealand distilleries sourcing their bottles from international suppliers and manufactures. Imported bottles are transported worldwide to reach New Zealand where they are filled with product, which is approximately 60% water, before they are distributed domestically as well as internationally. In this scenario there is a lot of wasted effort to transport bottles across the globe and filled primarily with water for exportation, which is not sustainable. This also increases the amount of logistics transportation that is required and has limiting factors in today's changing environment, such as the recent COVID-19 pandemic. Where new restraints and high pressures have developed across international trade systems effecting all supply and distribution routes globally.

An additional challenge to monitor is current global trends which have seen the fast rise in low and non-alcohol spirits and beverages with the International Wines and Spirits Record reporting that low-and-no 'spirits' grew their volume sales by 32.7% in 2020, even with the closure of bars around the world (International Wine and Spirits Record, 2021). With the increasing demand for low-and-no 'spirits' it is important to observe these trends and innovate alongside and ahead to maintain market traction and placement.

Current techniques used to develop new gin flavours requires multiple distillation iterations through a still which can be a lengthy process and consume considerable energy and effort; producing flavours and products that do not always hit the mark. A significant disadvantage of this method is the time required for each trial variation for the different composition of botanicals, although this is somewhat expected for any product development.

This investigative research project will determine whether the development of a concentrated gin 'hotshot' could be a solution to the challenges listed. The purpose of the gin 'hotshot' is to be a stable flavour concentrate that contains all the flavour and textural compounds of the final gin, but at elevated concentrations. This concentrated solution would match the original gin flavour and texture profile once it has been diluted with water and ethanol in market to 40% ABV while saving on distribution costs. Utilising a concentrated 'hotshot' will additionally expand further market potential within the low alcohol gin market through solely using water as the dilutant. The 'hotshot' could also be used as base for the development of new products and be blended with other 'top-note' concentrates made the same way but in smaller quantities to reduce new flavour development time.

The development of a concentrated 'hotshot' will require an in-depth understanding of the distillation process and each individual micro-process along with the key flavour compounds and the botanicals they are commonly extracted from. A key limitation in making this concentrate is using only techniques prescribed for manufacture of London Dry gin to retain classification.

To initialise this research, individual botanicals and their flavour compounds will be examined to determine the best method to extract the essential oils, this may include distillation, or solvent extraction. Suitable analytical chemistry methods will need to be selected or developed to be able to identify and quantify the flavour compounds in the gin samples. To judge the success of the project, the recombined 'hotshot' must have a similar composition of significant compounds to the conventional gin specimen but more concentrated.

1.2 Research Aim

This study aims to research whether it is possible to develop a concentrated gin 'hotshot' that is more concentrated in flavour and can match the original gin specimen flavour profile when diluted with water and ethanol.

1.2.1 Research Questions

The following are the research questions that this study will plan to answer:

- What are the significant micro-processes that extract flavour compounds in the gin manufacture process?

- At what stage are the flavour compounds extracted from the botanicals?
- What are the key influences on analyte extraction during gin manufacture?
- How can the flavour compounds be accurately identified and quantified?
- Is there a linear relationship between botanical ratio and flavour compound concentration?

1.2.2 Specific Objectives

The specific objectives that this study plans to achieve:

- To develop an understanding of the current methods used to distil and analyse gin.
- To identify the individual micro-processes that make up the overall distillation process.
- To develop a robust method that can identify and quantify the flavour compounds in gin samples.
- To develop experimental apparatus that can simulate the different stages of gin distillation.
- To characterise the key flavour compound extraction micro-processes.
- To determine the significant analyte extraction parameters.
- To determine the relationship between botanical ratio and flavour compound concentration.

1.3 Implications of Recent Literature

During the tail end of this project new literature was published by Black (2022) and Pauley and Hodel (2023) which mentioned a gin manufacture technique called ‘multi-shot’. Multi-shot is a technique utilised by some distilleries whereby increasing the botanical ratio they produce a gin that requires dilution with water and spirit to reach the flavour profile. This recent work answers the main question of this study which was whether production of a concentrated gin ‘hotshot’ is possible.

Using this keyword ‘multi-shot’, all published work was re-examined revealing only three mentions in regard to multi-shot gin, two published within the last 12 months (Black, 2022; Pauley & Hodel, 2023) and one published in 2019 (Qian et al., 2019). These publications only include a paragraph mentioning what it is, and no in-depth research exists. Mention was found in home-distiller forums over the last decade which proves that this manufacture method has existed but, has not been reported until recently. The recent articles on gin multi-shot technique indicates that this is an emerging practice in the gin industry and is an area worthy of more investigation.

The research by Black (2022) and, Pauley and Hodel (2023) was found at the end of the write up of this project. That is why mention of multi-shot will not be found in the literature review, except as a coda. The title of this project was changed retrospectively from gin ‘hotshot’ to ‘multi-shot’ to match the industry standard and make it easier to access. In this study the two terms are used interchangeably.

1.4 Thesis Structure

The structure of the thesis is made up of a series of chapters.

Chapter 1 has a brief overview of the project which includes the research aim, research questions and specific objectives.

Chapter 2 contains a detailed literature review, focusing on gin distillation, gin flavour compound analysis and the current research gaps.

Chapter 3 discusses how the key analytes were determined and how the equipment was calibrated using internal and external standardisation.

Chapter 4 develops the sample preparation method to prepare the gin samples for GC analysis. This chapter also contains gas chromatography method development.

Chapter 5 discusses maceration, macerate distillation, condensed vapour percolation and vapour infusion experimental methods and results.

Chapter 6 contains the conclusions and suggested future work.

Chapter 2: Literature Review

2.1 Introduction

This thesis aims to determine whether the development of a concentrated gin 'hotshot' is possible, and the investigation begins by examining and discussing relevant literature. The gin manufacture and distillation processes are discussed first in sections 2.2 – 2.4. Then in sections 2.5 – 2.7 the volatile organic compounds that are found in gin are discussed along with current and past analysis methods. Sections 2.8 – 2.9 discuss analytical chemistry techniques that are commonly used to increase the precision and accuracy of VOC analysis. Lastly, 2.10 covers the conclusions of the literature review and the research gaps.

2.2 Background of Gin

Gin is defined as "a colourless to pale straw-coloured alcoholic spirit distilled from grain or malt and flavoured with juniper berries and a variety of herbs and spices" (Oxford English Dictionary, 2017). While this may have been true, gin is now made from a neutral spirit which has been fermented from a range of carbohydrate bases and is not limited to grain or malt and is produced in a variety of different colours. To find where gin originated from you must trace it back through its roots of genever to medicinal tonics.

The origin of gin lies with its root spirit 'genever' which started as a medicine used by Italian Benedictine monks who experimented with healing tinctures and elixirs. They are credited with creating the first "gin" which was a juniper-based distilled tincture to heal bladder and kidney problems in the 11th century (Coates, 2004). The Dutch were not far behind and were also early enjoyers of juniper and were recorded as using juniper infused distilled beverages to treat a host of ailments in what they called "genever" from the 13th century (Van Schoonenberghe, 1999). However, it was not until the 16th century when the English were fighting alongside the Dutch that genever made its way to England and gained traction (Nuttall, 2020). This history is now commonly accepted as the true origin of gin however two individuals are often falsely accredited with its invention (Solmonson, 2012; Tlusty, 1998; Youngman, 2022). In the late 16th century Professor Sylvius de Bouve in Holland was known to sell juniper-based tonics however evidence now proves juniper-based drinks already existed (Stephenson, 2016). He is also commonly mistaken for Dr Franciscus Sylvius, a German doctor who shares half of the same name (they are often confused for

the same person) and who are both falsely accredited with inventing gin. Even though it has now been proven that genever existed long before either Professor Sylvius de Bouve or Dr Franciscus Sylvius, there are still authors claiming otherwise (Buglass, 2011; Mohammadi, 2010; Pauley & Maskell, 2017).

Genever became a drink of the wealthy after William the Orange took the British throne and regularly drank genever (Lessenich, 2015). After harsh laws were imposed on imported alcohol, distillers slightly changed how they made genever and created – gin, basically a cheaper version that grew massively in popularity and in turn started the London gin craze of the 18th century (Vivant, 1992). The gin craze was a period in the 18th century during which gin become incredibly cheap and led to a rise in violence and debauchery (Cleal, 2016). The government tried to control the damage that gin was causing by passing five separate gin acts to control the consumption, which did eventually succeed (Warner & Ivis, 2000).

Gin next rose in popularity due to the British army when they were given quinine in the form of tonic water to combat malaria. Soldiers then mixed this with gin which helped mask the bitter taste (Simonetti et al., 2022). Gin took off in America during the 1920's during the prohibition. Due to it being easy and cheap to make, though this did not always produce a plateable gin, which led to the creation of many gin cocktails as people were finding different ways to mask the unpleasant taste (Barnett, 2012).

Gin has experienced a resurgence over the past 30 years across the world, which has led to the rise of many artisan distilleries that specialise in unique flavours and types of gin (Cropp, 2021). Gin is on the rise for many of the same reasons that has made it popular in the past; it is cheap to make, does not require any aging while being very versatile and can be enjoyed in a range of cocktails.

New Zealand is one of the only western countries that allow the distillation of personal alcohol which has led to a rise in experienced home distillers who have spent years distilling in their garages. When the gin resurgence began, they realised they could turn their hobby commercial and New Zealand now boasts over 120 individual distilleries specialising in gin (McDonald, (2022).

There are seven main types of gin, London Dry, Plymouth, Old Tom, Genever, Navy Strength, Aged and New Western (Lev-Tov, 2021). London Dry is a legal style of gin and defined in the EU as made by distillation of natural botanicals using neutral alcohol of 96% and nothing can be added after distillation except water and have a final concentration of no less than 37.5% (Buck et al., 2020; Buglass, 2011).

2.3 Gin Manufacture

The process to manufacture gin has three main processes, fermentation, distillation, and redistillation (Aumatell, 2012). The first fermentation and distillation process are to produce a neutral spirit that will act as the ethanol base for the gin.

2.3.1 Neutral Spirit

A carbohydrate base is first fermented to create an alcoholic wash; this alcoholic wash is distilled to concentrate and neutralise the spirit. The desirable base spirit to be used is any neutral spirit that imparts little flavour into the end spirit with the key flavours coming solely from the botanicals (Willkie et al., 1937). According to Pauley and Maskell (2017) in modern distilleries the neutral spirit wash is not fermented from a specific base however wheat, maize and molasses are the most common. They also state that the neutral spirit base can be made a from carbohydrate sources such as barley, rice, potatoes, sugar cane, grapes and even by-products such as whey. The resulting spirit is neutral however there are still subtle flavours that come through and distilleries now take particular care in picking a base that is right for the flavour profile they are looking for (Willkie et al., 1937).

Aylott (2003) mentions that recent trends show that many distilleries outsource their neutral spirit production to a different company to streamline production and reduce costs. In New Zealand, many companies source their ethanol from Lactanol, the potable ethanol product of Fonterra Co-operative Dairy Company of Auckland, NZ, which uses whey as its carbohydrate base and produces as much as 18 million litres per year of potable and fuel grade ethanol (Ling, 2008). The dissolved solids in whey permeate are high in lactose which cannot be fermented by *Saccharomyces cerevisiae*, instead a special strain of *Kluyveromyces marxianus* yeast is used to ferment the lactose and produce ethanol (Silveira, et al., 2005). This ethanol is then concentrated via distillation and a rectifying column to achieve 96% ABV and an ultra-neutral spirit is obtained which is perfect for London dry gin manufacture (Aumatell, 2012).

The neutral spirit is then diluted with water and is ready to be redistilled with the botanicals to create the final product. Botanicals are infused into the neutral spirit during a redistillation stage which uses liquid and vapour phase aqueous ethanol to extract flavours (Hodel et al., 2020). This redistillation process is the most important part of the gin production process and is the step where the ethanol is concentrated again, and the bulk of the flavour is extracted and concentrated. The

three most common methods of infusing gin with the botanicals, are maceration/steeping, vapour infusion and vacuum distillation (Hodel et al., 2019).

In this project Lactanol ethanol is used as the base spirit and therefore the fermentation and first distillation stages of gin manufacture are out of scope. Instead, the focus is on the techniques involved in infusing botanical flavours into the base spirt.

2.3.2 Maceration

Maceration is the process of steeping botanicals in an ethanol-water mixture (often 40-60% ABV) to extract volatiles; depending on the botanical they may be dried, cut, ground or be fresh and whole. This process is usually done within the still prior to distillation and will be immediately distilled after the maceration period of 12-24 hours. The extracted flavours will interact in the still over time, in a significant manner as reported by Willkie et al. (1937), this leads to a rounded flavour profile which can be desirable in the final product. Maceration is usually followed by distillation which leaves the water-soluble compounds with high volatilities and undesirable characteristics to remain in the still.

When botanicals are steeped without distillation there is no selection between low and high volatile compounds creating a low-quality product which no commercial distillery would use (Headlands Distilling Co., 2020). However, it is a low-tech method to produce gin and used by some consumers to make gin at home. For example, Kavilanz (2013) showed that there has been a rise in gin kits for consumers to make gin in the comfort of their own home with no specialized equipment. These packs contain a selection of botanicals, which may include juniper berries, coriander, citrus peel, orris root and cassia among others; neutral spirit not included. The kits are designed to allow the consumer to create their own unique blend by allowing the botanicals to steep and extract flavour into the neutral spirit, usually vodka. This simple method allows many keen gin drinkers to create their own flavour blend and gain a deeper understanding of where the flavours come from in gin. The steeping technique does not meet the London Dry gin requirements, as no distillation with the botanicals occurs.

2.3.3 Vapour Infusion

Vapour infusion uses a distillation method with a botanical basket suspended above the liquid in the still or before the condenser (Hodel et al., 2020). In this method the botanicals will only be in contact

with the vapour and any condensate that forms on the botanicals. This keeps the botanicals at a lower temperature than botanicals in the more water-rich pot and limits some of the 'cooked' flavours otherwise possible (Hodel et al., 2019). These flavours may be desirable and the flavours from vapour infusion are not necessarily better or worse than maceration it just produces a different set of flavour compounds and flavour profile. There has been research by Hodel et al. (2020), and Hodel et al. (2019) that show vapour infusion leads to a higher effectiveness at extracting monoterpenes. A technique that is also used is coupling vapour infusion with maceration together to get a balance of flavour compounds.

2.3.4 Vacuum Distillation

Vacuum distillation is a more expensive method than maceration or vapour infusion however it is used by some craft distilleries to delicately extract flavours from botanicals (Headlands Distilling Co., 2020). Wojciechowska (2015), states that in vacuum distillation the pressure is lowered to allow ethanol to vaporize at room temperature. However, that is only with a full vacuum, a partial vacuum can be applied where ethanol will boil around 57°C (Watson & Suomatainen, 1984). Under a full or partial vacuum, the entire distillation process can then be kept at a lower temperature and reduces heat degradation of the botanicals and extracted compounds. This can allow the selective extraction of certain compounds while not extracting compounds that are present in high temperature processes. Greer et al. (2008), found that vacuum distilled gin was noted as being more flowery and pleasant than conventional distilled gin by a sensory panel.

2.3.5 Low or No Alcohol Gin

The market increase in non/low alcohol spirits has seen a steady increase with the global market increasing from US$74m in 2017 to US$143m in 2021 and predicted to have a ten-year CAGR of 10.45% (Beeson, 2023). This has led to many distilleries producing no/low alcohol spirits and New Zealand gin distilleries are no exception. The non-alcoholic 'gins' are technically not true London Dry gin since they do not contain alcohol above 37.5%, the legal requirement for it to be classed as such.

Lambrianidis (2021) discusses the two common methods that produce no and low alcohol gins. Low alcohol gins are typically made using the same process as traditional gin however the distillate will be redistilled many times to separate the alcohol from the flavour compound. Another method to get a non-alcoholic gin is to macerate the botanicals solely in water to extract the flavours that are

usually present in gin. These sorts of gins are high in the water-soluble compounds that are expected in gin but are lacking the ethanol-soluble compounds. Alternatively, they can be made by using the same process as traditional gin however when the distillate is being diluted it will not stop at 40% but will be further diluted until it reaches the low alcohol ABV goal. In this case a higher botanical ratio is needed to retain the flavour profile.

Some distilleries also add in something to mimic the alcohol taste which is referred as heat. This is often some form of capsaicin which gives the sensation of the alcohol heat.

2.4 Distillation

The distillation process is a separation method that relies on a disparity in volatilities between two components (Ray & Das, 2020). Smith and Jobson (2000) discuss how distillation is the most common method of separating homogeneous mixtures by utilizing a selective boiling and condensation process. They go on to state that this can lead to near complete separation of the components leaving almost pure substances in the range of up to 96% and much higher than many alternative separation processes. Keller (2014) also states that distillation is an extremely effective separation process however its main disadvantage is its high energy use. In 2009 approximately 19% of Europe's total energy consumption was used by the chemical industry sector and 40% of this was for separation processes, most of it specifically for distillation processes (Keller, 2014). This matches what Javed et al. (2022) has found; that distillation processes account for 40 – 50% of the total plant operating cost in refining and chemical processes. Within the food industry distillation is used on a much smaller scale and requires a smaller but still substantial amount of energy. Therefore, the development of a method that allows the still to be run less often by creating a concentrated gin base will also have large energy savings.

2.4.1 Gin Distillation Process

Gin distillation begins with a spirit that has already been rectified to create a pure and neutral spirit (Aumatell, 2012). See section 2.3.1 for more details on neutral spirit. Smaller distilleries use batch distillation however the larger distilleries use continuous operation since it can lead to reduction in vessel volumes and reduced cost (Stitt & Rooney, 2010).

Batch distillation begins by the addition of a liquid into the kettle that contains two substances, A and B, that have different volatility. In the case of gin distillation, the two substances are ethanol, also commonly referred to as ethyl alcohol, and water. As the liquid begins to boil a vapor will be produced, richer in the more volatile component in accord with Raoult's law (Guggenheim, 1937). The vapor produced passes through the botanicals if it is vapour infusion and through the condenser and is removed from the system which leads to the liquid in the kettle becoming progressively richer in the less volatile component. An increase in the proportion of the less volatile component in the liquid leads to an increase in boiling point of the mixture and causes the temperature of the vapor to also increase (Lower, 2022). As the distillation continues the ratio of the components change and there is an increasing proportion of the less volatile component in the vapor.

In relation to the distillation of an aqueous-ethanol solution, initially, the liquid has a low ethanol concentration and high water concentration. When the solution is heated the vapor produced initially has a high ethanol concentration and low water concentration. The, at first ethanol-rich, vapour moves from the kettle through to the condenser where it is cooled by water, often by a plate or coil heat exchanger. As the run progresses more of the vapour is condensed, removing a higher proportion of ethanol from the system. This leads to an increase in water concentration in the liquid and as a result in the vapour. Thereby, as the content of water in the vapour increases the distillate ethanol concentration (ABV) decreases as time progresses.

Reflux is the process of returning some of the condensate to the system to increase the separation of the compounds. This is very common in industrial distillation processes, where high purity is required. Reflux can be returned directly into the kettle, or it can be forced back down the column percolating through packing or over plates to increase the contact area between the vapour and the liquid. This is called forced reflux and is the main design factor in column stills. Some of the rising vapour condenses on the liquid surface. Some of the re-heated reflux liquid evaporates. This has the effect of multiple distillations in one pass. The longer the column, the more steps are involved and the more complete the separation of different compounds due to the difference in their physicochemical properties (Halvorsen & Skogestad, 2000). The other type of reflux is passive reflux. Passive reflux is condensation caused by the difference in temperature between the system and the environment.

2.4.2 Still Design

This review will focus on the two most common still types, the column still and pot still since they are used for gin distillation. These still types are also the most common in the spirits industry and are used for the majority of mainstream products. There are other still types, where their unique design benefits the product such as the Charente still used for cognac production (Aylott, 2003).

2.4.2.1 Pot Still

A pot still is traditionally used to distil whiskey and brandy and may produce a spirit anywhere from 40-60% ABV since there is only one distillation stage (Watson & Suomatainen, 1984). A pot still only achieves a partial separation and therefore retains more flavour compared to a column still which has multiple distillation stages and can strip some of the flavour but ends up with a much more concentrated product, anywhere up to 96%. Pot stills often have a spirit helmet or onion head that serves to reduce foaming and add passive reflux into the system.

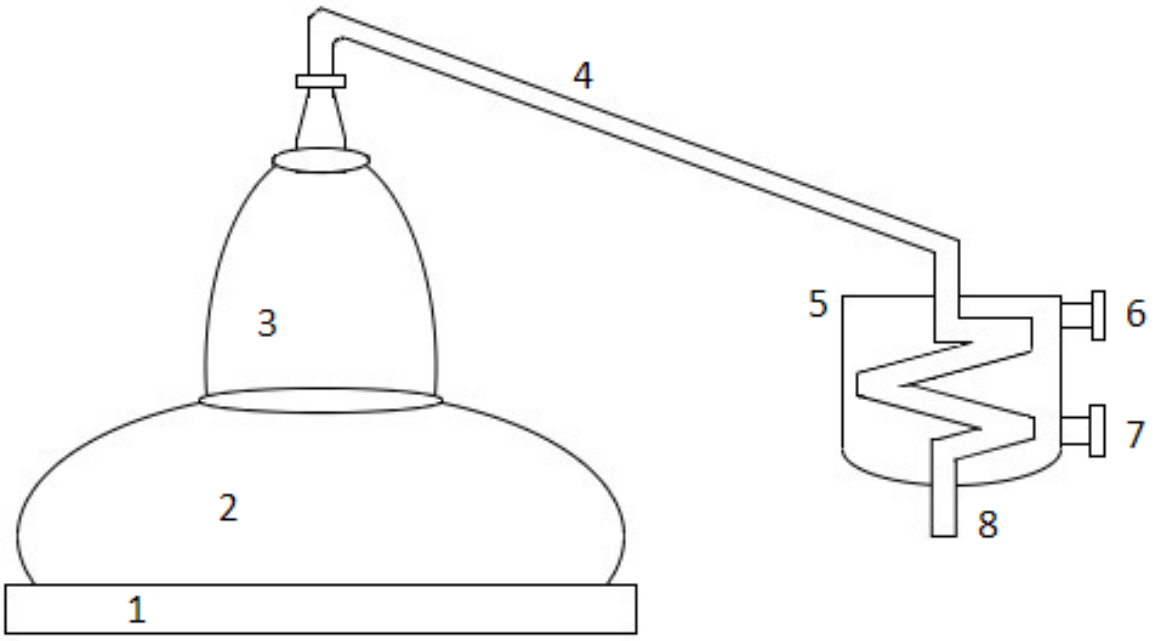

Figure 1: This is a simple pot still diagram that shows 8 possible components. Pot stills vary greatly in design, and some will not share the same components or even look alike.

1	Heating source	5	Condenser tank
2	Kettle	6	Coolant inlet
3	Helmet	7	Coolant outlet

4	Condenser tube/lyne arm	8	Distillate outlet

In Figure 1 a basic pot still diagram is shown. This pot still consists of a heating source, a kettle with a helmet (1,2,3). The kettle is connected to the condenser with a condenser tube (also called lyne arm) (4) and a coil condenser where the distillate is condensed with coolant (5,6,7,8,).

2.4.2.2 Column/Reflux Still

In a column still the vapour condenses and evaporates multiple times as it passes from the kettle through the column to the condenser. This creates a multiple distillation effect in a single column which increases the purity. The taller the column or the more stages, the higher the purity of the distillate. Column stills are often used to produce vodka, rum or any spirit that is pure with a very high alcohol percentage this method will retain less flavour and will then need to be diluted to the desired ABV (Claus & Berglund, 2005). They carry on saying that this is due to column stills achieving higher separation of more volatile compounds which leaves the less volatile flavour compounds in the still.

In Figure 2, the main components of a reflux still are shown. The condenser and kettle can be identical to a pot still however the main characteristic is the column which is designed to force reflux. The dephlegmator at the top of the column partially condenses the vapour to aid in the separation of ethanol and water. The condensate trickles back down over the packing or plates causing vapour to condensate and evaporate multiple times to give the effect of multiple distillations in a single run.

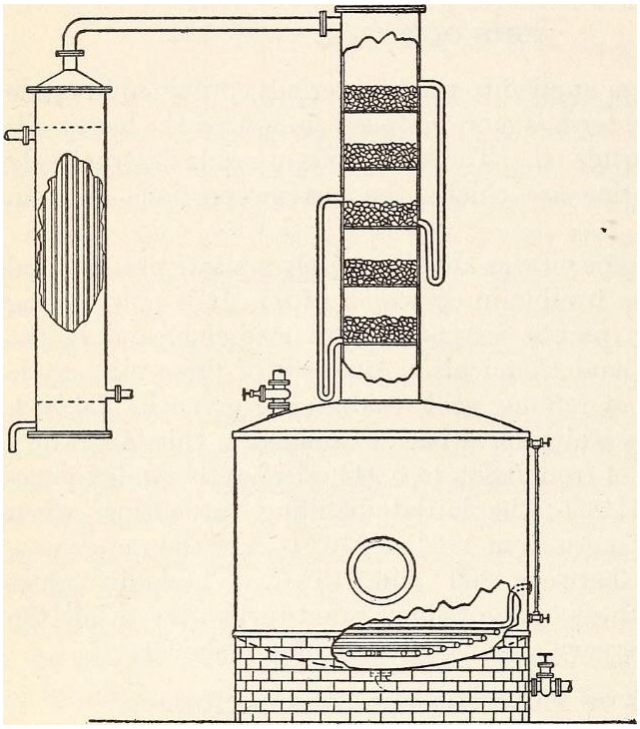

Figure 2: Simplistic column still schematic showing an example with copper chip packing within the column, due to the copper chips more distillations can be achieved in one pass leading to a purer more concentrated spirit (image from Veitch, F. P., 1911).

2.4.3 Cuts

The gin distillate produced can be separated into four distinct stages or 'cuts' depending on when it is produced (Kosar, 2017). Kosar lists the segments as foreshots, followed by heads, hearts and tails. Each segment is treated differently to ensure a desirable tasting and safe spirit is produced.

Some literature only discusses three cuts and include foreshots within the heads cut (McBain, 1986). Combining these two cuts can be dangerous as the key step is to discard foreshots due to it potentially containing methanol and acetaldehyde, while heads are usually retained and added into the next wash (Léauté, 1990).

The first section of distillate that is produced is known as the 'foreshots' and is usually discarded as it contains undesirable compounds possibly including methanol during the first distillation which is a health risk (Md et al., 2013). In consequent distillations it is unlikely for methanol to be present however the foreshots can often still contain undesirable flavour compounds so is often discarded and put through an ethanol recovery process.

The next section is known as the 'heads' and is high in low boiling impurities such as acetate, acetone and ethyl acetate which have unpleasant characteristics (Williams & Strauss, 1976). Often distillers will reuse their heads in the next run where some of the alcohol can be recovered.

The 'hearts' follow from the heads and are known as being the smoothest and containing pleasant flavours and is the premium product (Spaho, 2017). The hearts have the cleanest tasting product with desirable aroma and flavour compounds.

The 'tails' are the last part of the run and contain other alcohols such as butanol and propanol that have a higher boiling point to ethanol. As distillation progresses the vapour phase begins to have a higher component of water which is able to carry over longer molecules such as fatty and oil compounds that are unwanted (Spaho, 2017). Similar to the heads, tails can be added back into future runs to recover alcohol.

Aylott et al. (2003) states that some distillers combine their heads and tails to create 'feints' which then go through a separate alcohol recovery process to minimize loss as opposed to adding it into other runs.

2.5 Volatile Organic Compounds Present in Gin

The volatile organic compounds (VOC) that are present in gin and contribute the flavour and aroma originate from the botanicals, with the biggest proportion being extracted from juniper berry, the signature gin botanical present in the largest amount (Aylott, 2003). The VOC are referred to interchangeably by several names; essential oils since VOC are a component of the botanicals essential oil, congeners -defined as a minor component in alcoholic beverages.

There are many different botanicals that may appear in gin, however there are several that are almost always present: juniper berries, coriander seeds, angelica root/orris root and a citrus component. There are many factors that affect the composition of VOC in botanicals as to be expected in a natural product exposed to different climates, soil, predation, and other environmental factors (Chatzopoulou & Katsiotis, 1995). However, it is still useful to know what range the compositions will be within, and the compositions have been found for many of the main botanicals however the actual value will vary depending on afore mentioned factors.

Table 1: Composition by mass of the most common VOC found within the botanicals. The compounds are expressed as a percentage of total essential oil from each botanical. These were the only compounds present across four or more botanicals with the compound present in the highest concentration being α-pinene, limonene and linalool.

Compound	Composition of botanical essential oil (%)							
	Juniper berry[(1)]	Coriander seeds[(2)]	Kaffir lime leaf[(3)]	Angelica root[(4)]	Orris root[(5)]	Orange powder[(6)]	Black peppercorns[(7)]	Cardamon pods[(8)]
α-Pinene	51.4	7.98	3.6	32.69	-	1.31	7.3	1.15
α-Terpineol	-	0.25	7.6	-	-	0.34	0.5	5.06
β-Myrcene	8.3	1.18	0.3	5.87	-	3.25	2.6	-
β-Pinene	5	0.57	10.9	1.87		1.76	19	-
γ-Terpinene	0.2	5.93	trace	1.23	-	-	0.2	-
Camphene	0.8	1.31	0.3	1.53	-	-	0.2	-
Limonene	5.1	2.92	4.7	6.59	-	86.36	29.7	-
Linalool	0.1	66.4	2.8	-	-	2.19	2.1	5.33
p-Cymene	0.9	1.65	5.6	0.38	-	-	0.5	-
Sabinene	5.8	0.33	-	0.92	-	0.51	1.4	3.52
Citronellal	-	-	2.7	-	-	-	-	-
Other	22.4	11.45	64.2	48.92	100	4.28	36.5	84.94

[1]Höferl et al., (2014). [2]Misharina, (2001). [3]Waikedre et al., (2010). [4]Pasqua, (2003). [5]Mykhailenko, (2018). [6]Farahmandfar et al, (2020). [7]Orav et al, (2004). [8]Leela et al., (*2008*).

Table 1 uses the results from several studies to show how many of the botanicals have the same volatile organic compounds. The botanicals compared include Juniper berries, coriander seeds, kaffir lime leaf, angelica root, orris root, orange powder, black peppercorns and cardamon pods. A table showing entire composition of common botanicals can be found in Appendix A.1.1, Table 41.

Something that immediately stands out from Table 1 is that orris root does not share any of the common VOC with the other botanicals. This is due to orris root being added not as a primary source of flavour but as a flavour "fixative" (Broom, 2015). Compounds within orris root reduce the volatility of the volatile compounds from the other botanicals and bind the compounds more tightly into the solution (Maier, 1970). Orris root still contributes to the flavour profile and has a distinct earthy characteristic that adds to a gin's base notes. Angelica root is also considered a flavour fixative but has a slightly stronger profile and shares many of the VOC shown in Table 1. Both of these botanicals are counted as essential botanicals for their fixative properties and are commonly found in gin recipes (Ahmad et al., 2001).

Butola and Vashistha (2013) stated that they are also used in the perfume industry for aroma fixing and Broom lists orris root essential oil as one of the most expensive ingredients in the perfume industry.

2.5.1 Odour Threshold

Knowing the composition of the botanicals is only understanding half of the impact each will have since the aroma or flavour threshold for each compound is different. There has been research done into the odour threshold of common gin VOC by Clutton and Evans, (1978), Hodel et al., (2020) and Buck et al., (2020), however, there is a great variance in the results they have found. This could be due to changes in botanicals, from storage, age, or source. The studies were all tested over a range of aqueous-ethanol concentrations which would also have had an effect however the values are far from agreement, and they need to be further studied.

Table 2: Odour threshold data for five of the main compounds found by Clutton & Evans, (1978).

Compound	**Odour threshold (ug/L in 20% ethanol)**
β-Pinene	3,500
β-Myrcene	1,000
Limonene	6,500
γ-Terpinene	1,500
Linalool	9,000

Table 3: Odour threshold data for four of the main compounds calculated by Hodel et al., (2020).

Compound	**Odour threshold (µg/L in 8% ethanol)**
α-Pinene	2.5-62
β-Myrcene	15
Limonene	4-229
γ-Terpinene	1000

Table 4: Odour threshold data for three of the main compounds calculated by Buck et al., (2020).

Compound	**Odour threshold (ug/L in 45% ethanol)**
β-Myrcene	101
Limonene	2804
Linalool	24

2.6 Volatile Organic Compound Extraction

The compounds that are present in gin and contribute to the flavour can be called botanical congeners, these are the minor compounds that occur as a result of the distillation and fermentation process (Aylott, 2013). These congeners can be classified as volatile organic compounds (VOC) and are components of essential oils. During the gin distillation process there are several physicochemical processes in action that extract the VOC. The processes can be broken down to maceration, hydro-distillation and vapour/steam distillation (Hodel et al., 2020). The predominant source of literature is from water distillations in the extraction of essential oils and the gin distillation aqueous-ethanol system does not have extensive published work. Notable work into vapour infusion distillation parameters on the extraction of compounds from *Juniperus communis L* has been done by Hodel et al. (2020). Hodel et al. found that doubling of the botanical ratio of *Juniperus communis L* led to only 1.5 times increase of extracted compounds. This is an important finding however there is still a research gap for the other botanicals and distillation processes. The following sections 2.6.1-2.6.4 focus on the processes that occur during commercial spirit distillation that extract VOC. There are other methods used to extract VOC such as spinning cone column extraction, supercritical CO_2 and microwave assisted extraction however, since they are not used in commercial distilleries they will not be covered in this study and do not comply with London Dry Gin conventions.

2.6.1 Maceration

Maceration occurs before the distillation has begun while the juniper berries are soaking in the ethanol and water solution at room temperature which initiates the essential oil extraction. According to Naviglio et al. (2019) maceration is based on two physicochemical processes: diffusion

and osmosis. Osmosis is the movement of solvent particles across a semipermeable membrane from a region of low solute concentration to a region of higher solute concentration (Baumgarten & Feher, 2001). Diffusion is the movement of particles from high concentration to low concentration (Park et al., 2014). Naviglio et al. (2019) also discuss factors that can increase the extraction rate: granulometry, temperature and affinity. Granulometry is the size and surface area of the material, a decrease in granulometry leads to an increased extraction rate. The temperature can be increased to decrease the extraction time. Selection of a solvent with higher affinity towards the compounds of interest will increase effectiveness.

Once distillation begins, the solution temperature increases, and the process becomes water distillation (hydro-distillation).

2.6.2 Hydro-Distillation

Hydro-distillation is the simplest form of distillation and is the oldest method to separate essential oils from plants (Yalavarthi & Thiruvengadarajan, 2013). In hydro-distillation the botanicals are immersed completely in the solution which is brought to a boil. This is the distinguishing feature of this process, the direct contact between the boiling liquid and the botanicals. The solution in the kettle is increased from room temperature to the boiling point of the mixture, for an aqueous-ethanol mixture at atmospheric pressure and 40% ABV this occurs at 80.9°C (Tanthapanichakoon & Jian, 2012). The botanicals must be constantly mixed throughout the boiling process to prevent clusters forming and the denser botanicals from sinking to the heat source and causing thermal degradation. Clusters will limit the amount of wetting and surface area and decrease the extraction rate (Aguilera, 2003).

The main processes occurring during hydro-distillation of plant matter are hydro-diffusion, hydrolysis and thermal degradation (Fagbemi et al., 2021; Oreopoulou et al., 2019). The diffusion of water and essential oil through plant membranes is known as hydro-diffusion (Vian et al., 2008). A recent study by Handa (2008) explains that as the temperature increases the volatile oils within the solid first dissolve into solvent that is already present within the botanical cells. The study goes on to explain that the water and essential oil mixture then permeates the saturated membranes to the outer edge of the plant via osmosis. The limiting factor for the rate of extraction is the extent of the oil's solubility in solvent and not by their volatility (Handa, 2008). The longer the botanicals are in contact with the solvent the more wettability will increase, and the solvent will begin to diffuse into

the cells. This increases the rate that the oils will diffuse out of the cells as the solvent is in contact with more material.

Hydrolysis also begins to occur when components of essential oils come into contact with water. Hydrolysis is a chemical reaction between organic compounds and water leading to the decomposition of both compounds (Britannica, 2022). The main essential oil component that undergoes hydrolysis are esters which react with water to form alcohols and acids. A study by AL-Hilphy (2017) states that the rate of hydrolysis is affected by two factors: temperature and concentration of water. He continues that the higher the concentration of water the higher the concentration of alcohol and acids produced and the lower the total yield of essential oil. The longer the contact between water and oil is maintained the longer hydrolysis will continue for which makes it one of the main disadvantages of hydro-distillation (Handa, 2008). In the case of spirit hydrolysis, the decomposition product is often inevitable and ultimately desired and part of the flavour profile.

Thermal degradation occurs as the temperature rises and is highly important as many oil components are unstable at the higher temperatures (Evon, 2014). The most common method of hydro-distillation is done at atmospheric pressure where the temperature cannot be reduced. One of the key characteristics in vacuum distillation is that it can operate at low temperatures which stops heat decomposition. One factor which helps prevent heat decomposition in hydro-distillation is an agitator which keeps the botanicals moving and prevents them resting against the heating surface (Handa, 2008).

These three processes are occurring simultaneously and affect each other. Summarising from Handa (2008), AL-Hilphy (2017) and Evon (2014), a way to increase yield is to decrease the temperature, reduce the concentration of water to reduce hydrolysis and reduce botanical particle size to reduce the distance the oil-solvent mixture needs to permeate to reach the outside of the material to ensure maximum yield is achieved.

A disadvantage of hydro-distillation is that it does not lead to complete extraction and due to the high heat that the botanicals experience thermal degradation resulting in lower purity product (AL-Hilphy, 2017; Handa, 2008). A large portion of the water-soluble compounds are also left behind in the residue once the distillation has finished due to their high boiling points. This can be desired if the water-soluble compounds have unpleasant flavours and aromas.

2.6.3 Vapour/Steam Distillation

The third process is when the vapour passes through the botanical basket and over the botanicals which is a process known as vapour/steam distillation and has four stages: oil release, vaporization, mass transfer and condensation (Meireles, 2008). The main physicochemical processes that occur during these stages are hydro-diffusion, hydrolysis, and decomposition by heat. Hydro-diffusion, hydrolysis, diffusion, and osmosis have been explained in detail in the above section. This section will instead focus on the four stages that extract the VOC from the botanicals.

Another process occurring during steam distillation is percolation, this occurs when the vapour condenses on the botanicals and slowly trickles down extracting VOC along the way. Condensed vapour percolation extraction is driven by osmosis and diffusion (Coldea & Mudura, 2015). These are the same processes present in maceration however the temperature will be lower than in the boiling solution. This may lead to slightly less heat degradation and extraction of slightly more delicate flavours. There is little research on percolation extraction in the gin manufacture process and there is potential to optimize it.

2.6.3.1 Oil Release

During steam distillation of solid botanicals, some of the VOC is not accessible to the steam and the VOC must transfer out of the solid before it can be vapourised. The mechanism that moves the VOC depends upon the location within the solid as well as the characteristics of the botanical. Seeds, roots and fruits typically exhibit a different mechanism from leaves and flowers (Cerpa et al., 2009).

The VOC are usually found within the glandular trichomes on the surface of leaves and flowers. The liquid oil must permeate through the membrane and cuticle via exudation until it reaches the surface where it is vaporized (Sovová & Aleksovski, 2006). They go on to say that the oil release from trichomes happens at a much faster rate than from the matrix found in seeds and roots. As the botanical is heated the trichomes can burst releasing the oils to permeate through the solid and be dissolved in the water.

The mechanism for seeds, roots and fruits assumes that since a solid displays an isotropic behaviour with a uniform distribution of oils, diffusion inside the solid matrix can be assumed (Cerpa et al., 2009). Sovana and Aleksovski (2006), confirmed this with research into hydro-distillation of aniseed and coriander seeds. The oil release stage from solid material can be very slow and is often the

controlling stage in distillation (Katiyar, 2017). Katiyar states that since diffusion inside the botanical is the main resistance to VOC extraction, seeds and roots are often crushed.

2.6.3.2 Vaporization

Vaporization occurs at the liquid-vapour boundary. Molecules of the component move from the liquid to vapour phase according to their volatilities. The composition of each component is described by the vapour-liquid equilibrium expression as explained by Swami et al. (2008).

$$y_i P = \frac{x_i \gamma_i f_i^o}{\widehat{\phi}_i} \quad (1)$$

Where P is the total or operation pressure, x_i and y_i are the molar fractions of each component in the liquid and vapor phases, respectively, y_i is the activity coefficient of component i in the liquid phase, f_i^o is the standard state fugacity of pure component i, and $\widehat{\phi}_i$ is the fugacity coefficient of component i in the vapor phase.

2.6.3.3 Mass Transfer and Condensation

Molecules at the liquid-vapour boundary move into the steam vapour via mass transfer mechanisms that includes diffusion and convective mass transfer (Cerpa et al., 2009). The vapour oil molecules are carried by the vapour into a heat exchanger where they are condensed.

2.7 Analysis

Gin is made up of an aqueous ethanol solution with a water component, ethanol component and volatile organic compounds. Good London Dry gin is clear – all components are in solution. These separate components are able to be analysed via different methods that allow for identification and quantification of all components.

2.7.1 Ethanol and Water

There are several methods used to calculate the water and ethanol concentration within the gin. One of the simplest methods is by the measurement of the density of the solution followed by a conversion to alcohol by volume (ABV) by consulting a published table (Pečar & Doleček, 2005). The density can be measured by hydrometers, pycnometers, and digital density meters or density meter analyser (DMA). Anton Paar is a market leader in density measurement and has a large range of DMA's that are tailored for use in the alcohol industry measuring beer, wine, cider, spirits, liqueurs, wort or wash (Alcohol Meter, 2023). Commercial labs usually use more advance analytical chemistry methods such as high-performance liquid-chromatography (HPLC), gas chromatography (GC) or spectrophotometers. These methods can be less laborious and more accurate than traditional density analysis. The customs department also has guidelines on what are the acceptable methods for commercial operations within New Zealand. The approved methods for spirits are headspace or liquid injection gas chromatography, near infra-red spectroscopy, distillate analysis by gravimetric measurement or oscillating U-tube type density meter, or hydrometric testing using an OIML hydrometer of the British Standard BS 5470 (New Zealand Customs Service, 2013).

2.7.2 Volatile Compounds

Identification and quantification of VOC within gin samples has been done many times in the literature with an array of work done by Kelly et al. (1999), Clutton and Evans (1978), Dussort et al (2012) and Buck et al. (2020). They analysed gin VOC on a gas chromatograph coupled with a flame ionization detector (GC-FID), gas chromatograph coupled to mass spectrometer (GC-MS), and gas chromatograph coupled with olfactory (GC-O or GC-MS/O). The sample first had to be prepared with the two main methods requiring the raw sample to be extracted into a solvent or absorbed onto a coated needle in headspace solid phase microextraction (HS-SPME) before injection into a gas chromatograph.

2.7.2.1 Gas Chromatography

Gas chromatography (GC) has become the industry standard for the separation and analysis of volatile compounds within gases, liquids and solids (McNair et al., 2019). Basic GC componentry is shown in Figure 3.

Separation is achieved by partitioning the analytes between an inert gaseous mobile phase and a solid or liquid-on-solid stationary phase. The sample must be injected into the mobile phase (carrier gas) which is passed through the stationary phase. The carrier gas is an inert gas such as helium, nitrogen, hydrogen or argon. The stationary phase is within a thin glass or metal column inside the GC oven and will either be a capillary column or a packed column. The capillary column has a thin liquid coating on the inner surface of the column while the packed column has a fully packed column of fine particles. The temperature of the oven is controlled, and the eluent is monitored by a computerised detector. The analytes will separate themselves based on their relative vapour pressure and affinities for the stationary phase (McNair et al., 2019).

The sample is first vaporized in a heated injector before it is carried into the column and the oven temperature programme is initiated. As the analytes pass through the column, they are separated by two factors; sample volatility: the more volatile analytes are vaporized more quickly and move through the column quicker, and analyte affinity: polar analytes will interact more strongly with columns that are also polar (Piantanida & Barron, 2014). Once the analytes have been separated due to their volatility and affinity they elute sequentially and are quantified by the detector. Several key pieces of information are recorded, the retention time which is the time taken for the analytes to elute, as well as the chromatogram plot of detector intensity versus time. Each individual analyte will have a specific retention time which can be used to help identify it; the retention time of an analyte also depends on the column and temperature programme used.

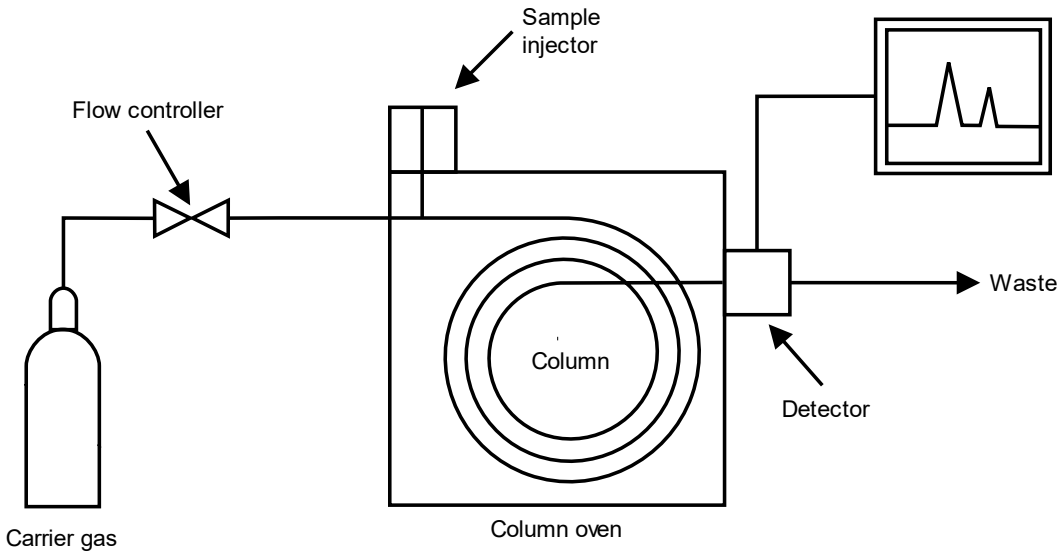

Figure 3: Simplistic line diagram of gas chromatograph. The carrier gas passes through a flow controller and into the column within the GC oven. The sample is injected into the column and the different compounds elute and are registered by the detector which converts the data into a chromatograph (image by Offnfopt, 2015).

The GC produces a chromatogram which is a graph with retention time on the x-axis which shows the amount of time taken for each analyte to pass through the column and reach the detector as well as the abundance on the y-axis (Thet & Woo, 2020). The response is shown on the y-axis. This reflects the concentration of analyte present and is calculated, in the case of a conventional Flame Ionisation Detector, from the current that flows through the flame when the compounds elute from the column in the carrier gas. The peaks on the graph correspond with the time that analyte took to reach the detector. Many factors affect the retention time of analytes such as column type, temperature programme etc, which makes comparing analysis between labs difficult without the use of a standardizing equation such as Kovats retention index which makes retention times into system-independent constants (Zenkevich, 2010).

Research by McNair et al. (2019) states that the most common detector for GC is the flame ionization detector (FID) because of its ability to perform across a wide operating range and being inexpensive. The FID is suited for, and has a high sensitivity for, detecting compounds that contain carbon atoms which are found within almost all organic compounds. The other most common detectors are the thermal conductivity cell (TCD) and the electron capture detector (ECD).

To select the appropriate detector, it is important to consider; does it have adequate sensitivity, have correct selectability for the analytes, and have good stability for the instrument (McNair et al., 2019). The predominant detector found in literature for the analysis of gin is the FID detector for the quantitation of compounds while the mass spectrometry and olfactory detector are commonly used for the identification of compounds (Buck et al., 2020; Clutton & Evans, 1978).

Gas chromatography-olfactometry (GC-O) or GC-MS-O is a technique that combines GC with a human assessor to detect different odour compounds (Delahunty et al., 2006). The GC separates the compounds before an olfactometer human assessor detects the odour activity as each compound elutes. Delahunty et al. discuss how this is a powerful analysis method especially when determining the odour threshold of compounds. Dussort et al. (2012) agrees stating that GC-O is the premier tool to determine the odour threshold values for volatile compounds. Dussort et al. continues that less than 5% of the volatile compounds affects the products aroma. Therefore GC-O is only able to categorize 5% of the compounds.

A gin sample must go through sample preparation before it is able to be analysed via gas chromatography. One of the main sample preparation objectives is to decrease water concentration. Before compounds move through the column they are vaporised, and water expands by roughly a factor of 1600 when it turns from a liquid to gas. This large expansion can lead to fluctuations in

pressure and decrease detection of other analytes due to peak splitting or tailing and even damage the column therefore water removal is important in GC sample preparation.

2.7.2.2 Gas Chromatography – Mass Spectrometry

A common method to identify unknown congeners in spirits is via direct liquid injection into a gas chromatography – mass spectrometer (GC-MS) as utilised by Hodel et al. (2019), Namara et al. (2007), and Stupak et al. (2017). Mass spectrometry is an analytical tool, coupled to GC, which is capable of measuring mass-to-charge ratio (*m/z)* of sample ions which allows for the identification of unknown compounds (Glish & Vachet, 2003). The result of measuring m/z is presented in a mass spectrum which shows the m/z ratio of the ions against their intensity. This spectrum is used to determine the isotropic signature of the sample and to identify the chemical compounds present.

A mass spectrometer consists of three main components: an ionization source, mass analyser and ion detection system. In a GC-MS system the GC separates a sample into its individual compounds based on their elution time and then passes through an ionization source which converts the compounds to gas-phase ions, so they can be analysed (Glish & Vachet, 2003). In Figure 4 below the basic components can be seen, the ion detection system is after the mass analyser but is not shown.

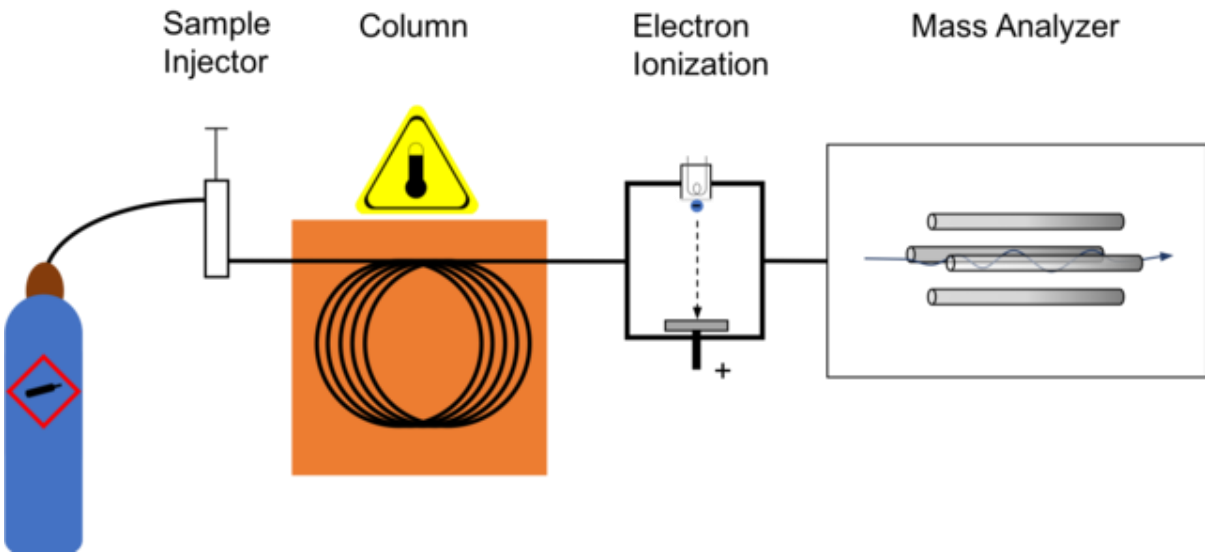

Figure 4: Simplified diagram of a GC-MS machine showing the pathway of the carrier gas. The sample will be injected into the column. A computer will be connected to the mass analyser which will be used to analyse the data and identify the compounds (image by Kkmurray, 2018).

2.7.2.3 Solid Phase Microextraction

Solid phase microextraction (SPME) is a solvent free sample preparation method that can be used to collect, concentrate, and analyse volatile compounds (Ouyang, 2012).

SPME allows for rapid analysis of volatile compounds based on the principle of adsorption/absorption and desorption. It is merely a stage of sample preparation before the sample can be analysed via gas chromatography. SPME was developed to improve upon limitations inherent in solid phase extraction and liquid-liquid extraction. It reduces solvent use (is a solvent-free method) and was designed for the analysis of volatile and semi-volatile components of wastewater but is now commonly used for the analysis of food and beverages. SPME can either be direct immersion into a liquid (DI-SPME), placed in the headspace above the liquid or solid sample (HS-SPME), or the fibre is protected by a membrane (M-SPME).

SPME is based on the partition equilibrium of analytes between the sample matrix and the extraction phase. The extraction phase is coated on a fibre or capillary tube and the most common are polydimethylsiloxane (PDMS), polyacrylate, divinylbenzene (DVB), carboxen (CAR), or a combination of these polymers PDMS-DVB, DVB-CAR and DVB-CAR-PDMS (Van Hout et al, 2003). The analytes partition between the sample liquid/gas phase and the extraction phase on the fibre until equilibrium is reached. The fibre is then placed into a separating instrument where desorption occurs, and analysis begins. One disadvantage of this method is the high cost of the fibres and can range from $300 to more than $1000 per fibre that may last 50-100 injections.

In gin analysis HS-SPME is a common preparation method with Biernacka and Wardencki (2012), Einfalt (2020), and Vichi et al. (2005), using this method to prepare gin samples before analysis via gas chromatography.

2.7.2.4 Other Analytical Techniques

While analysis will be performed on some form of GC the sample preparation is very important to optimise VOC analysis. Several different techniques are commonly used, liquid-liquid extraction, HS-SPME and SPME. However, there are several less common techniques that are also used to prepare gin samples for analysis. Wei (2018) used stir bar sorptive extraction (SBSE) coupled with GC-MS. (SBSE) was introduced in 1999 as a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. Vichi et al. (2008) analysed the

diterpenoids present in eight commercial gins using direct immersion solid-phase microextraction (DI-SPME) coupled to GC-MS.

2.8 Partition Coefficient

A partition coefficient is "the ratio of the concentration of a substance in one medium or phase (C_1) to the concentration in a second phase (C_2) when the two phases are at equilibrium" (Schlosser et al., 2010). Therefore, the partition coefficient (K_{PC}) is equal to:

$$K_{PC} = \left(\frac{C_1}{C_2}\right) \tag{2}$$

The partition coefficient directly affects how the compounds partition between the mobile phase and stationary phase in gas chromatography and hence effect when they elute and their retention time.

The VOC in the gin will never solely remain in the liquid phase. They are initially only found in the liquid however immediately after distillation they will begin partitioning into the vapour phase until equilibrium is reached. For this reason, it is important that product is bottled as quickly as possible to decrease the amount of VOC partitioning into the vapour phase and reducing the amount of flavour compounds in the gin. In a bottle there is only a small amount of headspace and equilibrium is reached quickly with minimum loss from the liquid phase.

Another factor affecting loss of compounds from the liquid phase is how volatile the compounds are as the more volatile compounds will evaporate at a quicker rate. Aylott (1995) has studied the loss of key flavour compounds from a bottle of gin that is left open over a 7-day period. Aylott found that out of the seven monitored compounds the concentration of, four decreased to almost 0 while three compounds stayed close to their initial concentration. Aylott concludes that this is purely due to α-pinene, β-myrcene, limonene and γ-terpinene being much more volatile than camphor, linalool and geranyl acetate.

There are methods to increase the partition coefficient and so the concentration in the headspace, these include heating and the addition of salt (salting out). For gas chromatography analysis methods such as HS-SPME, increasing the concentration in the headspace will allow for more compounds to be identified and salting out can enhance the sensitivity by up to 10 times (Kolb,

2000). This technique is often reported in literature for the analysis of gin volatiles and utilised by Zhao et al (2013) and Buck et al (2020) for HS-SPME analysis and by Dussort et al (2012) for GC-O analysis. There is evidence that the salting out effect can be effective for liquid-liquid extraction (LLE) as reported by Majors (2009), he states that it is most effective for increasing the concentration of polar analytes in the solvent. There are fewer reports of it being a successful technique when used with LLE of gin flavour compounds. Clutton and Evans (1978) reported no increase in recovery of analytes after investigation into 14 salt compounds.

The concentration of VOC in one phase can be determined if the partition coefficient and the concentration in the other phase is known. Partition coefficients are usually determined experimentally although theoretical estimates can be calculated for simple binary systems however, due to the high number of variables theoretical estimations include a large margin of error. To calculate it experimentally the concentration of the solute must be measured across both phases.

2.9 Sample Preparation

2.9.1 Water Removal

Since water is an unwanted component in gas chromatography there are several methods utilised to remove it.

Fractional freezing is a method used to separate substances that have a difference in melting points, this method can also be called freeze distillation or freeze concentration. This method can be used to separate water which has a melting point of 0°C and ethanol with a melting point of -114.1°C. Pickering (2000) describes the freeze concentration process capable of separating water from grape wine, though states that it is a delicate and expensive process.

Another method is using a chemical dehydration reaction. Padalia et al. (2013) and Narayanankutty et al. (2021) dried essential oil samples from hydro-distillation using anhydrous Na_2SO_4 before analysis on GC. Water removal can also be done using silica gel or calcium carbonate which will also absorb the water.

The most common method in gin analysis is to do a liquid extraction into a solvent that is immiscible with water and then perform analysis on the solvent.

2.9.2 Liquid-Liquid Extraction

Due to the large amount of water that is present in raw gin samples it is not recommended that samples be analysed directly by GC (Kuhn, 2002). A method to solve this issue is to extract the volatiles that are desired to be analysed into another solvent that can then be injected into the GC without the presence of water. Many different solvents are used in essential oil extraction and will be selected depending on the properties of the analytes of interest. Kyle (2017) states that the analytes must be soluble in the solvent and will ideally have high partition coefficients in the solvent. He goes on to state that the disadvantages of the LLE method is that a large solvent volume is required and has a longer extraction time. A polar solvent will only extract the polar compounds leaving many desirable compounds in the aqueous phase. Often to ensure complete extraction a mixture of polar and non-polar solvents need to be used which increases the cost and time. In the case of gin VOC analysis only the key compounds are required to be identified and quantified. The key compounds are relatively non-polar which means they are soluble in non-polar solvents and are mostly insoluble in water. A non-polar solvent will not extract as much of the polar compounds however they are not important for this study.

Common solvents used in the extraction of essential oils include hexane, chloroform, dimethyl sulfoxide (DMSO) and dichloromethane (DCM). Dimethyl sulfoxide (DMSO) is known to be an effective solvent for heavy aromatics. DMSO is a polar compound and is usually used for the extraction of compounds from non-polar phases. Since DMSO is miscible with water, the water will still have to be removed post extraction. Chloroform is also commonly used and is reported by Hossain and Shah (2015) as a successful solvent for the extraction of essential oil from *Merremia borneensis*. Dichloromethane is another popular solvent for the use of extracting essential oils as shown by Buck et al. (2020). While methanol is rarely used due to its low boiling point it is sometimes combined with DCM in 2:1 ratio to aid in the extraction of both polar and non-polar compounds. Another common solvent for liquid-liquid extraction prior to GC analysis is n-hexane. n-Hexane is well suited to GC analysis due to its low boiling point allowing it to move rapidly through the column. It is also immiscible in water making straightforward separation possible.

Clutton and Evans (1978) analysed and contrasted the suitability of solvents that are best suited for extracting essential oils found in gin. They judged solvents on eight attributes: low affinity for solute, ability to remain selective towards all major flavour compounds, be readily available and economic, be available in a pure stable state, have a low boiling point, be inert towards flavour components and ethanol-water, have a different density from 40% ethanol and being immiscible from ethanol.

After assessing suitable solvents Aylott (1995) and Vichi et al. (2008) use n-hexane to extract botanical congeners from gin and it is reported as an effective solvent for the extraction of essential oils from other materials by Danh et al. (2013).

2.9.3 Blanks and Samples

There are many methods to foster accuracy and precision while performing analysis on the GC-MS. Two methods are the use of blanks and samples. For example, reference samples, control samples, spiked samples, replicate samples and instrument blank, method blank, trip blank, field blank, and equipment blank.

Blanks should ideally have none or extremely low amount of analyte present otherwise by definition they are not blanks. EPA (US) standards (3) define acceptable blanks as needing analyte concentration at less than half of the low limit concentration (Raynie, 2018). The most common blanks include method blank which uses an analyte free matrix which is then processed in the exact same manner as the samples to determine any sources of contamination. This can cover instrument blank and equipment blank as they can overlap. Method blank is commonly utilised in GC analysis to check whether there is any column bleed in between samples or whether the solvent is contaminated.

2.10 Calibration of System

2.10.1 External Standardisation

External standardisation (ESTD) as a calibration method is one of the most common methods to increase precision, with Turner et al. (2019) stating that ESTD is the most straightforward method to increase precision in quantitative analysis. In scientific research it is important to be able to determine the concentration of an unknown sample and this can be done with a calibration curve generated using ESTD.

Calibration curves allows the unknown concentration to be predicted by understanding the instrument response to a set of known concentrations (Dolan, 2009). A range of samples must be prepared that cover a range of concentrations around the expected concentration of the unknown sample. That is, they cannot be much larger or much smaller than the unknown concentration.

Calibration curves cannot be extrapolated as the response factor can change with concentration. The chromatogram can be integrated to find the area of the standard peaks that will be used to plot the calibration curve, area vs concentration. While the purpose is generally to determine the unknown concentration, calibration curves are also used to calculate the limit of detection (LOD) and limit of quantitation (LOQ) (Turner et al, 2019).

2.10.2 Internal Standardisation

Since GC analysis is highly sensitive to small changes, the same two samples analysed on different days can result in different results due to external factors (Henshaw, 2017). This can be due to column degradation, temperature differences, gas flow rate and column failings. While these want to be kept the same through trials there will inevitably be uncontrollable factors that will slightly shift the analyte response area. Using an internal standard allows for the highest precision by accounting for slight difference in GC parameters (Turner et al., 2019) as well as accounting for volume and sampling errors (McNally et al, (2015).

Internal standard is defined as a compound that is not one of the analytes of interest, but it is added to all samples and calibration samples in a known amount to increase the precision and accuracy (Dolan, 2009). It provides a known basis against which unknown compounds can be ratioed. The accurate concentration determination of analytes of interest is determined by the relative response factor between the internal standard and the analyte (Magee & Herd, 1999).

Internal standards are commonly used in gas chromatography to increase accuracy however care needs to be taken when selecting the appropriate compound to ensure accurate results (Hiatt, 2011). It must not be present in the sample at any concentration, must not interact with the peak of any analytes of interest, the retention time must be like that of the other analytes and all recovery problems must be strictly proportional in order to accurately scale the responses (Coleman & Vanatta, 2005). Vekiari et al. (2002) used octyl acetate when analysing the essential oil extracted from the leaves and peel of a Cretan lemon. This was used because it does not appear naturally in the sample and is similar chemically to the main compounds being analysed. Aylott (1995) used 3-pentanol as an internal standard when characterising gin volatiles while Hodel et al. (2019) used 3-octanol for quantification of gin. The most common internal standards in wine quantitative and qualitative analysis are 2-octanol and 4-methyl-2-pentanol due to their chemical similarity to monoterpenes (Pati et al., 2021). This ensures that they elute in the right range. Due to the high

number of monoterpenes also present in gin, 2-octanol will be suitable for gin internal standardization.

To calculate the concentration of an unknown analyte using GC analysis the response factor must first be calculated. The response factor relates the concentration and the peak area.

$$RF = \frac{Peak\ area}{Concentration} \tag{3}$$

The response factor of an analyte and the response factor for the internal standard can be used to determine the relative response factor between the compounds.

$$RRF = \frac{RF_A}{RF_{IS}} \tag{4}$$

A series of dilutions containing known concentrations of the IS and analyte can then be used to calculate the RRF which is used in equation 5 to calculate the unknown concentration of the analyte in a sample.

$$Concentration\ A = \frac{1}{RF_{IS}} \times \frac{1}{RRF} \times Peak\ area\ A \tag{5}$$

2.10.3 Kovats Retention Index

The Kovats retention index (KRI), is a method used to convert GC retention times into system independent constants (Zenkevich, 2010). As outlined in this report GC analysis is highly dependent on numerus variables which leads to differences in retention times within the same run of samples. The Kovats retention index (KRI) removes any variation caused by different equipment, column length, film thickness or type, carrier gas type and pressure. This allows for the comparison of results between different machines and laboratories. While out of the scope of this study, the KRI would be beneficial for future researchers if they wanted to continue this study or use the results found in this study and be able to relate and compare to results on another gas chromatograph machine.

2.11 Conclusions and Research Gaps

The gin distillation process is comprised of three stages, initial fermentation of carbohydrate base to create a wash, distillation of this wash to create neutral spirit and secondary distillation to infuse the neutral spirit with flavour. All the key flavour compounds are imbued during the secondary distillation process and the micro-processes that occur to extract compounds are maceration, macerate distillation, condensed vapour percolation and vapour/steam distillation.

The main botanicals that are found in most London dry gins are: juniper berries, coriander seeds, angelica root/orris root and a citrus component. The flavour compounds extracted from these botanicals and any additions give gin its distinctive taste. Each compound has a different odour threshold with some compounds requiring a much higher concentration before it is noticeable compared to others. There is much contradictory research done on odour thresholds for the compounds of interest, but that research is outside the scope of this study.

The analysis of gin volatiles has been done many times with many different results. This is mainly due to the wide range of different VOC found in gin due to the different botanicals now being used in modern gin distillation. The analysis technique most used is gas chromatography however the equipment coupled to the GC varies from FID, MS, O and largely depends on the planned analysis. GC-MS is the optimal method for compound identification and GC-FID is the optimal method for quantification and GC-MS/O is ideal for flavour threshold investigation.

HS-SPME sample preparation has been used to great success however it is not the only method with liquid-liquid extraction also being very common. Direct injection was not recommended due to the high concentration of water. The high cost of HS-SPME fibres and their fragility is a big factor when choosing this method. Liquid-liquid extraction is a far cheaper method and will be investigated further to determine its suitability for research.

In summary much is known about gin manufacture and what compounds contribute the most to the distinctive flavour. There have also been many analysis methods used to identify and quantitate the vast number of different VOC within gin. What has not been researched is how does each individual micro-process extract flavour compounds from the botanicals and which is the most significant. Characterising the micro-processes will allow them to be optimized to create a gin ‘hotshot’. Some research is available for the relationship extraction rate and botanical ratio for juniper berry in vapour infusion however there is no literature on other botanicals or on the other processes and the key parameters influencing the extraction rate.

2.12 Coda to Literature Review: Emergence of Recent Work on "Multi-Shot Gin"

Recent reports by Black (2022) and, Pauley and Hodel (2023) show that some distilleries have experimented with two production methods: one-shot production and multi-shot production. One-shot production is when the distillate only requires dilution with water to reach required dilution whereas multi-shot requires dilution with water and spirit to reach the required flavour and ABV concentration. Multi-shot production has a much higher ratio of botanicals to create a higher concentrated flavour distillate. This multi-shot method is what this study initially called a gin 'hotshot' and was investigating whether industrial production was possible. This literature shows that not only is it possible but that it has started to be adopted as a production method in the gin industry. This confirms it as an emerging practice, but the lack of available literature proves that it is worthy of further investigation. This literature answers the research aim to this study however the research questions will still be beneficial in building up essential literature and understanding around this new production method.

Chapter 3: Key Analyte Determination and Calibration of Equipment

3.1 Introduction

The investigation into the influence that the separate distillation micro-processes have on the extraction rate begins with the determination of the key analytes of interest. Identified compounds could then be quantified over each individual process and the influence of different parameters investigated. In Chapter 2 it is discussed that there are no agreed odour threshold values for any of the flavour compounds in gin, so the key analytes were determined based on relative peak area.

After the key analytes had been determined the equipment was calibrated via internal and external standardisation. Once calibrated the GC-MS and GC-FID could identify and quantify the selected analytes.

3.2 Gas Chromatography – Mass Spectrometry Method Development

3.2.1 Gas Chromatography-Mass Spectrometry

3.2.1.1 Materials and Methods

The equipment used for GC-MS method development was a Shimadzu GCMS-QP2010 Ultra GC/MS equipped with a Shimadzu AOC-20s Autosampler. The column was a TG-5MS (30 m x 0.25 mm x 0.25 µm) with a 5% phenyl base sourced from Thermo Fisher Scientific, Auckland. Two methods were investigated trialling two different time-temperature programs. The following conditions were used across both time-temperature programmes; helium was used as the carrier gas with a flow rate of 1.09 mL/min and the split ratio was set at 20:1 with an injection amount set at 4 µL. The interface temperature was 280°C and the ion source temperature was 175°C with a solvent cut time of 1.8 minutes. The scan range was 35-500 m/z .

A short method was devised with the injection temperature set at 260°C with an initial oven temperature of 35°C and a 5 min hold time. The temperature was then increased to 300°C at the rate of 16°C/min with a hold time of 1.44 minutes for a total length of 23 minutes.

Secondly the longer method tested had an injection temperature of 260°C and an initial oven temperature of 55°C. The temperature was then increased to 60°C by 1°C/min with a 1-minute hold time, then increased to 65°C at 5°C/min and then to 110°C at 10°C/min with a 10 min hold time followed by increasing to 120°C at 10°C/min with 3 min hold, then increased to 250°C at 10°C/min and finally to 320°C at 40°C/min with a 3 min hold for a total run time of 43.5 minutes. The time-temperature program is shown in Figure 5.

The first sample analysed each session was a hexane blank. Initially the samples were injected in triplicate until the precision of the equipment was sufficiently well characterised and then singular injections were used.

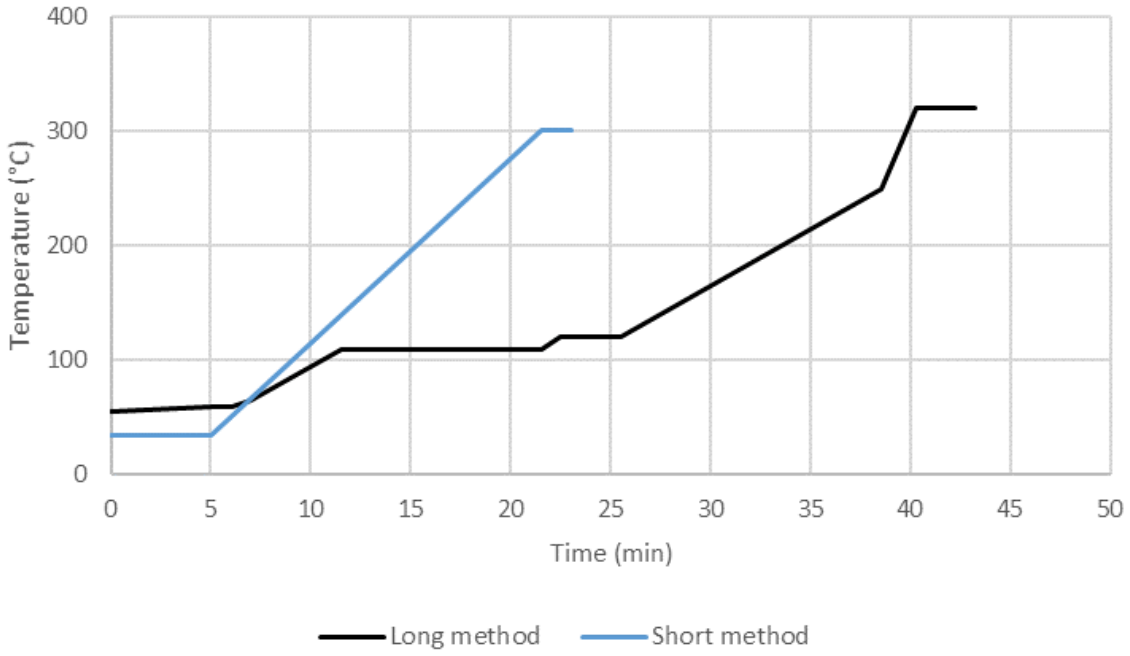

Figure 5: The two different time-temperature programmes; short: 23 minutes and long: 43.5 minutes long. The longer method was more successful at eluting a greater number of compounds for identification.

3.2.1.2 Results and Discussion

From the literature GC-MS is a common technique used for identification of volatiles in gin samples. However, some clear differences between GC methods in the literature were noted, namely differences in total method time and different time-temperature steps. Two contrasting methods were investigated, initially a long method that had multiple slower time-temperature steps, based

on work done by Hodel et al. (2019), and a short method with only one ramping temperature step, based on work done by Namara et al. (2007). In comparison the long method was 43.5 minutes and the short method 23 minutes. This time difference would make a substantial impact on the duration of this project due to the 600 samples that needed to be analysed by the GC-MS and GC-FID. The time-temperature method should be efficient but still identify and quantify the highest number of compounds on the GC-MS. During qualitative analysis only the first 30 peaks were considered significant and attempted to be identified.

There was a significant difference in compounds identified across the two methods. The results found that the longer more gradual method led to more compounds being identified on the GC-MS. While some of the compounds may have had a similar vaporisation temperature the determining factor is the shorter time-temperature program is unable to resolve the components properly.

Table 5: Number of compounds identified using GC-MS analysis from hexane extraction between the different time-temperature programme using the NIST library. A full list of the identified compounds can be found in Appendix A.1.3, Table 43.

Method	Compounds identified
Long method	28
Short method	20

Quantification of compounds was investigated via GC-MS analysis however it did not prove to be a sensitive method. The same sample injected multiple times led to a wide spread of values largely increasing the error. GC-MS is a valuable identification tool however it is not optimal equipment for quantification therefore GC-FID analysis was investigated.

3.2.2 Gas Chromatography – Flame Ionization Detector

3.2.2.1 Materials and Methods

The equipment used for quantitation analysis was an Agilent 7890A gas chromatograph (Agilent Technologies, US) with a flame ionization detector coupled to an Agilent 7683B autosampler. The column was a SUPELCOWAX 10 (30 m x 0.32 mm x 0.5 µm) fused silica capillary column obtained from Sigma-Aldrich, Auckland. The Agilent systems were controlled by Agilent Chemstation (Version

E.02.02.1431). The carrier gas was helium with a flow rate of 5 mL/min the injection temperature was 260°C with a volume of 2 µL and split ratio of 20:1.

The initial GC-MS method required the maximum number of analytes to be determined however the method was then shortened once it was found that all key analytes eluted within the first 20 minutes of the program. The temperature ramp after 23.5 minutes was increased from 10/min to 40/min decreasing the total time from 43.5 minutes to 27.5 minutes. This minimized the wait time between each sample and allowed for analysis to progress at a faster rate.

The GC-FID time-temperature program was set to: oven temperature of 55°C with 3 minute hold time, increased to 60°C by 5°C/min hold for 1 minute, then increased to 110°C at 10°C/min with 10 minute hold time, then increased to 120°C at 10°C/min with a 3 minute hold time, and finally increased to 270°C at 40°C/min. The time-temperature program is shown below in Figure 6.

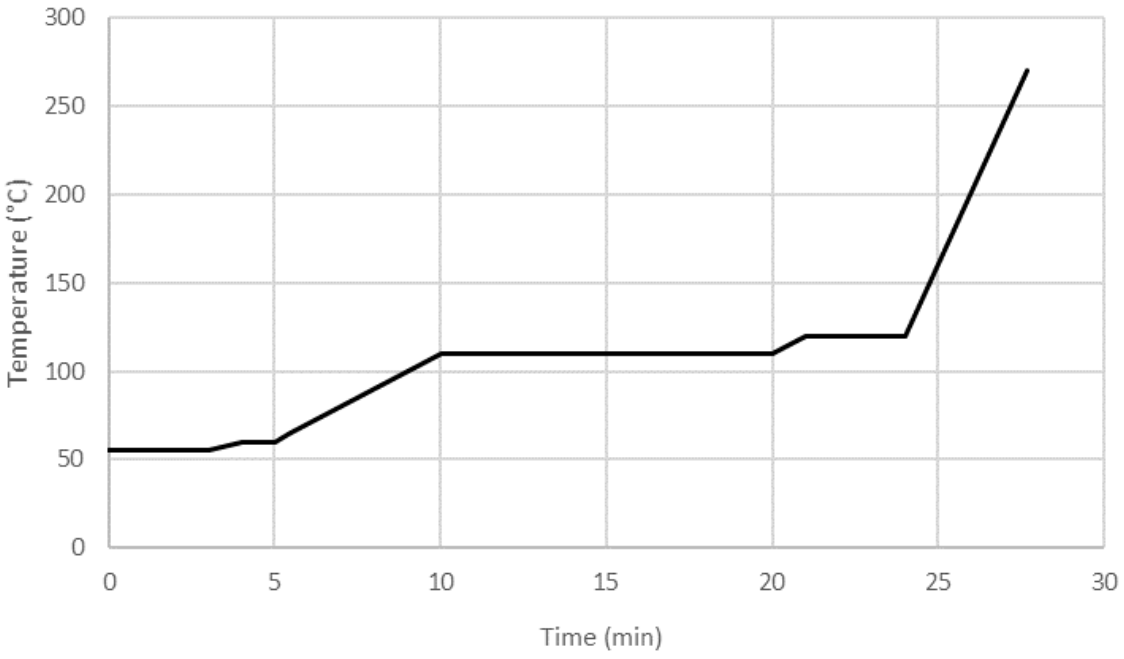

Figure 6: The time-temperature programme used for GC-FID analysis. It was adapted from the method used for GC-MS analysis, by increasing the temperature ramp and reducing the time.

The first sample analysed each session was a hexane blank and a mixed standard blank. Initially the samples were injected in triplicate until the precision of the equipment was sufficiently well characterised and then singular injections were used.

3.3 Determination of Key Analytes

3.3.1 Materials and Methods

The base gin samples at 40% and 79% ethanol by volume were obtained from BeGin Distilling, New Plymouth, New Zealand and were stored at -18°C. The gin samples were extracted into a solvent over 17 hours in a 3:1 ratio before analysis by GC-MS (full method in section 4.2.3). The analysis was performed on a Shimadzu GCMS-QP2010 Ultra gas chromatograph equipped coupled with a Shimadzu AOC-20s Auto Sampler. The column was a TG-5MS (30 m x 0.25 mm x 0.25 µm) with a 5% phenyl base sourced from Thermo Fisher Scientific, Waltham, Massachusetts, USA. The GC materials consisted of 2 mL short thread clear glass vials, 0.2 mL clear glass micro-inserts and 9mm ultraclean short thread silicone caps obtained from Thermo Fisher Scientific, Waltham, Massachusetts, USA. Three pipette sizes were used: Gilson-Pipetman P1000 100-1000 µL, Helena Labortaries-Quickpette 10-50 µL and Thermo Labsystems-Finnpipette 1000-5000 µL.

The determination of key analytes was determined by analysing the GC-MS results from the solvent selection investigation. During the solvent selection investigation (section 4.2.2) 28 compounds were identified across all four solvents by the Shimadzu GC-MS, with hexane having the highest number identified. The 28 compounds identified by hexane are shown in Appendix A.1.3, Table 43. Some compounds were only present in select solvents due to their affinity, yet the compounds with the largest peaks were common across all successful solvents. The compounds with the 10 largest peak areas are shown in Table 6, a further 18 compounds were identified in hexane but not considered possible key analytes since their peak areas combined for less than 16% of total peak area. It is noted in Chapter 2 that odour threshold is an important factor however many compounds are lacking relevant research or compounds have conflicting values therefore odour threshold was not considered for this study.

The 10 compounds were then compared on availability and cost. The cost analysis shown in Table 6 was done using data available from Sigma Aldrich (https://www.sigmaaldrich.com/NZ/en). Table 6 lists the 10 compounds with largest peak area using hexane which was determined to be the best in the solvent selection investigation in section 4.2.2.2.1.

Table 6: This table lists the compounds with the biggest peak areas identified in hexane. Based on this table the key analytes were determined based on economic viability and availability.

Compound	Peak Response	Total Peak %	Price ($/mL)
α-Pinene	71448224	30.46	$24.00/mL
Sabinene	39479237	16.83	$4,860.00/g
β-Myrcene	27995365	11.94	$846.00/g
Limonene	15933723	6.79	$17.8/mL
Citronellal	10908216	4.65	$1.43/mL
Germacrene D	7269434	3.10	$58,050.00/g
γ-Terpinene	6634336	2.83	$16.34/mL
β-Pinene	6336476	2.70	$4,860.00/g
α-Thujene	5698486	2.43	NA
Terpinen-4-ol	4622198	1.97	$6,660.00/g

One compound α-Thujene was not available in a pure standard form and was ruled out. Sabinene, Germacrene D, β-Pinene and Terpinen-4-ol were also considered out of the economic bounds for this study and were disregarded. The remaining compounds were considered viable for this study and included five of the largest seven compounds based on peak area. The selected compounds were α-pinene, β-myrcene, limonene, γ-terpinene and citronellal.

Four of these compounds are amongst the most common reported in the literature discussed in Chapter 2. Citronellal was not on the list of common compounds and in the research examined it is only reported to be found in kaffir lime leaf (Waikedre et al., 2010). An important factor to consider is that the reported compositions may be different to what is found in the botanicals sourced in New Zealand.

3.4 Internal Standard

The internal standard selected was 2-octanol, which meets critical criteria outlined in Chapter 2. This standard is commonly used in the wine industry to measure monoterpenes, the same compounds found in high concentrations within gin.

3.5 Calibration

3.5.1 External and Internal Standardisation

3.5.1.1 Materials and Methods

Calibration for quantitation analysis was performed on an Agilent 7890A gas chromatograph (Agilent Technologies, US) coupled to flame ionization detector (FID). The column was a SUPELCOWAX 10 (30 m x 320 µm x 0.5 µm) fused silica capillary column obtained from Sigma-Aldrich, St. Louis, Missouri, USA. System was equipped with an Agilent 7683B autosampler. The external and internal standards α-pinene 98%, β-myrcene 97%, (R)-(+)-Limonene 97%, γ-terpinene 97%, citronellal 95% and 2-octanol 97% were sourced from Sigma-Aldrich, St. Louis, Missouri, USA. All chemicals were stored at room temperature in flammable stores except β-myrcene which was stored at -18°C. Henceforth (R)-(+)-Limonene is referred to as Limonene. Hexane (95%) was used as the solvent and was sourced from UNIVAR. The GC vials and pipettes mentioned in section 3.3.1 were also used for calibration methods. All weighing throughout this project was done on Sartorius Entris II analytical balance.

The key analytes were used to calibrate the GC-FID to allow for the quantification of the unknown samples. Calibration was done via internal standardisation and external standardisation. These methods were adapted from work by Turner et al. (2019). The retention time of the internal and external standards were first determined, and the key analyte peak identity was confirmed.

Firstly, the retention time was determined. A series of base solutions were prepared by diluting each standard in hexane to 300 $\pm$ 20 mg/L. The internal standard was diluted to 5000 $\pm$ 100 mg/L, the higher concentration was to prevent the dilution of analytes when added to the sample. These base solutions were individually injected into the GC-FID in triplicate to determine the compound's retention time. Secondly, to confirm that the peaks of the analytes of interest were correctly identified, five extracted gin samples were individually spiked with a small amount of the base solutions.

Once all retention times and identities of external standards were confirmed the external standards were combined into one mixed standard solution diluted in hexane (shown in Table 7). This was a viable method since they each had distinct retention times and would not interfere with each other. The previously prepared 300 ± 20 mg/L solutions were used as stock solutions to prepare a series of five solutions containing the five external standards at varying concentrations. Each solution was spiked with 30 µL of internal standard solution for an end concentration of 52 $\pm$ 2 mg/L of 2-octanol.

Table 7: This table displays the varying concentrations of each external standard and the constant internal standard concentration which was used for internal and external standardisation calibration.

	Concentration (mg/L)				
Compound	**Solution A**	**Solution B**	**Solution C**	**Solution D**	**Solution E**
Limonene	114.7	14.1	1.0	35.6	72.8
Citronellal	74.4	118.1	14.0	1.0	35.8
β-Myrcene	40.2	74.5	124.1	25.6	0.5
γ-Terpinene	0.9	37.1	74.6	121.0	14.0
α-Pinene	18.3	1.1	41.7	98.3	116.2
2-Octanol	50.2	51.2	55.1	50.4	54.4

The combined standard solutions shown in Table 7 were used to test the instruments response to varying levels of analyte (see Appendix A.1.4 for full GC analysis results for all solutions). Each analyte's response was then plotted as a function of concentration creating a series of external standard calibration curves and calibrating the instrument. Internal standardisation is the ratio (between the internal standard and analyte) that is plotted against the concentration to make the internal standardisation curve. The relative response factor is then calculated from the concentration and response.

$$RF = \frac{Peak\ area}{Concentration} \tag{6}$$

$$RRF = \frac{RF_A}{RF_{IS}} \tag{7}$$

3.5.1.2 Results and Discussion

Initially the retention time of the key analytes was calculated on the GC-FID by diluting the pure standard in hexane. The retention time on the GC-FID must be determined independently to the GC-MS due to difference in the column and operating parameters. These initial samples also served to give a starting estimate of the range required for calibration samples. The initial samples at 300 ± 20 mg/L had far larger peaks than the peak areas recorded in initial gin samples, so calibration concentrations were lowered to be less than 150 mg/L.

Table 8: The average retention time for the external and internal standard on the gas chromatography – flame ionization detector equipment.

Compound	**Retention time (± 0.02 min)**
α-Pinene	2.00
β-Myrcene	4.11
Limonene	4.93
γ-Terpinene	5.97
Citronellal	10.05
2-Octanol	9.12

Due to minute differences in pressure, gas flow or column wear between analysis runs, the retention times drift and will often be slightly different on different days or even on the same day. These shifts in retention time were very small with common values changing by a maximum of 0.02 min between different days and runs. One benefit from selecting the largest peaks is that even when retention time shifts the analyte is still easily identifiable.

Once the retention times were known the mixed standard solutions were analysed. Figure 7 shows one of the combined standard solutions analysed in triplicate. The first seven peaks are the solvent and standards and there are two contamination peaks present after 15 minutes. The gas chromatograph sharply increases at the end due to a large increase in oven temperature to flush compounds through the column.

The peak areas of each external standard were plotted as a function of concentration with the calibration curve for α-pinene shown in Figure 8. This allows for the concentration of α-pinene to be calculated in unknown solutions once the peak area is determined on the GC-FID. The other calibration curves for the external standards can be found in Appendix A.1.4

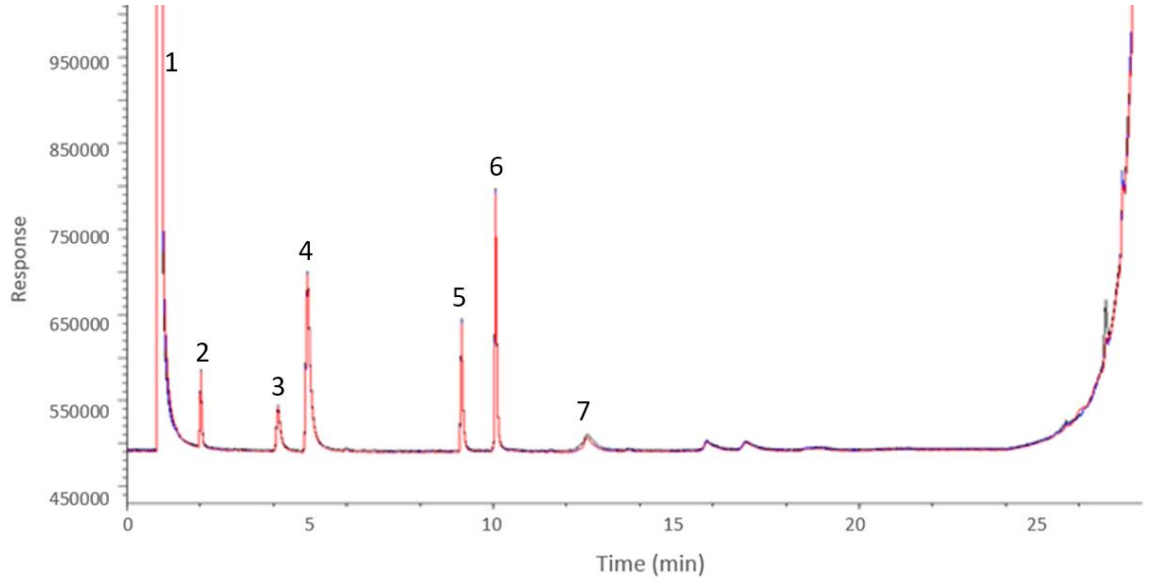

Figure 7: Triplicate injections of a single combined standard overlayed on each other. The first peak is the solvent hexane, and the following six peaks are the standard compounds. In order they are: α-pinene, β-myrcene, limonene, γ-terpinene, 2-octanol, and citronellal. There are two smaller peaks after 15-minutes which are deemed to be due to contamination. The increase after 24 minutes is due to the increase in temperature at the end of the program.

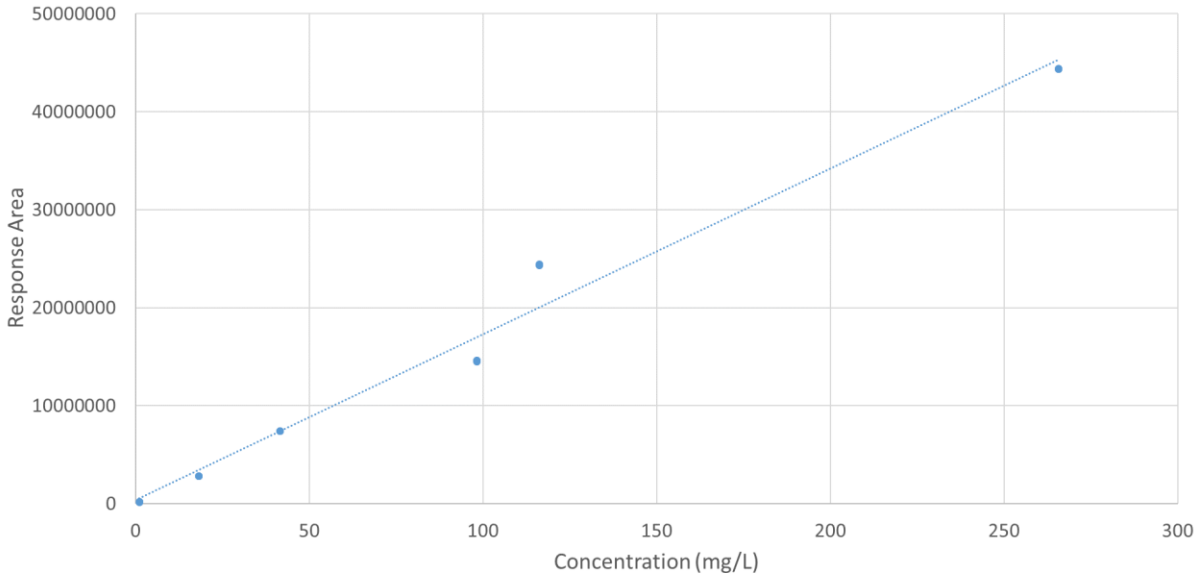

Figure 8: Calibration curve for α-pinene. Standard solutions were injected in triplicate and the points are superimposed. Graph had a function of y=169237x + 340644 and a R^2=0.98.

The external standardisation method is unable to account for slight variations in pressure and other variables that change between injections and runs. The internal standardisation method relates the

unknown concentration to the known concentration of an added internal standard compound accounting for small variations. The concentration of the internal standard and external standard in each mixed standard solution were used to calculate the relative response factor shown in Table 9.

Table 9: Relative response factor for each external standard calculated from the response to the internal standard 2-octanol.

Compound	RRF
α-Pinene	1.448
β-Myrcene	0.832
Limonene	1.272
γ-Terpinene	1.353
Citronellal	1.148

3.6 Key Analyte Concentration in Gin Specimen

The key analytes concentration in the original gin specimen was calculated to determine what elevated level the concentrated 'hotshot' product must reach. The gin specimen was extracted for 17 hours with hexane using an orbital shaker following the method in section 4.2.3. Analysis was performed on the GC-FID as outlined above in section 3.2. Three samples were extracted, and each sample was analysed in triplicate with the average concentration of the analyte in the gin sample shown in the table below. The concentrations align with literature since α-pinene and β-myrcene are the most common compounds to be present in the highest concentrations. Values in literature are slightly lower than calculated here with values by Hodel et al. (2020) and Einfalt (2020) reporting gins containing α-pinene concentrations of 30-40 mg/L. While a further 23 compounds were identified they were not able to be quantified since standards were not procured for them and they were not considered key analytes for this study. These other compounds do play an essential role in the flavour and aroma profile however, this study had limited time and chose to investigate the analytes with largest peak areas.

Current practice sees the distillate diluted from its initial 80% ABV to 40% ABV, effectively halving the analyte concentration. A 'hotshot' will require initial analyte concentrations over twice the values displayed in Table 10 before dilution with ethanol is also required to match desired levels. This is the minimum level, the ideal 'hotshot' will have even greater levels than double this concentration.

Table 10: Three 40% gin samples were extracted into hexane and the average concentration of the key analytes is shown. Triplicate injections of each sample were performed and error was calculated using standard error.

Compound	**Concentration (mg/L)**
α-Pinene	116 ± 5
β-Myrcene	57 ± 3
Limonene	18 ± 1
γ-Terpinene	8 ± 1
Citronellal	23 ± 1

3.6.1 Error

There were many possible sources of error during this study, the main causes were measurement error and analysis error. The concentration of the key analytes is calculated from the RRF from the internal standard. It is therefore critical that the measurements done to prepare the IS solution and the addition of the IS solution into all samples is done as precisely and accurately as possible. One method used to increase the precision was to maintain the same meticulous method throughout the preparation of each sample. This ensured that if there were any measurement errors it would be consistent throughout the study. The same pipettes and analytical balance were also used throughout to minimise possible occurrences of error.

The concentration error was calculated using the results from GC-FID analysis. The measured peak area should be consistent across multiple injections of the same sample however there was a spread which was used to calculate the error. The standard error (SE) is shown below, σ is standard deviation and n is number of samples.

$$SE = \frac{\sigma}{\sqrt{n}} \tag{6}$$

Due to time constraints some experiments were not done with replicates and so do not have a standard error calculated; these are displayed as a single value without an error estimate.

3.7 Conclusions

To conclude the key analytes which will be the focus of this study are α-pinene, β-myrcene, limonene, γ-terpinene and citronellal. While there are other contributors to the flavour and aroma profile, they were not considered for this study due to either economic, availability or concentration factors. The internal standard was selected to be 2-octanol due to its success as an IS for monoterpenes analysis.

The standards sourced from Sigma Aldrich were used to calibrate the equipment by internal and external standardisation methods. This allowed for the concentration of the key analytes in the gin specimen to be calculated as α-Pinene 116 ± 5 mg/L, β-Myrcene 57 ± 3 mg/L, Limonene 18 ± 1 mg/L, γ-Terpinene 8 ± 1 mg/L and Citronellal 23 ± 1 mg/L. A successful 'hotshot' will have initial analyte concentrations of at least twice those reported values before requiring dilution with water and ethanol. The ideal concentration will be much greater than double to significantly increase the efficiency.

A long and short GC-MS method was compared. It was found that the long method identified the most compounds. This method was used for all identification analysis. It was also used as the starting point for the GC-FID method development. After initial trialling on the GC-FID it was proven to adequately identify all key compounds. The method was then shortened to increase run efficiency since all key analytes eluted within the first 20 minutes.

Chapter 4: Gin Sample Preparation and Gas Chromatography Method Development

4.1 Introduction

As discussed in Chapter 2 raw gin samples are not recommended for direct injection into a GC due to their high water content. There are several gin sample preparation techniques that are present in literature. In this chapter several techniques are trialled with extensive investigation into LLE which was a very prevalent method in recent gin analysis and essential oil studies.

In literature there is an abundance of research reported which analyses the volatile compounds of gin using combinations of GC-FID and GC-MS analysis (Einfalt, 2020; Robbat Jr, et al. 2011). Each study has a unique time-temperature programme and method parameters. This chapter also investigates and develops an optimal GC-FID and GC-MS method for analysis of the gin specimen compounds.

4.2 Sample Preparation

4.2.1 Possible Approaches

As discussed in Chapter 2 there are many methods available to remove water from aqueous ethanol solutions in preparation for GC analysis. One such method is fractional freezing. An insulated Eppendorf tube with an aluminium rod through the centre was used for fractional freezing trials. The insulation slowed the freezing process while the aluminium rod helped to encourage freezing from the centre. The gin sample was allowed to slowly freeze in a -60°C freezer before being thawed in a -20°C freezer. This method was repeated with a small amount of the water rich component remaining frozen at each stage. This slowly increased the ABV of the sample over many stages. This was a very time-consuming method and required a large minimum sample volume due to available equipment. It was also unknown what proportion of the analytes would remain in the discarded aqueous phase and therefore be unable to be measured. For these reasons fractional freezing was ruled out as a viable method and was not continued with for the remainder of this study.

In literature the two most common methods to prepare gin sample was via HS-SPME or liquid-liquid extraction (LLE). HS-SPME was trialled using a 50/30 µm DVB/CAR/PDMS fibre (Supleco,

Pennsylvania, USA) to determine whether it was a viable method. This one trial proved that it would work however there was no headspace autosampler coupled with any of the GC equipment available. Manual injection is possible; however, it increases technician error and is very labour intensive. This reason coupled with the high economic cost of fibres meant that HS-SPME was not investigated in depth for this study. In the following section the LLE method is investigated in depth with several solvents, extraction times and ratios tested.

4.2.2 Liquid-Liquid Extraction Investigation

4.2.2.1 Materials and Methods

Primary materials to begin experimentation consisted of base gin distillate, of which samples of 40% and 79% ABV were obtained from BeGin Distilling, New Plymouth, New Zealand. Gin samples were stored at -18°C to maximise preservation. XNS (ultra-neutral spirit) grade ethanol at 96% ABV was obtained from Lactanol, Auckland, New Zealand. Extraction solvents used were hexane 95% (UNIVAR), chloroform (Thermo Fisher Scientific), dimethyl sulfoxide (UNIVAR) and Dichloromethane 99% (BDH). Purified and filtered water by reverse osmosis (RO) was obtained from the laboratory STULZ UltraWater reverse osmosis system (Frederick, Maryland, USA). Henceforth, all water utilised in experimentation and any mention of water is referring to RO water. Sodium chloride was sourced from UNIVAR. All weighing was done on Sartorius Entris II analytical balance. The three varying pipettes used were a Gilson-Pipetman P1000 100-1000 µL, Helena Labortaries-Quickpette 10-50 µL and Thermo Labsystems-Finnpipette 1000-5000 µL.

Extraction method techniques utilised the Heidolph Unimax 1010 orbital shaker at 183 RPM. All health and safety requirements were followed, and solvents were handled within an Ecoair Fume Cupboard, Thermoplastic Engineering Limited, Porirua, New Zealand. The same GC methods and materials were used as mentioned in section 3.5.1.

The LLE investigation was comprised of two stages, primarily: solvent selection, salt addition and solvent ratio, and secondly extraction time and extraction efficiency. The method developed was adapted from Clutton and Evans (1978).

To test the suitability of solvents, 30 mL of 40% ABV gin was extracted by 10 mL of solvent over 17 hours on an orbital shaker. The gin was diluted to 40% ABV if necessary, through the addition of water. The solvents investigated were chloroform, DCM, hexane and DMSO. After extraction the solvent containing the analytes was separated via a separating funnel or by pipette. DMSO, which is

not immiscible with water, was separated from water using a rotary evaporator. The rotary evaporator used was a Buchi Rotavapor R110, Buchi, Switzerland, using reduced pressure and 70°C water bath. The solvent was then placed straight into GC vials or into micro-inserts within the vials depending on the volume being analysed. All samples were analysed by Shimadzu GC-MS and assessed on number of compounds identified and their peak area.

Table 11: To determine the most effective solvent to extract gin volatiles, fours solvents were tested, with and without the addition of NaCl. Raw gin without any solvent was also tested at 40% ABV and 80% ABV. The following table displays the volumes and masses.

Solvent	Gin (40% ABV) (mL)	Hexane (mL)	Salt (g)
DCM	30	10	6.91
DCM	30	10	0
Hexane	30	10	6.87
Hexane	30	10	0
Chloroform	30	10	6.92
Chloroform	30	10	0
DMSO	30	10	6.91
DMSO	30	10	0
NONE-Raw gin 40%	30	10	0
NONE-Raw gin 80%	30	10	0

The effectiveness of salt addition was tested by repeating each solvent extraction with the addition of sodium chloride for a final concentration of 0.173 g/mL. Raw sample without solvent extraction was also investigated with 40% ABV and 80% ABV raw gin direct analysis. Literature shows that the addition of NaCl, up to 20%, leads to a decrease of the partition coefficient and an increase in extraction efficiency (Poll & Flink, 1984). The solvent ratio was investigated by changing the volumes of gin sample to solvent with ratios of 1:3 1:5, 1:7 tested.

Next steps included extraction time and extraction efficiency; the extraction method shown above was followed for this investigation. A spiked ethanol solution (shown in Table 12) was prepared with similar concentration of key analytes similar to what is found in the gin specimen. This solution was used as a mock gin to determine the extraction efficiency. Four different extraction periods were trialled: 1-hour, 5-hour, 17-hour and 24-hour. The internal standard 2-octanol was added prior to GC

analysis directly into the vial. This solution was used to calculate efficiencies since it contained a known concentration of analytes.

Table 12: To investigate extraction time and extraction efficiency a spiked ethanol solution was prepared with approximate concentrations of 115 mg/L. This was called a mock gin.

Compound	**Concentration (mg/L)**
α-Pinene	115.8
β-Myrcene	86.6
Limonene	110.8
γ-Terpinene	114.7
Citronellal	118.5

4.2.2.2 Results and Discussion

4.2.2.2.1 Solvent Selection

Solvent suitability was based on two factors, the ability to extract multiple compounds and the magnitude of compounds extracted. Samples were analysed by GC-MS and assessed by the number of compounds identified and their relative peak areas. It was judged that compounds did not need to be fully quantified but compared respective to their peak area, therefore an internal standard was not added. Additionally, the effect of salt addition was assessed by the same criteria.

One factor that was initially not accounted for was the age of the gin. Initial testing took place over several months and the initial gin sample were not expressing many compounds when analysed by the GC-MS. This was primarily hypothesized to be due to poor solvent suitability, however it was later considered that some fraction of VOC was lost over time due to inadequate storage conditions. To alleviate any experimental corruption fresh gin was acquired, the same range of solvents were tried resulting in a much higher analyte response.

It was assumed that the peak area of the key analytes had a strong linear relationship with its concentration which was later proven by the calibration curves calculated in section 3.5.1.2. During the solvent extraction investigation, a bigger peak area displayed that the solvent is more selective towards that compound. This is due to the affinity between the solvent and analyte. Shown below,

20% by weight NaCl was added during the extraction process and compared to an extraction sample using the same solvent, ratio and time.

Table 13: The number of compounds identified and relative response area across four different solvents. The addition of NaCl was also trialled which did not show an increase in peak area.

		VOC	Response				
Solvent	**Salt**	**identified**	**α-pinene**	**β-myrcene**	**Limonene**	**γ-terpinene**	**Citronellal**
Base gin (40%)	No	13	10,113,909	4,675,869	2,714,617	1,192,888	2,466,679
Base gin (80%)	No	23	27,676,044	11,860,415	6,494,615	2,802,697	5,512,604
DCM	No	12	43,877,865	18,193,784	10,445,433	4,346,668	7,556,392
	Yes	14	33,732,107	14,420,066	8,315,767	3,498,466	5,686,338
Hexane	No	28	71,448,224	27,995,365	15,933,723	6,634,336	10,908,216
	Yes	24	66,723,969	25,592,412	15,032,139	6,178,455	10,118,541
Chloroform	No	12	21,158,610	8,508,951	5,063,326	2,977,215	2,278,467
	Yes	10	18,410,221	7,341,040	4,391,784	2,852,420	920,726
Dimethyl sulfoxide	No	0	0	0	0	0	0
	Yes	0	0	0	0	0	0

Table 13 expresses solvents that have highest selectivity towards key analytes, the predominant being hexane, which was able to identify 28 compounds. It is also clear that the addition of salt lowered the response area of the key analytes for each solvent. The addition of salt is to encourage the “salting out” effect which lowers the partition coefficient and can increase the extraction of analytes. There are several claims that salt must always be added to headspace analysis to promote the salting out effect which increases the vapour phase concentration and makes the headspace analysis easier (Fiorini et al., 2015; Valente et al., 2013). Clutton and Evans (1978) investigated addition of salt in liquid-liquid gin extraction and reported their investigation into 17 different salts to facilitate ‘salting out’ which did not increase the recovery of VOC from their gin samples. The salt may have promoted the ‘salting out’ affect and increased the concentration in the headspace leading to a lower concentration in the liquid and in the solvent phase after extraction, due to the small volume of available headspace this would not produce a significant change. Salt is used to decrease the solubility of hydrophilic compounds in the aqueous phase and thus increase the partition of analytes into the organic solvent phase. Since the solution is an aqueous-ethanol mixture the addition of salt may have led to the compounds have an increased solubility in the mixture due

to the presence of ethanol and consequently decreasing the extraction of compounds into hexane. In all future extraction samples salt was omitted since there was no evidence that it increased the peak areas of any key analytes. It is recommended that if future work is done on headspace analysis that salt addition should be investigated again to see whether it is successful at increasing analyte concentration in the headspace.

It is interesting that DMSO did not show any peaks, this could have been due to all the VOC being evaporated by the rotary evaporator. DMSO is a more labour-intensive method due to the water evaporation step and therefore, it was not investigated further.

The influence salt addition has on analyte extraction into hexane was also investigated by analysis on GC-FID to confirm the results found via GC-MS analysis. GC-FID analysis allowed for accurate quantification of compounds and direct comparisons between concentration values with and without salt. Hexane was the only solvent trialled since it had been selected as the optimal and most promising solvent, all further extractions utilised hexane. Table 14 details that the hexane extractions completed without the presence of salt resulted in a higher analyte extraction. This evidence proves that salt should not be added to the liquid-liquid extractions in this study to increase extraction efficiency.

Table 14: The addition of salt during extraction with hexane was also trialled before analysis on the GC-FID and it was shown to not be beneficial to increase peak area.

	Salt		**No salt**	
Compound	**Peak area**	**Concentration (mg/L)**	**Peak area**	**Concentration (mg/L)**
α-Pinene	31147507	71.1	31777816	77.1
β-Myrcene	12970243	51.5	13699257	57.9
Limonene	26940823	70.0	27684703	76.5
γ-Terpinene	28005326	68.4	28566946	74.2
Citronellal	20866010	60.0	21925357	67.1

Table 15: The ratio of solvent to gin was trialled with three different ratios. The results are expressed as efficiency by amount recovered into hexane. The 1:3 ratio proved to have the highest recovery efficiency.

Compound	**1:5 ratio 40% ABV Recovery efficiency (%)**	**1:7 ratio 40% ABV Recovery efficiency (%)**	**1:3 ratio 40% ABV Recovery efficiency (%)**

α-Pinene	50	52	67
β-Myrcene	51	53	67
Limonene	53	56	69
γ-Terpinene	50	51	65
Citronellal	43	34	57

The ratio of solvent to gin-sample was adapted from Clutton and Evans (1978) who used a ratio of 10 mL solvent to 30 mL gin. In this study two other ratios were also trialled using smaller volumes. Ratios of 1 mL to 5 mL and 1 mL to 7 mL were investigated to determine whether increasing the ratio could improve extraction recovery. The efficiency was calculated using the spiked ethanol solution with the results for different extraction ratios shown in Table 15. Increasing the ratio has the potential to increase the partition coefficient which effects the extracted ethanol in the solvent. Resulting further into more analytes being present in the ethanol and not in the solvent that was analysed. While this may have been the factor, it is a complex process and investigating the properties of the analytes and their affinity for ethanol and hexane will not be investigated further. The solvent ratio of 3 mL sample to 1 mL solvent was the most efficient and was selected for future extractions.

4.2.2.3 Extraction Time

The spiked ethanol solution which was prepared as the mock gin was used for investigation into the optimal extraction time. A 17-hour extraction time was recommended in literature and was used for initial testing (Clutton & Evans, 1978). In section 4.2.2.4 the extraction efficiency is calculated at only 56 - 69%, this led to different extraction times to be trialled to determine whether this would increase the efficiency. The analyte recovered in extraction after 1, 5, 17 and 24 hours is shown below.

Table 16: The extraction efficiency expressed as percent recovery of target analyte. Extraction duration results for 1-, 5-, 17- and 24-hour periods.

	% Recovery			
Sample	**1 hour**	**5 hours**	**17 hours**	**24 hours**
α-Pinene	47.9	51.3	66.6	67.7
β-Myrcene	48.2	49.7	66.8	68.5

Limonene	49.9	52.7	69.0	71.1
γ-Terpinene	46.5	50.5	64.7	66.0
Citronellal	38.4	41.3	56.6	59.7

The results showed that as time progressed more analyte was extracted. The 17-hour extraction was almost as efficient as the 24-hour extraction. The hexane may have reached its saturation point around the 17-hour mark which led to only a small increase in concentration of the key analytes. The analyte that remains in the solution could potentially be extracted again with fresh hexane, which would extract a greater percent of total analyte. This would be good to investigate in a future study but was not a focus for this study.

A method to increase the speed of the extraction without requiring more time would have been to increase the RPM of the orbital shaker, however this was not explored for several reasons. The current apparatus could not increase past 183 RPM before specialized vial holders were necessary. The 17-hour period also allowed samples that began extraction at the end of a workday to then be ready at the start of the following day. This was considered an optimal extraction period and other methods to decrease this time was not deemed necessary.

4.2.2.4 Extraction Efficiency

The mock gin solution shown in Table 12 above was also used to calculate the extraction efficiency. Average extraction concentration from 3 mL of spiked ethanol solution into 1 mL hexane over 17 hours is shown below.

Table 17: The average extraction efficiency from the mock gin solution.

Compound	**Efficiency (%)**
α-Pinene	66.6
β-Myrcene	66.8
Limonene	69.0
γ-Terpinene	64.7
Citronellal	56.6

An average efficiency was determined at 65% for analyte recovery from the ethanol solution into the solvent. This efficiency is lower than was expected and further investigation into possible contributing factors was carried out. It was possible that analyte was reacting with the material or lost prior to the extraction process therefore the mock gin sample was spiked pre-extraction to determine whether there was any loss at these stages.

Table 18: The spiked ethanol solution was spiked with analytes pre-extraction and the final concentration was lower than the expected concentration which indicates that the loss is due to extraction efficiency as the values correlate with earlier values.

Compound	**Initial concentration (mg/L)**	**Mass added (µg)**	**Expected final concentration (mg/L)**	**Final concentration (mg/L)**
Limonene	86	190	276	183
γ-Terpinene	91	168	259	170
Citronellal	82	206	288	162

The results shown in Table 18 express sample spiking pre-extraction, where the concentration increased by a lower amount than expected for the mass of analyte added for 100% extraction. An extraction efficiency of 66%, 66% and 56% for limonene, γ-terpinene, and citronellal respectively were calculated. These values are similar to the values previously calculated in the extraction efficiency calculation.

The above results are assuming that the analytes present within the solution are single molecules. This may be slightly different to the actual chemical structures within the gin samples. This would be due to the VOC being present in micelles or molecular pairs which, when analysed by gas chromatography is not detected separately and skews the response. Freshly added compounds would not have time to form these chemical structures and would not be present in the extraction efficiency investigation. While the same cannot be known for the gin samples in this study it is assumed that the GC-FID detected response is proportional to the entire concentration within the sample allowing comparison of distillation techniques and the effect of changing process parameters.

4.2.3 Optimal Extraction Method for Sample Preparation

In summary, section 4.2 investigates appropriate methods to prepare gin samples prior to GC analysis. It was determined that for this study LLE would be the selected method. The following method was developed throughout the LLE investigation and used in all extractions forthcoming.

Samples were extracted into 95% hexane at a 3:1 ratio in small glass vials. These glass vials were shaken for a nominal period of 17 hours on an orbital shaker at 183 RPM. Vials were left to settle for 30 minutes before separation by pipette in a fume cupboard. Extracted solvent was placed directly into GC vials on an analytical balance where the internal standard (IS) could be added when required. The amount of IS added was recorded to calculate the RRF values and determine the concentrations of the unknown analytes. The GC vials were kept in a -18°C environment until ready to be analysed via gas chromatography. To improve accuracy and limit variation the same method was used for each extraction and addition of internal standard.

A method to increase the speed of the extraction and hence time required could have been to increase the RPM of the orbital shaker, however this was not explored for several reasons. The current apparatus could not increase past 183 RPM before specialized vial holders were necessary. The 17-hour period also allowed samples that began extraction at the end of a workday to then be ready at the start of the following day. This was considered an optimal time period and other methods to decrease this time was not deemed necessary.

4.3 Conclusions

In summary it was found that HS-SPME was a viable method however without an auto-sampler at the available research facilities it is a very manual process which increases the possibility of technician error and greatly increases the time required. This factor coupled with the larger economic cost ruled out HS-SPME as the sample preparation method. Fractional freezing was also considered but ruled out due to the time required to remove water and the possibility that key compounds may also be removed. LLE was selected since it was favourable economically and time efficient while being a recommended method in literature.

From the literature five solvents were investigated to determine which had the highest affinity for the key analytes. Hexane was able to extract the highest concentration of compounds and selected as the solvent of choice at a ratio of 1:3. The selected extraction time was 17-hours, while 24 hours led to marginally higher results it was less efficient and fewer samples were able to be extracted per

day. The extraction efficiency was found to be significantly lower than expected. Possible methods to increase this were not trialled in this study due to time constraints. In the future increasing the surface area or temperature may lead to a better efficiency. Alternatively using multiple washes of hexane could be explored to increase extraction efficiency.

Chapter 5: Maceration and Distillation Investigation

5.1 Introduction

In Chapter 2 the significant processes were determined to be maceration, macerate distillation, condensed vapour percolation and vapour infusion. In this chapter they are characterised and investigated to determine how they influence the extraction of key analytes and how this may be optimized to produce a high-concentration gin. During early stages of analysis, it was discovered that the ethanol had gotten contaminated.

5.2 Ethanol Contamination

Samples taken from maceration and distillation time 0, before distillation or before botanicals were added, showed significant concentrations of the key analytes. Further investigation on the GC-MS identified not only the five key analytes but also several others that were common VOC found in gin samples, see Appendix A.1.7. To investigate the cause of the contamination the ethanol, water, hexane, and glassware were tested to determine the source.

Table 19: The concentrations of analytes in the 'pure' ethanol solution that proved that the ethanol had been contaminated.

Compound	**Sample 1 (mg/L)**	**Sample 2 (mg/L)**	**Sample 3 (mg/L)**
α-Pinene	4.7	4.2	6.6
β-Myrcene	3.8	3.5	5.4
Limonene	3.1	0.3	4.3
γ-Terpinene	2.2	2.0	2.8
Citronellal	0.1	0.1	0.4

As shown in Table 19 the concentrations were significant. After ruling out the hexane, water and glassware the only possible source was the ethanol. After inquiry it was found that the container used to transport fresh ethanol to the university had previously held concentrated gin and was the source of the contamination. The trials that had been done using the contaminated ethanol were still considered useful data as the baseline values was subtracted from subsequent concentrations.

This did lead to some negative concentrations which was not possible, and those values were corrected to 0 mg/L. The negative values are thought to have been due to loss of compounds in the headspace or chemical reactions which lowered the concentration in the solution below the baseline ethanol contamination value.

5.3 Maceration

The maceration process in gin manufacture is the least complex and does not need to require any distillation. In industrial gin manufacture hybrid manufacture combines maceration and vapour infusion. This technique often only utilises macerated juniper berry as the significant flavour compounds are extracted in vapour infusion. Therefore, juniper berry was the sole botanical selected for the maceration investigation.

5.3.1 Materials and Methods

Due to the low complexity of the maceration method experimental trials were conducted in 500 mL Duran laboratory screw-top bottles. Some of the compounds may have been UV sensitive and the Duran bottles were kept in a cardboard box. Water, ethanol, and juniper berries could be easily added and monitored in these bottles making it a suitable vessel. XNS (ultra-neutral spirit) grade ethanol at 96% was obtained from Lactanol, Auckland, New Zealand. The juniper berries were sourced from BeGin distilling, New Plymouth, New Zealand. Purified and filtered water by reverse osmosis (RO) was obtained from the laboratory STULZ UltraWater reverse osmosis system (Frederick, Maryland, USA). The maceration samples were extracted and then analysed by GC-FID as detailed in section X, Y. Three parameters were investigated, botanical ratio, solvent concentration, and material state.

Figure 9: Maceration experimental trial. Bottle on the left has macerated for 1 hour, bottle on the right has macerated for 48 hours.

An initial base trial was done with base level parameters to have a set level to compare the other levels to. The base trial had 5.8 g of juniper berry and 350 mL of 58% ABV aqueous ethanol solvent, this is a juniper berry concentration of 16.6 g/L of solvent. Two increased botanical ratios were compared to this trial, 33.0 g/L and 63.1 g/L of juniper berry in 58% ABV aqueous ethanol solvent. Next stage was investigating the influence solvent concentration has on analyte extraction. Two different solvent concentrations were compared to the base trial, all containing 16.6 g/L of juniper berry and solvent concentrations of 78% and 96% ABV. The final parameter investigated was changing the material state, first trial had juniper berry placed under vacuum and second trial used crushed juniper berries using a mortar and pestle, both using juniper berries at a concentration of 16.6 g/L. The berries were lightly crushed with a mortar and pestle until each individual berry was ruptured. The berries were put under vacuum by placing the berries in a Büchner flask with a bung in the top neck and connecting the hose barb to the laboratory vacuum system. This laboratory system was comprised of dual vacuum pumps in duty/standby mode providing a nominal vacuum of - 68 kPa $\pm$ 12 kPa.

The material state trials were all macerated in the same solvent volume and concentration. Samples for all trials were taken at time 0, 1, 3, 24, 49, 72 (hours) and the samples extracted into hexane and analysed on the GC-FID and GC-MS as outlined in section 3.2.3 and 3.2.1 respectively. See Appendix A.1.8 for full experimental data including internal standard concentrations.

Table 20: Maceration experimental data for botanical ratio and solvent concentration. The mass of juniper berries and volumes of ethanol and water used are listed. Juniper berry expressed as grams per litre of aqueous ethanol solution.

Component	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 7	Trial 8
Juniper berry (g/L)	16.6	33.0	63.1	16.5	16.5	16.6	16.6
Ethanol (96%) (mL)	210	210	210	280	350	210	210
Water (mL)	140	140	140	70	0	140	140
Material state	Whole	Whole	Whole	Whole	Whole	Crushed	Vacuum

5.3.2 Results and Discussion

5.3.2.1 Botanical Ratio

Three different botanical ratios were investigated for maceration: 16.6 g/L, 33.0 g/L, and 63.1 g/L in 58% ABV aqueous ethanol over 72 hours. Each data point is a single sample that was analysed by a single injection by GC-FID. Single samples were done to allow for a greater range of variables to be investigated, this allowed the study to test all the main variables that play a role in the distillation process instead of a comprehensive study into only a few variables. The baseline value of analyte in the contaminated ethanol was calculated and subtracted from subsequent values. Over the 72-hour period γ-terpinene and citronellal were only present in small quantities, if at all. These compounds did not show a significant increase in concentration. This aligns with what is found in literature for citronellal since Höferl et al. (2014) and Falcão et al. (2018) reported it not being present in juniper berries at all.

As seen in Table 21, 22 and 23 limonene had no concentration detected in trial 1 or trial 3 which had the largest botanical ratio. In trial 2 after one hour the concentration had reached 2.1 mg/L, this only increases to 4.8 mg/L after a further 71 hours. It is unusual to see such a large increase after a short amount of time and then no significant increase the rest of the measured time. This sudden increase is therefore ruled as an error. Limonene is reported as making up 5.1% of juniper berry essential oil. It is then surprising that it has essentially no increase in concentration over the 72 hours. This could be due to the same reasons as stated for γ-terpinene, that the compound is not near the surface of the material and will take longer to be extracted.

γ-Terpinene is reported as making up 0.2% of the essential oil extracted from juniper berry by Höferl et al. (2014). γ-Terpinene concentration only increased to 0.4 mg/L after 72 hours for trial 1 and trial

3. This could be due to where the compound is found within the material and not enough time had passed for it to begin to be extracted. The trial containing the highest ratio of juniper berry, 63.1 g/L, did not report any increase in concentration over time. This leads to the theory that the concentrations that were calculated may have been an error. The low concentrations are negligible especially when considering the measurement error. Limonene reported a significant concentration in the 33.0 g/L juniper ratio compared to no concentration reported in the largest ratio 63 g/L. Limonene may also be an error and should be repeated.

Both α-pinene and β-myrcene showed an increase in concentration over the 72 hours. The higher ratio of botanicals showed the largest increase in concentration over time as expected and for α-pinene this was from approximately from 0 to 8.6 mg/L and for β-myrcene was from 0 to 4.2 mg/L.

Table 21: Concentration of key analytes over time for largest botanical ratio: 63.1 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol.

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**49 hours**	**72 hours**
α-Pinene	0.0	0.0	0.0	3.6	9.0	8.6
β-Myrcene	0.0	0.0	0.0	0.6	2.4	4.2
Limonene	0.0	0.0	0.0	0.0	0.0	0.0
γ-Terpinene	0.0	0.0	0.0	0.0	0.0	0.0
Citronellal	0.0	0.0	0.2	0.0	0.0	0.0

Table 22: Concentration of key analytes over time for botanical ratio: 33.0 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol.

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**49 hours**	**72 hours**
α-Pinene	0.0	2.2	0.8	2.8	3.4	6
β-Myrcene	0.0	0.6	0.4	1.4	1.0	5.4
Limonene	0.0	2.2	3.6	4.4	2.2	4.8
γ-Terpinene	0.0	0.2	0.0	0.0	0.0	0.4
Citronellal	0.0	0.6	0.5	0.0	0.0	0.0

Table 23: Concentration of key analytes over time for smallest botanical ratio: 16.6 g/L of juniper berries in 350 mL 58% ABV aqueous ethanol

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**49 hours**	**72 hours**
α-Pinene	0.0	1.8	1.2	0.0	2.4	6.0
β-Myrcene	0.0	2.2	0.8	3.0	0.2	2.6
Limonene	0.0	0.6	0.2	0.0	0.0	1.0
γ-Terpinene	0.0	0.6	0.0	0.0	0.0	0.4
Citronellal	0.0	0.0	0.0	1.5	0.0	0.0

From these results it is evident that the concentration of analyte extracted is insignificant or non-existent for several key analytes. This indicates that the base maceration process alone is not capable of extracting the high level of analyte required for the 'hotshot'. This ties in with literature since maceration is coupled with distillation to be effective and solely maceration is not recommended for commercial use. The main process in maceration is allowing the solvent to permeate the berries to speed up extraction once the solution is heated in distillation. The juniper berries also act as important nucleation sites to help control the boil. Compounds extracted into aqueous ethanol during maceration will evaporate during the boil and ultimately end up in the condensing gin.

To create a 'hotshot' gin the analyte concentration must be greatly increased from base levels. Due to the low extract rates in maceration even greatly increasing the botanical ratio would not be very effective. This extraction rate will increase when the solution is heated for distillation and will be further discussed in section 5.4.

5.3.2.2 Solvent Concentration

The influence solvent concentration had on the extraction rate was investigated with three 350 mL aqueous ethanol solutions of 58%, 78% and 96% (ABV) each containing 16.6 g/L of juniper berry. The different solvent ratios led to different extraction rates depending on the analyte and solvent ratio. Citronellal, and γ-terpinene did not show any increase with 96% solvent however limonene, β-myrcene and α-pinene showed increasing analyte concentration with an increase in solvent

concentration as shown below. The results for the 58% ABV trial can be found in Table 23 in the previous section.

Table 24: Concentration of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry.

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**48 hours**	**72 hours**
α-Pinene	0.0	0.0	0.0	0.0	6.1	13.4
β-Myrcene	0.0	0.0	0.0	0.0	3.7	7.8
Limonene	0.0	0.0	0.0	0.0	0.2	1.4
γ-Terpinene	0.0	0.0	0.0	0.0	0.0	0.7
Citronellal	0.0	0.0	0.0	0.0	0.0	0.0

As the solvent concentration was increased α-Pinene showed a much higher extraction compared to 58% ABV. The higher solvent ratios reached similar analyte concentrations with 78% reaching 14.5 mg/L and 96% reaching 13.4 mg/L. This is much greater than only 6 mg/L for 58%. β-myrcene also showed an increase in analyte concentration with increasing solvent ratio. The highest extraction was from 78% with 8.8 mg/L however 96% was not far behind with 7.8 mg/L. Both ratios were much higher than 58% with only 2.6 mg/L extracted. Limonene did show an increase with higher solvent ratios with 78% showing the greater increase at 2.8 mg/L compared to 1.4 mg/L for 96% solvent. A solvent ratio of 78% ABV also led to an increase of γ-Terpinene concentration whereas for the other ratios no concentration was recorded. Citronellal was still not present which is expected. The final concentration at 72 hours for the separate solvent concentrations is displayed in Figure 10 below.

Table 25: Key analyte concentration in macerated solution over time with 16.6 g/L of juniper berry in 78% ABV aqueous ethanol

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**48 hours**	**72 hours**
α-Pinene	0.0	0.8	2.2	5.3	4.5	14.5
β-Myrcene	0.0	0.6	1.8	3.1	2.7	8.8
Limonene	0.0	0.6	1.4	1.4	0.8	2.8
γ-Terpinene	0.0	0.5	1.0	0.7	0.2	1.2
Citronellal	0.0	0.0	1.2	0.0	0.0	0.0

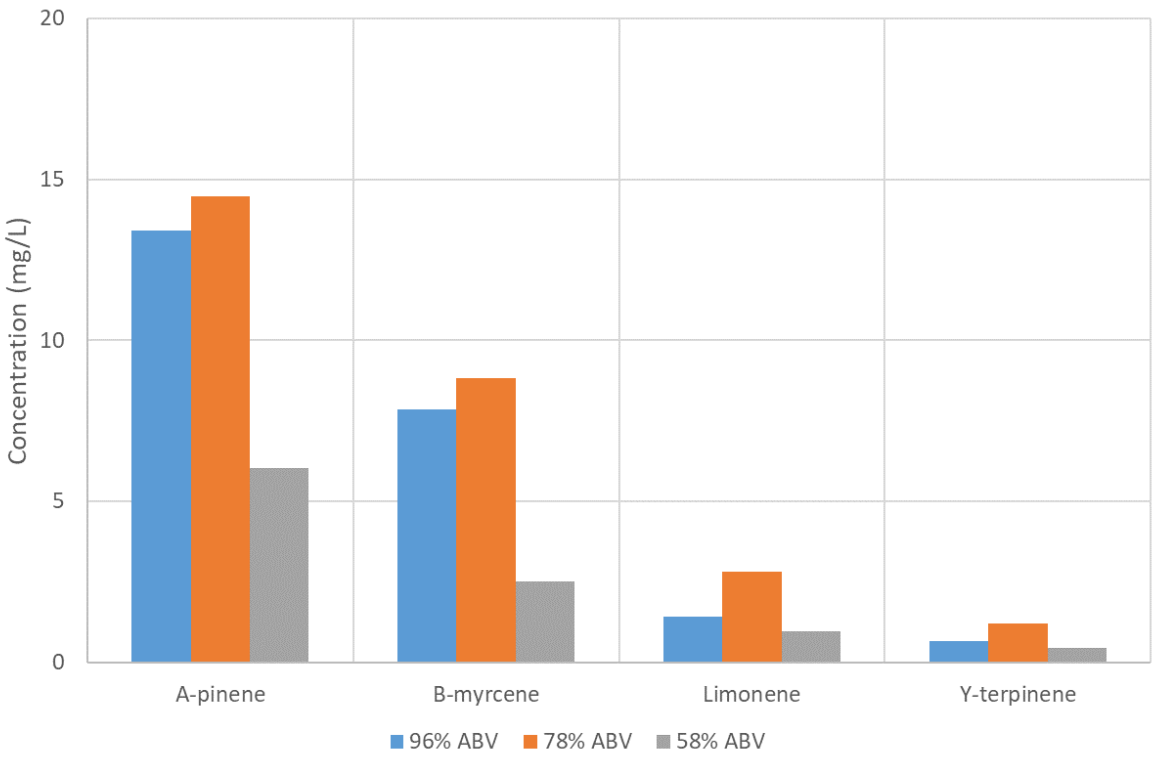

Figure 10: Concentration of key analytes after 72 hours in macerated solution for three experiments of different solvent concentration. These were analysed on the GC-FID with single injections from single sample.

In summary it was found that a solvent ratio of 78% ABV lead to the greatest extraction rates for the majority of compounds. Many compounds showed minimal increases in concentration until at least 24 hours had passed. This was likely due to the time required for the solvent to permeate the solid and then the time for the compound to diffuse out into the bulk solution. This aligns with literature as it is recommended to grind solids to decrease particle size and increase interfacial area to increase rate of diffusion and osmosis to speed up and increase extraction yield (Moncada et al., 2016).

An increase in solvent concentration does lead to an increase in analyte extraction however too high a ratio leads to decreasing returns. To develop a 'hotshot', macerating botanicals in 78% ABV solution would increase the extraction rate from the base trial. The significant length of time required until compounds are extracted is a disadvantage since this would occupy equipment possibly needed for other processes which make this a barrier for commercial viability.

5.3.2.3 Botanical State

This section investigates how the state of the botanical influences the extraction process. Berries were macerated whole, under vacuum and crushed. The results for the whole berries base trial can be found in Table 2. It was hypothesized that the vacuum would help the solvent permeate the material by removing all air and increasing the contact area between berry and solvent.

Table 26: Concentration of key analytes over time in macerated solution with 16.6 g/L of crushed juniper berries in 58% ABV aqueous ethanol.

	Concentration (mg/L)					
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**48 hours**	**72 hours**
α-Pinene	0.0	3.1	10.2	27.5	42.7	61.8
β-Myrcene	0.0	1.8	5.5	3.5	24.4	35.2
Limonene	0.0	0.3	1.3	3.1	4.9	7.5
γ-Terpinene	0.0	0.2	0.4	0.4	0.7	1.3
Citronellal	0.0	0.0	0.0	0.0	0.0	0.0

The crushed berries released the highest concentration of α-pinene compared to either whole or under vacuum. This is likely due to the crushed material have a far greater surface area exposed to the solvent which increases the extraction process. Crushing the berries increased the extraction rate of key compounds however it may also increase the extraction rate of unwanted compounds which were not measured here.

Interestingly while both β-myrcene and limonene had similar responses with the crushed sample leading to greater concentrations, γ-terpinene did not show such a response and did not show any difference in concentration over the different botanical states. The concentration remains steady over the entire time period. There was no significant change in concentration and the 24-hour value for whole berries is likely an error.

Table 27: Concentration of key analytes over time in macerated solution with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol.

	Concentration (mg/L)				
Compound	**0 hours**	**1 hour**	**3 hours**	**24 hours**	**48 hours**
α-Pinene	0.0	0.0	0.0	1.4	2.1
β-Myrcene	0.0	0.1	0.0	0.9	1.4
Limonene	0.0	0.1	0.0	0.1	0.2
γ-Terpinene	0.0	0.0	0.0	0.0	0.0
Citronellal	0.0	0.0	1.2	0.0	0.0

The berries that were analysed under a vacuum were testing whether the absence of air would lead to an increase in extraction rate. When the air that is present in small pockets on the surface of the berry or just under the surface of the berry is removed by the vacuum it is replaced by the solvent. This increases the wetting which should lead to increased extraction rates as the surface area between the solid and liquid is increased. This experiment did not show a significant increase of concentration for any of the key analytes extracted from berries under vacuum over the time period analysed. Naviglio et al. (2019) and Katiyar (2017) report that often the limiting factor of VOC extraction is the distance oils have to diffuse to reach the outer surface of the botanicals. Increasing the contact area between the botanical and the solvent does not decrease the distance the oils will have to diffuse therefore it is not unexpected that juniper berries under vacuum did not have an increased extraction rate.

Crushing led to the greatest increase in analyte concentration and it is recommended that solid material, roots and seeds, are crushed before maceration to decrease the distance that oils have to diffuse and increase extraction rate. This leads to a far greater extraction rate than whole berries. It is possible that the beneficial outcome of the maceration step is not to extract compounds, but to permeate the solvent into the berries which may result in a far greater and quicker extraction during the distillation stage. A future study that investigates the affect these variables have on the concentration of the analytes after distillation is needed to fully understand their role in the distillation process. This study proves that a vacuum does not lead to increased extraction rates from maceration alone. However, it is possible a vacuum may reduce the time required for maceration, further studies are required to confirm this. If it was a viable option, it would also involve additional cost and equipment to create and tolerate the vacuum. It is also possible that saturating internal air

pockets with solvent might decrease the anti-bumping effect of juniper berries during the subsequent boil.

5.4 Macerate Distillation

In industrial gin manufacture maceration is always combined with distillation to extract a greater percent of the compounds. The distillation of the macerated solution is called macerate distillation. This section investigates the distillation stage that occurs after maceration with juniper berries as the sole botanical in the kettle.

5.4.1 Materials and Methods

The distillation experiments in the next few sections all utilised similar apparatus in different configurations. The experimental apparatus that was used for macerate distillation is shown in Figure 11.

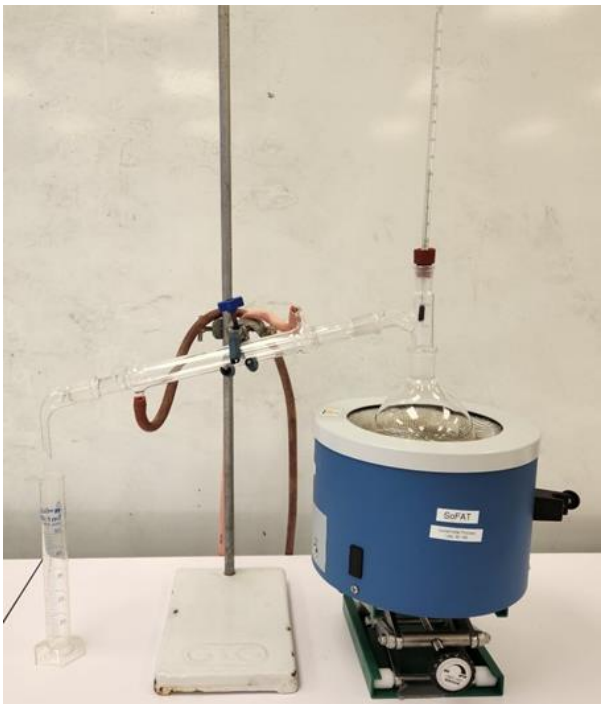

Figure 11: The experimental apparatus for macerate distillation was comprised of a lab jack, heating mantle, thermometer and Quickfit glassware. There is little chance for reflux since most of the condensate flow directly out of the condenser into the collection vessel.

The CMU0500/EX1 500 mL heating mantel and remote controller MC242 were sourced from Electrothermal, Vernon Hills, Illinois, USA. The heating mantle was raised on a laboratory jack for elevation. A 500 mL round-bottom beaker was used to hold the solution to be distilled and this was connected to a three-way joint with a thermometer and condenser attached. The condenser led to 50 mL measuring cylinder to measure the distillate.

The distillation was done in a fume cupboard due to the presence of flammable ethanol fumes and a sparkless heating mantle was used with the switch outside the fume hood. The heating mantle's max heating setting was used until the solution started to boil and it was then reduced by 50%. All maceration solutions were made up to 350 mL 58% ABV and there were two separate investigations, first was change in botanical ratio; four different amounts of juniper berries, 8.5 g/L, 16.8 g/L, 33.16 g/L and 57.3 g/L which were macerated for 24 hours. Secondly the maceration time prior to distillation was investigated by comparing three different periods, 0 hours, 24 hours and 72 hours to the same botanical and solvent ratio. The 0-hour macerated trial added the dry juniper berries immediately prior to distillation. The 24 and 72 hour macerated solutions were added directly into the kettle with the berries.

Table 28: Weights and volumes of juniper berry, ethanol and water for macerate distillation experiments comparing botanical ratio. Juniper berry expressed as grams per litre of aqueous ethanol solution.

Component	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
Juniper berry (g/L)	8.5	16.8	33.1	57.3
Ethanol (mL)	210	210	210	210
Water (mL)	140	140	140	140

Table 29: The effect of maceration time was compared by comparing 0 hours, 24 hours and 72 hours to the same botanical and solvent ratio. The solvent ratio remained constant for each trial at 350 mL 60% ABV.

Component	**Trial 1**	**Trial 3**	**Trial 2**
Juniper berry (g/L)	16.7	16.8	16.6
Maceration time (hour)	0	24	72
Ethanol (mL)	210	210	210
Water (mL)	140	140	140

Every 40 mL of distillate the measuring cylinder was changed, and two sets of samples were taken from that allotment, the first was then extracted for GC analysis using the method mentioned in

section 3.2.3 and the second sample was diluted for ethanol analysis using the method mentioned in 5.7.1. The temperature and time were also recorded at the point that every sample was taken. The initial and final state of the kettle was also sampled for analysis via GC.

5.4.2 Results and Discussion

5.4.2.1 Maceration Time

The first investigation was into the influence that the maceration period prior to distillation had on the concentration of extracted analytes. There was a significant difference between berries that had not been macerated at all compared to 24 and 72 hour macerated trials. There was also minimal reported increase in analyte concentration between 24 and 72 hours. Citronellal was not discussed since it is not present in juniper berries which was confirmed in section 5.3.

Table 30: Concentration of key analytes in distillate after distillation using berries macerated over different lengths of time. The berries were in the kettle and were in direct contact with the boiling liquid.

	Concentration (mg/L)		
Compound	**0 hours**	**24 hours**	**72 hours**
α-Pinene	18.3	35.5	34.6
β-Myrcene	8.8	12.9	15.1
Limonene	0.7	1.7	2.2
γ-Terpinene	0.7	1.0	1.3

These results do prove that maceration time is a significant factor in analyte extraction with a vast increase between 0 hours and 24 hours. This increased period of time allows the solvent to permeate the solid material and begin the extraction process. This is a limiting factor and why solid material is often crushed before essential oil extraction. The concentration of the analytes in the kettle post-boil was also analysed and did not find anything significant with little or none of the key analytes still present. This shows that all the compound that was extracted from the berries in the kettle is carried through during the distillation into the distillate.

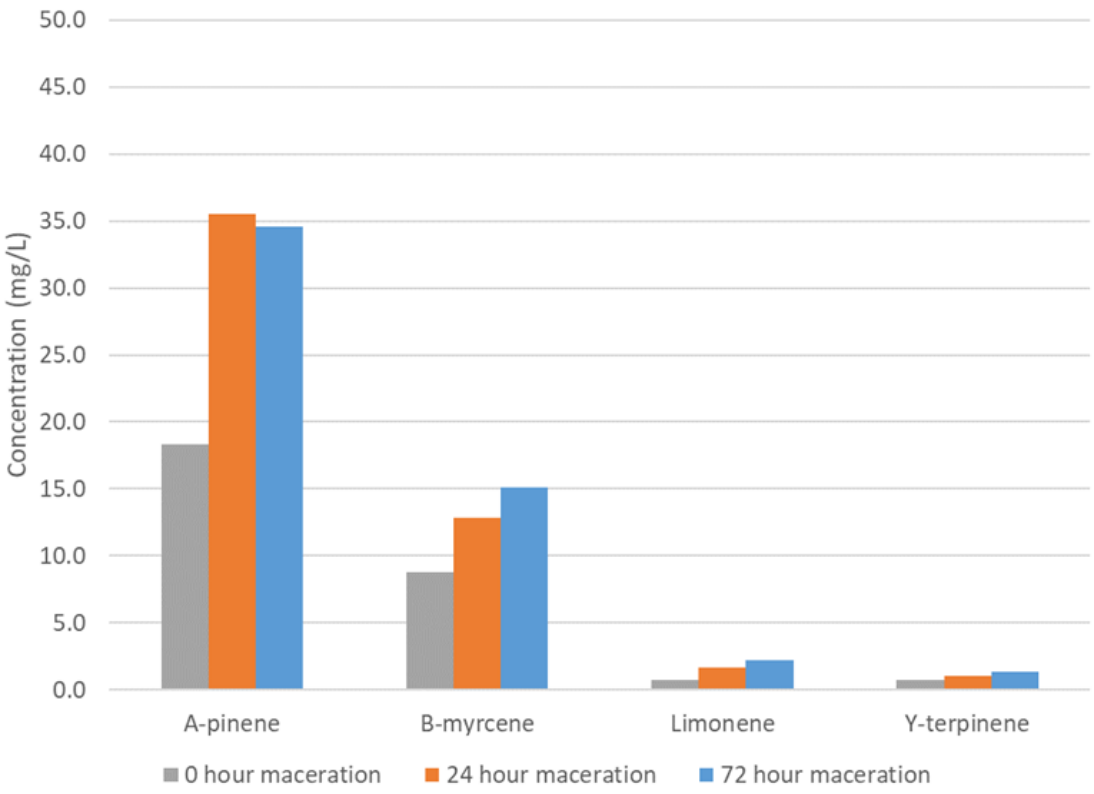

Figure 12: Concentration of key analytes in distillate after distillation using berries maceration for three different lengths of time. The samples were analysed by GC-FID with single injection of single sample. Citronellal showed no increase and was not included on this graph.

Further research should be done to determine whether a lower time also achieves similar results to the 24- and 72-hour maceration trials. It is possible that a shorter time period could produce the same analyte yield but be more time efficient. A further investigation could also crush juniper berries prior to maceration and distillation and compare to the yields from whole berries to determine the extraction efficiency and how much analyte remains in the crushed berry after macerate distillation and whether it is more efficient.

5.4.2.2 Botanical Ratio

In total four botanical ratios were investigated to the same solvent volume and concentration. This was aiming to determine the relationship between botanical mass and extracted analyte. As expected, the larger the botanical ratio the greater the concentrated of extracted analyte. The increase from 8.3 g/L to 16.6 g/L led to much higher analyte extraction for all key compounds. There was decreasing returns when doubling the botanical ratio from 33.1 g/L to 63.1 g/L only led to a small increase shown in Table 31. The increase of from 33.1 g/L to 63.1 g/L led to a much smaller

increase in analyte extraction compared to when the analyte was increased by from 8.3 g/L to 16.6 g/L. This result agrees with a study by Hodel et al. (2020) who found that increasing juniper berry ratio in distillation led to diminishing returns.

A factor that may have contributed to the decreased efficiency is the area in the maceration process. As the botanical ratio was increased more botanicals in the same area and more were touching and not completely submerged during maceration. As shown in section 5.4.2.1, maceration time directly affects the amount extracted and if a portion of the botanicals were not in contact with the solvent extraction rate will be decreased. Another factor is the overcrowding in the kettle during distillation. The same kettle size was used through the investigation which became crowded with juniper berries at the highest ratio. The area available decreased the amount individual berries moved around the kettle during the boil which may have affected the analyte extraction.

Table 31: Key analytes concentration in macerate distillate for 8.3 g/L, 16.6 g/L, 33.1 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 58% ABV aqueous ethanol.

	Concentration (mg/L)			
Compound	**8.3 g/L**	**16.6 g/L**	**33.1 g/L**	**57.1 g/L**
α-Pinene	16.0	35.5	47.0	48.2
β-Myrcene	7.9	12.9	19.9	24.1
Limonene	1.1	1.7	3.5	2.7
γ-Terpinene	0.4	1.0	1.7	1.3
Citronellal	0.5	0.5	0.5	0.3

5.5 Condensed Vapour Percolation

In an industrial gin manufacture process as the vapour passes over the botanicals some of the vapour will begin to condense and fall back through the botanicals. This process is called condensed vapour percolation (CVP). Now the entire botanical recipe will be used more analytes will be present and in greater quantities. In industrial gin manufacture reflux, forced or passive, is not directed to fall over the botanicals, however many still configurations could be easily modified to allow this to happen. This investigation is aiming to determine whether this is an efficient process and percolation through the botanicals should be increased.

5.5.1 Materials and Methods

The condensed vapour percolation method utilised a custom-built distillation apparatus that allowed for the vapour to be routed through different pathways to trial different micro processes. A Technical Glassware Products (TGP), Dunedin, New Zealand, reactor column with custom-made stainless-steel end caps was used to house the botanical basket. The end caps were custom made machined stainless steel to fit with Quickfit glassware. The column used to house the botanical basket is shown in Figure 13A and B. In CVP the vapour flow was routed to enter above the basket and enter directly into the condenser before the condensed vapour percolated over the botanicals. The Quickfit glassware used were a 500 mL round bottom boiling flask, condenser, three-way distilling adapter, adapter with two parallel necks and bend receiver. The botanicals: juniper berries, coriander seeds, angelica root, kaffir lime leaves, black pepper corns, orange powder and orris root were sourced from BeGin Distilling, New Plymouth, New Zealand. The apparatus was able to be adjusted to have the vapour enter above or below the botanicals, in Figure 14 the apparatus is set up in the condensed vapour percolation mode. In the experimental photos the insulation was removed from the column to display the heat trace and end caps. Thermometers were also able to be added to key areas within the process, above the kettle and or before the condenser in vapour infusion mode.

Figure 13A and 13B: Shows the reactor column used to house the botanical basket, the column is wrapped in heat trace. The stainless-steel caps are also visible at either end of the column. The botanical basket is also visible within the column on the right.

In the condensed vapour percolation arrangement, the vapour will not pass over the botanicals, instead it will be condensed, and the condensate will pass over the botanicals. This was to isolate the process that occurs during gin distillation where the vapour condenses on the botanicals.

Figure 14: Condensed vapour percolation experimental apparatus. The glass column had a botanical basket at the top just below the vapour inlet. Top of shot is the condenser where the vapour condensed before falling over the botanicals and out the bottom of the column into the measuring cylinder. Since the column is not above the kettle there is a lower level of reflux than steam distillation.

To bring up the glassware to operating temperature 300 mL of water was boiled for 10 minutes before operation. The botanicals basket was then placed into the glass column and the kettle filled with the aqueous ethanol solution and boiling chips before the heating mantel was switched to max heat. Once it had started boiling the setting was reduced to 50%. Samples of the distillate were collected in a measuring cylinder in 40 mL segments. The measuring cylinder was switched every 40 mL and each segment was both extracted into hexane for GC analysis and diluted for ethanol concentration analysis, see section 3.2.3 and 5.7.1 for full method. The kettle was also sampled at the initial and final stage to be analysed GC and ethanol concentration.

Table 32: The distillate segment that each sample was taken from, and the volume needed. Each sample was taken from a 40 mL distillate segment other than the final distillate sample which was of the entire yield. The kettle was sampled pre- and post-boil. GC analysis was used to quantify the analyte concentration and the HPLC analysis calculated the ABV.

Sample	Distillate segment	Kettle	GC analysis		HPLC analysis	
			Volume of sample (mL)	Volume of water (mL)	Volume of sample (mL)	Volume of water (mL)
1	-	Pre-boil	2	1	0.2	1.8
2	0-40 mL	-	1.5	1.5	0.2	1.8
3	40-80 mL	-	1.5	1.5	0.2	1.8
4	80-120 mL	-	1.5	1.5	0.2	1.8
5	120-160 mL	-	1.5	1.5	0.2	1.8
6	160-200 mL	-	1.5	1.5	0.2	1.8
7	200-238 mL	-	1.5	1.5	-	-
8	0-238 mL	-	1.5	1.5	0.2	1.8
9	-	Post-boil	3	0	-	-

The GC samples were diluted to 40 % ABV and a volume of 3 mL. The HPLC samples were diluted to 10% and a volume of 2 mL. The initial kettle ABV was 60% therefore 2 mL of sample and 1 mL of water were combined for a final sample of 3 mL at 40% ABV.

Table 33: The entire botanical ratio for condensed vapour percolation is listed below. The botanical ratio was doubled in between trials while keeping the solvent ratio the same. The solvent ratio remained constant for each trial at 350 mL 60% ABV.

Botanical	Full recipe (g/L)	Three quarter recipe (g/L)	Half recipe (g/L)	One quarter recipe (g/L)
Juniper berry	80.3	60.1	40.1	20.4
Kaffir lime leaf	20.2	15.0	10.1	5.0
Orange powder	4.1	3.0	2.1	1.0
Coriander seed	2.0	1.5	1.0	0.5
Angelica root	2.1	1.5	1.0	0.5
Orris root	2.0	1.5	1.0	0.5
Black peppercorn	2.1	1.5	0.9	0.5
Cardamon pods	1.6	1.1	0.9	0.5

The average concentration of distillate was 80% so 1.5 mL of sample and 1.5 mL of water was needed for a final sample of 3 mL at 40% ABV. The kettle was below 40% ABV and was analysed without the fortification of ethanol. The trials used a 350 mL aqueous ethanol base at 60% ABV and a botanical ratio shown in Table 33.

5.5.2 Results and Discussion

The distillate was divided into seven segments. The first six were 40 mL each and the final was the total distillate. Each segment was individually analysed to determine the concentration of each analyte as the distillation progressed. This investigation was comparing the entire botanical recipe which meant citronellal would begin. It is not a component of juniper berry and was not expected to be present earlier.

The results showed that the analytes were extracted at different stages of the distillation process. The highest concentration of α-pinene, β-myrcene and limonene was obtained towards the end of the boil, during the 160-200 mL segment. This is approximately two-thirds of the set yield. Set yield was determined by assuming that the average ABV of the distillate was 80% and the set point was 90% yield of total ethanol in the system which was at approximately 238 mL of distillate.

In Figure 15 both largest peaks are from α-pinene and β-myrcene in the 160-200 mL segment. This is progressing towards the end of the distillation process and may even be past the start of the tails cut. This study did not include sensory evaluation of the distillate to differentiate between the heads, hearts, and tails. In future research selecting a compound that only comes through at the start of each segment could be a simple method of distinguishing the segments. Another method is by sensory analysis of the distillate however this is a more complicated approach. A full sensory panel requires a lot of training and cost and was outside the scope of this study. The cuts can be made when the vapour reaches a certain temperature however this can easily change due to numerous factors including environmental which make this hard to keep consistent. The total concentration of analytes in the distillate is shown in Figure 16 below and shows that the higher botanical ratio leads to a higher concentration of analyte in the distillate as expected.

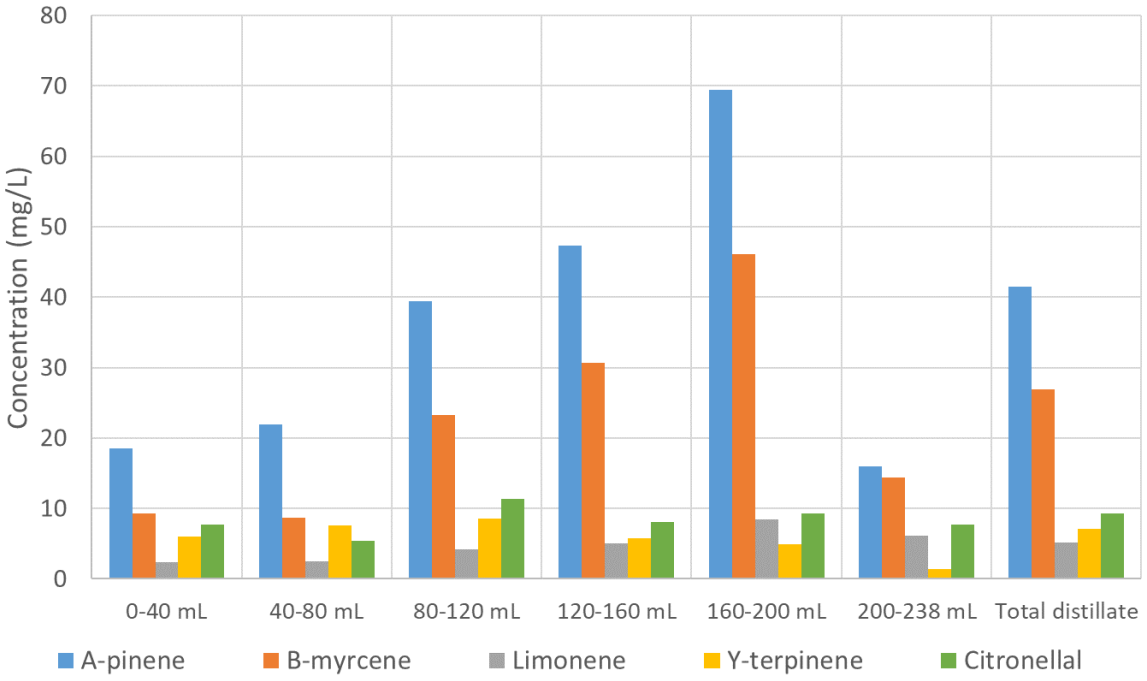

Figure 15: Analyte concentration in distillate segments in full botanical ratio experiment.

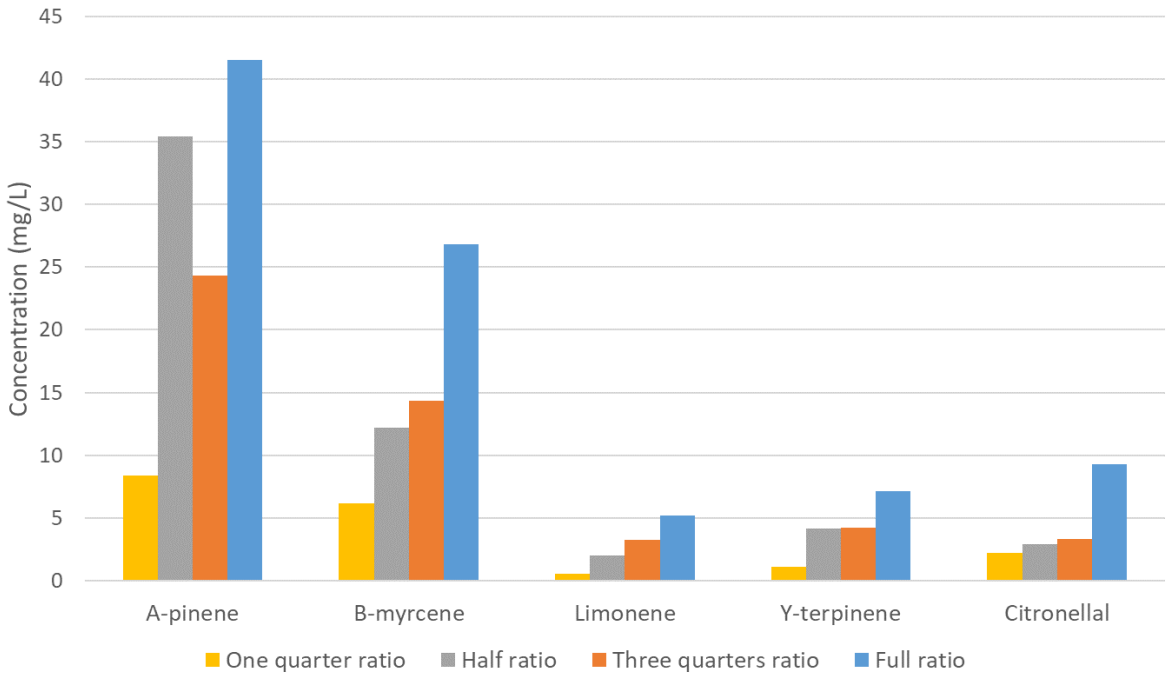

Figure 16: Concentration of key analytes in total distillate of all botanical ratios for condensed steam distillation. Total distillate was 238 mL. Samples were analysed by GC-FID and were single injection from single sample.

The amount of botanical is still producing efficient analyte concentrations therefore the botanical ratio could still be increased until it becomes less efficient. The concentrations for the analytes were much lower than for the most efficient trials for maceration or macerate distillation even for trials

that included a higher mass of botanicals. This shows that increasing the condensed vapour percolation process would not be an efficient method to create a 'hotshot' gin. While it is possible that the efficiency could be increased from the results in this study if the process was optimized, it is unlikely to increase to a point where it is more efficient than the other processes.

5.6 Vapour Infusion

The aqueous-ethanol vapour extraction method, or vapour infusion (VP) as it is referred to in gin manufacture, involves suspending the botanicals in a basket in the vapour pathway. The botanicals are only in contact with the vapour and any condensate. This study has already investigated the condensate and this section will focus on the vapour infusion extraction process.

5.6.1 Materials and Methods

Vapour infusion produced by rearranging the apparatus used in condensed vapour distillation experiments. The new configuration has the vapour entering the glass column directly underneath the botanicals and the vapour passes over the botanicals before reaching the condenser. A thermometer above the kettle and before the condenser were utilized to monitor the system. The insulation was removed for the photos to demonstrate where the basket was located.

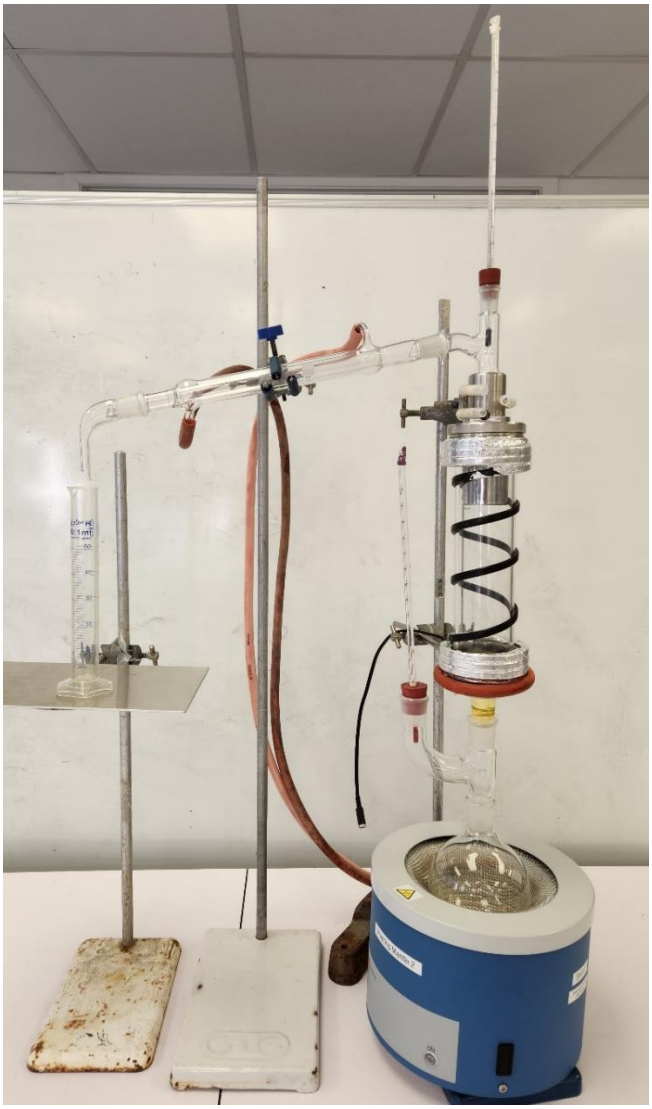

Figure 17: Vapour infusion experimental apparatus. In this arrangement the vapour entered the bottom of the column before passing over the botanicals and into the condenser at the top of the frame. Condensate can fall back into the kettle since the column is directly above it and this leads to a higher level of reflux.

In the heating mantle the 500 mL round bottom flask was connected to an adapter with two parallel necks to connect to the glass column and thermometer. There was 15 W/m heat trace wrapped in insulation around the glass column to increase the temperature and control condensation. Above the column was a distilling adapter and another thermometer and condenser. The two temperatures were recorded for each segment of distillate. Samples were taken and prepared by the same process as condensed vapour distillation; the distillate was divided into 40 mL segments that were separated as they were produced until a GC analysis and ethanol analysis sample was taken. Analysis methods for these techniques can be found in section 3.2.3 and 5.7.1. Each segment was recombined, and the total distillate was also analysed. For further details see Table 34. The initial and final state of the kettle was also sampled and analysed. The heating mantle's max heating setting was used until the

solution started to boil before it was reduced by 50%. Boiling chips were added into the kettle to add nucleation sites to initiate boiling. The sample segments and volumes are shown in Table 34 and in Table 35 the botanical ratios can be found. Each trial used a 350 mL aqueous ethanol base at 60% ABV.

Table 34: The distillate segment that each sample was taken from, and the volume needed. Each sample was taken from a 40 mL distillate segment other than the final distillate sample which was of the entire yield. The kettle was sampled pre- and post-boil. GC analysed was used to quantify the analyte concentration and the HPLC analysis calculated the ABV.

			GC analysis		HPLC analysis	
Sample	**Distillate segment**	**Kettle**	**Volume of sample (mL)**	**Volume of water (mL)**	**Volume of sample (mL)**	**Volume of water (mL)**
1	-	Pre-boil	2	1	0.2	1.8
2	0-40 mL	-	1.5	1.5	0.2	1.8
3	40-80 mL	-	1.5	1.5	0.2	1.8
4	80-120 mL	-	1.5	1.5	0.2	1.8
5	120-160 mL	-	1.5	1.5	0.2	1.8
6	160-200 mL	-	1.5	1.5	0.2	1.8
7	200-238 mL	-	1.5	1.5	-	-
8	0-238 mL	-	1.5	1.5	0.2	1.8
9	-	Post-boil	3	0	-	-

Table 35: The entire botanical ratio for vapour infusion trials is listed below. The botanical ratio was increased in between trials while keeping the solvent ratio the same. The solvent ratio remained constant for each trial at 350 mL 60% ABV. Juniper berry expressed as grams per litre of aqueous ethanol solution.

Botanical	**Full recipe (g/L)**	**Three quarter recipe (g/L)**	**Half recipe (g/L)**	**One quarter recipe (g/L)**
Juniper berry	80.5	60.1	40.1	20.4
Coriander seed	20.1	15.0	10.0	5.0
Kaffir lime leaf	4.2	3.1	2.1	1.0
Angelica root	2.0	1.5	1.0	0.5
Orange powder	2.2	1.5	1.0	0.5
Black peppercorn	2.0	1.5	1.0	0.5
Orris root	2.1	1.6	1.0	0.5
Cardamon pods	1.7	1.1	0.9	0.4

5.6.2 Results and Discussion

The vapour infusion sampling and analysis was executed by the same method used in condensed vapour percolation explained in section 5.5. It was not possible to prevent passive reflux causing condensate to form on the botanicals however it was minimised by heating the botanical chamber before commencing experimental and the heat trace wire and insulation wrapped around the glass column kept the apparatus at temperature. This set up was unable to prevent all passive reflux however it was reduced as much as possible.

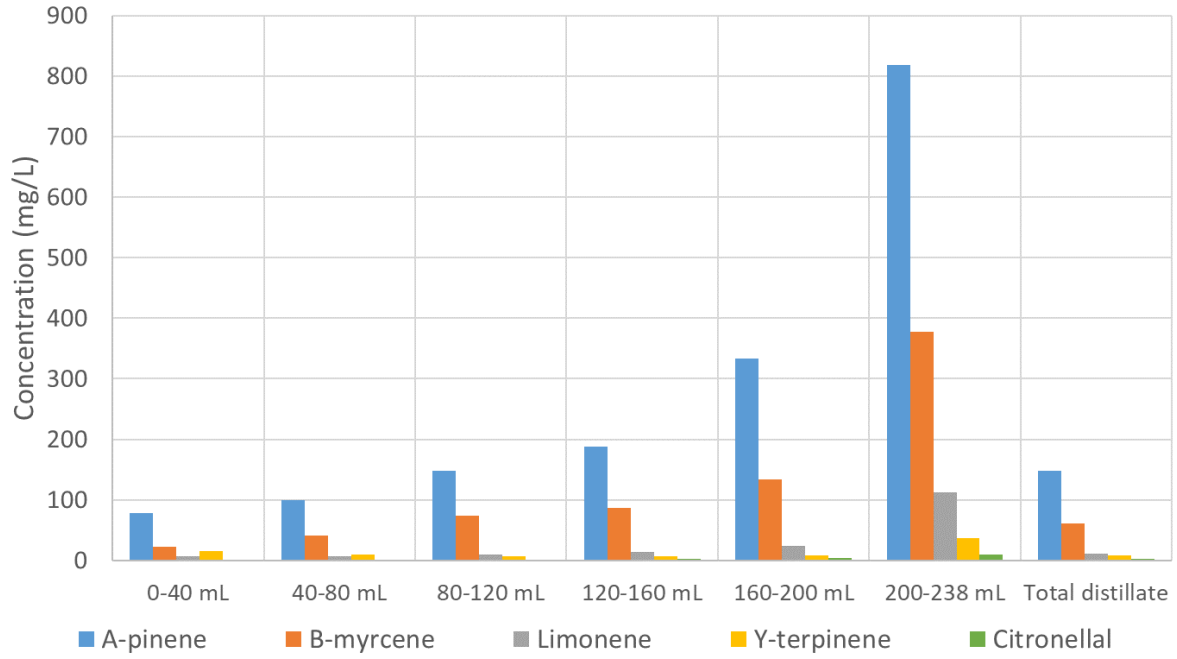

Figure 18: Concentration of key analytes in distillate for full botanical ratio for each segment from vapour infusion experiments.

In Figure 18 the concentration of each analyte for reach distillate segment is plotted for the full botanical ratio experiment. From this graph it is clear that analyte concentration peaked during the 200-238 mL segment. This is towards the end of the distillation and would likely be considered part of the tails. This high concentration is why it is important to recover ethanol and analytes from tails when possible. Most common method is adding it into the next distillation run. α-Pinene had the largest concentration in the distillate with 148 mg/L. This is much larger than condensed steam percolation or macerate distillation with 41 mg/L and 48 mg/L respectively. It is important to consider that macerate distillation only had a singular botanical, juniper berry, and a lower mass than the juniper present in CVP or VI in the full botanical ratio compared here. Figure 18 clearly shows that α-pinene is present in the largest concentration followed by β-myrcene. The

predominant botanical in these trials is juniper berry which the two highest constituents of are α-pinene and β-myrcene so this is expected.

In Table 36 the analyte concentration in the total distillate is displayed. It shows that there is not a large increase in analyte extracted between the three-quarter ratio and full ratio. One of the factors that could be causing this is how the botanicals were situated within the botanical's basket. The botanicals were contained in a stainless-steel basket within the glass column this was held in place by a rubber O-ring in between the column and stainless-steel cap. This limited the volume available for the botanicals and they had to be packed into the contained for the full ratio experiment. This may have limited the contact area between the vapour and the botanicals which decrease the concentration of extracted analytes. In a future experiment finding a basket that could sit lower in the column would generate more volume for the botanicals.

Table 36: Concentration of the key analytes in the total distillate yield of 238 mL for all botanical ratios for vapour infusion experiments.

Compound	Concentration (mg/L)			
	Full botanical ratio	**Three quarters botanical ratio**	**Half botanical ratio**	**One quarter botanical ratio**
α-Pinene	148.1	142.1	88.2	30.7
β-Myrcene	61.0	61.8	36.2	16.2
Limonene	11.2	11.3	6.3	3.7
γ-Terpinene	7.8	9.1	5.5	2.1
Citronellal	2.0	7.4	4.0	3.8

Figure 19 shows the concentration of α-pinene for each distillate segment across all botanical ratios. The full ratio had much higher concentrations for most of the segments other than in the total distillate where it was almost identical to the three quarters ratio. This is a lower value than expected when comparing the other values on this graph. The three quarters ratio value at the 200-238 mL segment also seems to be a lower value than expected when compared to the neighbouring values. This was reflected in almost all experiments as shown in Figure 20 with the β-myrcene concentration. The internal standard addition could have been measured wrong or there may have been an error in the extraction process or GC analysis. There were many opportunities for readings to appear like they were an error throughout the course of this study. They were minimised wherever possible but what is recommended in the future is that experiments are repeated

especially for obvious outliers to produce more robust data. Unfortunately, this was unable to be completed for this project due to the time limited nature.

In Figure 19 the segment of distillate with the highest level of α-pinene was in the 200-238 mL segment. This was mirrored for β-myrcene, limonene, γ-terpinene and citronellal. What was interesting with γ-terpinene was that the second highest segment was the first segment 0-40 mL. This suggested that γ-terpinene is much quicker to extract than the other compounds, potentially due to where in the material the compound is located.

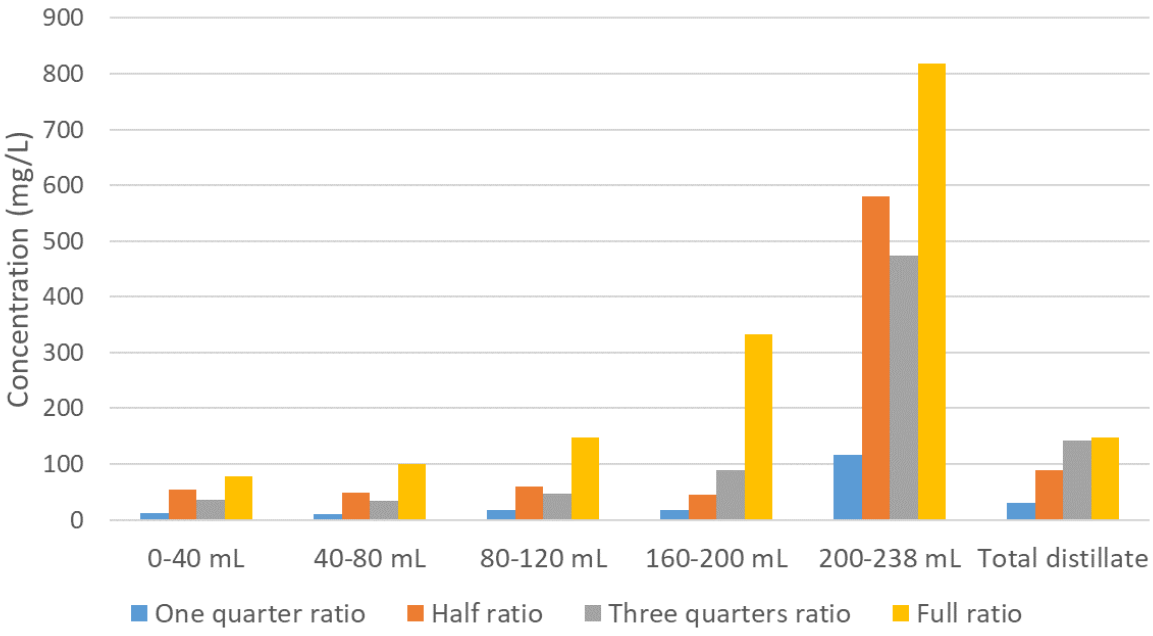

Figure 19: Concentration of α-pinene in distillate segments for all botanical ratios for vapour infusion distillation experiments. The samples were collected in 40 mL segments with the total distillate sample taken from the full 238 mL yield. Analysed by GC-FID and were single injection from single sample.

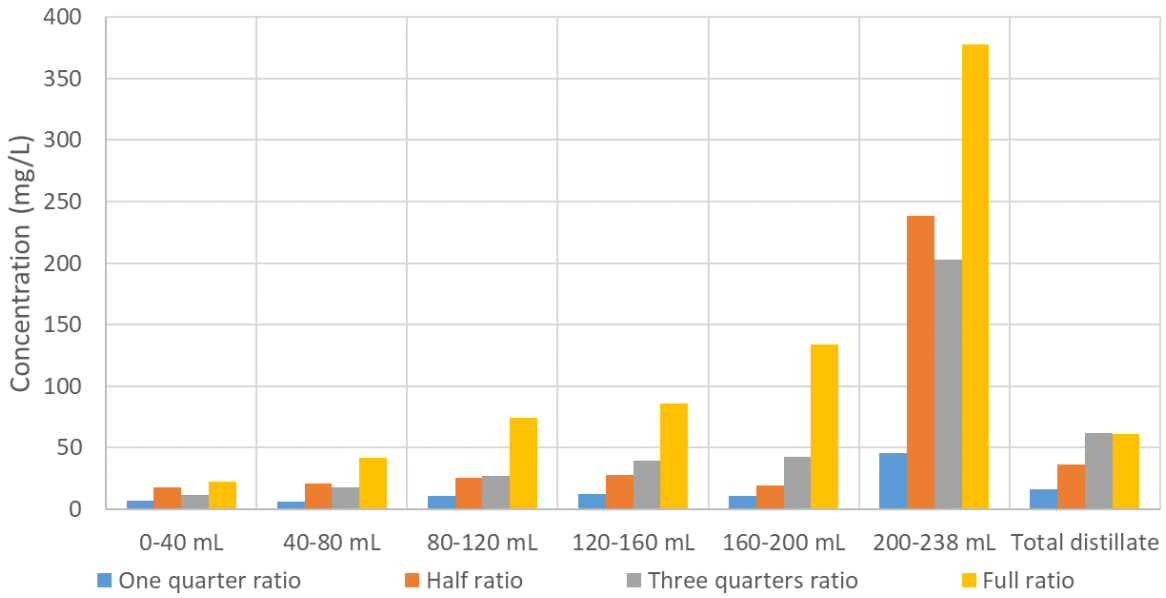

Figure 20: Concentration of β-myrcene in distillate segments for all botanical ratios for aqueous-ethanol vapour distillation experiments. The samples were collected in 40 mL segments with the total distillate sample taken from the full 238 mL yield. Analysed by GC-FID and were single injection from single sample.

To conclude vapour infusion led to the highest extraction rates out of the micro-processes investigated in this study. The advantage of steam distillation is that the botanicals are only in contact with the vapour, which has a higher ABV and a lower temperature than the boiling liquid. This reduces heat degradation and hydrolysis resulting in a higher overall extraction concentration compared to macerate distillation. This agrees with the findings of AL-Hilphy (2017) who states that the higher the concentration of water and the higher the temperature the lower the yield of essential oils. Handa (2008) also states that a disadvantage of macerate distillation is that it does not lead to complete extraction. The botanicals in vapour infusion are also in contact with the higher ABV solvent, which leads to increased extraction rates as shown in section 5.3.2.2 Solvent Concentration where 78% ABV and 96% ABV had higher extraction rates than 58% ABV. The trials that macerated in higher ABV solution led to a higher extraction rate, therefore the higher ABV vapour ~80% should lead to greater extraction rates than the other processes which have a lower ABV. Condensed vapour percolation did expose the botanicals to a higher ABV solvent however the overall temperature was much lower, decreasing thermal degradation but also reducing the overall reaction rate and there was lower passive reflux which resulted in a lower extraction rate. Vapour infusion is the most efficient process and should be focused on when developing a 'hotshot' gin.

While it currently is the most efficient it could potentially still be improved by crushing or milling the larger botanicals prior to distillation. In commercial manufacture maceration, macerate distillation

and vapour infusion are normally combined into a hybrid method which would increase the total analyte concentration.

The botanical ratio should be further increased and investigated to determine whether it is beneficial to increase it further or whether the returns are starting to diminish. Repeats of experimental work is also recommended especially where a value is an obvious outlier.

5.7 Ethanol Concentration and Distillate Volume

It was expected that the ethanol concentration of the distillate would vary across the three distillation processes due different arrangement of kettle, column, and condenser. Therefore, the ethanol concentration was calculated for each process.

5.7.1 Materials and Methods

Ethanol concentration analysis was determined using a Shimadzu LC-20A system equipped with an Aminex HPX-87H column sourced from BIO-RAD, Hercules, California, USA. Ethanol was filtered using syringe-driven filters sourced from BIOFIL, Gangzhou, China, were 13 mm nylon membrane with 0.22 µm pore size.

Ethanol analysis was done on an individual distillate segment used in the previous experiments. That was a total of seven samples, every 40 mL segment and including the total distillate. Distillate samples were taken and diluted by a factor of 1:10 with water. The diluted sample was then filtered through 0.22 µm nylon membrane BIOFIL filter before being frozen at -18°C until analysed on the HPLC.

Ethanol concentration analysis was determined using a reversed phase HPLC system Shimadzu 20A series. The system was comprised of a LC-20AD pump, SIL-20A HT autosampler, RID-20A refractive index detector, and CTO-20AC column oven. The column was a Aminex HPX-87H sourced from BIO-RAD kept at 60°C with an isocratic mobile phase consisting of 5mM H_2SO_4 with a flow rate of 0.6 mL/min. The system was calibrated using standard solutions of 10% V/v of pure ethanol. The HPLC system was controlled using Lab Solutions Lite version: 5.101.

5.7.2 Results and Discussion

The ethanol concentration and distillate volume were recorded and plotted below as a function of time. The maceration distillation was significantly quicker than the other two processes however they all had similar ethanol concentration levels.

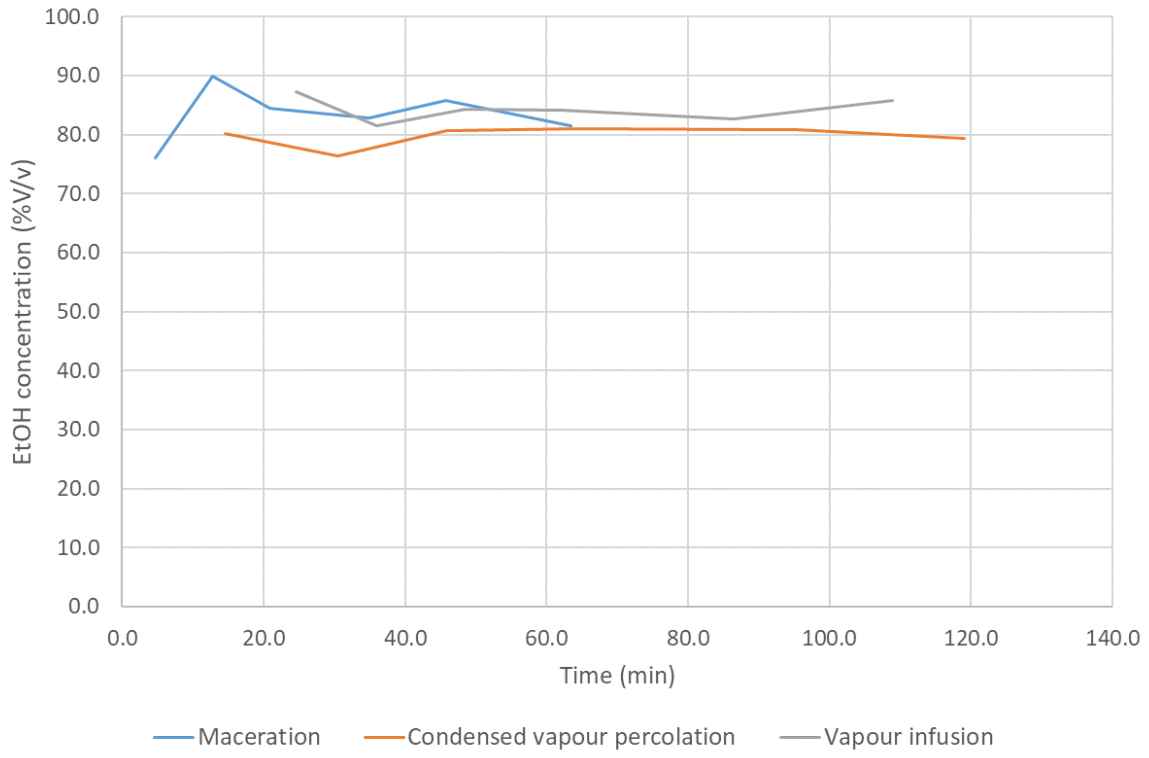

Figure 21: The ethanol concentration of the three separate process as a function of time. The ethanol concentration was calculated for two trials of each process that were then averaged, and that value displayed on this chart.

The maceration process may have been quicker than the other processes due to there being less thermal mass involved since the vapour did not have to pass through the glass column and stainless-steel ends. The vapour infusion experimental set up also had the glass column directly above the kettle which allowed for any condensate that formed on the column or botanical basket to fall back into the kettle as a form of uncontrollable reflux. This was not possible in either of the other processes. For maceration there was no column, and the vapour directly entered the condenser after exiting the kettle. In condensed vapour percolation the condensation that did not reach the condenser would fall into the collection vessel at the bottom of the column since the vapour entered from the side. The uncontrollable reflux would lead to a slightly higher ABV as shown by the results.

Table 37: Average ABV of the final distillate of the three micro processes.

Process	ABV of total distillate
Maceration	82
Condensed vapour percolation	79
Vapour infusion	86

The volume of distillate as a function of time is shown below. Maceration is clearly the fastest process to reach the yield setpoint of 238 mL. As explained above it was likely quicker due to the smaller thermal mass and reduced passive reflux since there was no column.

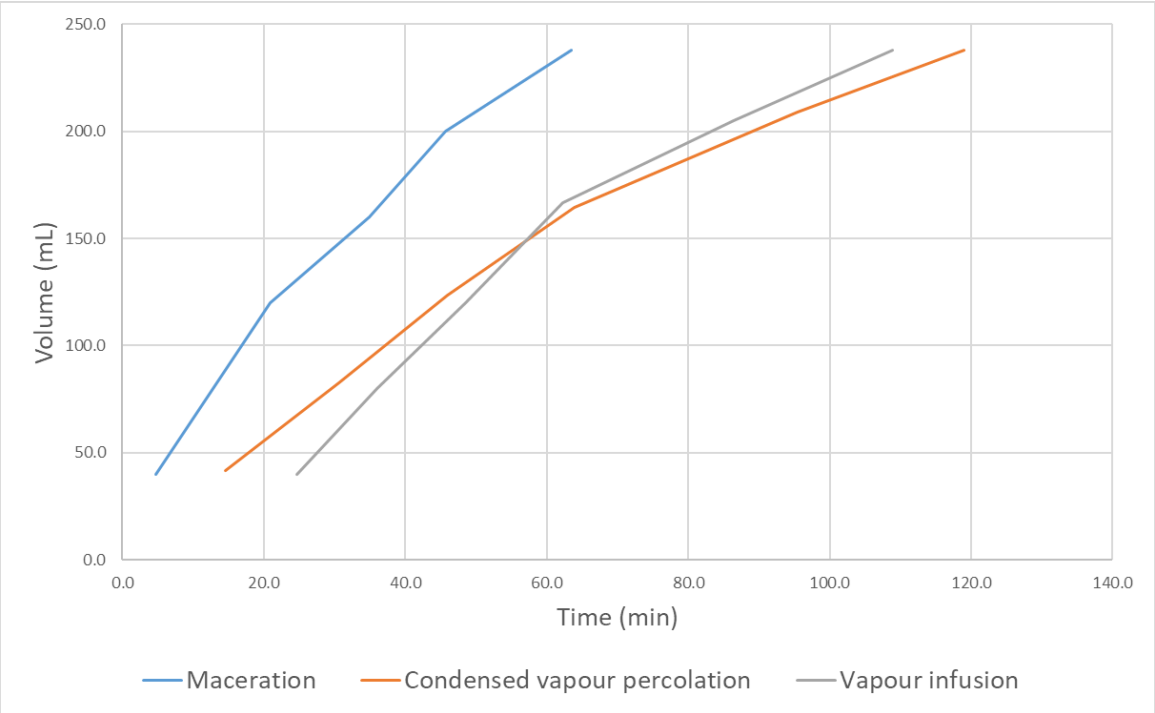

Figure 22: Rate of distillate produced as a function of time across the three processes. Maceration distillation had a much quicker production rate and condensed vapour percolation, and vapour infusion were very similar.

5.8 Analyte Extraction Effectiveness

The extraction effectiveness was compared across the different distillation processes by first determining the total analyte in fresh juniper berry and then the amount remining in spent berries after the completion of the different processes.

5.8.1 Materials and Methods

To calculate the total amounts of compound in fresh juniper berries they were first heavily crushed with a mortar and pestle and then extracted into 3mL of hexane. Spent berries from macerate distillation, condensed vapour percolation and vapour infusion were first dried in a convection air oven at 54°C overnight and then crushed and extracted into hexane. The samples were then analysed on the GC-FID and compared.

Table 38: The weight of the berries used for analyte extraction effectiveness calculation along with the weight of hexane.

	Berry weight (g)	**Hexane (mL)**	**State**
Sample 1	0.3019	2.0711	Fresh, crushed
Sample 2	0.329	2.0192	Spent, dried, crushed

In order to compare the extraction effectiveness across the different process three experiments were done containing only the main botanical: juniper berry.

Table 39: The mass of juniper berry used across effectiveness trials.

Trial	**Juniper berry (g)**	**Ethanol (mL)**	**Water (mL)**
Macerate distillation	28.09	210	140
Condensed vapour percolation	28.06	210	140
Vapour infusion	28.04	210	140

5.8.2 Results and Discussion

The extraction efficiency after distillation from juniper berries was calculated by comparing fresh (unused) berries and the amount of analyte in the distillate. Firstly, the moisture content was determined to be 14.34% dry basis. This closely matched previous results in literature of 14.04% dry basis by Altuntas (2015). It was assumed that all available analyte was extracted into hexane however it is possible there was some not recovered from the fresh berry. It is also possible that ethanol and hexane would have different recovery efficiencies however this value is not known and

will be assumed to be the same for this study. Citronellal was not included in this investigation since it was not present in juniper berry.

Table 40: Percentage of analyte recovered in distillate from the three processes.

Compound	Analyte extracted into distillate from macerate distillation (%)	Analyte extracted into distillate from condensed vapour percolation (%)	Analyte extracted into distillate from vapour infusion (%)
α-pinene	39	22	58
β-myrcene	28	21	53
Limonene	18	18	62
γ-terpinene	37	61	-

γ-Terpinene had a measurement error for vapour infusion leading to a zero-concentration value. Macerate distillation had similar recovery rates to CVP for β-myrcene and limonene but a much higher rate for α-pinene. Interestingly CVP had a very high extraction rate for γ-terpinene, it would have been interesting to compare the recovery to VI however the investigation will have to be repeated for further analysis. The recovery rates for vapour infusion were much higher than the other processes for the other three analytes. Since VI proved to be the most efficient process it can be theorised that the recovery for γ-terpinene would be higher than CVP if the experiment was repeated.

This comparison is only comparing the analyte recovery from juniper berry. While juniper berry makes up the majority of the botanical bill the other botanicals also play a significant role. Further research will have to be done into the analyte recovery from the other botanicals. From the literature it is theorised that the solid materials, seeds and roots, may have lower recovery than leaves, flowers and powder, since the compounds take longer to transfer out of the material.

The recovery percentage was quite low which shows that there is still a large portion of analyte remaining in the berry. This is currently lost analyte and continuing processing the botanicals will also extract numerous unwanted compounds that will taint the flavour of the distillate. Hence why tails are not included in the final product. An ethanol recover process includes adding the tails into future runs which also recovers some of the desired analytes. This could be investigated further to determine how much the recovery could be increased before extracting undesirable compounds.

5.9 Conclusion

In conclusion this chapter was investigating the separate micro processes that extract analytes during the gin manufacture process. Maceration proved to be an inefficient process even over a substantial period of 72 hours. Increasing the maceration solvent ABV from 58% to 78% led to an increase in all analyte concentrations. However, the largest increase was by changing the material state. The extraction rate was increased substantially by crushing the juniper berries, leading to an increase of over 20 times for α-pinene and β-myrcene from the base trial.

In industrial gin manufacture maceration is followed by distillation. Coupling these processes proved to be more efficient at extracting analytes from the whole juniper berries than in maceration or un-macerated distillation alone. It was not as efficient as crushed berry maceration. Future work investigating the extraction rate of crushed berries in macerate distillation would be useful to understand how much more efficient it is. From the analyte recovery values there is still analyte remaining in the juniper berry after the completion of the process but crushing may increase total recovery.

Condensed vapour percolation led to the lowest extraction rates for many analytes however was efficient at extracting γ-terpinene. This was confirmed in the analyte extraction effectiveness investigation where γ-terpinene had the highest recovery rate for CVP. Due to the overall low performance from CVP it is not a viable method to create a ‘hotshot’ gin and instead the focus should be on the vapour infusion and macerate distillation.

Vapour infusion led to the highest extraction rates across the different processes investigated. The results of investigating the different botanical ratios prove that the ratio could still be further increased before diminishing returns are reached. Manufacture of a gin ‘hotshot’ should focus on the vapour infusion process to reach the high levels of analyte concentration needed. In this investigation vapour infusion was investigated as an individual process whereas in industrial gin manufacture this process is often combined with macerate distillation. Optimizing and including macerate distillation would lead to a greater concentration of analytes in the distillate and be closer to producing an industrial ‘hotshot’ gin.

Chapter 6: Conclusions and Recommended Further Research

6.1 Conclusions

In conclusion it was found that production of a concentrated gin 'hotshot' is possible. In light of recent literature, it is evident that it is an emerging process in the gin industrial that they are calling multi-shot gin. The recent literature does not characterise the process and there are still many aspects that need to be investigated. This study has begun to investigate the parameters the influence key analyte extraction and how they can be optimized to create an efficient multi-shot gin.

Throughout this study gas chromatography was found to be a suitable analysis method for the volatile organic compounds found in gin. Utilising both GC-FID and GC-MS equipment allowed for identification and quantification of analytes of interest. There was great success in the GC analysis however it was also produced many outliers which led to experiments needing to be repeated if time allowed. Sample preparation was found to be the most important factor in obtaining precise results and multiple analytical chemistry techniques were used. Utilising the internal standardisation method and the external standardisation method allowed for the concentrations of key analytes to be determined in the gin samples. The optimal solvent was found to be hexane at 1 part per 3 part of sample with an average extraction efficiency of 65% for the key analytes.

The four analyte extraction processes: maceration, macerate distillation, condensed vapour percolation and vapour infusion were successfully investigated to understand the process and when in the timecourse the analytes of interest were extracted. There was a clear relationship between the botanical ratio and analyte concentration in the distillate. Doubling the amount of botanical did not lead to approximately double the concentration of analyte. There were diminishing returns when this was investigated for maceration and macerate distillation however that point had not been reached for condensed vapour percolation and vapour infusion.

To develop a multi-shot gin the highest efficiency process needs to be used and vapour infusion was the most efficient. It was found that the extraction efficiency was much higher for vapour infusion compared to maceration distillation and condensed vapour percolation. Combing maceration and vapour infusion would increase the total analyte concentration especially if the maceration process was optimized.

In maceration, increasing the solvent concentration to 78% ABV proved successful in increasing the analyte extraction rate. However, the most successful method was crushing the berries which led to a much higher extraction rate than any of the other factors.

There was not adequate time to complete repeats of key experiments that would have been beneficial for the understanding of the holistic distillation process. Repeats are important at improving precision of experiments “duplicates are good, but triplicates are better” (D. James, Personal Communication, Oct 1, 2021). Several results could be clarified with repeats of condensed steam extractions and steam distillation experiments since they appear to be obvious outliers.

This study has shown that it is possible to create a concentrated multi-shot gin and begins to understand the processes that are occurring during gin distillation. The research completed begins the process of characterising the main micro-processes and the key analytes. Further investigation needs to be done to determine whether even higher botanical ratios might be supported before extraction efficiency drops away. It is clear that vapour infusion has the highest efficiency and a hybrid method with optimized macerate distillation should be focused on in further studies to get maximum yield.

6.2 Recommendations for Future Work

While outside the scope of this study conducting flavour threshold investigations into the analytes within gin would allow for better understanding of what analytes contribute the most to the flavour. While there is some literature on this topic it is conflicting and not does not correlate. That investigation would be an entire study by itself which is why it was not attempted during this project.

In future work a second internal standard could be added to the sample prior to extraction to determine the extraction efficiency for each sample. This would improve the accuracy, especially if there are a lot of factors influencing the extraction rate. It was not deemed necessary for this project since the extraction efficiency values did not shift significantly throughout the experimental trials.

Vapour infusion was found to be the most efficient process with the current botanical ratios. The botanical ratios need to be further increased to determine when the extraction efficiency begins to diminish.

The analyte extraction efficiency investigation gave an understanding of the efficiency of the different processes for juniper berry however these could be repeated for each botanical to determine whether the placement of botanicals could be optimized. The maceration and macerate distillation experiments could also be repeated with every botanical to better compare with the other processes.

Investigation into how to recover analyte from spent botanicals after high botanical ratio distillations without extracting undesirable compounds would be beneficial. Spent botanicals are rarely used however the results have shown that there is often still a high concentration of analyte in the botanicals. A longer distillation may result in undesirable compounds in the distillate which may produce a larger proportion of tails. Potentially macerating them for the next distillation could be trialled.

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Appendices

Appendix A

A.1.1 Composition of Essential Oil from Botanicals

Table 41: Composition of essential oil of common botanicals shown in alphabetical order expressed in mass percentage. This table shows that while many botanicals are composed of the same compounds the majority are only present in one or two botanicals. A small fraction is still unidentified for each botanical other than orange powder.

	Composition of botanical essential oil (%)							
Compound	**Juniper berry**	**Coriander seeds**	**Kaffir lime leaf**	**Angelica root**	**Orris root**	**Orange powder**	**Black peppercorn**	**Cardamon pods**
(E)-β-Caryo-phyllene	-	-	-	-	-	-	14	-
(E)-β-Farnesene	-	-	-	-	-	-	0.7	-
(E)-β-Ocimene	0.1	-	-	-	-	-	-	-
2-Methoxy-4-vinylphenol	-	-	-	-	0.25	-	-	-
4-Terpineol	-	0.14	-	-	-	-	-	2.19
α-Bisabolol	-	-	-	-	-	-	0.2	-
α-Cadinol	-	-	-	-	-	-	trace	-
α-Cayophyllene	-	-	-	0.38	-	-	-	-
α-Copaene	-	-	0.3	-	-	-	-	-
α-Copaene	-	-	-	1.32	-	-	-	-
α-Cubebene	-	-	-	-	-	-	1.6	-
α-Farnesene	-	-	-	-	-	-	0.2	-
α-Farnesol	-	-	-	-	-	-	trace	-
α-Fenchene	0.2	-	-	-	-	-	-	-
α-Irone	-	-	-	-	2.85	-	-	-
α-Myrcene	-	-	-	-	-	-	-	1.87
α-Phellandrene	-	-	0.4	-	-	-	-	-
α-Phellandrene	-	-	-	3.74	-	-	2.2	-

α-Pinene	51.4	7.98	3.6	32.69	-	1.31	7.3	1.15
α-Pinene oxide	0.1	-	-	-	-	-	-	-
α-Selinene	-	-	-	-	-	-	0.3	-
α-Terpinene	0.1	-	-	-	-	-	0.1	-
α-Terpineol	-	0.25	7.6	-	-	0.34	0.5	5.06
α-Terpinolene	-	-	-	1.84	-	-	-	-
α-Terpiny-lacetate	-	-	-	-	-	-	-	40.54
α-Thujene	0.9	-	trace	0.62	-	-	0.2	-
β-Bisabolene	-	-	-	-	-	-	0.2	-
β-Car-3-ene	-	-	0.2	-	-	-	-	-
β-Caryophyllene	-	-	-	-	-	0.18	-	-
β-Elemene	-	-	-	-	-	-	0.3	-
β-Eudesmol	-	-	0.3	-	-	-	1.1	-
β-Isometilionone	-	-	-	-	0.21	-	-	-
β-Myrcene	8.3	1.18	0.3	5.87	-	3.25	2.6	-
β-Ocimene	-	-	-	-	-	0.85	-	-
β-Phellandrene	0.5	-	-	3.43	-	-	0.3	-
β-Pinene	5	0.57	10.9	1.87	-	1.76	19	-
β-Selinene	-	-	-	-	-	-	0.3	-
γ-Terpinene	0.2	5.93	trace	1.23	-	-	0.2	-
δ-3-Carene	0.2	-	-	17.07	-	-	10.6	-
δ-Cadinol	-	-	-	-	-	-	trace	-
δ-Cardinene	-	-	-	-	-	-	0.6	-
Benzophenone	-	-	-	-	1.11	-	-	-
Borneol	trace	-	0.6	-	-	-	-	-
Borneol acetate	-	-	0.2	1.37	-	-	-	-
Camphene	0.8	1.31	0.3	1.53	-	-	0.2	-
Campholen aldehyde	0.1	-	-	-	-	-	-	-
Camphor	-	4.42	-	-	-	-	-	-
Capric acid	-	-	-	-	14.5	-	-	-
Caprylic acid	-	-	-	-	1.72	-	-	-
Carvacrol	-	-	1.2	-	-	-	-	-

Caryophyllene oxide	-	-	0.5	-	-	-	0.8	-
cis-Cadina-1,4-diene	-	-	0.6	-	-	-	-	-
cis-p-Menth-2-en-ol	-	-	1.3	-	-	-	-	-
cis-Sabinene hydrate	0.1	-	-	-	-	-	-	-
cis-Verbenol	0.5	-	-	-	-	-	-	-
cis-β-Ocymene	-	-	-	1.8	-	-	-	-
Citronellal	-	-	2.7	-	-	-	-	-
Citronellol	-	-	6	-	-	-	-	-
Citronellyl acetate	-	-	2.2	-	-	-	-	-
Cubenol	-	-	0.2	-	-	-	-	-
Decanaol	-	-	-	-	-	0.39	-	-
Dihydrocarveol	-	-	-	-	-	-	trace	-
Dihydro-β-Irone	-	-	-	-	0.25	-	-	-
Endo-Fenchol	-	-	2.1	-	-	-	-	-
Eucalyptol	-	-	6.4	-	-	-	-	23.86
Eugenol	-	-	-	-	-	-	0.2	-
Geraniol	-	-	1.1	-	-	-	-	-
Geranyl acetate	-	3.4	-	-	-	0.38	-	2.13
Germacrene D	-	-	-	0.47	-	0.4	trace	-
Hedycaryol	-	-	0.1	-	-	-	0.4	-
Heneicosane	-	-	-	-	0.23	-	-	-
Heptacosane	-	-	-	-	0.59	-	-	-
Hexanal	trace	-	-	-	-	-	-	-
Isopulegol	-	-	1.6	-	-	-	-	-
Lauric acid	-	-	-	-	15.42	-	-	-
Ledol	-	-	-	-	-	-	trace	-
Limonene	5.1	2.92	4.7	6.59	-	86.36	29.7	-
Linalool	0.1	66.4	2.8	-	-	2.19	2.1	5.33
Linalool oxide	-	0.78	-	-	-	-	-	-
Linalyl acetate	-	1.44	-	-	-	1.35	-	-

Myristic acid	-	-	-	-	56	-	-	-
Myristic acid, methyl ester	-	-	-	-	0.17	-	-	-
Myrtenol	-	-	1.2	-	-	-	trace	-
Nerol	-	-	-	-	-	-	-	5.24
Nerolidol	-	-	-	-	-	0.29	-	1.07
Neryl acetate	-	-	0.2	-	-	-	-	-
Octanol	-	-	-	-	-	0.44	-	-
Palmitic acid	-	-	-	-	1.13	-	-	-
p-Cymen-8-ol	-	-	0.5	-	-	-	-	-
p-Cymene	0.9	1.65	5.6	0.38	-	-	0.5	-
Pentacosane	-	-	-	-	2.29	-	-	-
Perillene	0.1	-	-	-	-	-	-	-
p-Menth-8-en-3-ol	-	-	2.6	-	-	-	-	-
p-Mentha-2,4(8)-diene	-	-	3.8	-	-	-	-	-
Sabinene	5.8	0.33	-	0.92	-	0.51	1.4	3.52
Squalene	-	-		-	0.69	-	-	-
Terpinen-4-ol	-	-	13	-	-	-	0.3	-
Terpinolene	0.4	-	-	-	-	-	0.6	-
Thuja-2,4(10)-diene	0.2	-	-	-	-	-	-	-
τ-Muurolol	-	-	-	-	-	-	0.1	-
Toluene	trace	-	-	-	-	-	-	-
trans-2,6-γ-Irone	-	-	-	-	1.22	-	-	-
trans-b-Ocymene	4.83	-	-	4.83	-	-	-	-
trans-b-Pharnesene	0.26	-	-	0.26	-	-	-	-
trans-Pinocarveol	0.3	-	-	-	-	-	-	-
trans-Piperitol	-	-	0.8	-	-	-	-	-
trans-p-Menth-2-en-1-ol	trace	-	2.1	-	-	-	-	-

trans-p-Menth-6-ene-2,8-diol	-	-	1	-	-	-	-	-
trans-Sabinene hydrate	0.1	-	-	0.91	-	-	trace	-
Tricosane	-	-	-	-	0.89	-	-	-
Tridecanoic acid	-	-	-	-	0.21	-	-	-
Unidentified	13.41	1.3	11	10.88	0.27	-	1.2	8.04

A.1.2 Chemical Properties

Table 42: Chemical properties of the standards and solvents used in this study. All data sourced from https://pubchem.ncbi.nlm.nih.gov/.

Compound	**Boiling point (°C)**	**Density (g/cm^3)**	**Molecular weight (g/mol)**	**Formula**	**IUPAC name**
α-Pinene	156	0.858	136.23	$C_{10}H_{16}$	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
β-Myrcene	167	0.791	136.23	$C_{10}H_{16}$	7-methyl-3-methylideneocta-1,6-diene
Limonene	176	0.842	136.23	$C_{10}H_{16}$	1-methyl-4-prop-1-en-2-ylcyclohexene
γ-Terpinene	183	0.850	136.23	$C_{10}H_{16}$	1-methyl-4-propan-2-ylcyclohexa-1,4-diene
Citronellal	208	0.857	154.25	$C_{10}H_{18}O$	3,7-dimethyloct-6-enal
2-Octanol	179	0.819	130.23	$C_8H_{18}O$	octan-2-ol
Hexane	69	0.655	86.18	C_6H_{14}	hexane
Ethanol	78	0.789	46.07	C_2H_6O	ethanol
Chloroform	61	1.479	119.37	$CHCl_3$	chloroform
DCM	40	1.326	84.93	CH_2CL_2	dichloromethane
DMSO	189	1.100	78.14	C_2H_6OS	methylsulfinylmethane

A.1.3 GC-MS Compound Identification Data

Table 43: Gin extracted into hexane and analysis via Shimadzhu GC-MS with a stepped time-temperature programme that took 43-minutes identified 28 compounds.

Compound	Retention time	Peak area	Area (%)
α-Thujene	7.645	5698486	2.43
α -Pinene	7.946	71448224	30.46
Sabinene	10.015	39479237	16.83
β-Pinene	10.123	6336476	2.7
β -Myrcene	10.909	27995365	11.94
3-Carene	11.582	1656388	0.71
α -Terpinene	11.825	2928570	1.25
p-Cymene	12.107	1530891	0.65
Limonene	12.242	15933723	6.79
Eucalyptol	12.311	2441774	1.04
γ-Terpinene	13.186	6634336	2.83
Terpinolen	13.982	2299158	0.98
Linalool	14.334	2569124	1.1
Terpineol-cis-beta	15.030	789094	0.34
Citronellal	15.617	10908216	4.65
Terpinen-4-ol	16.343	4622198	1.97
Verbenyl ethyl ether	16.476	1332488	0.57
Cis-sabinene hydrate	17.564	1065931	0.45
α -Terpinene acetate	23.983	2504117	1.07
β -Elemene	26.449	1784253	0.76
Caryophyllene	27.989	4094679	1.75
γ -Elemene	28.937	848220	0.36
α -Caryophyllene	29.853	2031167	0.87
Germacrene D	30.938	7269434	3.1
β -Selinene	31.105	714159	0.3
Germacrene B	31.458	1126364	0.48
δ-Cadinene	32.242	2636824	1.12
α -Selinene	33.044	3965660	1.69

Table 44: Gin extracted into hexane and analysis via Shimadzhu GC-MS with a single ramped time-temperature programme that took 23-minutes identified 20 compounds.

Compound	Retention time	Peak area	Area (%)
α-Thujene	8.809	6112115	3.32
α -Pinene	8.908	24644782	13.38
Sabinene	9.508	21780271	11.82
β -Myrcene	9.741	31975415	17.37
3-Carene	10.009	2110122	1.15
Limonene	10.247	17161409	9.31
Eucalyptol	10.283	2180908	1.18
γ-Terpinene	10.611	6976907	3.79
Linalool	11.084	2914938	1.58
Citronellal	11.635	11804723	6.41
Terpineol-cis-beta	11.751	1270952	0.69
Terpinene 4-acetate	11.958	6226687	3.38
Terpinyl acetate	13.534	2716274	1.47
Germacrene B	14.290	1641703	0.89
α-Caryophyllene	14.525	2704687	1.47
Germacrene D	14.738	7993989	4.34
β-Seliene	14.793	1054194	0.57
δ-Cadinene	15.023	2712801	1.47
γ-Elemene	15.360	2257614	1.23

A.1.4 External Standard Calibration

Table 45: For initial retention time determination a single analyte was diluted in hexane and analysed via GC-FID. The following concentrations were used.

Compound	External standard (g)	Hexane (g)	Concentration (mg/L)
Limonene	0.0076	16.5617	291.5
Citronellal	0.008	16.5496	300.7
β-Myrcene	0.0054	11.0212	311.2

γ-Terpinene	0.0046	9.7088	300.9
α-Pinene	0.0064	10.2426	400.9
2-Octanol	0.0081	18.5825	276.8

Combined Standard Solution A

Combined standard solutions were prepared by combining different weights of the single standard solutions shown in Table 45.

Table 46: Concentrations of five external standards and one internals standard for the first combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times.

Compound	**External standard solution added (g)**	**Concentration (mg/L)**	**Peak area**		
			A	**B**	**C**
Limonene	1.299	114.7	18295685	18551338	17772485
Citronellal	0.8164	74.4	10347029	10396777	10219601
β-Myrcene	0.4264	40.2	3965035	4056638	3857488
γ-Terpinene	0.0096	0.9	221278	167485	139599
α-Pinene	0.1505	18.3	2826287	2785105	2800272
2-Octanol	0.5989	50.2	6161455	6088491	6070674

$$RF_{IS} = \frac{Peak\ area}{[IS]} \quad (8)$$

Table 47: Relative response factor for the internal standard for combined standard solution A.

Injection	$\boldsymbol{RF_{IS}}$
A	122659
B	121207
C	120852

Combined Standard Solution B

Table 48: Concentrations of five external standards and one internals standard for the second combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times.

Compound	External standard solution added (g)	Concentration (mg/L)	Peak area		
			A	B	C
Limonene	0.1579	14.1	2051111	2073152	1777761
Citronellal	1.2818	118.1	16354217	16153716	14399070
β-Myrcene	0.8085	74.5	7829969	8049124	6998478
γ-Terpinene	0.402	37.1	6044227	6043606	5334930
α-Pinene	0.009	1.1	195379	198512	157857
2-Octanol	0.604	51.2	6440849	6422233	5556987

Table 49: Relative response factor for the internal standard for combined standard solution B.

Injection	RF_{IS}
A	125691
B	125327
C	108442

Combined Standard Solution C

Table 50: Concentrations of five external standards and one internals standard for the third combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times.

Compound	External standard solution added (g)	Concentration (mg/L)	Peak area		
			A	B	C
Limonene	0.0109	1.0	235144	200276	224287
Citronellal	0.1519	14.0	1897046	1941914	1945836
β-Myrcene	1.3013	124.1	12679280	13115954	12656888
γ-Terpinene	0.809	74.6	12052429	12234682	12235968

α-Pinene	0.3393	41.7	7405430	7410922	7372603
2-Octanol	0.65	55.1	6645315	6698178	6712100

Table 51: Relative response factor for the internal standard for combined standard solution C.

Injection	$\boldsymbol{RF_{IS}}$
A	120503
B	121462
C	121714

Combined Standard Solution D

Table 52: Concentrations of five external standards and one internals standard for the fourth combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times.

Compound	**External standard solution added (g)**	**Concentration (mg/L)**	**Peak area**		
			A	**B**	**C**
Limonene	0.3984	35.6	5051799	5423025	5385426
Citronellal	0.0104	1.0	153863	172336	147497
β-Myrcene	0.2682	25.6	2524303	2394937	2413858
γ-Terpinene	1.312	121.0	19444333	19475836	19000170
α-Pinene	0.8	98.3	14620520	14583019	14463751
2-Octanol	0.594	50.4	5931480	6026675	5883203

Table 53: Relative response factor for the internal standard for combined standard solution D.

Injection	$\boldsymbol{RF_{IS}}$
A	117699
B	119588
C	116741

Combined Standard Solution E

Table 54: Concentrations of five external standards and one internals standard for the fifth combined standard solution. The peak areas are also shown. The solution was prepared once and injected three times.

Compound	External standard solution added (g)	Concentration (mg/L)	Peak area		
			A	B	C
Limonene	0.815	72.8	11485370	11502707	11075960
Citronellal	0.4013	35.8	5235182	4975780	5393203
β-Myrcene	0.006	0.5	76088	69551	87072
γ-Terpinene	0.1572	14.0	2354053	2278573	2235231
α-Pinene	1.3011	116.2	24380654	24292811	24414781
2-Octanol	0.6093	54.4	6307048	6117164	6302777

Table 55: Relative response factor for the internal standard for combined standard solution E.

Injection	RF_{IS}
A	115898
B	112408
C	115819

Calibration Curves

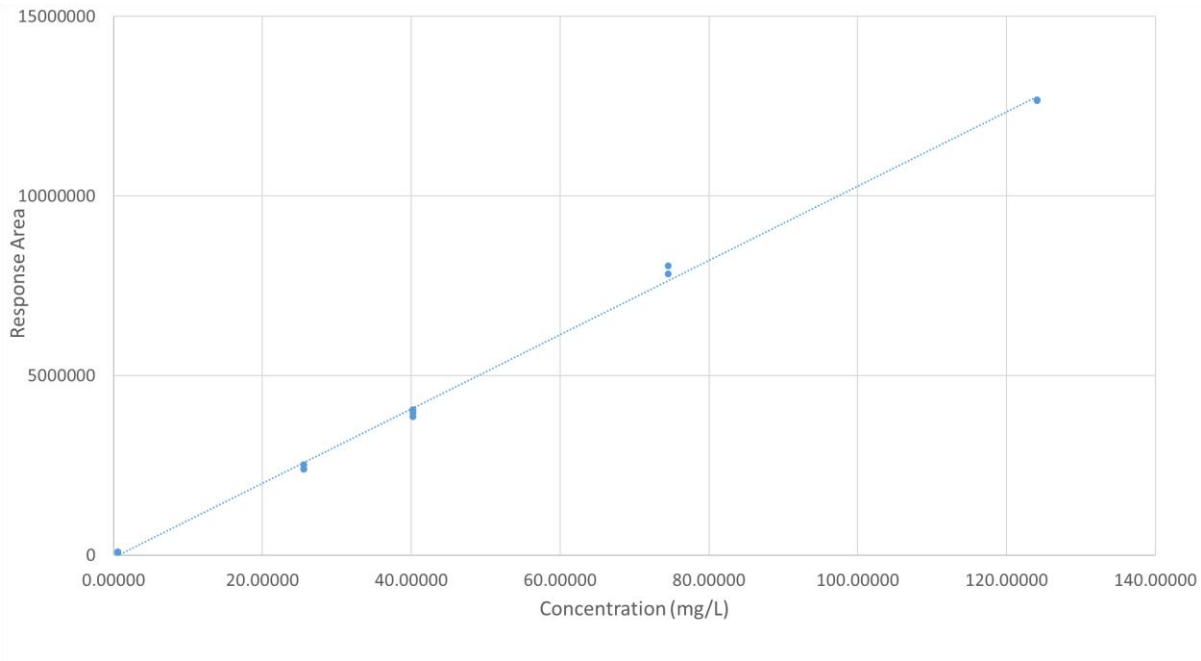

Figure 23: Calibration curve for β-myrcene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=102091x + 32741 and a R^2=0.998

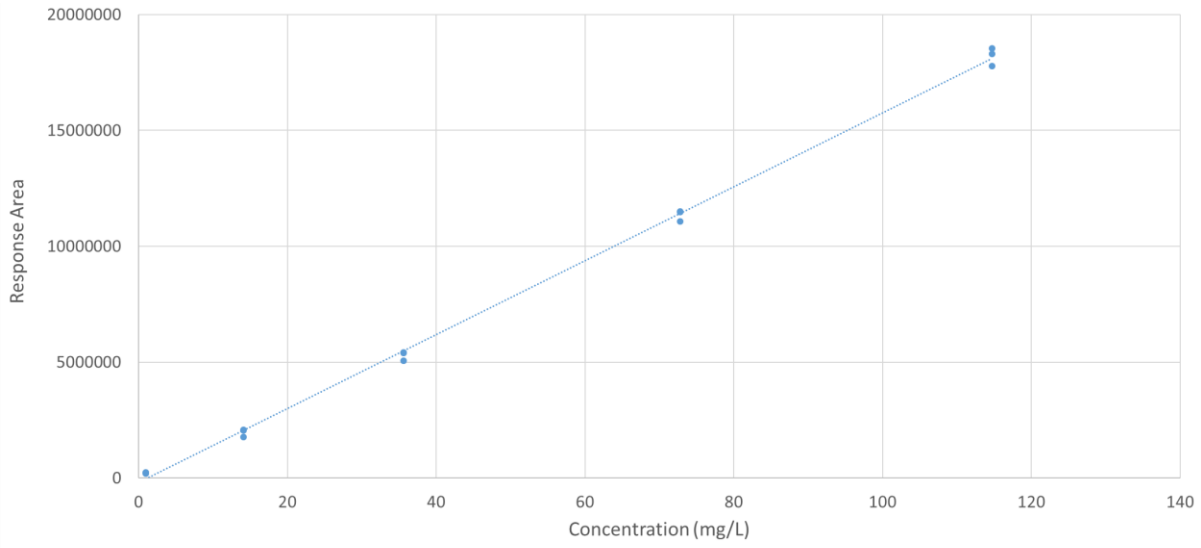

Figure 24: Calibration curve for limonene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=156370x + 66915 and a R^2=0.9979.

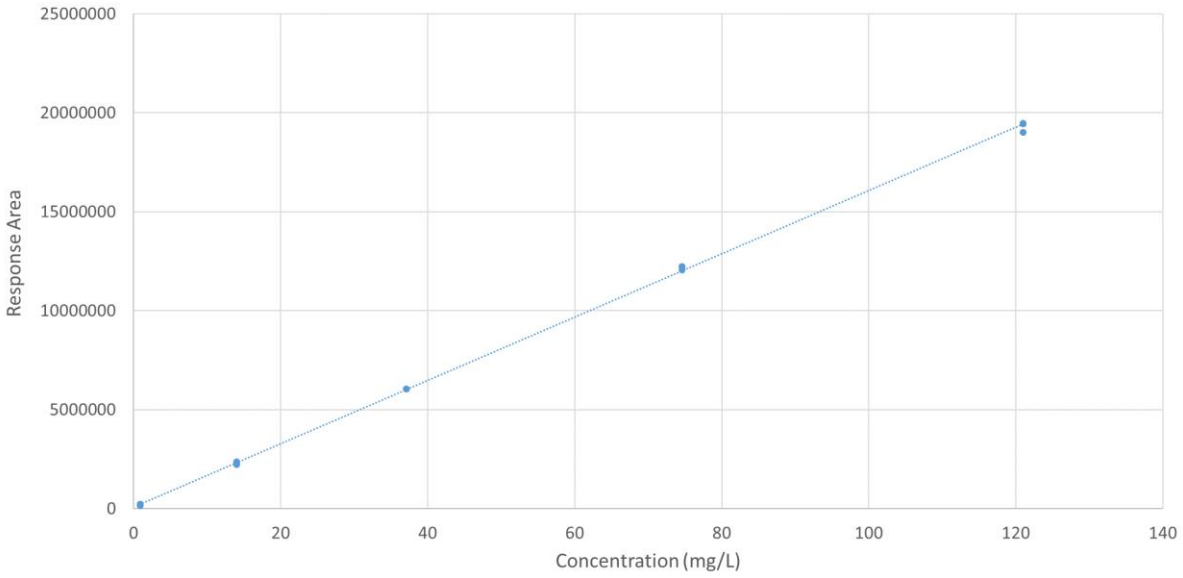

Figure 25: Calibration curve for γ-terpinene. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=159770x + 84731 and a R^2=0.9996.

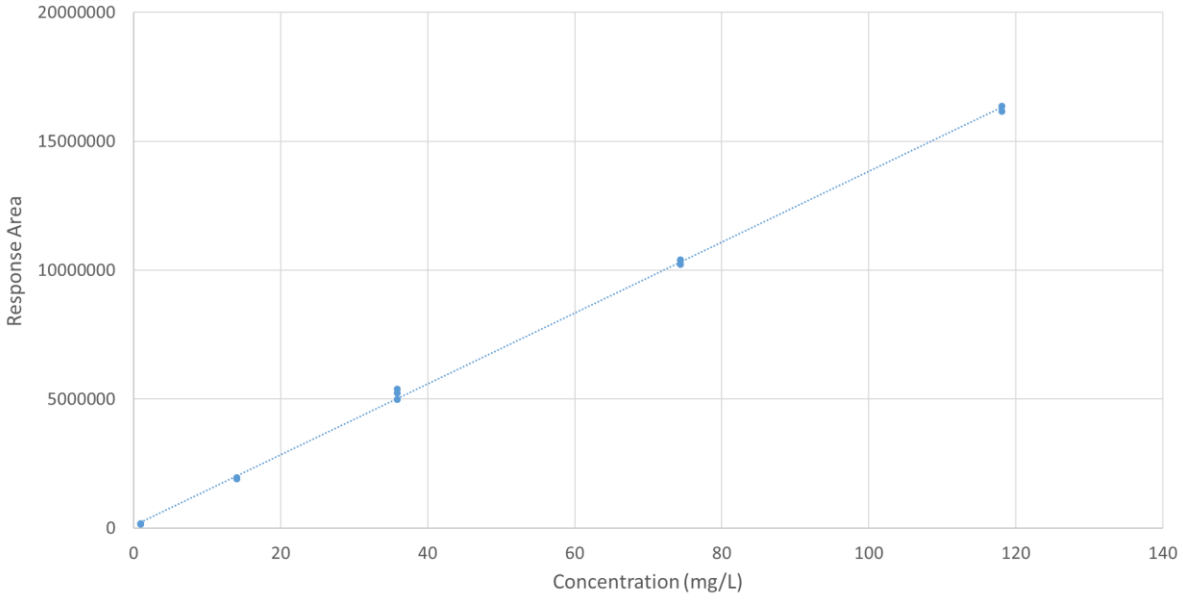

Figure 26: Calibration curve for citronellal. Standard solutions were injected in triplicate and the points are super imposed. Graph had a function of y=137536x + 85892 and a R^2=0.9993.

A.1.5 Internal Standard Calibration

Table 56: Multiple internal standard solutions were prepared over the course of this project, and they are shown below.

Solution	2-octanol (g)	Hexane (g)	Concentration (mg/L)

1	0.0244	3.0010	5132
2	0.0245	3.0031	5150
3	0.0282	3.0435	5844
4	0.0248	3.1183	5021

Table 57: The concentration and response area for 2-octanol that was used to determine the calibration curve for the Agilent GC-FID.

Concentration	**Response area**		
(mg/L)	**A**	**B**	**C**
276.7	37550644	37543707	37274725
226.7	26373138	26479386	26421115
120.8	15171482	14846350	15209839
59.1	7583185	7456419	7707202
30.3	4141122	4060335	3972184
1.2	163424	198045	167370

A.1.6 Concentration of Analytes in Gin

Table 58: Concentration of internal standard within the three gin samples.

Sample	**Sample weight**	**IS solution #**	**IS weight**	**Internal standard concentration (mg/L)**
A	0.2714	3	0.0033	70.1
B	0.2775	3	0.0051	105.3
C	0.2788	3	0.0040	82.5

Table 59: Peak areas and concentrations for analytes in Juno gin.

	Peak area			**Concentration (mg/L)**		
Compound	**A**	**B**	**C**	**A**	**B**	**C**
α-Pinene	26842305	25724912	27451522	105.5	115.3	127.7
β-Myrcene	7459985	7285313	7816845	51.0	56.9	63.3
Limonene	3829654	3579019	3713196	16.6	17.7	19.1
γ-Terpinene	1685808	1662842	1848638	7.3	8.2	9.5

Citronellal	3487479	3464769	3589326	20.3	23.0	24.7

A.1.7 Ethanol Contamination

Table 60: This table shows the compounds that were identified in the ethanol contamination investigation. Several compounds were identified but were unable to be quantified due to lack of standards and are labelled as trace amounts.

	Concentration (mg/L)		
Compound	**Trial 1**	**Trial 2**	**Trial 3**
α-Pinene	4.7	4.2	6.6
β-Myrcene	3.8	3.5	5.4
Limonene	3.1	0.3	4.3
γ-Terpinene	2.2	2.0	2.8
Citronellal	0.1	0.1	0.4
α-thujene	-	-	Trace
3-Carene	-	Trace	Trace
Camphor	Trace	-	Trace
Sabinene	Trace	Trace	trace
δ-Cadinene	Trace	-	Trace
Germacrene D	Trace	-	Trace

A.1.8 Maceration

Botanical Ratio

Where applicable the initial concentration found in the ethanol was subtracted from all subsequent samples. This resulted in some negative values since many values were close to zero hence all negative values were corrected to 0 mg/L. Concentrations have been adjusted for extraction efficiency and volume ratio.

Table 61: Sample properties and internal standard concentration data.

Trial	**Sample**	**IS solution #**	**Time (hour)**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
16.6 g/L juniper berry	1Ma	1	0	0.4500	0.0058	65.2

	2Ma	1	1	0.3246	0.0053	82.3
	3Ma	1	3	0.3034	0.0035	58.4
	4Ma	1	24	0.4026	0.0076	94.9
	5Ma	1	49	0.4556	0.0064	71.0
	6Ma	1	72	0.4536	0.0077	85.5
33.0 g/L juniper berry	1Mb	1	0	0.4414	0.0054	61.9
	2Mb	1	1	0.4871	0.0053	55.2
	3Mb	1	3	0.5165	0.0064	62.7
	4Mb	1	24	0.4920	0.0080	82.0
	5Mb	1	49	0.4556	0.0064	75.0
	6Mb	1	72	0.3986	0.0077	97.1
57.1 g/L juniper berry	1Mc	1	0	0.3840	0.0062	81.4
	2Mc	1	1	0.5127	0.0029	28.8
	3Mc	1	3	0.3713	0.0159	210.4
	4Mc	1	24	0.3925	0.0057	73.3
	5Mc	1	49	0.4820	0.0053	55.7
	6Mc	1	72	0.4508	0.0055	61.8

Solvent Concentration

Table 62: Sample properties and internal standard concentration data for crushed and vacuum botanical trials.

Trial	**Sample**	**IS solution #**	**Time (hour)**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
16.6 g/L juniper berry macerated in 78% ABV	ConcA1	2	0	0.4922	0.0033	27.2
	ConcA2	2	1	0.5183	0.0049	38.2
	ConcA3	2	3	0.4803	0.0086	71.7
	ConcA4	2	24	0.4414	0.0077	69.9

	ConcA5	2	48	0.4476	0.0023	20.8
	ConcA6	2	72	0.4753	0.0040	34.1
16.6 g/L of juniper berry macerated in 96% ABV	ConcB1	2	0	0.455	0.0063	55.7
	ConcB2	2	1	0.6194	0.0033	21.6
	ConcB3	2	3	0.4305	0.0045	42.2
	ConcB4	2	24	0.6181	0.0032	21.0
	ConcB5	2	48	0.463	0.0042	36.7
	ConcB6	2	72	0.4923	0.0053	43.6

Table 63: Peak area of key analytes in 78% ABV macerated solution with 16.6 g/L of juniper berry.

	Peak area					
Compound	**ConcA1**	**ConcA2**	**ConcA3**	**ConcA4**	**ConcA5**	**ConcA6**
Limonene	1228808	1152595	1354725	1453384	1908224	1776371
Citronellal	12861	0	60706	0	0	0
β-Myrcene	945126	829412	1101664	1683764	2832712	3082775
γ-Terpinene	710563	806589	898737	790546	897030	847069
α-Pinene	1907221	1735460	2314022	4663975	7354163	8531753
2-Octanol	13978295	11155529	16298135	16916871	9193839	6351203

Table 64: Key analyte concentration in macerated solution over time with 16.6 g/L of juniper berry at 78% ABV.

	Concentration (mg/L)					
Compound	**ConcA1**	**ConcA2**	**ConcA3**	**ConcA4**	**ConcA5**	**ConcA6**
Limonene	0.0	0.6	1.4	1.4	0.8	2.8
Citronellal	0.0	0.0	1.2	0.0	0.0	0.0
β-Myrcene	0.0	0.6	1.8	3.1	2.7	8.8
γ-Terpinene	0.0	0.5	1.0	0.7	0.2	1.2
α-Pinene	0.0	0.8	2.2	5.3	4.5	14.5

Table 65: Peak area of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry.

	Peak area					
Compound	**ConcB1**	**ConcB2**	**ConcB3**	**ConcB4**	**ConcB5**	**ConcB6**
Limonene	1697840	1564049	1701537	1698994	1852060	2081415
Citronellal	0	0	0	0	0	0
β-Myrcene	1359740	1257237	1376056	2239227	2913145	3641123
γ-Terpinene	1129534	1013409	1071993	1083281	1141887	1238614
α-Pinene	2778200	2654084	2731942	4779476	7205270	9826531
2-Octanol	13629757	6306155	11665346	12242928	9104018	8501747

Table 66: Concentration of key analytes in 96% ABV macerated solution with 16.6 g/L of juniper berry.

	Concentration (mg/L)					
Compound	**ConcB1**	**ConcB2**	**ConcB3**	**ConcB4**	**ConcB5**	**ConcB6**
Limonene	0.0	0.0	0.0	0.0	0.2	1.4
Citronellal	0.0	0.0	0.0	0.0	0.0	0.0
β-Myrcene	0.0	0.0	0.0	0.0	3.7	7.8
γ-Terpinene	0.0	0.0	0.0	0.0	0.0	0.7
α-Pinene	0.0	0.0	0.0	0.0	6.1	13.4

Botanical State

Table 67: Sample properties and internal standard concentration data for crushed and vacuum botanical trials.

Trial	**Sample**	**IS solution #**	**Time (hour)**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
16.6 g/L juniper berry crushed	CRS1	2	0	0.4822	0.0072	60.0
	CRS2	2	1	0.5398	0.0032	24.0
	CRS3	2	3	0.4550	0.0062	54.8
	CRS4	2	24	0.5027	0.0062	49.7
	CRS5	2	48	0.5043	0.0042	33.7
	CRS6	2	72	0.4441	0.0038	34.7

16.6 g/L of juniper berry under vacuum	Vac1	2	0	0.4850	0.0052	43.3
	Vac2	2	1	0.5000	0.0217	169.6
	Vac3	2	3	0.5190	0.0078	60.4
	Vac4	2	24	0.4519	0.0074	65.7
	Vac5	2	48	0.4922	0.0039	32.1

Table 68: Peak areas of key analytes in macerated solution over time with 16.6 g/L of crushed juniper berry in 58% ABV aqueous ethanol.

	Peak Area					
Compound	**CRS1**	**CRS2**	**CRS3**	**CRS4**	**CRS5**	**CRS6**
Limonene	961133	1308563	1484622	2772124	3680413	4040923
Citronellal	0	0	0	0	0	0
β-Myrcene	777234	1882237	2801347	2070945	10122580	11324965
γ-Terpinene	563971	769141	627946	679065	814635	934338
α-Pinene	1502470	4856931	8340623	21733171	30301930	34186291
2-Octanol	21441885	8753522	13459539	12799340	7943409	6264570

Table 69: Concentration of key analytes in macerated solution with 16.6 g/L g of crushed juniper berries in 58% ABV aqueous ethanol.

	Concentration (mg/L)					
Compound	**CRS1**	**CRS2**	**CRS3**	**CRS4**	**CRS5**	**CRS6**
Limonene	0.0	0.3	1.3	3.1	4.9	7.5
Citronellal	0.0	0.0	0.0	0.0	0.0	0.0
β-Myrcene	0.0	1.8	5.5	3.5	24.4	35.2
γ-Terpinene	0.0	0.2	0.4	0.4	0.7	1.3
α-Pinene	0.0	3.1	10.2	27.5	42.7	61.8

Table 70: Peak areas of key analytes in macerated solution over time with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol.

	Peak area				
Compound	**Vac1**	**Vac2**	**Vac3**	**Vac4**	**Vac5**
Limonene	996516	922459	1009601	1015750	1176138
Citronellal	0	0	0	0	0
β-Myrcene	854298	781410	827416	1182322	1504858
γ-Terpinene	681840	553000	627496	672347	563532
α-Pinene	1941390	1530750	1732473	2828575	3610867
2-Octanol	10909250	37090757	18362633	15937993	8389281

Table 71: Concentration of key analytes in macerated solution over time with 16.6 g/L of juniper berry under vacuum in 58% ABV aqueous ethanol.

	Concentration (mg/L)				
Compound	**Vac1**	**Vac2**	**Vac3**	**Vac4**	**Vac5**
Limonene	0.0	0.1	0.0	0.1	0.2
Citronellal	0.0	0.0	1.2	0.0	0.0
β-Myrcene	0.0	0.1	0.0	0.9	1.4
γ-Terpinene	0.0	0.0	0.0	0.0	0.0
α-Pinene	0.0	0.0	0.0	1.4	2.1

A.1.9 Macerate Distillation

Table 72: Sample properties and internal standard concentration data for macerate distillation for different botanical ratio trials.

Trial	**IS solution #**	**Sample**	**Sample location**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
8.3 g/L juniper berry 24 hour macerated in 60% ABV	2	MBA1	Kettle pre-boil	0.3360	0.0045	67.9
	2	MBA2	Total distillate	0.3526	0.0049	70.5
	2	MBA3	Kettle post-boil	0.3358	0.0050	75.4

16.6 g/L juniper berry 24 hour macerated in 60% ABV	2	MBB1	Kettle pre-boil	0.4590	0.0056	62.0
	2	MBB2	Total distillate	0.3423	0.0061	90.0
	2	MBB3	Kettle post-boil	0.3880	0.0062	80.9
33.1 g/L juniper berry 24 hour macerated in 60% ABV	1	MBC1	Kettle pre-boil	0.3155	0.0055	88.1
	1	MBC2	Total distillate	0.3558	0.0051	72.7
	1	MBC3	Kettle post-boil	0.2757	0.0054	98.8
63.1 g/L juniper berry 24 hour macerated in 60% ABV	4	MBD1	Kettle pre-boil	0.2874	0.0029	50.1
	4	MBD2	Total distillate	0.2874	0.0039	67.1

Table 73: Key analytes peak area in macerate distillate for 8.3 g/L and 16.6 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV aqueous ethanol.

	Peak area					
Compound	**MBA1**	**MBA2**	**MBA3**	**MBB1**	**MBB2**	**MBB3**
α-Pinene	1452422	7097954	-	743374	14788778	-
β-Myrcene	983712	2013717	542163	-	3075263	-
Limonene	94886	424335	-	-	635336	92948
γ-Terpinene	-	154978	-	-	379363	41383
2-Octanol	12454416	10727766	12114523	9362946	12885516	11300127
Citronellal	156201	152893	183176	157995	148470	165151

Table 74: Key analytes concentration in macerate distillate for 8.3 g/L and 16.6 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV.

	Concentration (mg/L)					
Compound	**MBA1**	**MBA2**	**MBA3**	**MBB1**	**MBB2**	**MBB3**
Limonene	0.2	1.1	0.0	0.0	1.7	0.3
Citronellal	0.4	0.5	0.6	0.5	0.5	0.6
β-Myrcene	3.2	7.9	2.0	0.0	12.9	0.0
γ-Terpinene	0.0	0.4	0.0	0.0	1.0	0.1
α-Pinene	2.7	16.0	0.0	1.7	35.5	0.0

Table 75: Key analytes peak area in macerate distillate for 33.0 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV.

	Peak Area				
Compound	**MBC1**	**MBC2**	**MBC3**	**MBD1**	**MBD2**
α-Pinene	832278	20509520	691823	4500620	24895634
β-Myrcene	913871	5001189	489845	1825722	7154016
Limonene	85388	1375895	110440	209691	1279353
γ-Terpinene	40099	678016	54468	162679	624464
2-Octanol	14981580	10898269	13859964	11320466	11897860
Citronellal	171086	141100	161546	120150	118087

Table 76: Key analytes concentration in macerate distillate for 33.0 g/L and 57.1 g/L of juniper berries macerated for 24 hours in 350 mL 60% ABV.

	Concentration (mg/L)				
Compound	**MBC1**	**MBC2**	**MBC3**	**MBD1**	**MBD2**
α-Pinene	1.7	47.0	1.7	6.8	48.2
β-Myrcene	3.2	19.9	2.1	4.8	24.1
Limonene	0.2	3.5	0.3	0.4	2.7
γ-Terpinene	0.1	1.7	0.1	0.3	1.3
Citronellal	0.5	0.5	0.6	0.3	0.3

A.1.10 Condensed Vapour Percolation Extraction

Table 77: Internal standard data for condensed vapour percolation trials with different botanical ratios.

Trial	IS solution #	Sample	Distillate sample segment	Sample (g)	IS (g)	Concentration (mg/L)
Quarter botanical recipe	4	CSFI	Kettle pre-boil	0.2950	0.0039	65.4
	4	CSF1	0-40 mL	0.3013	0.0029	47.8
	4	CSF2	40-80 mL	0.2847	0.0033	57.4
	4	CSF3	80-120 mL	0.2710	0.0032	58.5
	4	CSF4	120-160 mL	0.2859	0.0024	41.7
	4	CSF5	160-200 mL	0.2805	0.0029	51.3
	4	CSF6	200-238 mL	0.3003	0.0035	57.8
	4	CSFF	0-238 mL	0.2911	0.0036	61.2
Half botanical recipe	3	CSDI	Kettle pre-boil	0.2973	0.0032	53.4
	3	CSD1	0-40 mL	0.2789	0.0037	65.6
	3	CSD2	40-80 mL	0.2750	0.0040	71.9
	3	CSD3	80-120 mL	0.2935	0.0032	54.1
	4	CSD4	120-160 mL	0.2822	0.0058	101.0
	4	CSD5	160-200 mL	0.2826	0.0032	56.1
	4	CSD6	200-238 mL	0.3028	0.0038	62.1
	4	CSDF	0-238 mL	0.2859	0.0032	55.5
Three quarter recipe	4	CSGI	Kettle pre-boil	0.2815	0.0029	51.1
	4	CSG1	0-40 mL	0.2832	0.0032	56.0
	4	CSG2	40-80 mL	0.2918	0.0034	57.7
	4	CSG3	80-120 mL	0.2752	0.0033	59.4
	4	CSG4	120-160 mL	0.2817	0.0034	59.8
	4	CSG5	160-200 mL	0.2920	0.0032	54.3

	4	CSG6	200-238 mL	0.2676	0.0034	62.9
	4	CSGF	0-238 mL	0.2844	0.0034	59.2
Full recipe	4	CSEI	Kettle pre-boil	0.3038	0.0032	52.3
	4	CSE1	0-40 mL	0.2971	0.0055	91.1
	4	CSE2	40-80 mL	0.2808	0.0027	47.7
	4	CSE3	80-120 mL	0.2709	0.0040	72.9
	4	CSE4	120-160 mL	0.3058	0.0030	48.7
	4	CSE5	160-200 mL	0.2874	0.0035	60.3
	4	CSE6	200-238 mL	0.3028	0.0039	63.7
	4	CSEF	0-238 mL	0.2704	0.0039	71.3

Table 78: Key analytes peak area for condensed vapour percolation with quarter botanical recipe.

	Peak area							
Compound	**CSFI**	**CSF1**	**CSF2**	**CSF3**	**CSF4**	**CSF5**	**CSF6**	**CSFF**
α-Pinene	1476885	1734948	4495349	2628708	3183837	8356946	1469605	4208131
β-Myrcene	1052894	-	1524898	1408446	1722120	3385943	1097561	1784777
Limonene	65064	126727	360326	181858	199941	430841	83594	260310
γ-Terpinene	67143	152283	1109744	598950	293755	347254	50129	496936
2-Octanol	10672966	9627413	10926397	8951772	10976289	9186833	10375203	10577663
Citronellal	189315	193063	529878	924746	860181	1126782	446311	750971

Table 79: Key analytes concentration for condensed vapour percolation with quarter botanical recipe.

	Concentration (mg/L)							
Compound	**CSFI**	**CSF1**	**CSF2**	**CSF3**	**CSF4**	**CSF5**	**CSF6**	**CSFF**
α-Pinene	3.1	3.0	8.1	5.9	4.2	16.0	2.8	8.4
β-Myrcene	3.9	-	4.8	5.5	3.9	11.3	3.7	6.2
Limonene	0.2	0.1	0.7	0.5	0.3	0.9	0.2	0.6
γ-Terpinene	0.2	0.1	2.2	1.5	0.4	0.7	0.1	1.1
Citronellal	0.6	0.5	1.4	3.1	1.7	3.2	1.3	2.2

Table 80: Key analytes peak area for condensed vapour percolation with half botanical recipe.

	Peak area							
Compound	**CSDI**	**CSD1**	**CSD2**	**CSD3**	**CSD4**	**CSD5**	**CSD6**	**CSDF**
α-Pinene	-	3256555	7329465	5957309	6863652	8459318	16576927	15623571
β-Myrcene	-	811085	1673673	2119355	2832865	3818516	5082203	3085081
Limonene	-	265395	775775	696656	555538	704529	1381643	9473322
γ-Terpinene	-	664180	3000455	1857891	728668	441274	429909	1665189
2-Octanol	11255270	12737224	13530271	11875881	14554240	8547685	9111757	8402750
Citronellal	172386	234543	790386	1126450	959798	725874	753474	863584

Table 81: Key analytes concentration for condensed vapour percolation with half botanical recipe.

	Concentration (mg/L)							
Compound	**CSDI**	**CSD1**	**CSD2**	**CSD3**	**CSD4**	**CSD5**	**CSD6**	**CSDF**
α-Pinene	-	5.8	13.4	9.3	16.4	19.1	38.8	35.4
β-Myrcene	-	2.5	5.3	5.8	11.8	15.0	20.7	12.2
Limonene	-	0.5	1.6	1.2	1.5	1.8	3.6	23.8
γ-Terpinene	-	1.3	6.0	3.2	1.9	1.1	1.1	4.2
Citronellal	0.4	0.6	2.1	2.6	3.4	2.4	2.6	2.9

Table 82: Key analytes peak area for condensed vapour percolation with three quarter botanical recipe.

	Peak area							
Compound	**CSGI**	**CSG1**	**CSG2**	**CSG3**	**CSG4**	**CSG5**	**CSG6**	**CSGF**
α-Pinene	-	5972961	14790897	10292823	13524602	22734061	5258213	13025854
β-Myrcene	-	1776154	2919898	3218771	4390377	7181124	2866488	4403943
Limonene	-	547286	3530031	1332289	1539418	2419264	1009379	1586360
γ-Terp-inene	-	1239286	4385721	2262374	1073914	945129	241399	2030592
2-Octanol	10426966	11213461	10269086	11112016	11176409	11181914	11737916	10889117
Citronellal	141678	175701	600595	1265882	1516848	1671107	1046946	1194770

Table 83: Key analytes concentration for condensed vapour percolation with three quarter botanical recipe.

	Concentration (mg/L)							
Compound	**CSGI**	**CSG1**	**CSG2**	**CSG3**	**CSG4**	**CSG5**	**CSG6**	**CSGF**
α-Pinene	-	10.2	28.6	18.9	24.9	38.0	9.7	24.3
β-Myrcene	-	5.3	9.8	10.3	14.0	20.9	9.2	14.3

Limonene	-	1.0	7.5	2.7	3.1	4.5	2.1	3.3
γ-Terpinene	-	2.3	9.3	4.6	2.2	1.7	0.5	4.2
Citronellal	0.4	0.1	1.7	3.4	4.1	4.1	2.9	3.3

Table 84: Key analytes peak area for condensed vapour percolation with full botanical recipe.

	Peak area							
Compound	**CSEI**	**CSE1**	**CSE2**	**CSE3**	**CSE4**	**CSE5**	**CSE6**	**CSEF**
α-Pinene	-	5331584	13185364	15356353	25760999	31187255	6496401	16208520
β-Myrcene	-	1528932	3013234	5210952	9585992	11880923	3353749	6022031
Limonene	-	610218	1334121	1487494	2478980	3411308	2266622	1839279
γ-Terpinene	-	1578880	4138082	3036134	2822331	2008413	513579	2532625
2-Octanol	1251110	9025904	9875245	9768905	9100971	9305610	8898494	9562274
Citronellal	-	1491842	2183055	2971572	2964060	2825729	2106993	2440221

Table 85: Key analytes concentration for condensed vapour percolation with full botanical recipe.

	Concentration (mg/L)							
Compound	**CSEI**	**CSE1**	**CSE2**	**CSE3**	**CSE4**	**CSE5**	**CSE6**	**CSEF**
α-Pinene	-	18.5	21.9	39.4	47.4	69.4	16.0	41.5
β-Myrcene	-	9.2	8.7	23.3	30.7	46.1	14.4	26.9
Limonene	-	2.3	2.4	4.2	5.0	8.4	6.2	5.2
γ-Terpinene	-	6.0	7.6	8.6	5.7	4.9	1.4	7.2
Citronellal	-	7.7	5.4	11.3	8.1	9.3	7.7	9.3

A.1.11 Vapour Infusion

Table 86: Internal standard data for vapour infusion trials with different botanical ratios.

Trial	**IS solution #**	**Sample**	**Distillate sample segment**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
Quarter botanical recipe	4	SDDI	Kettle pre-boil	0.2755	0.0039	70.0
	4	SDD1	0-40 mL	0.3098	0.0037	59.2

	4	SDD2	40-80 mL	0.2907	0.0033	56.3
	4	SDD3	80-120 mL	0.2942	0.0039	65.6
	4	SDD4	120-160 mL	0.3000	0.0039	64.3
	4	SDD5	160-200 mL	0.2971	0.0032	53.4
	4	SDD6	200-238 mL	0.2959	0.0037	61.9
	4	SDDF	0-238 mL	0.2883	0.0037	63.5
Half botanical recipe	4	SDBI	Kettle pre-boil	0.2755	0.0039	70.0
	4	SDB1	0-40 mL	0.3098	0.0037	59.2
	4	SDB2	40-80 mL	0.2907	0.0033	56.3
	4	SDB3	80-120 mL	0.2942	0.0039	65.6
	4	SDB4	120-160 mL	0.3	0.0039	64.3
	4	SDB5	160-200 mL	0.2971	0.0032	53.4
	4	SDB6	200-238 mL	0.2959	0.0037	61.9
	4	SDBF	0-238 mL	0.2883	0.0037	63.5
Three quarter recipe	4	SDEI	Kettle pre-boil	0.2854	0.0037	64.2
	4	SDE1	0-40 mL	0.2957	0.0030	50.3
	4	SDE2	40-80 mL	0.2877	0.0033	56.8
	4	SDE3	80-120 mL	0.2932	0.0031	52.4
	4	SDE4	120-160 mL	0.2843	0.0030	52.3
	4	SDE5	160-200 mL	0.2982	0.0032	53.2
	4	SDE6	200-238 mL	0.2955	0.0031	52.0
	4	SDEF	0-238 mL	0.2828	0.0035	61.3
Full recipe	4	SDCI	Kettle pre-boil	0.2967	0.0030	54.5
	4	SDC1	0-40 mL	0.2730	0.0030	51.5
	4	SDC2	40-80 mL	0.2795	0.0029	47.9
	4	SDC3	80-120 mL	0.3004	0.0029	52.7

4	SDC4	120-160 mL	0.3107	0.0033	51.1
4	SDC5	160-200 mL	0.2814	0.0029	48.8
4	SDC6	200-238 mL	0.2948	0.0029	70.5
4	SDCF	0-238 mL	0.2734	0.0039	50.2

Table 87: Key analytes peak area for vapour infusion with quarter botanical recipe.

	Peak area							
Compound	**SDDI**	**SDD1**	**SDD2**	**SDD3**	**SDD4**	**SDD5**	**SDD6**	**SDDF**
α-Pinene	921949	5141691	5131568	5989591	6447375	9274196	57210187	12684883
β-Myrcene	-	1691007	1893112	2070866	3068841	3260113	12714991	3842736
Limonene	-	622674	491527	364107	458016	907789	9891929	1368464
γ-Terpinene	-	1409847	1019305	556493	435394	397603	1558640	804670
2-Octanol	9116161	8388015	9769336	7566887	9308984	9522458	10374032	9030542
Citronellal	160127	274423	600166	810588	982537	1331236	2985347	1057760

Table 88: Key analytes concentration for vapour infusion with quarter botanical recipe.

	Concentration (mg/L)							
Compound	**SDDI**	**SDD1**	**SDD2**	**SDD3**	**SDD4**	**SDD5**	**SDD6**	**SDDF**
α-Pinene	2.4	12.5	10.2	17.8	15.3	17.9	117.3	30.7
β-Myrcene	-	7.1	6.5	10.7	12.7	10.9	45.4	16.2
Limonene	-	1.7	1.1	1.2	1.2	1.9	22.4	3.7
γ-Terpinene	-	3.8	2.2	1.8	1.1	0.8	3.5	2.1
Citronellal	0.6	1.0	1.8	3.6	3.5	3.8	9.1	3.8

Table 89: Key analytes peak area for vapour infusion with half botanical recipe.

	Peak area							
Compound	**SDBI**	**SDB1**	**SDB2**	**SDB3**	**SDB4**	**SDB5**	**SDB6**	**SDBF**
α-Pinene	-	24264984	21886893	25647843	28474761	22375502	257135040	38402099
β-Myrcene	-	4580415	5497551	6337487	7163430	5568785	60683567	9040092
Limonene	-	1678450	1196414	1311730	1579463	1250732	18974271	2475509
γ-Terpinene	-	4136414	1857135	1102597	1074073	729692	7376276	2178684
2-Octanol	10302683	9201290	8828344	9720831	9916440	9187160	9423473	9497736
Citronellal	-	368866	550799	678026	1047274	1293674	6927938	1175793

Table 90: Key analytes concentration for vapour infusion with half botanical recipe.

	Concentration (mg/L)							
Compound	**SDBI**	**SDB1**	**SDB2**	**SDB3**	**SDB4**	**SDB5**	**SDB6**	**SDBF**
α-Pinene	-	107.7	96.3	119.5	127.6	89.8	1166.5	177.4
β-Myrcene	-	17.6	21.0	25.6	27.8	19.4	238.5	36.2
Limonene	-	4.1	2.9	3.4	3.9	2.8	47.3	6.3
γ-Terpinene	-	10.1	4.5	2.8	2.6	1.6	18.4	5.5
Citronellal	-	1.2	1.8	2.3	3.5	3.8	23.2	4.0

Table 91: Key analytes peak area for vapour infusion with three quarters botanical recipe.

	Peak area							
Compound	**SDEI**	**SDE1**	**SDE2**	**SDE3**	**SDE4**	**SDE5**	**SDE6**	**SDEF**
α-Pinene	-	19834682	18763234	26114756	34911801	48946980	254873781	70020908
β-Myrcene	-	3641894	5782646	8632174	10415931	13644739	62602052	17492867
Limonene	-	2199000	1901835	2132233	2668406	3437856	17280191	5045662
γ-Terpinene	-	7477709	3465605	2143602	1644805	1716931	5736855	4048905
2-Octanol	10581346	9381393	10831637	10095735	8321089	10135335	9609445	10374251
Citronellal	157791	525493	1101834	1438300	1901104	2965494	7086927	2466209

Table 92: Key analytes concentration for vapour infusion with three quarters botanical recipe.

	Concentration (mg/L)							
Compound	**SDEI**	**SDE1**	**SDE2**	**SDE3**	**SDE4**	**SDE5**	**SDE6**	**SDEF**
α-Pinene	-	36.6	33.8	46.6	75.5	88.3	474.2	142.1
β-Myrcene	-	11.7	18.2	26.8	39.2	42.9	202.8	61.8
Limonene	-	4.5	3.8	4.2	6.4	6.9	35.5	11.3
γ-Terpinene	-	15.2	6.9	4.2	3.9	3.4	11.8	9.1
Citronellal	0.5	1.4	2.9	3.8	6.1	7.9	19.5	7.4

Table 93: Key analytes peak area for vapour infusion with full botanical recipe.

	Peak area							
Compound	**SDCI**	**SDC1**	**SDC2**	**SDC3**	**SDC4**	**SDC5**	**SDC6**	**SDCF**
α-Pinene	3015244	38017575	52912513	82688357	93164678	181726093	475496161	84095872
β-Myrcene	-	6192529	12815805	22213400	24438033	41959171	125984833	19882472
Limonene	3460766	2853964	3602909	5191310	6105979	11569139	59303045	5735826
γ-Terpinene	40949	6883153	4535193	3384166	3091459	4496791	19232972	4018989
2-Octanol	8946277	9132952	9428879	9186427	8937520	9573751	9742674	13751773
Citronellal	144580	399505	532492	659859	1025128	1460722	3685977	782825

Table 94: Key analytes concentration for vapour infusion with full botanical recipe.

	Peak area							
Compound	**SDCI**	**SDC1**	**SDC2**	**SDC3**	**SDC4**	**SDC5**	**SDC6**	**SDCF**
α-Pinene	5.8	77.9	99.2	148.2	188.7	333.5	818.8	148.1
β-Myrcene	-	22.1	41.9	74.5	86.2	134.1	377.8	61.0
Limonene	7.3	6.5	7.5	10.3	13.7	23.5	112.9	11.2
γ-Terpinene	0.1	15.6	9.4	6.7	6.9	9.1	36.5	7.8
Citronellal	0.4	1.2	1.5	1.8	3.1	4.0	9.4	2.0

A.1.12 Process Analyte Extraction Effectiveness

Table 95: Internal standard data for process extraction effectiveness with 28 g of juniper berry.

Trial	**IS solution #**	**Sample**	**Distillate sample segment**	**Sample (g)**	**IS (g)**	**Concentration (mg/L)**
Macerate distillation	4	MJBI	Kettle pre-boil	0.2822	0.0034	59.7
	4	MJBF	0-238 mL	0.2851	0.0048	83.0
Condensed vapour percolation extraction	4	CJBI	Kettle pre-boil	0.2923	0.0032	54.3
	4	CJBF	0-40 mL	0.2849	0.0034	59.1
Vapour infusion	4	SJBI	Kettle pre-boil	0.2892	0.0031	53.2
	4	SJBF	0-40 mL	0.3031	0.0034	55.6

Table 96: Key analytes peak area for process extraction effectiveness with 28 g of juniper berry.

	Peak area					
Compound	**MJBI**	**MJBF**	**CJBI**	**CJBF**	**SJBI**	**SJBF**
α-Pinene	15384858	56610737	1892429	3965039	704582	36195668

β-Myrcene	4425173	14949708	1179350	2194780	667113	8497203
Limonene	566232	2707861	67568	345507	-	1701298
γ-Terpinene	136687	1110131	58668	143029	-	544041
2-Octanol	11116396	278578	10522066	10570472	9237200	10334153
Citronellal	132099	146247	155067	133608	136988	160381

Table 97: Key analytes concentration for process extraction effectiveness with 28 g of juniper berry.

	Concentration (mg/L)					
Compound	**MJBI**	**MJBF**	**CJBI**	**CJBF**	**SJBI**	**SJBF**
α-Pinene	28.4	5794.7	3.4	7.6	1.4	66.9
β-Myrcene	14.2	2664.8	3.6	7.3	2.3	27.4
Limonene	1.2	306.4	0.1	0.7	-	3.5
γ-Terpinene	0.3	125.3	0.1	0.3	-	1.1
Citronellal	0.4	22.2	0.4	0.4	0.4	0.4

A.1.13 Moisture content

Table 98: Moisture content analysis for juniper berries sourced from BeGin Distilling. The moisture content was conducted in triplicate and the average moisture content was 14.34% (d.b.).

Sample	**Wet berries (g)**	**Dried berries (g)**	**Water weight (g)**	**Moisture content (% d.b)**
A	2.9926	2.6172	0.3754	14.34
B	3.0526	2.6723	0.3803	14.23
C	3.0449	2.6608	0.3841	14.44